(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization

International Bureau





(10) International Publication Number WO 2016/086197 A1

(43) International Publication Date 2 June 2016 (02.06.2016)

(51) International Patent Classification: C12Q 1/68 (2006.01)

(21) International Application Number:

PCT/US2015/062787

(22) International Filing Date:

25 November 2015 (25.11.2015)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/084,127 25 November 2014 (25.11.2014) US

- (71) Applicant: THE BRIGHAM AND WOMEN'S HOS-PITAL, INC. [US/US]; 75 Francis Street, Boston, MA 02115 (US).
- (72) Inventors: EBERT, Benjamin, Levine; 47 Greenough Street, Brookline, MA 02445 (US). JAISWAL, Siddhartha; The Brigham and Women's Hospital, Inc., 75 Francis Street, Boston, MA 02115 (US). KATHIRESAN, Sekar; The Broad Institute Inc., 415 Main Street, Cambridge, MA 02142 (US).
- (74) Agents: KOWALSKI, Thomas, J. et al.; Vedder Price P.C., 1633 Broadway, New York, NY 10019 (US).
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available); ARIPO (BW. GH. GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))



(54) Title: METHOD OF IDENTIFYING AND TREATING A PERSON HAVING A PREDISPOSITION TO OR AFFLICTED WITH A CARDIOMETABOLIC DISEASE

(57) Abstract: The invention relates to method for identifying and selecting a subject with increased risk of developing a cardiometabolic disease and optionally, providing a personalized medicine method, which may involve sequencing at least part of a genome of one or more cells in a blood sample of the subject and identifying from said sequencing one or more mutations in one or more somatic mutations.

METHOD OF IDENTIFYING AND TREATING A PERSON HAVING A PREDISPOSITION TO OR AFFLICTED WITH A CARDIOMETABOLIC DISEASE

RELATED APPLICATIONS AND INCORPORATION BY REFERENCE

[0001] This application claims benefit of and priority to US provisional patent application Serial No. 62/084,127 filed November 25, 2014.

[0002] The foregoing applications, and all documents cited therein or during their prosecution ("appln cited documents") and all documents cited or referenced in the appln cited documents, and all documents cited or referenced herein ("herein cited documents"), and all documents cited or referenced in herein cited documents, together with any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention. More specifically, all referenced documents are incorporated by reference to the same extent as if each individual document was specifically and individually indicated to be incorporated by reference.

FIELD OF THE INVENTION

[0003] The present invention relates to identifying individuals with a predisposition to cardiovascular disease. In particular, the invention relates to method for identifying and selecting a subject with increased risk of developing a cardiometabolic disease and optionally a hematological cancer, and in some instances, providing a personalized medicine method.

BACKGROUND OF THE INVENTION

[0004] Cancer is thought to arise via stepwise acquisition of genetic or epigenetic changes that transform a normal cell (Nowell PC. Science 1976;194:23-8). Hence, the existence of a premalignant state bearing only the initiating lesions may be detectable in some individuals with no other signs of disease. For example, multiple myeloma (MM) is frequently preceded by monoclonal gammopathy of unknown significance (MGUS) (Kyle RA et al. The New England journal of medicine 2002;346:564-9), and chronic lymphocytic leukemia (CLL) is commonly preceded by monoclonal B-lymphocytosis (MBL) (Rawstron AC et al. The New England journal of medicine 2008;359:575-83).

1

[0005] Several lines of evidence have suggested that clonal hematopoiesis due to an expansion of cells harboring an initiating driver mutation might be an aspect of the aging hematopoietic system. Clonal hematopoiesis in the elderly was first demonstrated in studies that found that approximately 25% of healthy women over the age of 65 have a skewed pattern of X-chromosome inactivation in peripheral blood cells (Busque L et al. Blood 1996;88:59 65, Champion KM et al. British journal of haematology 1997;97:920 6) which in some cases is associated with mutations in TET2 (Busque L et al. Nature genetics 2012;44:1179-81). Largescale somatic events such as chromosomal insertions and deletions and loss of heterozygosity (LOH) also occur in the blood of ~2% of individuals over the age of 75 (Jacobs KB et al. Nature genetics 2012;44:651-8, Laurie CC et al. Nature genetics 2012;44:642-50). Pre-leukemic hematopoietic stem cells (HSCs) harboring only the initiating driver mutation have been found in the bone marrow of patients with AML in remission (Jan M et al. Science translational medicine 2012;4:149ra18, Shlush LI et al. Nature 2014;506:328-33). Furthermore, a substantial proportion of the population carries cells with t(14;18) translocations, although the lesion is generally present in fewer than 1 in 1000 cells (Roulland S et al. t(14;18) Journal of clinical oncology: official journal of the American Society of Clinical Oncology 2014;32:1347-55).

[0006] Recent sequencing studies have identified a set of recurrent mutations in several types of hematological malignancies (Mardis ER et al. The New England journal of medicine 2009;361:1058-66, Bejar R et al. The New England journal of medicine 2011;364:2496-506, Papaemmanuil E et al. The New England journal of medicine 2011;365:1384-95, Walter et al. Leukemia 2011;25:1153-8, Welch JS et al. Cell 2012;150:264-78, Cancer Genome Atlas Research N. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. The New England journal of medicine 2013;368:2059-74, Papaemmanuil E et al. Blood 2013;122:3616-27; quiz 99, Walter MJ et al. Leukemia 2013;27:1275-82, Zhang J et al. Proceedings of the National Academy of Sciences of the United States of America 2013;110:1398-403, Morin RD et al. Nature 2011;476:298-303, Lenz G et al. Science 2008;319:1676-9, Lohr JG et al. Proceedings of the National Academy of Sciences of the United States of America 2012;109:3879-84, Neumann M et al. Blood 2013;121:4749-52). However, the frequency of these somatic mutations in the general population is unknown. Citation or identification of any document in this application is not an admission that such document is available as prior art to the present invention.

SUMMARY OF THE INVENTION

[0007] The present invention relates to Applicants' hypothesis that somatically acquired single nucleotide variants (SNVs) and small insertions/deletions (indels) might be increasingly detectable in the blood of otherwise healthy individuals as a function of age.

[0008] The invention relates to method for identifying and selecting a subject with increased risk of developing a cardiometabolic disease and optionally a hematological cancer, which may comprise the steps of: (a) sequencing at least part of a genome which may comprise one or more genes selected from the group consisting of *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *JAK2* and *SF3B1* of one or more cells in a blood sample of the subject, (b) identifying from said sequencing one or more mutations in one or more genes selected from the group consisting of *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *JAK2* and *SF3B1*, wherein presence of said mutation(s) indicates an increased risk of developing a cardiometabolic disease and optionally a hematological cancer.

[0009] The invention also relates to a method for identifying and selecting a subject with an increased risk of developing a cardiometabolic disease and optionally a hematological cancer and providing a personalized medicine method, said method which may comprise the steps of (a) sequencing at least part of a genome which may comprise one or more genes selected from the group consisting of *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *JAK2* and *SF3B1* of one or more cells in a blood sample of the subject, (b) identifying from said sequencing one or more mutations in one or more genes selected from the group consisting of *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *JAK2* and *SF3B1*, wherein presence of said mutation(s) indicates an increased risk of developing a cardiometabolic disease and optionally a hematological cancer, and (c) initiating a treatment or monitoring regimen to suppress said mutation(s) in the subject, thereby decreasing risk of developing a cardiometabolic disease and optionally a hematological cancer.

[0010] The presence of said mutation(s) in the above embodiments may indicate an increase in red blood cell distribution width (RDW).

[0011] The cardiometabolic disease may be atherosclerosis, coronary heart disease (CHD) or ischemic stroke (IS) or type 2 diabetes (T2D).

[0012] In embodiments wherein an increased risk for hematological cancer is also screened in addition to a cardiometabolic disease, the hematological cancer may be a leukemia, a lymphoma, a myeloma or a blood syndrome. The leukemia may be an acute myeloid leukemia (AML), chronic myelogenous leukemia (CML) or chronic lymphocytic leukemia (CLL). The

blood syndrome may be myelodysplastic syndrome (MDS) or myeloproliferative neoplasm (MPN).

[0013] The one more cells in the blood sample may be derived from hematopoietic stem cells (HSCs), committed myeloid progenitor cells having long term self-renewal capacity or mature lymphoid cells having long term self-renewal capacity.

[0014] In some embodiments the part of the genome that is sequenced may be an exome. In other embodiments, the sequencing may be whole exome sequencing (WES) or targeted gene sequencing..

[0015] In an advantageous embodiment, the subject is a human. In other embodiments, the human may exhibit one or more risk factors of being a smoker, having a high level of total cholesterol or having high level of high-density lipoprotein (HDL).

[0016] The mutations of at least *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *JAK2* and *SF3B1* may be frameshift mutations, nonsense mutations, missense mutations or splice-site variant mutations.

[0017]If the mutation is in DNMT3A, the mutation may advantageously be a mutation in exons 7 to 23. In a particularly advantageous embodiment, the mutation in DNMT3A is a mutation selected from the group consisting of P307S, P307R, R326H, R326L, R326C, R326S, R366P, R366H, R366G, A368T, F414L, F414S, F414C, C497Y, Q527H, Q527P, Y533C, G543A, G543S, G543C, L547H, L547P, L547F, M548I, M548K, G550R, W581R, W581G, W581C, G646V, G646E, L653W, L653F, V657A, V657M, R659H, Y660C, R676W, R676Q, G685R, G685E, G685A, D686Y, D686G, G699R, G699S, G699D, P700S, P700R, P700Q, D702N, D702Y, V704M, V704G, I705F, I705T, I705S, C710S, S714C, N717S, N717I, P718L, R720H, R720G, Y724C, R729Q, R729W, R729G, F731L, F732del, F732S, F732L, F734L, F734C, Y735C, Y735N, Y735S, R736H, R736C, R736P, L737H, L737V, L737F, L737R, A741V, R749C, R749L, F751L, F752del, F752C, F752L, F752I, F752V, L754R, L754H, F755S, F755I, F755L, M761I, M761V, G762C, S770W, S770P, R771Q, F772I, F772V, L773R, E774K, E774D, D781G, R792H, G796D, G796V, N797Y, N797H, P799R, P799H, R803S, P804S, P804L, S828N, K829R, Q842E, P849L, D857N, W860R, F868S, G869S, G869V, M880V, S881R, S881I, R882H, R882P, R882C, R882G, Q886R, G890D, L901R, L901H, P904L, F909C and A910P.

[0018] If the mutation is in *TET2*, the mutation is advantageously selected from the group consisting of S282F, N312S, L346P, S460F, D666G, P941S, and C1135Y.

[0019] If this mutation is in *ASXL1*, the mutation is advantageously a mutation in exon 11-12.

[0020] If the mutation is in TP53, the mutation is advantageously a mutation selected from the group consisting of S46F, G105C, G105R, G105D, G108S, G108C, R110L, R110C, T118A, T118R, T118I, L130V, L130F, K132Q, K132E, K132W, K132R, K132M, K132N, C135W, C135S, C135F, C135G, O136K, O136E, O136P, O136R, O136L, O136H, A138P, A138V, A138A, A138T, T140I, C141R, C141G, C141A, C141Y, C141S, C141F, C141W, V143M, V143A, V143E, L145O, L145R, P151T, P151A, P151S, P151H, P152S, P152R, P152L, T155P. R158H, R158L, A159V, A159P, A159S, A159D, A161T, A161D, Y163N, Y163H, Y163D, Y163S, Y163C, K164E, K164M, K164N, K164P, H168Y, H168P, H168R, H168L, H168Q, M169I, M169T, M169V, T170M, E171K, E171Q, E171G, E171A, E171V and E171D, V172D, V173M, V173L, V173G, R174W, R175G, R175C, R175H, C176R, C176G, C176Y, C176F, C176S, P177R, P177R, P177L, H178D, H178P, H178Q, H179Y, H179R, H179Q, R181C, R181Y, D186G, G187S, P190L, P190T, H193N, H193P, H193L, H193R, L194F, L194R, I195F, I195N, I195T, V197L, G199V, Y205N, Y205C, Y205H, D208V, R213Q, R213P, R213L, R213Q, H214D, H214R, S215G, S215I, S215R, V216M, V217G, Y220N, Y220H, Y220S, Y220C, E224D, I232F, I232N, I232T, I232S, Y234N, Y234H, Y234S, Y234C, Y236N, Y236H, Y236C, M237V, M237K, M237I, C238R, C238G, C238Y, C238W, N239T, N239S, S241Y, S241C, S241F, C242G, C242Y, C242S, C242F, G244S, G244C, G244D, G245S, G245R, G245C, G245D, G245A, G245V, G245S, M246V, M246K, M246R, M246I, N247I, R248W, R248G, R248Q, R249G, R249W, R249T, R249M, P250L, I251N, L252P, I254S, I255F, I255N, 1255S, L257Q, L257P, E258K, E258Q, D259Y, S261T, G262D, G262V, L265P, G266R, G266E, G266V, R267W, R267Q, R267P, E271K, V272M, V272L, R273S, R273G, R273C, R273H, R273P, R273L, V274F, V274D, V274A, V274G, V274L, C275Y, C275S, C275F, A276P, C277F, P278T, P278A, P278S, P278H, P278R, P278L, G279E, R280G, R280K, R280T, R280I, R280S, D281N, D281H, D281Y, D281G, D281E, R282G, R282W, R282Q, R282P, E285K, E285V, E286G, E286V, E286K, K320N, L330R, G334V, R337C,R337L, A347T, L348F, T377P.

[0021] If the mutation is in *JAK2*, the mutation is advantageously selected from the group consisting of N533D, N533Y, N533S, H538R, K539E, K539L, I540T, I540V, V617F, R683S,

R683G, del/ins537---539L, del/ins538---539L, del/ins540---543MK, del/ins540---544MK, del/ins541- -543K, del542---543, del543---544 and ins11546---547.

[0022] If the mutation is in *SF3B1*, the mutation is advantageously selected from the group consisting of G347V, R387W, R387Q, E592K, E622D, Y623C, R625L, R625C, H662Q, H662D, K666N, K666T, K666E, K666R, K700E, V701F, A708T, G740R, G740E, A744P, D781G and E783K.

[0023] Accordingly, it is an object of the invention to not encompass within the invention any previously known product, process of making the product, or method of using the product such that Applicants reserve the right and hereby disclose a disclaimer of any previously known product, process, or method. It is further noted that the invention does not intend to encompass within the scope of the invention any product, process, or making of the product or method of using the product, which does not meet the written description and enablement requirements of the USPTO (35 U.S.C. §112, first paragraph) or the EPO (Article 83 of the EPC), such that Applicants reserve the right and hereby disclose a disclaimer of any previously described product, process of making the product, or method of using the product.

[0024] It is noted that in this disclosure and particularly in the claims and/or paragraphs, terms such as "comprises", "comprised", "comprising" and the like can have the meaning attributed to it in U.S. Patent law; e.g., they can mean "includes", "included", "including", and the like; and that terms such as "consisting essentially of" and "consists essentially of" have the meaning ascribed to them in U.S. Patent law, e.g., they allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention.

[0025] These and other embodiments are disclosed or are obvious from and encompassed by, the following Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] The following detailed description, given by way of example, but not intended to limit the invention solely to the specific embodiments described, may best be understood in conjunction with the accompanying drawings.

[0027] Figure 1. Prevalence of somatic mutation by age. Colored bands represent 50th, 75th, and 95th percentiles.

[0028] Figure 2. Characteristics of called somatic variants. A) Ten most frequently mutated genes. B) Number of subjects with 1, 2, 3, or 4 called variants. C) Percentages of type of single nucleotide base pair changes seen in the called variants. D) Allele fractions of called somatic variants. Allele fraction defined as variant reads divided by variant plus reference reads. For variants on the X chromosome in men this number was divided by 2.

[0029] Figure 3. Development of hematologic malignancies. A) Forest plot for risk of developing hematologic malignancy in those with somatic mutations overall and with VAF≥0.10, relative to those without mutations. Diamond represents the results of fixed-effects meta-analysis for the 2 cohorts, and horizontal lines are 95 percent confidence intervals. Hazard ratios were estimated by competing risks regression with death as the competing risk. The analysis includes adjudicated cancer information from MEC and unadjudicated information by annual subject interview in JHS. For interview data, leukemia, lymphoma, multiple myeloma, blood cancer, and spleen cancer were considered hematologic malignancy. All models included age groups (less than 50, 50-59, 60-69, greater than 70), diabetes status, and sex as covariates. B) Cumulative incidence plot for developing hematologic malignancy. Curves were generated from competing risks data with death as the competing risk. C) VAF in subjects that did or did not develop hematologic malignancy, p-value from Wilcoxon test.

[0030] Figure 4. Effect of somatic mutations on all-cause mortality. A) Forest plot for all-cause mortality risk associated with having a somatic clone. Diamond represents the results of fixed-effects meta-analysis of all cohorts, and horizontal lines are 95 percent confidence intervals. All models included age groups (less than 60, 60-69, 70-79, 80-89, and 90 or greater), diabetes status, and sex as covariates in a Cox proportional hazards model. Botnia includes Helsinki-sib and Diabetes-reg. B) Kaplan-Meier survival curves from the same cohorts as above, with p-values from log rank test. Left panel is those younger than 70 at time of DNA ascertainment, and right panel is those 70 or older. C) Cox proportional hazards model for all-cause mortality for those with or without mutations stratified by normal or high RDW. Diamond represents the results of fixed-effects meta-analysis of all cohorts, and horizontal lines are 95 percent confidence intervals. All models included age groups (less than 60, 60-69, 70-79, 80-89, and 90 or greater), diabetes status, and sex as covariates.

[0031] Figure 5. Association of somatic mutations with incident cardiovascular disease. A-B) Cumulative incidence plots for incident coronary heart disease (CHD) (A) and ischemic

stroke (B). Curves were generated from competing risks data with death as the competing risk. Those with prevalent events were excluded from the analyses. C-D) Forest plots for risk of developing incident CHD (C) and ischemic stroke (D) in those with somatic mutations. Diamond represents the results of fixed-effects meta-analysis using beta-coefficients from competing risks regressions for both cohorts, and horizontal lines are 95 percent confidence intervals. Age groups (less than 50, 50-59, 60-69, and 70 or greater), T2D status, sex, systolic blood pressure groups (less than 140 mm Hg, 140-160 mm Hg, and greater than 160 mm Hg), and body mass index groups (less than 25, 25-35, and greater than 35) were included as categorical covariates in the competing risks regression models, with death as the competing risk. Those with prevalent events were excluded from the analyses.

[0032] Figure 6. Characteristics of *DNMT3A* variants. A) Frequency of *DNMT3A* nonsense, frameshift, and splice-site variants called as somatic by age group. B) Frequency of R882 and non-R882 missense variants called as somatic by age group. C) Allele fraction of called *DNMT3A* variants by mutation type.

[0033] Figure 7. Co-mutations. A) Co-mutation plot, individuals are represented by columns. Black rectangles represent mutated genes, red rectangles represent 2 separate mutations in the same gene. B) Correlation plot for variant allele fraction (VAF) from the 49 subjects with 2 mutations.

[0034] Figure 8. Factors associated with clonality A) Frequency of mutation for males and females by age group. For those 60 or older, being male is associated with having a detectable clone (OR 1.3, 95% CI 1.1-1.5, p=0.005 by multivariable logistic regression using age, sex, T2D and BMI as covariates). B) Frequency of mutation for those with and without type 2 diabetes by age group. C) Frequency of mutation for non-Hispanics, Hispanics, and South Asians by age group.

[0035] Figure 9. Mutations by ethnic backgroundNumber of mutations for each gene stratified by ethnic background.

[0036] Figure 10. Blood counts for individuals with and without detectable mutations. Dots represent individuals. Box represents 25th and 75th percentiles, line in box represents median. Whiskers represent 5th and 95th percentiles. For listed genes, individuals only had mutations in that gene, and not other genes. Dashed red lines represent 11.5% and 14.5%, the normal ranges for RDW. Abbreviations: WBC-white blood cell count, PLT-platelet count, RDW-red cell

distribution width, MCV-mean corpuscular volume. Individuals were from Jackson Heart Study, Longevity Genes Project, Botnia, Helsinki-sib, or Malmo-sib.

[0037] Figure 11. Kaplan-Meier Curves for overall survival by cohorts.

[0038] Figure 12. Kaplan-Meier curves for individuals with or without clones, stratified by high (≥14.5%) or normal (<14.5%) red cell distribution width. Hash marks represent censored individuals. Individuals were from Jackson Heart Study or Longevity Genes Project.

[0039] Figure 13. Risk model for coronary heart disease and ischemic stroke. Regression parameters are same as shown for Figure 5C and 5D.Hazard ratios were estimated using competing risks regression with death as the competing risk. P-values are derived from the Fine-Gray test. Individuals with prior coronary heart disease (CHD) were excluded for CHD analysis, and individuals with prior ischemic stroke were excluded for stroke analysis. A) Coronary heart disease, B) ischemic stroke.

[0040] Figure 14. Mutation validation and serial samples. A) Eighteen variants were validated using targeted, amplicon based re-sequencing ("Rapid Heme Panel", see Methods). Comparison of VAF between the two methods is shown for the 18 variants. WES, whole exome sequencing. RHP, Rapid Heme Panel. B) Peripheral blood DNA from 4 to 8 years after the initial DNA collection was available for 13 subjects in JHS who had detectable mutations on exome sequencing. As described in the Supplementary Methods, amplicon-based targeted resequencing was performed for a panel of 95 genes. Graphs represent individual subjects, with mutation variant allele frequency (VAF) from the initial and later time points shown. All of the initial mutations detected on exome sequencing were still present in the later sample, and 2 subjects had acquired new mutations.

[0041] Figure 15. Risk of cancer, cardiovascular, or other death associated with clonality. Hazard ratios obtained by competing risks regression, with death by other causes as the competing risk. Results shown are risk associated with clonality. For cancer deaths, only non-hematologic cancers were included. Cardiovascular deaths included strokes (hemorrhagic or ischemic) and fatal myocardial infarction. All regressions included age groups (less than 50, 50-59, 60-69, 70 or older), diabetes status, and gender as covariates. Individuals were from Botnia, FUSION, MEC, and Jackson Heart Study. For Jackson Heart Study, only cardiovascular outcomes were adjudicated (all other deaths were considered unadjudicated).

[0042] Figure 16. (A) Forest plot for odds ratio (OR) of having a somatic mutation in those presenting with myocardial infarction (MI) compared to those without MI (referent). Results are shown for 2 cohorts (ATVB and PROMIS) and stratified by age groups. (B) Counts for number of mutations seen in cases versus controls for the most frequently mutated genes.

[0043] Figure 17. (A) Low power sections of the aortic root from recipients of Tet2-/marrow (top row) or control marrow (bottom row) are shown at three time-points after initiation of diet. The left and center columns are oil red O stained, and the right column is Masson's trichrome. (B) Spleen (top row, H&E) and digit (bottom row, oil red O) histology are shown in mice after 14 weeks on diet. Note the large fat deposits (white arrowheads) and sheets of lipid-laden macrophages in the tissues (black arrowheads) of Tet2-/- recipients.

[0044] Figure 18. Risk of cardiovascular events from carrying a somatic clonal mutation. Risk if cardiovascular events (myocardial infarction, coronary revascularization, or stroke) in each cohort and fixed effects meta-analysis are displayed. Effect estimates are derived from a Cox proportional hazards model after accounting for age, sex, ethnicity, diabetes mellitus, smoking, hypertension, LDL cholesterol. There was no evidence of heterogeneity between the effect estimates in the two cohorts (P for heterogeneity = 0.79).

[0045] Figure 19. Coronary arterial calcification quantity by somatic clonal mutation carrier status. Among participants in the BioImage cohort, coronary arterial calcification (CAC) quantity was obtained as the total Agatston score. The relative difference in CAC in somatic clonal mutation carriers compared to non-carriers is estimated from linear regression with log-transformation of CAC quantity.

DETAILED DESCRIPTION OF THE INVENTION

[0046] The incidence of hematological malignancies increases with age and is associated with recurrent somatic mutations in specific genes. Applicants hypothesized that such mutations would be detectable in the blood of some individuals not known to have hematological disorders.

[0047] Applicants analyzed whole exome sequencing data from peripheral blood cell DNA

of 17,182 individuals who were unselected for hematologic phenotypes. Applicants looked for somatic mutations by identifying previously characterized single nucleotide variants (SNVs) and small insertions (index), in 160, genes, recurrently, mutated in hematological

malignancies. The presence of mutations was analyzed for association to hematological phenotypes, survival, and cardiovascular events.

[0048] Detectable somatic mutations were rare in individuals younger than 40, but rose appreciably with age. At ages 70-79, 80-89, and 90-108 these clonal mutations were observed in 9.6% (220 out of 2299), 11.7% (37 out of 317), and 18.4% (19 out of 103) of individuals, respectively. The majority of the variants occurred in 3 genes: DNMT3A, TET2, and ASXL1. The presence of a somatic mutation was associated with increased risk of developing hematologic malignancy (HR 11, 95% confidence interval [95% CI] 3.9-33), increased all-cause mortality (HR 1.4, 95% CI 1.1-1.8), and increased risk of incident coronary heart disease (HR 2.0, 95% CI 1.2-3.4) and ischemic stroke (HR 2.6, 95% CI 1.4-4.8).

[0049] Clonal hematopoiesis of indeterminate potential (CHIP) is a common pre-malignant condition in the elderly, and is associated with increased risk of transformation to hematologic malignancy and increased all-cause mortality, possibly due to increased cardiometabolic disease.

[0050] Cardiovascular disease is the leading cause of death worldwide. Given the association of somatic mutations with all-cause mortality beyond that explicable by hematologic malignancy and T2D, Applicants performed association analyses from two cohorts comprising 3,353 subjects with available data on coronary heart disease (CHD) and ischemic stroke (IS). After excluding those with prevalent events, Applicants found that those carrying a mutation had increased cumulative incidence of both CHD and IS (Figure 5A and 5B). In multivariable analyses that included age, sex, T2D, systolic blood pressure, and body mass index as covariates, the hazard ratio of incident CHD and IS was 2.0 (95% CI 1.2-3.5, P=0.015) and 2.6 (95% CI 1.3-4.8, P=0.003) in the individuals carrying a somatic mutation as compared to those without (Figure 5C and 5D, Figure 8).

[0051] For a subset of individuals, the traditional risk factors of smoking, total cholesterol, and high-density lipoprotein were also available; the presence of a somatic mutation remained significantly associated with incident CHD and IS even in the presence of these risk factors, and the risk was even greater in those with VAF≥0.10 (Supplementary Table S12). Elevated RDW and high-sensitivity C-reactive protein (hsCRP) have also been associated with adverse cardiac outcomes (Tonelli M et al. Circulation 2008;117:163-8, Ridker PM et al. The New England journal of medicine 2002;347:1557-65), possibly reflecting an underlying inflammatory cause. In a multivariable analysis of 1,795 subjects from JHS, those with a mutation and RDW≥14.5%

had a markedly increased risk of incident CHD, and this effect was independent of hsCRP (Supplementary Table S13).

[0052] Applicants find that somatic mutations leading to clonal outgrowth of hematopoietic cells are frequent in the general population. This entity, which Applicants term clonal hematopoiesis with indeterminate potential (CHIP), is present in over 10% of individuals over 70, making it one of the most common known pre-malignant lesions. The exact prevalence of CHIP is dependent on how cancer-causing mutations are defined and on the sensitivity of the technique used to detect mutations, and thus may substantially exceed this estimate. Unlike other pre-malignant lesions, CHIP appears to involve a substantial proportion of the affected tissue in most individuals; based on the proportion of alleles with the somatic mutation, Applicants find that a median of 18% of peripheral blood leukocytes are part of the abnormal clone. CHIP also persists over time; in all tested cases, the mutations were still present after 4 to 8 years.

[0053] The genes most commonly mutated in CHIP are *DNMT3A*, *TET2*, and *ASXL1*. This is consistent with previous studies that have found *DNMT3A* and *TET2* mutations to be frequent and early events in AML and MDS (Jan M et al. Science translational medicine 2012;4:149ra18, Shlush LI et al. Nature 2014;506:328-33, Papaemmanuil E et al. The New England journal of medicine 2011;365:1384-95, Welch JS et al. Cell 2012;150:264-78). Murine models of *DNMT3A* or *TET2* loss-of-function demonstrate that mutant HSCs have altered methylation patterns at pluripotency genes and a competitive advantage compared to wild-type HSCs, but mice rarely develop frank malignancy, and then only after long latency (Jeong M et al. Nature genetics 2014;46:17-23, Koh KP et al. Cell stem cell 2011;8:200-13, Challen GA et al. Nature genetics 2012;44:23-31, Moran-Crusio K et al. Cancer cell 2011;20:11-24). Similarly, Applicants' data show that humans with CHIP can live for many years without developing hematological malignancies, though they do have increased risk relative to those without mutations.

[0054] TET2 and DNMT3A are frequently mutated in some lymphoid malignancies, and the initiating event for such tumors may occur in a HSC (Neumann M et al. Blood 2013;121:4749-52, Quivoron C et al. Cancer cell 2011;20:25-38, Odejide O et al. Blood 2014;123:1293-6, Asmar F et al. Haematologica 2013;98:1912-20, Couronne L et al. The New England journal of medicine 2012;366:95-6). While it is most likely that these mutations occur in a HSC, it also

possible that they occur in committed myeloid progenitors or mature lymphoid cells that have acquired long-term self-renewal capacity.

[0055] The use of somatic mutations to aid in the diagnosis of patients with clinical MDS is becoming widespread. Applicants' data demonstrate that the majority of individuals with clonal mutations in peripheral blood do not have MDS or another hematological malignancy, nor do the majority develop a clinically diagnosed malignancy in the near term. At this time, it would be premature to genetically screen healthy individuals for the presence of a somatic clone, as the positive predictive value for current or future malignancy is low.

[0056] The invention relates to method for identifying and selecting a subject with increased risk of developing a cardiometabolic disease and optionally a hematological cancer, which may comprise the steps of: (a) sequencing at least part of a genome which may comprise one or more genes selected from the group consisting of *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *JAK2* and *SF3B1* of one or more cells in a blood sample of the subject, (b) identifying from said sequencing one or more mutations in one or more genes selected from the group consisting of *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *JAK2* and *SF3B1*, wherein presence of said mutation(s) indicates an increased risk of developing a cardiometabolic disease and optionally a hematological cancer.

[0057] The invention also relates to a method for identifying and selecting a subject with an increased risk of developing a cardiometabolic disease and optionally a hematological cancer and providing a personalized medicine method, said method which may comprise the steps of (a) sequencing at least part of a genome which may comprise one or more genes selected from the group consisting of *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *JAK2* and *SF3B1* of one or more cells in a blood sample of the subject, (b) identifying from said sequencing one or more mutations in one or more genes selected from the group consisting of *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *JAK2* and *SF3B1*, wherein presence of said mutation(s) indicates an increased risk of developing a cardiometabolic disease and optionally a hematological cancer, and (c) initiating a treatment or monitoring regimen to suppress said mutation(s) in the subject, thereby decreasing risk of developing a cardiometabolic disease and optionally a hematological cancer.

[0058] The presence of said mutation(s) in the above embodiments may indicate an increase in red blood cell distribution width (RDW).

[0059] The cardiometabolic disease may be atherosclerosis, coronary heart disease (CHD) or ischemic stroke (IS).

[0060] In embodiments wherein an increased risk for hematological cancer is also screened in addition to a cardiometabolic disease, the hematological cancer may be a leukemia, a lymphoma, a myeloma or a blood syndrome. The leukemia may be an acute myeloid leukemia (AML) or chronic myelogenous leukemia (CML). The blood syndrome may be myelodysplastic syndrome (MDS).

[0061] The one more cells in the blood sample may be hematopoietic stem cells (HSCs), committed myeloid progenitor cells having long term self-renewal capacity or mature lymphoid cells having long term self-renewal capacity.

[0062] In some embodiments the part of the genome that is sequenced may be an exome. In other embodiments, the sequencing may be whole exome sequencing (WES).

[0063] In an advantageous embodiment, the subject is a human. In other embodiments, the human may exhibit one or more risk factors of being a smoker, having a high level of total cholesterol or having high level of high-density lipoprotein (HDL).

[0064] The mutations of at least *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *JAK2* and *SF3B1* may be frameshift mutations, nonsense mutations, missense mutations or splice-site variant mutations.

If the mutation is in DNMT3A, the mutation may advantageously be a mutation in [0065] exons 7 to 23. In a particularly advantageous embodiment, the mutation in DNMT3A is a mutation selected from the group consisting of P307S, P307R, R326H, R326L, R326C, R326S, R366P, R366H, R366G, A368T, F414L, F414S, F414C, C497Y, Q527H, Q527P, Y533C, G543A, G543S, G543C, L547H, L547P, L547F, M548I, M548K, G550R, W581R, W581G, W581C, G646V, G646E, L653W, L653F, V657A, V657M, R659H, Y660C, R676W, R676Q, G685R, G685E, G685A, D686Y, D686G, G699R, G699S, G699D, P700S, P700R, P700Q, D702N, D702Y, V704M, V704G, I705F, I705T, I705S, C710S, S714C, N717S, N717I, P718L, R720H, R720G, Y724C, R729Q, R729W, R729G, F731L, F732del, F732S, F732L, F734L, F734C, Y735C, Y735N, Y735S, R736H, R736C, R736P, L737H, L737V, L737F, L737R, A741V, R749C, R749L, F751L, F752del, F752C, F752L, F752I, F752V, L754R, L754H, F755S, F755I, F755L, M761I, M761V, G762C, S770W, S770P, R771Q, F772I, F772V, L773R, E774K, E774D, D781G, R792H, G796D, G796V, N797Y, N797H, P799R, P799H, R803S, P804S, P804L, S828N, K829R, Q842E, P849L, D857N, W860R, F868S, G869S, G869V, M880V, S881R, S881I, R882H, R882P, R882C, R882G, Q886R, G890D, L901R, L901H, P904L, F909C and A910P.

[0066] If the mutation is in *TET2*, the mutation is advantageously selected from the group consisting of S282F, N312S, L346P, S460F, D666G, P941S, and C1135Y.

[0067] If this mutation is in *ASXL1*, the mutation is advantageously a mutation in exon 11-12.

[0068]If the mutation is in TP53, the mutation is advantageously a mutation selected from the group consisting of S46F, G105C, G105R, G105D, G108S, G108C, R110L, R110C, T118A, T118R, T118I, L130V, L130F, K132Q, K132E, K132W, K132R, K132M, K132N, C135W, C135S, C135F, C135G, Q136K, Q136E, Q136P, Q136R, Q136L, Q136H, A138P, A138V, A138A, A138T, T140I, C141R, C141G, C141A, C141Y, C141S, C141F, C141W, V143M, V143A, V143E, L145Q, L145R, P151T, P151A, P151S, P151H, P152S, P152R, P152L, T155P, R158H, R158L, A159V, A159P, A159S, A159D, A161T, A161D, Y163N, Y163H, Y163D, Y163S, Y163C, K164E, K164M, K164N, K164P, H168Y, H168P, H168R, H168L, H168Q, M169I, M169T, M169V, T170M, E171K, E171Q, E171G, E171A, E171V and E171D, V172D, V173M, V173L, V173G, R174W, R175G, R175C, R175H, C176R, C176G, C176Y, C176F, C176S, P177R, P177R, P177L, H178D, H178P, H178Q, H179Y, H179R, H179Q, R181C, R181Y, D186G, G187S, P190L, P190T, H193N, H193P, H193L, H193R, L194F, L194R, I195F, I195N, I195T, V197L, G199V, Y205N, Y205C, Y205H, D208V, R213Q, R213P, R213L, R213Q, H214D, H214R, S215G, S215I, S215R, V216M, V217G, Y220N, Y220H, Y220S, Y220C, E224D, I232F, I232N, I232T, I232S, Y234N, Y234H, Y234S, Y234C, Y236N, Y236H, Y236C, M237V, M237K, M237I, C238R, C238G, C238Y, C238W, N239T, N239S, S241Y, S241C, S241F, C242G, C242Y, C242S, C242F, G244S, G244C, G244D, G245S, G245R, G245C, G245D, G245A, G245V, G245S, M246V, M246K, M246R, M246I, N247I, R248W, R248G, R248Q, R249G, R249W, R249T, R249M, P250L, I251N, L252P, I254S, I255F, I255N, 1255S, L257Q, L257P, E258K, E258Q, D259Y, S261T, G262D, G262V, L265P, G266R, G266E, G266V, R267W, R267Q, R267P, E271K, V272M, V272L, R273S, R273G, R273C, R273H, R273P, R273L, V274F, V274D, V274A, V274G, V274L, C275Y, C275S, C275F, A276P, C277F, P278T, P278A, P278S, P278H, P278R, P278L, G279E, R280G, R280K, R280T, R280I, R280S, D281N, D281H, D281Y, D281G, D281E, R282G, R282W, R282Q, R282P, E285K, E285V, E286G, E286V, E286K, K320N, L330R, G334V, R337C,R337L, A347T, L348F, T377P.

[0069] If the mutation is in *JAK2*, the mutation is advantageously selected from the group consisting of N533D, N533Y, N533S, H538R, K539E, K539L, I540T, I540V, V617F, R683S, R683G, del/ins537---539L, del/ins538---539L, del/ins540---543MK, del/ins540---544MK, del/ins541- -543K, del542---543, del543---544 and ins11546---547.

[0070] If the mutation is in *SF3B1*, the mutation is advantageously selected from the group consisting of G347V, R387W, R387Q, E592K, E622D, Y623C, R625L, R625C, H662Q, H662D, K666N, K666T, K666E, K666R, K700E, V701F, A708T, G740R, G740E, A744P, D781G and E783K.

Aging is associated with a large increase in the prevalence of atherosclerosis and [0071] cancer. Applicants recently analyzed whole exome sequencing data from over 17,000 individuals who were unselected for hematological phenotypes and found that at least 10% of humans age 70 or older harbor a mutation in a known cancer-causing gene in their blood cells (Jaiswal et al., NEJM 2014). Surprisingly, the presence of these mutations was associated with an increased risk of myocardial infarction (hazard ratio [HR]=2.0) and ischemic stroke (HR=2.6) in ~3,000 individuals for whom long-term follow-up information was available. It is unknown if somatic mutations that cause clonal expansion of hematopoietic stem cells also affect the function of differentiated blood cells such as macrophages, which are considered to be important mediators of atherosclerosis. Preliminary mouse data indicates that at least one of these mutations (TET2) does indeed directly alter blood cell function to lead to accelerated atherosclerosis. This application seeks to definitively establish whether these somatic mutations in blood cells are causally linked to atherosclerosis. Applicants expand the initial findings by assessing whether mice bearing mutations in DNMT3A, TET2, or JAK2 in their blood cells have accelerated atherosclerosis. Applicants probe the molecular mechanism of accelerated atherosclerosis in these mice. Applicants look for alterations in expression of liver X receptor (LXR), peroxisome proliferator activated receptor gamma (PPARG), and lipopolysaccharide (LPS) target genes in mutant macrophages using RNA-seq, DNase footprinting, and bisulfite sequencing. Applicants identify and validate therapeutic targets in macrophages with these somatic mutations using drug screens in newly created cell-line models.

[0072] Atherosclerosis is the leading cause of death in the United States; however, little is known about non-lipid risk factors in humans. This application relates to a mechanism behind

the proposed causal association between these somatic mutations in blood cells and atherosclerosis.

[0073] Cancer is thought to arise via the stepwise acquisition of genetic or epigenetic mutations that transform a normal cell (P. C. Nowell, Science 194, 23-28 (1976), J. S. Welch et al., Cell 150, 264-278 (2012)). For most hematological cancers, the genes that are frequently mutated are now known (R. Bejar et al., N Engl J Med 364, 2496-2506 (2011, N. Cancer Genome Atlas Research, Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med 368, 2059-2074 (2013), E. Papaemmanuil et al., Blood 122, 3616-3627; quiz 3699 (2013), T. Haferlach et al., Leukemia 28, 241-247 (2014)). Applicants hypothesized that these mutations would be detectable in the blood cells of some individuals not known to have hematological disorders, and that the presence of these mutations would represent a premalignant state. To test this hypothesis, Applicants examined whole exome sequencing data from large cohorts who were unselected for hematological phenotypes (S. Jaiswal et al., N Engl J Med 371, 2488-2498 (2014), G. Genovese et al., N Engl J Med 371, 2477-2487 (2014), M. Xie et al., Nat Med 20, 1472-1478 (2014)). The DNA source for these exomes was peripheral blood cells. Applicants found that ~10% of individuals over the age of 70 harbored a detectable mutation in their blood cells (Figure 1). The majority of the mutations could be accounted for by loss-of-function mutations in 3 genes, DNMT3A, TET2, and ASXL1, while activating mutations in JAK2 were the fifth most common mutation. These mutations are common in myeloid malignancies. Compared to those without mutations, persons who harbored these mutations were over 10 times more likely to develop a hematologic malignancy over the next several years, confirming that this condition is a bona fide pre-malignant entity (D. P. Steensma et al., Blood 126, 9-16 (2015)).

[0074] Surprisingly, the presence of these mutations was also associated with an increased risk of mortality that could not be explained by hematological malignancy alone. Applicants found that those with mutations had a higher rate of having a fatal myocardial infarction (MI) or ischemic stroke. When Applicants looked at risk of developing incident coronary heart disease or ischemic stroke, Applicants found that the presence of mutations was an even stronger risk factor than smoking, hypertension, or elevated cholesterol (Figure 5).

[0075] To replicate these findings, Applicants examined whole exome sequencing data from two large case-control cohorts designed to study early-onset MI. The cases (n=4,405) were

individuals from Italy or Pakistan who had blood cells collected for DNA sequencing at the time of presentation of MI, and controls (n=3,006) were age-matched healthy individuals from the same populations. Applicants found an even greater risk associated with mutations in these cohorts; the presence of a mutation was over 8 times more likely in the MI cases as compared to age-matched controls for those 40 or younger, and 3 times more likely in those age 41-50 (Figure 16A). *DNMT3A*, *TET2*, *ASXL1*, and *JAK2* were the most frequently mutated genes, and were strongly enriched in the cases (Figure 16B).

[0076] These genetic studies have firmly established a statistical link between the presence of these mutations and atherosclerotic disease, but alone cannot establish causality. It is possible that this association is merely a correlation, and that the presence of mutations is a molecular marker of aging. However, a few observations suggest a causal relationship. First, Applicants' human genetic data shows a dose-response relationship between the size of the mutant clone and risk of atherosclerotic disease. There is a greatly increased risk of incident coronary heart disease (HR=4.4) in those with a mutant allele fraction of ≥ 0.10 (meaning $\geq 20\%$ of the blood cells harbor the mutation, including the macrophages in vessel walls), while those with clones smaller than this size had no increased risk. Second, there is a large body of evidence to suggest that macrophages are important in the development of atherosclerosis (K. J. Moore et al., Nat Rev Immunol 13, 709-721 (2013)). They are the most prevalent cell type within vessel wall lesions, and are critical for reverse cholesterol transport (RCT), the process by which excess lipid is exported from tissues for clearance by the liver (M. Cuchel et al., Circulation 113, 2548-2555 (2006)). Third, inflammation and alterations in blood cell parameters are linked to adverse cardiovascular outcomes in epidemiological studies (R. Ross, N Engl J Med 340, 115-126 (1999), P. Libby, Nature 420, 868-874 (2002), P. Libby et al., Am J Med 116 Suppl 6A, 9S-16S (2004), P. M. Ridker et al., N Engl J Med 347, 1557-1565 (2002), M. Tonelli et al., Circulation 117, 163-168 (2008), M. Madjid et al., J Am Coll Cardiol 44, 1945-1956 (2004)).

[0077] Wild-type mice have much lower baseline cholesterol and triglyceride levels than humans in modern societies. Therefore, a standard experimental model is to use mice lacking low-density lipoprotein receptor (*Ldlr-/-*) (E. Maganto-Garcia et al., Current protocols in immunology / edited by John E. Coligan ... [et al.] Chapter 15, Unit 15 24 11-23 (2012)). These mice rapidly develop atherosclerosis when fed a high cholesterol diet. By transplanting bone

marrow into *Ldlr-*/- mice from a genetically defined mouse strain, one can test the effect of a genetic perturbation in hematopoietic cells on the development of atherosclerosis.

In general, studies of experimental atherosclerosis have revealed two relevant processes that can be influenced by macrophages: 1) reverse cholesterol transport, and 2) the local inflammatory milieu. The importance of macrophage reverse cholesterol transport is exemplified by mice in which both liver X receptor genes have been knocked out in the hematopoietic compartment (*LXRαβ-/-*) (R. K. Tangirala et al., Proceedings of the National Academy of Sciences of the United States of America 99, 11896-11901 (2002)). Recipients transplanted with marrow from these mice develop accelerated atherosclerosis and have a lipid accumulation phenotype in several tissues. LXRs are a class of nuclear receptors expressed in macrophages, hepatocytes, and intestinal epithelium that activate transcription of genes involved in cholesterol transport and lipid metabolism when bound by ligand. In the absence of LXR mediated transcription, macrophages do not efficiently upregulate expression of the cholesterol transporters ABCA1 and ABCG1. This leads to a defect in exporting cholesterol to acceptor molecules such as high-density lipoprotein (HDL) and Apo-AI, ultimately resulting in foam cell formation in tissues such as the vessel wall, skin, and spleen.

[0079] The role of inflammation in atherosclerosis has also been well studied. Mice that are deficient in toll-like receptor (TLR)-2 (A. E. Mullick et al., J Clin Invest 115, 3149-3156 (2005)), TLR4 (K. S. Michelsen et al., Proceedings of the National Academy of Sciences of the United States of America 101, 10679-10684 (2004)), or inflammasome (P. Duewell et al., Nature 464, 1357-1361 (2010)) signaling have reduced atherosclerosis, whereas mice that lack the inflammatory repressor *BCL6* in macrophages have accelerated atherosclerosis (G. D. Barish et al., Cell Metab 15, 554-562 (2012)). Macrophage mediated inflammation results in recruitment of other immune cells and intimal hyperplasia, resulting in increased lesion size and instability. Additional evidence indicates that inflammatory signaling may also directly inhibit macrophage RCT (A. Castrillo et al., Mol Cell 12, 805-816 (2003)). Finally, activation of another class of nuclear receptors, PPARs, ameliorates atherosclerosis by increasing macrophage RCT via LXR activation (A. Chawla et al., Mol Cell 7, 161-171 (2001)), as well as by attenuating inflammation via induction of the transcriptional repressors *BCL6*, *NCOR1*, and *NCOR2* (G. Pascual et al., Nature 437, 759-763 (2005), A. Chawla, Circ Res 106, 1559-1569 (2010)).

[0800] To test the hypothesis that mutations in blood cells are causally linked to atherosclerosis, Applicants turned to defined mouse models. Applicants crossed Tet2^{fl/fl} mice to Vav1-Cre mice; the resultant Tet2^{fl/fl}, Vav1-Cre mice have exon 3 of TET2 deleted, leading to complete loss of Tet2 function in all of their hematopoietic cells. Previous studies have demonstrated that hematopoietic stem cells (HSCs) from these mice have a differentiation defect that results in a progressive increase in HSC frequency and consequent clonal expansion of the mutant cells in a competitive transplant setting (K. Moran-Crusio et al., Cancer cell 20, 11-24 Thus, this model system recapitulates the clonal advantage of TET2 mutant (2011)). hematopoietic cells seen in humans. Applicants then transplanted bone marrow from these mice, or control Vav1-Cre mice, into Ldlr-/- recipient mice and initiated high cholesterol diet. Tissues were then examined at several time points. The results from these preliminary experiments are clear; mice that received Tet2-/- bone marrow had not only increased lesion size in the aortic root (Figure 17A), but also striking numbers of lipid-laden macrophages in the spleen, skin, and peritoneal fluid (Figure 17B). This phenotype is remarkably similar to the one described for mice transplanted with $LXR\alpha\beta$ -/- marrow (R. K. Tangirala et al., Proceedings of the National Academy of Sciences of the United States of America 99, 11896-11901 (2002)). In summary, these results point to a marked deficit of reverse cholesterol transport in macrophages that lack TET2, of which one manifestation is accelerated atherosclerosis.

[0081] The present invention relates to determining the effect of mutations in *TET2*, *DNMT3A*, and *JAK2* on atherosclerosis development *in vivo*. Applicants has already generated or obtained models for *DNMT3A* loss-of-function (*Dnmt3a*^{fl/fl} mice) (S. Nguyen et al., Dev Dyn 236, 1663-1676 (2007)) and *JAK2* gain-of-function (floxed *Jak2* V617F knock-in heterozygous mice) (A. Mullally et al., Cancer cell 17, 584-596 (2010)). Unfortunately, there are no publicly available mouse models of *ASXL1* that mimic the mutations seen in humans, which are truncating mutations in exon 12. Applicants hypothesize that introducing these mutations into hematopoietic cells lead to accelerated atherosclerosis using the Ldlr-/- transplant system described above. Applicants perform the transplants described above and analyze mice after 5, 9, and 14 weeks on high-cholesterol (1.25%) diet. Lesion size and cellular composition, as well serum lipid profiles are measured. Applicants also examine lipid content of macrophages in the spleen, lung, liver, skin, intestines, and peritoneal fluid to determine if there is a global defect in macrophage RCT. It is important to note that the mice are not expected to develop a leukemic

phenotype within this time frame. Applicants also determine whether other cells within the hematopoietic compartment besides macrophages can contribute to the phenotype. Applicants cross the *TET2*, *DNMT3A*, and *JAK2* mutant mice to mice that have *CD2*-Cre (lymphoid specific) or *PF4*-Cre (platelet specific), then perform transplants into *Ldlr*-/- mice and determine the extent of atherosclerosis in each model after 9 weeks on diet. *Adgre1*-Cre mice, which express Cre in mature macrophages only, are used to test the hypothesis that the mutations do not necessarily need to occur in stem cells to exert a phenotype.

[0082] It is possible that macrophages are the cells responsible for the phenotype, but that the mutations must occur in stem cells in order to have a functional effect due to altered differentiation. If the mutant strains crossed with *Adgre1*-Cre do not have a phenotype, Applicants isolate monocyte precursors (J. Hettinger et al., Nat Immunol 14, 821-830 (2013)) from mutant Vav1-Cre mice and transplant these into recipient *Ldlr*-/- mice to test the hypothesis that monocytes/macrophages alone can recapitulate the phenotype.

[0083] The present application also relates to determining the mechanism by which mutations in *TET2*, *DNMT3A*, and *JAK2* lead to altered macrophage function and accelerated atherosclerosis. As detailed above, much experimental evidence indicates that macrophage function has a profound effect on the development of atherosclerosis. Applicants hypothesize that mutations in TET2, DNMT3A, and JAK2 lead to accelerated atherosclerosis by inhibiting macrophage reverse cholesterol transport, increasing macrophage-mediated inflammation, or both. Applicants characterize the transcriptional response of mutant macrophages to induction of RCT and inflammation by agonists of LXRs, PPARG, and TLR4.

[0084] To ensure robustness of results, Applicants utilize 2 types of primary macrophages: thioglycollate-induced peritoneal macrophages and bone-marrow derived macrophages grown *in vitro* in the presence of cytokines. The specific agonists to be used are GW3965 (LXR alpha/beta agonist), pioglitazone (PPARG agonist), and LPS (TLR4 agonist). Gene expression are assessed by RNA-sequencing. Gene set enrichment analysis are used to determine the extent of transcriptional changes for various classes of genes by comparing expression in mutant/wild-type cells that have been treated to mutant/wild-type cells that are not treated. For these experiments, gene expression are measured at two time-points after exposure to agonists to also assess the kinetics of the transcriptional response.

[0085] Applicants are uncovering the mechanistic link between the specific mutations and alterations in gene expression in response to the agonists listed above. Applicants perform DNase footprinting and bisulfite sequencing to molecularly determine the how mutant macrophages are altered in response to the specific agonists. Peritoneal macrophages are used in these experiments because they represent an in vivo baseline epigenetic state. DNase footprinting provides a powerful method to infer transcription factor binding in a genome-wide manner by identifying DNA sequence motifs that are protein bound. Bisulfite sequencing provides single base resolution of cytosine methylation. As TET2 and DNMT3A are enzymes that alter DNA methylation, it is likely that perturbing their function result in an abnormal epigenetic state. For example, TET2 converts 5-methylcytosine to 5-hydroxymethylcytosine, which ultimately leads to de-methylation. As methylation at promoters and enhancers anti-correlates with gene expression and transcription factor binding, Applicants hypothesize that loss of TET2 function results in abnormal methylation of cis-regulatory elements for LXR/PPARG targets, reduced binding of transcription factors at these elements, and ultimately attenuated expression of the target genes. DNMT3A, a de novo DNA methyltransferase, has a function that opposes that of TET2. Applicants hypothesize that de novo methylation at cis-regulatory elements of proinflammatory genes is necessary to attenuate an inflammatory response in macrophages, and that this regulatory check is lacking in DNMT3A null macrophages. Unlike TET2 and DNMT3A, JAK2 is unique in that it is an activator of STAT signaling, not an epigenetic regulator. STAT transcription factors are known to activate a variety of genes involved in the immune response. Applicants hypothesize that constitutive STAT signaling in the setting of JAK2 activating mutations results in a stronger/more protracted pro-inflammatory phenotype in mutant macrophages, which are determined by assessing the transcriptional response to LPS. Applicants are performing functional assays to assess the physiological effect of the mutations on macrophage activity. Applicants assess cholesterol efflux, cholesterol uptake, efferocytosis, LPS tolerance, and M1/M2 polarization in mutant and wild-type macrophages in response to the specific agonists. Applicants hypothesize that one or more of these processes are altered due to the mutations.

[0086] While DNase footprinting is a powerful technique, it relies upon knowing DNA motifs for specific transcription factors to accurately assign binding. Furthermore, enhancers are inferred from transcription factor binding, rather than by assessing chromatin marks. Therefore,

Applicants also consider chromatin immunoprecipitation sequencing for LXR alpha and beta, PPARG, RelA, RelB, c-REL, STAT3, and STAT5, as well as the canonical histone marks for enhancers (H3K4me1, H3K4me3, H3K27ac, H3K27me3). Finally, Applicants also consider assessment of 5-hydroxymethylcytosine as technology to detect this mark continues to improve.

[0087] The present invention also relates to identifying molecules and pathways that can reverse the pro-atherogenic phenotype of mutant macrophages. Applicants are identifying therapeutic targets for macrophages that have mutations in TET2, DNMT3A, or JAK2. Applicants hypothesize that specific molecules or pathways can be targeted to reverse some aspects of the pro-atherogenic phenotype of mutant macrophages. Applicants are testing whether existing agonists for LXR and PPARG can reverse the phenotype in the bone marrow transplant models. Mice are placed on drug after 8 weeks on high cholesterol diet, and plaque size are compared between treated/untreated and mutant/wild-type mice after an additional 12 weeks on diet. While these approaches may prove efficacious in mice, current LXR and PPARG agonists are not considered front-line drugs in humans because of side-effects and the potential for serious adverse events. Thus, Applicants are identifying novel targets that can reverse the phenotype specifically in mutant macrophages. Applicants utilize THP-1 cells, a monocytic leukemia cell line that retains the ability differentiate into macrophages and is responsive to LXR and PPARG agonists. Frameshift mutations in TET2 and DNMT3A are created by CRISP-Cas9, while JAK2 V617F are introduced lentivirally. Applicants also introduce artificial reporters that activate fluorescence by LXR agonists, PPARG agonists, or LPS (via NF-kB activation). Applicants are screening the cells using a highly targeted drug library of 481 molecules that affect distinct cellular pathways and processes (A. Basu et al., Cell 154, 1151-1161 (2013)). The read-out is an image-based assessment of fluorescence intensity from the reporter. The aim is to identify molecules that preferentially activate or repress the reporter in mutant, but not wild-type macrophages. Applicants are also testing individual compounds that show desired activity in the mouse bone marrow transplant model, as well as in *in vitro* assays in primary macrophages.

[0088] The small molecule approach described above may not yield any viable candidates for a variety of reasons. In this case, a second screen is performed using a CRISPR-Cas9/small-guide RNA system to inactivate all protein-coding genes. Briefly, THP-1 cells with reporter are transduced with Cas9 and a pooled library of small guide RNA molecules. After inducing macrophage differentiation, Applicants identify cells that activate or repress the reporter in

response to agonists. DNA sequencing is used to determine which guides are present, and the positive hits are further validated to ensure that they preferentially affect mutant cells.

With respect to general information on CRISPR-Cas Systems, components thereof, and delivery of such components, including methods, materials, delivery vehicles, vectors, particles, AAV, and making and using thereof, including as to amounts and formulations, all useful in the practice of the instant invention, reference is made to: US Patents Nos. 8,697,359, 8,771,945, 8,795,965, 8,865,406, 8,871,445, 8,889,356, 8,889,418, 8,895,308, 8,906,616, 8,932,814, 8,945,839, 8,993,233 and 8,999,641; US Patent Publications US 2014-0310830 (US App. Ser. No. 14/105,031), US 2014-0287938 A1 (U.S. App. Ser. No. 14/213,991), US 2014-0273234 A1 (U.S. App. Ser. No. 14/293,674), US2014-0273232 A1 (U.S. App. Ser. No. 14/290,575), US 2014-0273231 (U.S. App. Ser. No. 14/259,420), US 2014-0256046 A1 (U.S. App. Ser. No. 14/226,274), US 2014-0248702 A1 (U.S. App. Ser. No. 14/258,458), US 2014-0242700 A1 (U.S. App. Ser. No. 14/222,930), US 2014-0242699 A1 (U.S. App. Ser. No. 14/183,512), US 2014-0242664 A1 (U.S. App. Ser. No. 14/104,990), US 2014-0234972 A1 (U.S. App. Ser. No. 14/183,471), US 2014-0227787 A1 (U.S. App. Ser. No. 14/256,912), US 2014-0189896 A1 (U.S. App. Ser. No. 14/105,035), US 2014-0186958 (U.S. App. Ser. No. 14/105,017), US 2014-0186919 A1 (U.S. App. Ser. No. 14/104,977), US 2014-0186843 A1 (U.S. App. Ser. No. 14/104,900), US 2014-0179770 A1 (U.S. App. Ser. No. 14/104,837) and US 2014-0179006 A1 (U.S. App. Ser. No. 14/183,486), US 2014-0170753 (US App Ser No. 14/183,429); US 2015-0184139 (U.S. App. Ser. No. 14/324,960); 14/054,414 European Patent Applications EP 2 771 468 (EP13818570.7), EP 2 764 103 (EP13824232.6), and EP 2 784 162 (EP14170383.5); and PCT Patent Publications WO 2014/093661 (PCT/US2013/074743), WO 2014/093694 (PCT/US2013/074790), WO 2014/093595 (PCT/US2013/074611), WO 2014/093718 (PCT/US2013/074825), WO 2014/093709 (PCT/US2013/074812), WO 2014/093622 WO 2014/093635 (PCT/US2013/074691), WO (PCT/US2013/074667), 2014/093655 (PCT/US2013/074736), WO 2014/093712 (PCT/US2013/074819), WO 2014/093701 WO (PCT/US2013/051418), WO (PCT/US2013/074800), 2014/018423 2014/204723 (PCT/US2014/041790), WO 2014/204724 (PCT/US2014/041800), WO 2014/204725 (PCT/US2014/041803), WO 2014/204726 (PCT/US2014/041804), WO 2014/204727 (PCT/US2014/041806), WO 2014/204728 (PCT/US2014/041808), WO 2014/204729 (PCT/US2014/041809), WO 2015/089351 (PCT/US2014/069897), WO

2015/089354 (PCT/US2014/069902), 2015/089364 (PCT/US2014/069925), WO WO (PCT/US2014/070068), WO 2015/089427 2015/089462 (PCT/US2014/070127), WO 2015/089419 (PCT/US2014/070057), WO 2015/089465 (PCT/US2014/070135), WO 2015/089486 (PCT/US2014/070175), PCT/US2015/051691, PCT/US2015/051830. Reference is made to PCT application designating, inter alia, the United States, application No. PCT/US14/41806, filed June 10, 2014. Reference is made to PCT application designating, inter alia, the United States, application No. PCT/US14/41806, filed June 10, 2014.

[0090] Each of these patents, patent publications, and applications, and all documents cited therein or during their prosecution ("appln cited documents") and all documents cited or referenced in the appln cited documents, together with any instructions, descriptions, product specifications, and product sheets for any products mentioned therein or in any document therein and incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention. All documents (e.g., these patents, patent publications and applications and the appln cited documents) are incorporated herein by reference to the same extent as if each individual document was specifically and individually indicated to be incorporated by reference.

[0091] Also with respect to general information on CRISPR-Cas Systems, mention is made of the following (also hereby incorporated herein by reference):

- Multiplex genome engineering using CRISPR/Cas systems. Cong, L., Ran, F.A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P.D., Wu, X., Jiang, W., Marraffini, L.A., & Zhang, F. Science Feb 15;339(6121):819-23 (2013);
- ➤ RNA-guided editing of bacterial genomes using CRISPR-Cas systems. Jiang W., Bikard D., Cox D., Zhang F, Marraffini LA. Nat Biotechnol Mar;31(3):233-9 (2013);
- ➢ One-Step Generation of Mice Carrying Mutations in Multiple Genes by CRISPR/Cas-Mediated Genome Engineering. Wang H., Yang H., Shivalila CS., Dawlaty MM., Cheng AW., Zhang F., Jaenisch R. Cell May 9;153(4):910-8 (2013);
- ➤ Optical control of mammalian endogenous transcription and epigenetic states. Konermann S, Brigham MD, Trevino AE, Hsu PD, Heidenreich M, Cong L, Platt RJ, Scott DA, Church GM, Zhang F. Nature. 2013 Aug 22;500(7463):472-6. doi: 10.1038/Nature12466. Epub 2013 Aug 23;

➤ Double Nicking by RNA-Guided CRISPR Cas9 for Enhanced Genome Editing Specificity. Ran, FA., Hsu, PD., Lin, CY., Gootenberg, JS., Konermann, S., Trevino, AE., Scott, DA., Inoue, A., Matoba, S., Zhang, Y., & Zhang, F. Cell Aug 28. pii: S0092-8674(13)01015-5. (2013);

- ➤ DNA targeting specificity of RNA-guided Cas9 nucleases. Hsu, P., Scott, D., Weinstein, J., Ran, FA., Konermann, S., Agarwala, V., Li, Y., Fine, E., Wu, X., Shalem, O., Cradick, TJ., Marraffini, LA., Bao, G., & Zhang, F. Nat Biotechnol doi:10.1038/nbt.2647 (2013);
- ➤ Genome engineering using the CRISPR-Cas9 system. Ran, FA., Hsu, PD., Wright, J., Agarwala, V., Scott, DA., Zhang, F. Nature Protocols Nov;8(11):2281-308. (2013);
- ➢ Genome-Scale CRISPR-Cas9 Knockout Screening in Human Cells. Shalem, O., Sanjana, NE., Hartenian, E., Shi, X., Scott, DA., Mikkelson, T., Heckl, D., Ebert, BL., Root, DE., Doench, JG., Zhang, F. Science Dec 12. (2013). [Epub ahead of print];
- Crystal structure of cas9 in complex with guide RNA and target DNA. Nishimasu, H., Ran, FA., Hsu, PD., Konermann, S., Shehata, SI., Dohmae, N., Ishitani, R., Zhang, F., Nureki, O. Cell Feb 27. (2014). 156(5):935-49;
- ➤ Genome-wide binding of the CRISPR endonuclease Cas9 in mammalian cells. Wu X., Scott DA., Kriz AJ., Chiu AC., Hsu PD., Dadon DB., Cheng AW., Trevino AE., Konermann S., Chen S., Jaenisch R., Zhang F., Sharp PA. Nat Biotechnol. (2014) Apr 20. doi: 10.1038/nbt.2889,
- CRISPR-Cas9 Knockin Mice for Genome Editing and Cancer Modeling, Platt et al., Cell 159(2): 440-455 (2014) DOI: 10.1016/j.cell.2014.09.014,
- ➤ Development and Applications of CRISPR-Cas9 for Genome Engineering, Hsu et al, Cell 157, 1262-1278 (June 5, 2014) (Hsu 2014),
- ➤ Genetic screens in human cells using the CRISPR/Cas9 system, Wang et al., Science. 2014 January 3; 343(6166): 80–84. doi:10.1126/science.1246981,
- ➤ Rational design of highly active sgRNAs for CRISPR-Cas9-mediated gene inactivation, Doench et al., Nature Biotechnology published online 3 September 2014; doi:10.1038/nbt.3026, and

➤ In vivo interrogation of gene function in the mammalian brain using CRISPR-Cas9, Swiech et al, Nature Biotechnology; published online 19 October 2014; doi:10.1038/nbt.3055.

- ➤ Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex, Konermann S, Brigham MD, Trevino AE, Joung J, Abudayyeh OO, Barcena C, Hsu PD, Habib N, Gootenberg JS, Nishimasu H, Nureki O, Zhang F., Nature. Jan 29;517(7536):583-8 (2015).
- ➤ A split-Cas9 architecture for inducible genome editing and transcription modulation, Zetsche B, Volz SE, Zhang F., (published online 02 February 2015) Nat Biotechnol. Feb;33(2):139-42 (2015);
- ➤ Genome-wide CRISPR Screen in a Mouse Model of Tumor Growth and Metastasis, Chen S, Sanjana NE, Zheng K, Shalem O, Lee K, Shi X, Scott DA, Song J, Pan JQ, Weissleder R, Lee H, Zhang F, Sharp PA. Cell 160, 1246–1260, March 12, 2015 (multiplex screen in mouse), and
- ➤ In vivo genome editing using Staphylococcus aureus Cas9, Ran FA, Cong L, Yan WX, Scott DA, Gootenberg JS, Kriz AJ, Zetsche B, Shalem O, Wu X, Makarova KS, Koonin EV, Sharp PA, Zhang F., (published online 01 April 2015), Nature. Apr 9;520(7546):186-91 (2015).
- ➤ High-throughput functional genomics using CRISPR-Cas9, Shalem et al., Nature Reviews Genetics 16, 299-311 (May 2015).
- ➤ Sequence determinants of improved CRISPR sgRNA design, Xu et al., Genome Research 25, 1147-1157 (August 2015).
- ➤ A Genome-wide CRISPR Screen in Primary Immune Cells to Dissect Regulatory Networks, Parnas et al., Cell 162, 675-686 (July 30, 2015).
- ➤ CRISPR/Cas9 cleavage of viral DNA efficiently suppresses hepatitis B virus, Ramanan et al., Scientific Reports 5:10833. doi: 10.1038/srep10833 (June 2, 2015).
- > Crystal Structure of Staphylococcus aureus Cas9, Nishimasu et al., Cell 162, 1113-1126 (Aug. 27, 2015).
- ➤ BCL11A enhancer dissection by Cas9-mediated in situ saturating mutagenesis, Canver et al., Nature 527(7577):192-7 (Nov. 12, 2015) doi: 10.1038/nature15521. Epub 2015 Sep 16.

➤ Cpf1 Is a Single RNA-Guided Endonuclease of a Class 2 CRISPR-Cas System, Zetsche et al., Cell 163, 759-71 (Sep 25, 2015).

➤ Discovery and Functional Characterization of Diverse Class 2 CRISPR-Cas Systems, Shmakov et al., Molecular Cell, 60(3), 385–397 doi: 10.1016/j.molcel.2015.10.008 Epub October 22, 2015.

each of which is incorporated herein by reference, and discussed briefly below:

- Cong et al. engineered type II CRISPR/Cas systems for use in eukaryotic cells based on both Streptococcus thermophilus Cas9 and also Streptococcus pyogenes Cas9 and demonstrated that Cas9 nucleases can be directed by short RNAs to induce precise cleavage of DNA in human and mouse cells. Their study further showed that Cas9 as converted into a nicking enzyme can be used to facilitate homology-directed repair in eukaryotic cells with minimal mutagenic activity. Additionally, their study demonstrated that multiple guide sequences can be encoded into a single CRISPR array to enable simultaneous editing of several at endogenous genomic loci sites within the mammalian genome, demonstrating easy programmability and wide applicability of the RNA-guided nuclease technology. This ability to use RNA to program sequence specific DNA cleavage in cells defined a new class of genome engineering tools. These studies further showed that other CRISPR loci are likely to be transplantable into mammalian cells and can also mediate mammalian genome cleavage. Importantly, it can be envisaged that several aspects of the CRISPR/Cas system can be further improved to increase its efficiency and versatility.
- ➤ Jiang et al. used the clustered, regularly interspaced, short palindromic repeats (CRISPR)—associated Cas9 endonuclease complexed with dual-RNAs to introduce precise mutations in the genomes of *Streptococcus pneumoniae* and *Escherichia coli*. The approach relied on dual-RNA:Cas9-directed cleavage at the targeted genomic site to kill unmutated cells and circumvents the need for selectable markers or counter-selection systems. The study reported reprogramming dual-RNA:Cas9 specificity by changing the sequence of short CRISPR RNA (crRNA) to make single- and multinucleotide changes carried on editing templates. The study showed that simultaneous use of two crRNAs enabled multiplex mutagenesis. Furthermore, when the approach was used in combination with recombineering, in *S. pneumoniae*, nearly 100% of cells that were

recovered using the described approach contained the desired mutation, and in *E. coli*, 65% that were recovered contained the mutation.

- ➤ Wang *et al.* (2013) used the CRISPR/Cas system for the one-step generation of mice carrying mutations in multiple genes which were traditionally generated in multiple steps by sequential recombination in embryonic stem cells and/or time-consuming intercrossing of mice with a single mutation. The CRISPR/Cas system will greatly accelerate the *in vivo* study of functionally redundant genes and of epistatic gene interactions.
- ➤ Konermann *et al.* addressed the need in the art for versatile and robust technologies that enable optical and chemical modulation of DNA-binding domains based CRISPR Cas9 enzyme and also Transcriptional Activator Like Effectors.
- Ran et al. (2013-A) described an approach that combined a Cas9 nickase mutant with paired guide RNAs to introduce targeted double-strand breaks. This addresses the issue of the Cas9 nuclease from the microbial CRISPR-Cas system being targeted to specific genomic loci by a guide sequence, which can tolerate certain mismatches to the DNA target and thereby promote undesired off-target mutagenesis. Because individual nicks in the genome are repaired with high fidelity, simultaneous nicking via appropriately offset guide RNAs is required for double-stranded breaks and extends the number of specifically recognized bases for target cleavage. The authors demonstrated that using paired nicking can reduce off-target activity by 50- to 1,500-fold in cell lines and to facilitate gene knockout in mouse zygotes without sacrificing on-target cleavage efficiency. This versatile strategy enables a wide variety of genome editing applications that require high specificity.
- Hsu et al. (2013) characterized SpCas9 targeting specificity in human cells to inform the selection of target sites and avoid off-target effects. The study evaluated >700 guide RNA variants and SpCas9-induced indel mutation levels at >100 predicted genomic off-target loci in 293T and 293FT cells. The authors that SpCas9 tolerates mismatches between guide RNA and target DNA at different positions in a sequence-dependent manner, sensitive to the number, position and distribution of mismatches. The authors further showed that SpCas9-mediated cleavage is unaffected by DNA methylation and that the dosage of SpCas9 and sgRNA can be titrated to minimize off-target modification.

Additionally, to facilitate mammalian genome engineering applications, the authors reported providing a web-based software tool to guide the selection and validation of target sequences as well as off-target analyses.

- Ran et al. (2013-B) described a set of tools for Cas9-mediated genome editing via non-homologous end joining (NHEJ) or homology-directed repair (HDR) in mammalian cells, as well as generation of modified cell lines for downstream functional studies. To minimize off-target cleavage, the authors further described a double-nicking strategy using the Cas9 nickase mutant with paired guide RNAs. The protocol provided by the authors experimentally derived guidelines for the selection of target sites, evaluation of cleavage efficiency and analysis of off-target activity. The studies showed that beginning with target design, gene modifications can be achieved within as little as 1–2 weeks, and modified clonal cell lines can be derived within 2–3 weeks.
- Shalem *et al.* described a new way to interrogate gene function on a genome-wide scale. Their studies showed that delivery of a genome-scale CRISPR-Cas9 knockout (GeCKO) library targeted 18,080 genes with 64,751 unique guide sequences enabled both negative and positive selection screening in human cells. First, the authors showed use of the GeCKO library to identify genes essential for cell viability in cancer and pluripotent stem cells. Next, in a melanoma model, the authors screened for genes whose loss is involved in resistance to vemurafenib, a therapeutic that inhibits mutant protein kinase BRAF. Their studies showed that the highest-ranking candidates included previously validated genes NF1 and MED12 as well as novel hits NF2, CUL3, TADA2B, and TADA1. The authors observed a high level of consistency between independent guide RNAs targeting the same gene and a high rate of hit confirmation, and thus demonstrated the promise of genome-scale screening with Cas9.
- Nishimasu et al. reported the crystal structure of Streptococcus pyogenes Cas9 in complex with sgRNA and its target DNA at 2.5 A° resolution. The structure revealed a bilobed architecture composed of target recognition and nuclease lobes, accommodating the sgRNA:DNA heteroduplex in a positively charged groove at their interface. Whereas the recognition lobe is essential for binding sgRNA and DNA, the nuclease lobe contains the HNH and RuvC nuclease domains, which are properly positioned for cleavage of the complementary and non-complementary strands of the target DNA, respectively. The

nuclease lobe also contains a carboxyl-terminal domain responsible for the interaction with the protospacer adjacent motif (PAM). This high-resolution structure and accompanying functional analyses have revealed the molecular mechanism of RNA-guided DNA targeting by Cas9, thus paving the way for the rational design of new, versatile genome-editing technologies.

- Wu et al. mapped genome-wide binding sites of a catalytically inactive Cas9 (dCas9) from Streptococcus pyogenes loaded with single guide RNAs (sgRNAs) in mouse embryonic stem cells (mESCs). The authors showed that each of the four sgRNAs tested targets dCas9 to between tens and thousands of genomic sites, frequently characterized by a 5-nucleotide seed region in the sgRNA and an NGG protospacer adjacent motif (PAM). Chromatin inaccessibility decreases dCas9 binding to other sites with matching seed sequences; thus 70% of off-target sites are associated with genes. The authors showed that targeted sequencing of 295 dCas9 binding sites in mESCs transfected with catalytically active Cas9 identified only one site mutated above background levels. The authors proposed a two-state model for Cas9 binding and cleavage, in which a seed match triggers binding but extensive pairing with target DNA is required for cleavage.
- ➤ Platt *et al.* established a Cre-dependent Cas9 knockin mouse. The authors demonstrated *in vivo* as well as *ex vivo* genome editing using adeno-associated virus (AAV)-, lentivirus-, or particle-mediated delivery of guide RNA in neurons, immune cells, and endothelial cells.
- ➤ Hsu *et al.* (2014) is a review article that discusses generally CRISPR-Cas9 history from yogurt to genome editing, including genetic screening of cells.
- ➤ Wang *et al.* (2014) relates to a pooled, loss-of-function genetic screening approach suitable for both positive and negative selection that uses a genome-scale lentiviral single guide RNA (sgRNA) library.
- Doench *et al.* created a pool of sgRNAs, tiling across all possible target sites of a panel of six endogenous mouse and three endogenous human genes and quantitatively assessed their ability to produce null alleles of their target gene by antibody staining and flow cytometry. The authors showed that optimization of the PAM improved activity and also provided an on-line tool for designing sgRNAs.

Swiech *et al.* demonstrate that AAV-mediated SpCas9 genome editing can enable reverse genetic studies of gene function in the brain.

- ➤ Konermann *et al.* (2015) discusses the ability to attach multiple effector domains, e.g., transcriptional activator, functional and epigenomic regulators at appropriate positions on the guide such as stem or tetraloop with and without linkers.
- > Zetsche *et al.* demonstrates that the Cas9 enzyme can be split into two and hence the assembly of Cas9 for activation can be controlled.
- ➤ Chen *et al.* relates to multiplex screening by demonstrating that a genome-wide *in vivo* CRISPR-Cas9 screen in mice reveals genes regulating lung metastasis.
- ➤ Ran et al. (2015) relates to SaCas9 and its ability to edit genomes and demonstrates that one cannot extrapolate from biochemical assays. Shalem et al. (2015) described ways in which catalytically inactive Cas9 (dCas9) fusions are used to synthetically repress (CRISPRi) or activate (CRISPRa) expression, showing. advances using Cas9 for genome-scale screens, including arrayed and pooled screens, knockout approaches that inactivate genomic loci and strategies that modulate transcriptional activity.
- ➤ Shalem *et al.* (2015) described ways in which catalytically inactive Cas9 (dCas9) fusions are used to synthetically repress (CRISPRi) or activate (CRISPRa) expression, showing. advances using Cas9 for genome-scale screens, including arrayed and pooled screens, knockout approaches that inactivate genomic loci and strategies that modulate transcriptional activity.
- ➤ Xu et al. (2015) assessed the DNA sequence features that contribute to single guide RNA (sgRNA) efficiency in CRISPR-based screens. The authors explored efficiency of CRISPR/Cas9 knockout and nucleotide preference at the cleavage site. The authors also found that the sequence preference for CRISPRi/a is substantially different from that for CRISPR/Cas9 knockout.
- Parnas *et al.* (2015) introduced genome-wide pooled CRISPR-Cas9 libraries into dendritic cells (DCs) to identify genes that control the induction of tumor necrosis factor (Tnf) by bacterial lipopolysaccharide (LPS). Known regulators of Tlr4 signaling and previously unknown candidates were identified and classified into three functional modules with distinct effects on the canonical responses to LPS.

Ramanan *et al* (2015) demonstrated cleavage of viral episomal DNA (cccDNA) in infected cells. The HBV genome exists in the nuclei of infected hepatocytes as a 3.2kb double-stranded episomal DNA species called covalently closed circular DNA (cccDNA), which is a key component in the HBV life cycle whose replication is not inhibited by current therapies. The authors showed that sgRNAs specifically targeting highly conserved regions of HBV robustly suppresses viral replication and depleted cccDNA.

Nishimasu *et al.* (2015) reported the crystal structures of SaCas9 in complex with a single guide RNA (sgRNA) and its double-stranded DNA targets, containing the 5'-TTGAAT-3' PAM and the 5'-TTGGGT-3' PAM. A structural comparison of SaCas9 with SpCas9 highlighted both structural conservation and divergence, explaining their distinct PAM specificities and orthologous sgRNA recognition.

[0092] Mention is also made of Tsai et al, "Dimeric CRISPR RNA-guided FokI nucleases for highly specific genome editing," Nature Biotechnology 32(6): 569-77 (2014) which is not believed to be prior art to the instant invention or application, but which may be considered in the practice of the instant invention. Mention is also made of Konermann et al., "Genome-scale transcription activation by an engineered CRISPR-Cas9 complex," doi:10.1038/nature14136, incorporated herein by reference.

[0093] In general, the CRISPR-Cas or CRISPR system is as used in the foregoing documents, such as WO 2014/093622 (PCT/US2013/074667) and refers collectively to transcripts and other elements involved in the expression of or directing the activity of CRISPR-associated ("Cas") genes, including sequences encoding a Cas gene, a tracr (trans-activating CRISPR) sequence (e.g. tracrRNA or an active partial tracrRNA), a tracr-mate sequence (encompassing a "direct repeat" and a tracrRNA-processed partial direct repeat in the context of an endogenous CRISPR system), a guide sequence (also referred to as a "spacer" in the context of an endogenous CRISPR system), or "RNA(s)" as that term is herein used (e.g., RNA(s) to guide Cas9, e.g. CRISPR RNA and transactivating (tracr) RNA or a single guide RNA (sgRNA) (chimeric RNA)) or other sequences and transcripts from a CRISPR locus. In general, a CRISPR system is characterized by elements that promote the formation of a CRISPR complex at the site of a target sequence (also referred to as a protospacer in the context of an endogenous CRISPR system). In the context of formation of a CRISPR complex, "target sequence" refers to

a sequence to which a guide sequence is designed to have complementarity, where hybridization between a target sequence and a guide sequence promotes the formation of a CRISPR complex. A target sequence may comprise any polynucleotide, such as DNA or RNA polynucleotides. In some embodiments, a target sequence is located in the nucleus or cytoplasm of a cell. In some embodiments, direct repeats may be identified in silico by searching for repetitive motifs that fulfill any or all of the following criteria: 1. found in a 2Kb window of genomic sequence flanking the type II CRISPR locus; 2. span from 20 to 50 bp; and 3. interspaced by 20 to 50 bp. In some embodiments, 2 of these criteria may be used, for instance 1 and 2, 2 and 3, or 1 and 3. In some embodiments, all 3 criteria may be used. In some embodiments it may be preferred in a CRISPR complex that the tracr sequence has one or more hairpins and is 30 or more nucleotides in length, 40 or more nucleotides in length, or 50 or more nucleotides in length; the guide sequence is between 10 to 30 nucleotides in length, the CRISPR/Cas enzyme is a Type II Cas9 enzyme. In embodiments of the invention the terms guide sequence and guide RNA are used WO interchangeably in foregoing cited documents such as 2014/093622 (PCT/US2013/074667). In general, a guide sequence is any polynucleotide sequence having sufficient complementarity with a target polynucleotide sequence to hybridize with the target sequence and direct sequence-specific binding of a CRISPR complex to the target sequence. In some embodiments, the degree of complementarity between a guide sequence and its corresponding target sequence, when optimally aligned using a suitable alignment algorithm, is about or more than about 50%, 60%, 75%, 80%, 85%, 90%, 95%, 97.5%, 99%, or more. Optimal alignment may be determined with the use of any suitable algorithm for aligning sequences, non-limiting example of which include the Smith-Waterman algorithm, the Needleman-Wunsch algorithm, algorithms based on the Burrows-Wheeler Transform (e.g. the Burrows Wheeler Aligner), ClustalW, Clustal X, BLAT, Novoalign (Novocraft Technologies; available at www.novocraft.com), ELAND (Illumina, San Diego, CA), SOAP (available at soap.genomics.org.cn), and Maq (available at maq.sourceforge.net). In some embodiments, a guide sequence is about or more than about 5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 75, or more nucleotides in length. In some embodiments, a guide sequence is less than about 75, 50, 45, 40, 35, 30, 25, 20, 15, 12, or fewer nucleotides in length. Preferably the guide sequence is 10 - 30 nucleotides long. The ability of a guide sequence to direct sequence-specific binding of a CRISPR complex to a target sequence

may be assessed by any suitable assay. For example, the components of a CRISPR system sufficient to form a CRISPR complex, including the guide sequence to be tested, may be provided to a host cell having the corresponding target sequence, such as by transfection with vectors encoding the components of the CRISPR sequence, followed by an assessment of preferential cleavage within the target sequence, such as by Surveyor assay as described herein. Similarly, cleavage of a target polynucleotide sequence may be evaluated in a test tube by providing the target sequence, components of a CRISPR complex, including the guide sequence to be tested and a control guide sequence different from the test guide sequence, and comparing binding or rate of cleavage at the target sequence between the test and control guide sequence reactions. Other assays are possible, and will occur to those skilled in the art. A guide sequence may be selected to target any target sequence. In some embodiments, the target sequence is a sequence within a genome of a cell. Exemplary target sequences include those that are unique in the target genome. For example, for the S. pyogenes Cas9, a unique target sequence in a genome NNNNNNNNNNNNNXGG (N is A, G, T, or C; and X can be anything) has a single occurrence in the genome. A unique target sequence in a genome may include an S. pyogenes Cas9 target is A, G, T, or C; and X can be anything) has a single occurrence in the genome. For the S. thermophilus CRISPR1 Cas9, a unique target sequence in a genome may include a Cas9 target of site the form MMMMMMMNNNNNNNNNNNXXAGAAW where NNNNNNNNNNNNXXAGAAW (N is A, G, T, or C; X can be anything; and W is A or T) has a single occurrence in the genome. A unique target sequence in a genome may include an S. thermophilus of the CRISPR1 Cas9 site form target MMMMMMMMNNNNNNNNNNNNNXXAGAAW where NNNNNNNNNNNXXAGAAW (N is A, G, T, or C; X can be anything; and W is A or T) has a single occurrence in the genome. For the S. pyogenes Cas9, a unique target sequence in a genome may include a Cas9 target site of the is A, G, T, or C; and X can be anything) has a single occurrence in the genome. A unique target sequence in a genome may include an S. pyogenes Cas9 target site of the form T, or C; and X can be anything) has a single occurrence in the genome. In each of these

sequences "M" may be A, G, T, or C, and need not be considered in identifying a sequence as unique. In some embodiments, a guide sequence is selected to reduce the degree secondary structure within the guide sequence. In some embodiments, about or less than about 75%, 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 1%, or fewer of the nucleotides of the guide sequence participate in self-complementary base pairing when optimally folded. Optimal folding may be determined by any suitable polynucleotide folding algorithm. Some programs are based on calculating the minimal Gibbs free energy. An example of one such algorithm is mFold, as described by Zuker and Stiegler (Nucleic Acids Res. 9 (1981), 133-148). Another example folding algorithm is the online webserver RNAfold, developed at Institute for Theoretical Chemistry at the University of Vienna, using the centroid structure prediction algorithm (see e.g. A.R. Gruber et al., 2008, Cell 106(1): 23-24; and PA Carr and GM Church, 2009, Nature Biotechnology 27(12): 1151-62).

[0100] In general, a tracr mate sequence includes any sequence that has sufficient complementarity with a tracr sequence to promote one or more of: (1) excision of a guide sequence flanked by tracr mate sequences in a cell containing the corresponding tracr sequence; and (2) formation of a CRISPR complex at a target sequence, wherein the CRISPR complex comprises the tracr mate sequence hybridized to the tracr sequence. In general, degree of complementarity is with reference to the optimal alignment of the tracr mate sequence and tracr sequence, along the length of the shorter of the two sequences. Optimal alignment may be determined by any suitable alignment algorithm, and may further account for secondary structures, such as self-complementarity within either the tracr sequence or tracr mate sequence. In some embodiments, the degree of complementarity between the tracr sequence and tracr mate sequence along the length of the shorter of the two when optimally aligned is about or more than about 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 97.5%, 99%, or higher. In some embodiments, the tracr sequence is about or more than about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, or more nucleotides in length. In some embodiments, the tracr sequence and tracr mate sequence are contained within a single transcript, such that hybridization between the two produces a transcript having a secondary structure, such as a hairpin. In an embodiment of the invention, the transcript or transcribed polynucleotide sequence has at least two or more hairpins. In preferred embodiments, the transcript has two, three, four or five hairpins. In a further embodiment of the invention, the transcript has at most five hairpins. In a

hairpin structure the portion of the sequence 5' of the final "N" and upstream of the loop corresponds to the tracr mate sequence, and the portion of the sequence 3' of the loop corresponds to the tracr sequence Further non-limiting examples of single polynucleotides comprising a guide sequence, a tracr mate sequence, and a tracr sequence are as follows (listed 5' to 3'), where "N" represents a base of a guide sequence, the first block of lower case letters represent the tracr mate sequence, and the second block of lower case letters represent the tracr sequence, and the final poly-T sequence represents the transcription terminator: **(1)** ggcttcatgccgaaatcaacacctgtcattttatggcagggtgttttcgttatttaaTTTTT; (2) aaatcaacacctgtcattttatggcagggtgttttcgttatttaaTTTTTT; (3) aaatcaacacctgtcattttatggcagggtgtTTTTTT; **(4)** (5) gaaaaagtggcaccgagtcggtgcTTTTTT; ttgaaaaagtgTTTTTT; (6)

[0101] In some embodiments, candidate tracrRNA may be subsequently predicted by sequences that fulfill any or all of the following criteria: 1. sequence homology to direct repeats (motif search in Geneious with up to 18-bp mismatches); 2. presence of a predicted Rho-independent transcriptional terminator in direction of transcription; and 3. stable hairpin secondary structure between tracrRNA and direct repeat. In some embodiments, 2 of these criteria may be used, for instance 1 and 2, 2 and 3, or 1 and 3. In some embodiments, all 3 criteria may be used.

[0102] In some embodiments, chimeric synthetic guide RNAs (sgRNAs) designs may incorporate at least 12 bp of duplex structure between the direct repeat and tracrRNA.

[0103] For minimization of toxicity and off-target effect, it will be important to control the concentration of CRISPR enzyme mRNA and guide RNA delivered. Optimal concentrations of CRISPR enzyme mRNA and guide RNA can be determined by testing different concentrations in a cellular or non-human eukaryote animal model and using deep sequencing the analyze the extent of modification at potential off-target genomic loci. For example, for the guide sequence targeting 5'-GAGTCCGAGCAGAAGAAGAA-3' in the EMX1 gene of the human genome, deep sequencing can be used to assess the level of modification at the following two off-target loci, 1: 5'-GAGTCCTAGCAGGAGAAGAA-3' and 2: 5'-GAGTCTAAGCAGAAGAAGAA-3'. The concentration that gives the highest level of on-target modification while minimizing the level of off-target modification should be chosen for in vivo delivery. Alternatively, to minimize the level of toxicity and off-target effect, CRISPR enzyme nickase mRNA (for example S. pyogenes Cas9 with the D10A mutation) can be delivered with a pair of guide RNAs targeting a site of interest. The two guide RNAs need to be spaced as follows. Guide sequences and strategies to mimize toxicity and off-target effects can be as in WO 2014/093622 (PCT/US2013/074667).

[0104] The CRISPR system is derived advantageously from a type II CRISPR system. In some embodiments, one or more elements of a CRISPR system is derived from a particular organism comprising an endogenous CRISPR system, such as *Streptococcus pyogenes*. In preferred embodiments of the invention, the CRISPR system is a type II CRISPR system and the Cas enzyme is Cas9, which catalyzes DNA cleavage. Non-limiting examples of Cas proteins include Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 and Csx12), Cas10, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, homologues thereof, or modified versions thereof.

[0105] In some embodiments, the unmodified CRISPR enzyme has DNA cleavage activity, such as Cas9. In some embodiments, the CRISPR enzyme directs cleavage of one or both strands at the location of a target sequence, such as within the target sequence and/or within the complement of the target sequence. In some embodiments, the CRISPR enzyme directs cleavage of one or both strands within about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, 200, 500, or more base pairs from the first or last nucleotide of a target sequence. In some embodiments, a

vector encodes a CRISPR enzyme that is mutated to with respect to a corresponding wild-type enzyme such that the mutated CRISPR enzyme lacks the ability to cleave one or both strands of a target polynucleotide containing a target sequence. For example, an aspartate-to-alanine substitution (D10A) in the RuvC I catalytic domain of Cas9 from S. pyogenes converts Cas9 from a nuclease that cleaves both strands to a nickase (cleaves a single strand). Other examples of mutations that render Cas9 a nickase include, without limitation, H840A, N854A, and N863A. As a further example, two or more catalytic domains of Cas9 (RuvC I, RuvC II, and RuvC III or the HNH domain) may be mutated to produce a mutated Cas9 substantially lacking all DNA cleavage activity. In some embodiments, a D10A mutation is combined with one or more of H840A, N854A, or N863A mutations to produce a Cas9 enzyme substantially lacking all DNA cleavage activity. In some embodiments, a CRISPR enzyme is considered to substantially lack all DNA cleavage activity when the DNA cleavage activity of the mutated enzyme is about no more than 25%, 10%, 5%, 1%, 0.1%, 0.01%, or less of the DNA cleavage activity of the nonmutated form of the enzyme; an example can be when the DNA cleavage activity of the mutated form is nil or negligible as compared with the non-mutated form. Where the enzyme is not SpCas9, mutations may be made at any or all residues corresponding to positions 10, 762, 840, 854, 863 and/or 986 of SpCas9 (which may be ascertained for instance by standard sequence comparison tools). In particular, any or all of the following mutations are preferred in SpCas9: D10A, E762A, H840A, N854A, N863A and/or D986A; as well as conservative substitution for any of the replacement amino acids is also envisaged. The same (or conservative substitutions of these mutations) at corresponding positions in other Cas9s are also preferred. Particularly preferred are D10 and H840 in SpCas9. However, in other Cas9s, residues corresponding to SpCas9 D10 and H840 are also preferred. Orthologs of SpCas9 can be used in the practice of the invention. A Cas enzyme may be identified Cas9 as this can refer to the general class of enzymes that share homology to the biggest nuclease with multiple nuclease domains from the type II CRISPR system. Most preferably, the Cas9 enzyme is from, or is derived from, spCas9 (S. pyogenes Cas9) or saCas9 (S. aureus Cas9). StCas9" refers to wild type Cas9 from S. thermophilus, the protein sequence of which is given in the SwissProt database under accession number G3ECR1. Similarly, S pyogenes Cas9 or spCas9 is included in SwissProt under accession number Q99ZW2. By derived, Applicants mean that the derived enzyme is largely based, in the sense of having a high degree of sequence homology with, a wildtype enzyme, but

that it has been mutated (modified) in some way as described herein. It will be appreciated that the terms Cas and CRISPR enzyme are generally used herein interchangeably, unless otherwise apparent. As mentioned above, many of the residue numberings used herein refer to the Cas9 enzyme from the type II CRISPR locus in Streptococcus pyogenes. However, it will be appreciated that this invention includes many more Cas9s from other species of microbes, such as SpCas9, SaCa9, St1Cas9 and so forth. Enzymatic action by Cas9 derived from Streptococcus pyogenes or any closely related Cas9 generates double stranded breaks at target site sequences which hybridize to 20 nucleotides of the guide sequence and that have a protospacer-adjacent motif (PAM) sequence (examples include NGG/NRG or a PAM that can be determined as described herein) following the 20 nucleotides of the target sequence. CRISPR activity through Cas9 for site-specific DNA recognition and cleavage is defined by the guide sequence, the tracr sequence that hybridizes in part to the guide sequence and the PAM sequence. More aspects of the CRISPR system are described in Karginov and Hannon, The CRISPR system: small RNAguided defence in bacteria and archaea, Mole Cell 2010, January 15; 37(1): 7. The type II CRISPR locus from Streptococcus pyogenes SF370, which contains a cluster of four genes Cas9, Cas1, Cas2, and Csn1, as well as two non-coding RNA elements, tracrRNA and a characteristic array of repetitive sequences (direct repeats) interspaced by short stretches of non-repetitive sequences (spacers, about 30bp each). In this system, targeted DNA double-strand break (DSB) is generated in four sequential steps. First, two non-coding RNAs, the pre-crRNA array and tracrRNA, are transcribed from the CRISPR locus. Second, tracrRNA hybridizes to the direct repeats of pre-crRNA, which is then processed into mature crRNAs containing individual spacer sequences. Third, the mature crRNA:tracrRNA complex directs Cas9 to the DNA target consisting of the protospacer and the corresponding PAM via heteroduplex formation between the spacer region of the crRNA and the protospacer DNA. Finally, Cas9 mediates cleavage of target DNA upstream of PAM to create a DSB within the protospacer. A pre-crRNA array consisting of a single spacer flanked by two direct repeats (DRs) is also encompassed by the term "tracr-mate sequences"). In certain embodiments, Cas9 may be constitutively present or inducibly present or conditionally present or administered or delivered. Cas9 optimization may be used to enhance function or to develop new functions, one can generate chimeric Cas9 proteins. And Cas9 may be used as a generic DNA binding protein.

[0106] Typically, in the context of an endogenous CRISPR system, formation of a CRISPR complex (comprising a guide sequence hybridized to a target sequence and complexed with one or more Cas proteins) results in cleavage of one or both strands in or near (e.g. within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 50, or more base pairs from) the target sequence. Without wishing to be bound by theory, the tracr sequence, which may comprise or consist of all or a portion of a wild-type tracr sequence (e.g. about or more than about 20, 26, 32, 45, 48, 54, 63, 67, 85, or more nucleotides of a wild-type tracr sequence), may also form part of a CRISPR complex, such as by hybridization along at least a portion of the tracr sequence to all or a portion of a tracr mate sequence that is operably linked to the guide sequence.

[0107] An example of a codon optimized sequence, is in this instance a sequence optimized for expression in a eukaryote, e.g., humans (i.e. being optimized for expression in humans), or for another eukaryote, animal or mammal as herein discussed; see, e.g., SaCas9 human codon optimized sequence in WO 2014/093622 (PCT/US2013/074667). Whilst this is preferred, it will be appreciated that other examples are possible and codon optimization for a host species other than human, or for codon optimization for specific organs is known. In some embodiments, an enzyme coding sequence encoding a CRISPR enzyme is codon optimized for expression in particular cells, such as eukaryotic cells. The eukaryotic cells may be those of or derived from a particular organism, such as a mammal, including but not limited to human, or non-human eukaryote or animal or mammal as herein discussed, e.g., mouse, rat, rabbit, dog, livestock, or non-human mammal or primate. In some embodiments, processes for modifying the germ line genetic identity of human beings and/or processes for modifying the genetic identity of animals which are likely to cause them suffering without any substantial medical benefit to man or animal, and also animals resulting from such processes, may be excluded. In general, codon optimization refers to a process of modifying a nucleic acid sequence for enhanced expression in the host cells of interest by replacing at least one codon (e.g. about or more than about 1, 2, 3, 4, 5, 10, 15, 20, 25, 50, or more codons) of the native sequence with codons that are more frequently or most frequently used in the genes of that host cell while maintaining the native amino acid sequence. Various species exhibit particular bias for certain codons of a particular amino acid. Codon bias (differences in codon usage between organisms) often correlates with the efficiency of translation of messenger RNA (mRNA), which is in turn believed to be dependent on, among other things, the properties of the codons being translated and the

availability of particular transfer RNA (tRNA) molecules. The predominance of selected tRNAs in a cell is generally a reflection of the codons used most frequently in peptide synthesis. Accordingly, genes can be tailored for optimal gene expression in a given organism based on codon optimization. Codon usage tables are readily available, for example, at the "Codon Usage Database" available at www.kazusa.orjp/codon/ and these tables can be adapted in a number of ways. See Nakamura, Y., et al. "Codon usage tabulated from the international DNA sequence databases: status for the year 2000" Nucl. Acids Res. 28:292 (2000). Computer algorithms for codon optimizing a particular sequence for expression in a particular host cell are also available. such as Gene Forge (Aptagen; Jacobus, PA), are also available. In some embodiments, one or more codons (e.g. 1, 2, 3, 4, 5, 10, 15, 20, 25, 50, or more, or all codons) in a sequence encoding a CRISPR enzyme correspond to the most frequently used codon for a particular amino acid. [0108] In some embodiments, a vector encodes a CRISPR enzyme comprising one or more nuclear localization sequences (NLSs), such as about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more NLSs. In some embodiments, the CRISPR enzyme comprises about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more NLSs at or near the amino-terminus, about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more NLSs at or near the carboxy-terminus, or a combination of these (e.g. zero or at least one or more NLS at the amino-terminus and zero or at one or more NLS at the carboxy terminus). When more than one NLS is present, each may be selected independently of the others, such that a single NLS may be present in more than one copy and/or in combination with one or more other NLSs present in one or more copies. In a preferred embodiment of the invention, the CRISPR enzyme comprises at most 6 NLSs. In some embodiments, an NLS is considered near the N- or C-terminus when the nearest amino acid of the NLS is within about 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, 50, or more amino acids along the polypeptide chain from the N- or C-terminus. Non-limiting examples of NLSs include an NLS sequence derived from: the NLS of the SV40 virus large T-antigen, having the amino acid sequence PKKKRKV; the NLS from nucleoplasmin (e.g. the nucleoplasmin bipartite NLS with the sequence KRPAATKKAGQAKKKK); the c-myc NLS having the amino acid sequence PAAKRVKLD or RQRRNELKRSP; the hRNPA1 M9 NLS having the sequence NQSSNFGPMKGGNFGGRSSGPYGGGGQYFAKPRNQGGY; the sequence RMRIZFKNKGKDTAELRRRRVEVSVELRKAKKDEQILKRRNV of the IBB domain from importin-alpha; the sequences VSRKRPRP and PPKKARED of the myoma T protein; the

sequence POPKKKPL of human p53; the sequence SALIKKKKKMAP of mouse c-abl IV; the sequences DRLRR and PKQKKRK of the influenza virus NS1; the sequence RKLKKKIKKL of the Hepatitis virus delta antigen; the sequence REKKKFLKRR of the mouse Mx1 protein; the sequence KRKGDEVDGVDEVAKKKSKK of the human poly(ADP-ribose) polymerase; and the sequence RKCLQAGMNLEARKTKK of the steroid hormone receptors (human) glucocorticoid. In general, the one or more NLSs are of sufficient strength to drive accumulation of the CRISPR enzyme in a detectable amount in the nucleus of a eukaryotic cell. In general, strength of nuclear localization activity may derive from the number of NLSs in the CRISPR enzyme, the particular NLS(s) used, or a combination of these factors. Detection of accumulation in the nucleus may be performed by any suitable technique. For example, a detectable marker may be fused to the CRISPR enzyme, such that location within a cell may be visualized, such as in combination with a means for detecting the location of the nucleus (e.g. a stain specific for the nucleus such as DAPI). Cell nuclei may also be isolated from cells, the contents of which may then be analyzed by any suitable process for detecting protein, such as immunohistochemistry, Western blot, or enzyme activity assay. Accumulation in the nucleus may also be determined indirectly, such as by an assay for the effect of CRISPR complex formation (e.g. assay for DNA cleavage or mutation at the target sequence, or assay for altered gene expression activity affected by CRISPR complex formation and/or CRISPR enzyme activity), as compared to a control no exposed to the CRISPR enzyme or complex, or exposed to a CRISPR enzyme lacking the one or more NLSs.

[0109] Aspects of the invention relate to the expression of the gene product being decreased or a template polynucleotide being further introduced into the DNA molecule encoding the gene product or an intervening sequence being excised precisely by allowing the two 5' overhangs to reanneal and ligate or the activity or function of the gene product being altered or the expression of the gene product being increased. In an embodiment of the invention, the gene product is a protein. Only sgRNA pairs creating 5' overhangs with less than 8bp overlap between the guide sequences (offset greater than -8 bp) were able to mediate detectable indel formation. Importantly, each guide used in these assays is able to efficiently induce indels when paired with wildtype Cas9, indicating that the relative positions of the guide pairs are the most important parameters in predicting double nicking activity. Since Cas9n and Cas9H840A nick opposite strands of DNA, substitution of Cas9n with Cas9H840A with a given sgRNA pair should have

resulted in the inversion of the overhang type; but no indel formation is observed as with Cas9H840A indicating that Cas9H840A is a CRISPR enzyme substantially lacking all DNA cleavage activity (which is when the DNA cleavage activity of the mutated enzyme is about no more than 25%, 10%, 5%, 1%, 0.1%, 0.01%, or less of the DNA cleavage activity of the nonmutated form of the enzyme; whereby an example can be when the DNA cleavage activity of the mutated form is nil or negligible as compared with the non-mutated form, e.g., when no indel formation is observed as with Cas9H840A in the eukaryotic system in contrast to the biochemical or prokaryotic systems). Nonetheless, a pair of sgRNAs that will generate a 5' overhang with Cas9n should in principle generate the corresponding 3' overhang instead, and double nicking. Therefore, sgRNA pairs that lead to the generation of a 3' overhang with Cas9n can be used with another mutated Cas9 to generate a 5' overhang, and double nicking. Accordingly, in some embodiments, a recombination template is also provided. A recombination template may be a component of another vector as described herein, contained in a separate vector, or provided as a separate polynucleotide. In some embodiments, a recombination template is designed to serve as a template in homologous recombination, such as within or near a target sequence nicked or cleaved by a CRISPR enzyme as a part of a CRISPR complex. A template polynucleotide may be of any suitable length, such as about or more than about 10, 15, 20, 25, 50, 75, 100, 150, 200, 500, 1000, or more nucleotides in length. In some embodiments, the template polynucleotide is complementary to a portion of a polynucleotide comprising the target sequence. When optimally aligned, a template polynucleotide might overlap with one or more nucleotides of a target sequences (e.g. about or more than about 1, 5, 10, 15, 20, or more nucleotides). In some embodiments, when a template sequence and a polynucleotide comprising a target sequence are optimally aligned, the nearest nucleotide of the template polynucleotide is within about 1, 5, 10, 15, 20, 25, 50, 75, 100, 200, 300, 400, 500, 1000, 5000, 10000, or more nucleotides from the target sequence.

[0110] In some embodiments, one or more vectors driving expression of one or more elements of a CRISPR system are introduced into a host cell such that expression of the elements of the CRISPR system direct formation of a CRISPR complex at one or more target sites. For example, a Cas enzyme, a guide sequence linked to a tracr-mate sequence, and a tracr sequence could each be operably linked to separate regulatory elements on separate vectors. Or, RNA(s) of the CRISPR System can be delivered to a transgenic Cas9 animal or mammal, e.g., an animal or

mammal that constitutively or inducibly or conditionally expresses Cas9; or an animal or mammal that is otherwise expressing Cas9 or has cells containing Cas9, such as by way of prior administration thereto of a vector or vectors that code for and express in vivo Cas9. Alternatively, two or more of the elements expressed from the same or different regulatory elements, may be combined in a single vector, with one or more additional vectors providing any components of the CRISPR system not included in the first vector. CRISPR system elements that are combined in a single vector may be arranged in any suitable orientation, such as one element located 5' with respect to ("upstream" of) or 3' with respect to ("downstream" of) a second element. The coding sequence of one element may be located on the same or opposite strand of the coding sequence of a second element, and oriented in the same or opposite direction. In some embodiments, a single promoter drives expression of a transcript encoding a CRISPR enzyme and one or more of the guide sequence, tracr mate sequence (optionally operably linked to the guide sequence), and a tracr sequence embedded within one or more intron sequences (e.g. each in a different intron, two or more in at least one intron, or all in a single intron). In some embodiments, the CRISPR enzyme, guide sequence, tracr mate sequence, and tracr sequence are operably linked to and expressed from the same promoter. Delivery vehicles, vectors, particles, nanoparticles, formulations and components thereof for expression of one or more elements of a CRISPR system are as used in the foregoing documents, such as WO 2014/093622 (PCT/US2013/074667). In some embodiments, a vector comprises one or more insertion sites, such as a restriction endonuclease recognition sequence (also referred to as a "cloning site"). In some embodiments, one or more insertion sites (e.g. about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more insertion sites) are located upstream and/or downstream of one or more sequence elements of one or more vectors. In some embodiments, a vector comprises an insertion site upstream of a tracr mate sequence, and optionally downstream of a regulatory element operably linked to the tracr mate sequence, such that following insertion of a guide sequence into the insertion site and upon expression the guide sequence directs sequence-specific binding of a CRISPR complex to a target sequence in a eukaryotic cell. In some embodiments, a vector comprises two or more insertion sites, each insertion site being located between two tracr mate sequences so as to allow insertion of a guide sequence at each site. In such an arrangement, the two or more guide sequences may comprise two or more copies of a single guide sequence, two or more different guide sequences, or combinations of these. When multiple different guide

sequences are used, a single expression construct may be used to target CRISPR activity to multiple different, corresponding target sequences within a cell. For example, a single vector may comprise about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or more guide sequences. In some embodiments, about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more such guide-sequence-containing vectors may be provided, and optionally delivered to a cell. In some embodiments, a vector comprises a regulatory element operably linked to an enzymecoding sequence encoding a CRISPR enzyme, such as a Cas protein. CRISPR enzyme or CRISPR enzyme mRNA or CRISPR guide RNA or RNA(s) can be delivered separately; and advantageously at least one of these is delivered via a nanoparticle complex. CRISPR enzyme mRNA can be delivered prior to the guide RNA to give time for CRISPR enzyme to be expressed. CRISPR enzyme mRNA might be administered 1-12 hours (preferably around 2-6 hours) prior to the administration of guide RNA. Alternatively, CRISPR enzyme mRNA and guide RNA can be administered together. Advantageously, a second booster dose of guide RNA can be administered 1-12 hours (preferably around 2-6 hours) after the initial administration of CRISPR enzyme mRNA + guide RNA. Additional administrations of CRISPR enzyme mRNA and/or guide RNA might be useful to achieve the most efficient levels of genome modification. [0111] In one aspect, the invention provides methods for using one or more elements of a CRISPR system. The CRISPR complex of the invention provides an effective means for modifying a target polynucleotide. The CRISPR complex of the invention has a wide variety of utility including modifying (e.g., deleting, inserting, translocating, inactivating, activating) a target polynucleotide in a multiplicity of cell types. As such the CRISPR complex of the invention has a broad spectrum of applications in, e.g., gene therapy, drug screening, disease diagnosis, and prognosis. An exemplary CRISPR complex comprises a CRISPR enzyme complexed with a guide sequence hybridized to a target sequence within the target polynucleotide. The guide sequence is linked to a tracr mate sequence, which in turn hybridizes to a tracr sequence. In one embodiment, this invention provides a method of cleaving a target polynucleotide. The method comprises modifying a target polynucleotide using a CRISPR complex that binds to the target polynucleotide and effect cleavage of said target polynucleotide. Typically, the CRISPR complex of the invention, when introduced into a cell, creates a break (e.g., a single or a double strand break) in the genome sequence. For example, the method can be used to cleave a disease gene in a cell. The break created by the CRISPR complex can be

repaired by a repair processes such as the error prone non-homologous end joining (NHEJ) pathway or the high fidelity homology-directed repair (HDR). During these repair process, an exogenous polynucleotide template can be introduced into the genome sequence. In some methods, the HDR process is used modify genome sequence. For example, an exogenous polynucleotide template comprising a sequence to be integrated flanked by an upstream sequence and a downstream sequence is introduced into a cell. The upstream and downstream sequences share sequence similarity with either side of the site of integration in the chromosome. Where desired, a donor polynucleotide can be DNA, e.g., a DNA plasmid, a bacterial artificial chromosome (BAC), a yeast artificial chromosome (YAC), a viral vector, a linear piece of DNA, a PCR fragment, a naked nucleic acid, or a nucleic acid complexed with a delivery vehicle such as a liposome or poloxamer. The exogenous polynucleotide template comprises a sequence to be integrated (e.g., a mutated gene). The sequence for integration may be a sequence endogenous or exogenous to the cell. Examples of a sequence to be integrated include polynucleotides encoding a protein or a non-coding RNA (e.g., a microRNA). Thus, the sequence for integration may be operably linked to an appropriate control sequence or sequences. Alternatively, the sequence to be integrated may provide a regulatory function. The upstream and downstream sequences in the exogenous polynucleotide template are selected to promote recombination between the chromosomal sequence of interest and the donor polynucleotide. The upstream sequence is a nucleic acid sequence that shares sequence similarity with the genome sequence upstream of the targeted site for integration. Similarly, the downstream sequence is a nucleic acid sequence that shares sequence similarity with the chromosomal sequence downstream of the targeted site of integration. The upstream and downstream sequences in the exogenous polynucleotide template can have 75%, 80%, 85%, 90%, 95%, or 100% sequence identity with the targeted genome sequence. Preferably, the upstream and downstream sequences in the exogenous polynucleotide template have about 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the targeted genome sequence. In some methods, the upstream and downstream sequences in the exogenous polynucleotide template have about 99% or 100% sequence identity with the targeted genome sequence. An upstream or downstream sequence may comprise from about 20 bp to about 2500 bp, for example, about 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, or 2500 bp. In some methods, the exemplary upstream or downstream sequence have

about 200 bp to about 2000 bp, about 600 bp to about 1000 bp, or more particularly about 700 bp to about 1000 bp. In some methods, the exogenous polynucleotide template may further comprise a marker. Such a marker may make it easy to screen for targeted integrations. Examples of suitable markers include restriction sites, fluorescent proteins, or selectable markers. The exogenous polynucleotide template of the invention can be constructed using recombinant techniques (see, for example, Sambrook et al., 2001 and Ausubel et al., 1996). In a method for modifying a target polynucleotide by integrating an exogenous polynucleotide template, a double stranded break is introduced into the genome sequence by the CRISPR complex, the break is repaired via homologous recombination an exogenous polynucleotide template such that the template is integrated into the genome. The presence of a double-stranded break facilitates integration of the template. In other embodiments, this invention provides a method of modifying expression of a polynucleotide in a eukaryotic cell. The method comprises increasing or decreasing expression of a target polynucleotide by using a CRISPR complex that binds to the polynucleotide. In some methods, a target polynucleotide can be inactivated to effect the modification of the expression in a cell. For example, upon the binding of a CRISPR complex to a target sequence in a cell, the target polynucleotide is inactivated such that the sequence is not transcribed, the coded protein is not produced, or the sequence does not function as the wild-type sequence does. For example, a protein or microRNA coding sequence may be inactivated such that the protein or microRNA or pre-microRNA transcript is not produced. In some methods, a control sequence can be inactivated such that it no longer functions as a control sequence. As used herein, "control sequence" refers to any nucleic acid sequence that effects the transcription, translation, or accessibility of a nucleic acid sequence. Examples of a control sequence include, a promoter, a transcription terminator, and an enhancer are control sequences. The target polynucleotide of a CRISPR complex can be any polynucleotide endogenous or exogenous to the eukaryotic cell. For example, the target polynucleotide can be a polynucleotide residing in the nucleus of the eukaryotic cell. The target polynucleotide can be a sequence coding a gene product (e.g., a protein) or a non-coding sequence (e.g., a regulatory polynucleotide or a junk DNA). Examples of target polynucleotides include a sequence associated with a signaling biochemical pathway, e.g., a signaling biochemical pathwayassociated gene or polynucleotide. Examples of target polynucleotides include a disease associated gene or polynucleotide. A "disease-associated" gene or polynucleotide refers to any

gene or polynucleotide which is yielding transcription or translation products at an abnormal level or in an abnormal form in cells derived from a disease-affected tissues compared with tissues or cells of a non disease control. It may be a gene that becomes expressed at an abnormally high level; it may be a gene that becomes expressed at an abnormally low level, where the altered expression correlates with the occurrence and/or progression of the disease. A disease-associated gene also refers to a gene possessing mutation(s) or genetic variation that is directly responsible or is in linkage disequilibrium with a gene(s) that is responsible for the etiology of a disease. The transcribed or translated products may be known or unknown, and may be at a normal or abnormal level. The target polynucleotide of a CRISPR complex can be any polynucleotide endogenous or exogenous to the eukaryotic cell. For example, the target polynucleotide can be a polynucleotide residing in the nucleus of the eukaryotic cell. The target polynucleotide can be a sequence coding a gene product (e.g., a protein) or a non-coding sequence (e.g., a regulatory polynucleotide or a junk DNA). Without wishing to be bound by theory, it is believed that the target sequence should be associated with a PAM (protospacer adjacent motif); that is, a short sequence recognized by the CRISPR complex. The precise sequence and length requirements for the PAM differ depending on the CRISPR enzyme used, but PAMs are typically 2-5 base pair sequences adjacent the protospacer (that is, the target sequence) Examples of PAM sequences are given in the examples section below, and the skilled person will be able to identify further PAM sequences for use with a given CRISPR enzyme. In some embodiments, the method comprises allowing a CRISPR complex to bind to the target polynucleotide to effect cleavage of said target polynucleotide thereby modifying the target polynucleotide, wherein the CRISPR complex comprises a CRISPR enzyme complexed with a guide sequence hybridized to a target sequence within said target polynucleotide, wherein said guide sequence is linked to a tracr mate sequence which in turn hybridizes to a tracr sequence. In one aspect, the invention provides a method of modifying expression of a polynucleotide in a eukaryotic cell. In some embodiments, the method comprises allowing a CRISPR complex to bind to the polynucleotide such that said binding results in increased or decreased expression of said polynucleotide; wherein the CRISPR complex comprises a CRISPR enzyme complexed with a guide sequence hybridized to a target sequence within said polynucleotide, wherein said guide sequence is linked to a tracr mate sequence which in turn hybridizes to a tracr sequence. Similar considerations and conditions apply as above for methods of modifying a target

polynucleotide. In fact, these sampling, culturing and re-introduction options apply across the aspects of the present invention. In one aspect, the invention provides for methods of modifying a target polynucleotide in a eukaryotic cell, which may be *in vivo*, *ex vivo* or *in vitro*. In some embodiments, the method comprises sampling a cell or population of cells from a human or non-human animal, and modifying the cell or cells. Culturing may occur at any stage *ex vivo*. The cell or cells may even be re-introduced into the non-human animal or plant. For re-introduced cells it is particularly preferred that the cells are stem cells.

[0094] Indeed, in any aspect of the invention, the CRISPR complex may comprise a CRISPR enzyme complexed with a guide sequence hybridized to a target sequence, wherein said guide sequence may be linked to a tracr mate sequence which in turn may hybridize to a tracr sequence.

[0095] As used herein "diagnosis" or "identifying a patient having" refers to a process of determining if an individual is afflicted with, or has a genetic predisposition to develop, a cardiometabolic disease.

[0096] As used herein, a "companion diagnostic" refers to a diagnostic method and or reagent that is used to identify subjects susceptible to treatment with a particular treatment or to monitor treatment and/or to identify an effective dosage for a subject or sub-group or other group of subjects. For purposes herein, a companion diagnostic refers to reagents, such as DNA isolation and sequencing reagents, that are used to detect somatic mutations in a sample. The companion diagnostic refers to the reagents and also to the test(s) that is/are performed with the reagent.

[0097] The present invention may be applied to other diseases in addition to cardiometabolic diseases and diseases affiliated with cardiometabolic diseases. In the present application, atherosclerosis is considered a cardiometabolic disease. The methods of the present invention may also be utilized for the diagnosis, prediction and treatment of other cancers in addition to hematological cancer. Other diseases which may be diagnosed, predicted or treated by methods of the present invention include, but are not limited to, autoimmune diseases (such as arthritis), other diseases involving decreased immunity (such as severe infection), dementia (such as Alzheimer's disease), diabetes, hypertension, Progeroid syndromes and other diseases involving in premature aging (such as Alzheimer's disease and Parkinson's disease).

[0098] The terms "treat," treating," "treatment," and the like refer to reducing or ameliorating a cardiovascular disease or symptoms associated therewith. It will be appreciated that, although not precluded, treating a cardiovascular disease or the risk of developing a cardiometabolic disease does not require that the disease or the risk be completely eliminated.

[0099] In the context of the present invention, a "treatment" is a procedure which alleviates or reduces the negative consequences of a cardiometabolic disease. Many cardiometabolic disease treatments are known in the art, and some are set forth herein. Any treatments or potential treatments can be used in the context of the present invention.

[00100] A treatment is not necessarily curative, and may reduce the effect of a cardiovascular disease by a certain percentage over an untreated a cardiovascular disease. The percentage reduction or diminution can be from 10% up to 20, 30, 40, 50, 60, 70, 80, 90, 95, 99 or 100%.

[00101] Methods of treatment may be personalized medicine procedures, in which the DNA of an individual is analyzed to provide guidance on the appropriate therapy for that specific individual. The methods of the invention may provide guidance as to whether treatment is necessary, as well as revealing progress of the treatment and guiding the requirement for further treatment of the individual.

[00102] As used herein, "inhibiting the development of," "reducing the risk of," "prevent," "preventing," and the like refer to reducing the probability of developing a cardiometabolic disease in a patient who may not have a cardiometabolic disease, but may have a genetic predisposition to developing a cardiometabolic disease. As used herein, "at risk," "susceptible to," or "having a genetic predisposition to," refers to having a propensity to develop a cardiometabolic disease. For example, a patient having a genetic mutation in a gene associated with a cardiometabolic disease has increased risk (e.g., "higher predisposition") of developing the disease relative to a control subject having a "lower predisposition" (e.g., a patient without a genetic mutation in a gene associated with a cardiometabolic disease).

[00103] As used herein, "reduces," "reducing," "inhibit," or "inhibiting," may mean a negative alteration of at least 10%, 15%, 25%, 50%, 75%, or 100%.

[00104] As used herein, "increases" or "increasing" may mean a positive alteration of at least 10%, 15%, 25%, 50%, 75%, or 100%.

[00105] A "therapeutically effective amount" refers to the amount of a compound required to improve, inhibit, or ameliorate a condition of a patient, or a symptom of a disease, in a clinically

relevant manner. Any improvement in the patient is considered sufficient to achieve treatment. A sufficient amount of an active compound used to practice the present invention for the treatment of cardiovascular disease varies depending upon the manner of administration, the age, body weight, genotype, and general health of the patient. Ultimately, the prescribers or researchers will decide the appropriate amount and dosage regimen. Such determinations are routine to one of ordinary skill in the art.

[00106] From a therapeutic perspective, antihypertensives (such as diuretic medicines, beta-blocking agents, calcium-channel blockers, renin-angiotensin system agents), lipid-modifying medicines, anti-inflammatory agents, nitrates and antiarrhythmic medicines are considered strong candidates for a cardiometabolic disease treatment. Aspects of the invention relate to the administration of antihypertensives (such as diuretic medicines, beta-blocking agents, calcium-channel blockers, renin-angiotensin system agents), lipid-modifying medicines, nitrates and antiarrhythmic medicines separately to individuals in need thereof that may also possess different gene variants associated with a favorable response to each type of administration.

[00107] In other embodiments, treatment and/or prevention of cardiometabolic disease may involve aspirin, statins, steroidal or non-steroidal anti-inflammatory drugs, and/or epigenetic modifiers. The epigenetic modifiers may be non-specific DNA synthesis inhibitors, such as DNA methyltransferase inhibitors (such as, but not limited to 5-aza-2'-deoxycytidine or 5-azacytidine) or histone deacetylase inhibitors (such as varinostat, romidepsin, panobinostat, belinostat and entinostat).

Proprotein convertase subtilisin kexin 9 (PCSK9) is a member of the subtilisin serine [00108]protease family. PCSK9 is primarily expressed by the liver and is critical for the down regulation of hepatocyte LDL receptor expression. LDL-C levels in plasma are highly elevated in humans with gain of function mutations in PCSK9, classifying them as having severe hypercholesterolemia. When PCSK9 binds to the LDL receptor, the receptor is broken down and can no longer remove LDL cholesterol from the blood. If PCSK9 is blocked, more LDL receptors will be present on the surface of the liver and will remove more LDL cholesterol from the blood. Therefore, PCSK9 is an attractive target for CRISPR. PCS9K-targeted CRISPR may be formulated in a lipid particle and for example administered at about 15, 45, 90, 150, 250 and 400 http://www.alnylam.com/capella/wpμg/kg intraveneously (see, e.g., content/uploads/2013/08/ALN-PCS02-001-Protocol-Lancet.pdf).

Bailey et al. (J Mol Med (Berl). 1999 Jan;77(1):244-9) discloses insulin delivery by [00109]ex-vivo somatic cell gene therapy involves the removal of non-B-cell somatic cells (e.g. fibroblasts) from a diabetic patient, and genetically altering them in vitro to produce and secrete insulin. The cells can be grown in culture and returned to the donor as a source of insulin replacement. Cells modified in this way could be evaluated before implantation, and reserve stocks could be cryopreserved. By using the patient's own cells, the procedure should obviate the need for immunosuppression and overcome the problem of tissue supply, while avoiding a recurrence of cell destruction. Ex-vivo somatic cell gene therapy requires an accessible and robust cell type that is amenable to multiple transfections and subject to controlled proliferation. Special problems associated with the use of non-B-cell somatic cells include the processing of proinsulin to insulin, and the conferment of sensitivity to glucose-stimulated proinsulin biosynthesis and regulated insulin release. Preliminary studies using fibroblasts, pituitary cells, kidney (COS) cells and ovarian (CHO) cells suggest that these challenges could be met, and that ex-vivo somatic cell gene therapy offers a feasible approach to insulin replacement therapy. The system of Bailey et al. may be used/and or adapted to the CRISPR Cas system of the present invention for delivery to the liver.

The methods of Sato et al. (Nature Biotechnology Volume 26 Number 4 April 2008, [00110]Pages 431-442) may be applied to the CRISPR Cas system of the present invention for delivery to the liver. Sato et al. found that treatments with the siRNA-bearing vitamin A-coupled liposomes almost completely resolved liver fibrosis and prolonged survival in rats with otherwise lethal dimethylnitrosamine-induced liver cirrhosis in a dose- and duration-dependent manner. Cationic liposomes (Lipotrust) containing O,O'-ditetradecanoyl-N-(a-trimethylammonioacetyl) diethanolamine chloride (DC-6-14)cationic lipid, cholesterol and as a dioleoylphosphatidylethanolamine at a molar ratio of 4:3:3 (which has shown high transfection efficiency under serumcontaining conditions for in vitro and in vivo gene delivery) were purchased from Hokkaido System Science. The liposomes were manufactured using a freezedried empty liposomes method and prepared at a concentration of 1 mM (DC-16-4) by addition of double-distilled water (DDW) to the lyophilized lipid mixture under vortexing before use. To prepare VA-coupled liposomes, 200 nmol of vitamin A (retinol, Sigma) dissolved in DMSO was mixed with the liposome suspensions (100 nmol as DC-16-4) by vortexing in a 1.5 ml tube at 25 1C. To prepare VA-coupled liposomes carrying siRNAgp46 (VA-lip-siRNAgp46), a solution of

siRNAgp46 (580 pmol/ml in DDW) was added to the retinol-coupled liposome solution with stirring at 25 C. The ratio of siRNA to DC-16-4 was 1:11.5 (mol/mol) and the siRNA to liposome ratio (wt/wt) was 1:1. Any free vitamin A or siRNA that was not taken up by liposomes were separated from liposomal preparations using a micropartition system (VIVASPIN 2 concentrator 30,000 MWCO PES, VIVASCIENCE). The liposomal suspension was added to the filters and centrifuged at 1,500g for 5 min 3 times at 25 1C. Fractions were collected and the material trapped in the filter was reconstituted with PBS to achieve the desired dose for in vitro or in vivo use. Three injections of 0.75 mg/kg siRNA were given every other day to rats. The system of Sato et al. may be used/and or adapted to the CRISPR Cas system of the present invention for delivery to the liver by delivering about 0.5 to 1 mg/kg of CRISPR Cas RNA in the liposomes as described by Sato et al. to humans.

[00111]The methods of Rozema et al. (PNAS, August 7, 2007, vol. 104, no. 32) for a vehicle for the delivery of siRNA to hepatocytes both in vitro and in vivo, which Rozema et al. have named siRNA Dynamic PolyConjugates may also be applied to the present invention. Key features of the Dynamic Poly-Conjugate technology include a membrane-active polymer, the ability to reversibly mask the activity of this polymer until it reaches the acidic environment of endosomes, and the ability to target this modified polymer and its siRNA cargo specifically to hepatocytes in vivo after simple, low-pressure i.v. injection. SATA-modified siRNAs are synthesized by reaction of 5' aminemodified siRNA with 1 weight equivalents (wt eq) of Nsuccinimidyl-S-acetylthioacetate (SATA) reagent (Pierce) and 0.36 wt eq of NaHCO₃ in water at 4°C for 16 h. The modified siRNAs are then precipitated by the addition of 9 vol of ethanol and incubation at 80°C for 2 h. The precipitate is resuspended in 1X siRNA buffer (Dharmacon) and quantified by measuring absorbance at the 260-nm wavelength. PBAVE (30 mg/ml in 5mMTAPS, pH 9) is modified by addition of 1.5 wt % SMPT (Pierce). After a 1-h incubation, 0.8 mg of SMPT-PBAVE was added to 400 µl of isotonic glucose solution containing 5 mM TAPS (pH 9). To this solution was added 50 µg of SATA-modified siRNA. For the doseresponse experiments where [PBAVE] was constant, different amounts of siRNA are added. The mixture is then incubated for 16 h. To the solution is then added 5.6 mg of Hepes free base followed by a mixture of 3.7 mg of CDM-NAGand 1.9mg of CDM-PEG. The solution is then incubated for at least 1 h at room temperature before injection. CDM-PEG and CDM-NAG are synthesized from the acid chloride generated by using oxalyl chloride. To the acid chloride is

added 1.1 molar equivalents polyethylene glycol monomethyl ether (molecular weight average of 450) to generate CDM-PEG or (aminoethoxy)ethoxy-2-(acetylamino)-2-deoxy-β-D-glucopyranoside to generate CDM-NAG. The final product is purified by using reverse-phase HPLC with a 0.1% TFA water/acetonitrile gradient. About 25 to 50 μg of siRNA was delivered to mice. The system of Rozema et al. may be applied to the CRISPR Cas system of the present invention for delivery to the liver, for example by envisioning a dosage of about 50 to about 200 mg of CRISPR Cas for delivery to a human.

[00112] In an aspect the invention provides methods, regents and companion diagnostics for identifying and treating subjects at risk for, or having a PCSK9 mediated disorder. The invention can be used to select therapeutic compositions and dosages, to predict and monitor responses and outcomes. Subjects may be treated to prevent, delay the onset of, or ameliorate the PCSK9 mediated disorder by promoting or inhibiting PCSK9 activity.

[00113] PCSK9 inhibitors can be used to treat patients with familial hypercholesterolemia (FH), clinical atherosclerotic cardiovascular disease (CVD), and other disorders requiring lowering of LDL cholesterol (LDL-C). PCSK9 inhibitors include evolocumab (Repatha[™]), Praulent, and Alnylam. Evolocumab is a human monoclonal IgG2 antibody which binds to PCSK9 and inhibits circulating PCSK9 from binding to the low density lipoprotein (LDL) receptor (LDLR), preventing PCSK9-mediated LDLR degradation and permitting LDLR to recycle back to the liver cell surface. By inhibiting the binding of PCSK9 to LDLR, evolocumab increases the number of LDLRs available to clear LDL from the blood, thereby lowering LDL-C levels. Evolocumab can be administered alone or in combination with other agents. Evolocumab is used as an adjunct to diet and maximally tolerated statin therapy.

[00114] The liver X receptor (LXR) is a member of the nuclear receptor family of transcription factors and is related to nuclear receptors such as the PPARs, FXR and RXR. Liver X receptors (LXRs) are nuclear receptors involved in regulation of lipid metabolism, cholesterol homeostasis, and inflammatory responses in the central nervous system. Two isoforms of LXR have been identified and are referred to as LXR α and LXR β . LXR α expression is restricted to liver, kidney, intestine, fat tissue, macrophages, lung, and spleen and is highest in liver, whereas LXR β is expressed in almost all tissues and organs. Defects contribute to the pathogenesis of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, and Huntington's disease.

[00115] In an aspect the invention provides methods, regents and companion diagnostics for identifying and treating subjects at risk for, or having an LXR mediated disorder. Subjects are identified and can be treated to prevent, delay the onset of, or ameliorate the LXR mediated disorder by activating or inhibiting LXR activity. LXR agonists (e.g., hypocholamide, T0901317, GW3965, or N,N-dimethyl-3beta-hydroxy-cholenamide (DMHCA)) are useful to reduce the cholesterol level in <u>serum</u> and <u>liver</u> and inhibits the development of <u>atherosclerosis</u> in murine disease models. Certain LXR <u>agonists</u> (e.g. GW3965) improve <u>glucose</u> tolerance in a model of diet-induced <u>obesity</u> and <u>insulin</u> resistance by regulating <u>genes</u> involved in <u>glucose</u> metabolism in liver and adipose tissue.

[00116] Peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes and play essential roles in the regulation of cellular differentiation, development, and metabolism. There are three main forms: PPAR α ; PPAR β / δ , and PPAR γ . The PPAR γ 1 isoform is expressed in the spleen, intestine, and white adipose tissue, the PPAR γ 2 is preferentially expressed in white and brown fat, and PPAR γ 2 is most abundantly in fat cells, and plays a pivotal role in fat cell differentiation and lipid storage. Hereditary disorders of all PPARs have been described, leading to, for example, lipodystrophy, and insulin resistance.

[00117] In an aspect, the invention provides methods, reagents and diagnostics for identifying, monitoring, and treating inherited disorders. In certain embodiments, the disorders may have single genetic components. Advantageously, the invention is useful for complex multifactorial disorders that do not have a single genetic cause, such as, but not limited to, heart disease, diabetes, and obesity. Thus, multiple gene loci can be evaluated and treatments designed or adjusted accordingly. Accordingly, a subject's disease or disorder can be diagnosed as to relative contributions of variations in multiple genes. Accordingly, patient-specific treatments cam be selected involving multiple drugs in specific combinations and/or dosages. For example, depending on the presence of gene variants at genetic loci that determine propensity to develop a metabolic disease, patient-specific combinations and dosages of drugs to treat diabetes can be selected. According to the invention, in one embodiment, a patient suffering from a particular disease is diagnosed to determine the presence or absence of genetic variants associated with a disease or condition, and a treatment regime assigned accordingly. In particular, treatments that are likely to be effective or to which a subject is likely to be most sensitive are selected. In

another embodiment, treatments which are predicted to result in unwanted patient-specific side effects are avoided.

[00118] Advantageously, the invention provides methods, reagents and diagnostics for identifying, monitoring, and treating diseases or disorders that arise spontaneously or develop over time. In certain embodiments, such disorders are clonal. By clonal it is meant that there is an aspect to the disease that results from appearance or enlargement (or disappearance) of a population of cells. One non-limiting example is a clonal population of cells that arises through a spontaneous mutation. Another non-limiting example is a clonal population of cells that arises from an external stimulation. For example, an autoimmune disorder may arise or be exacerbated by expansion or activation of a population of immune cells. Yet another example is loss of a highly diverse population of cells leaving a population arising from a small number of clones. For example, it has been observed that clonal diversity of immune cells capable of mounting an immune response diminishes with age. In certain embodiments, there is activation or inhibition of expression of one or more genes that develops over time. For example, expression levels can be monitored by measuring transcription or evaluating DNA methylation.

[00119] In an embodiment of the invention, a cholesterol level is monitored and a treatment to reduce cholesterol is initiated. One or more of *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *JAK2* and *SF3B1* is tested for sequence and a cholesterol medication is selected accordingly, taking into account the alleles of *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *JAK2* and *SF3B1* present in the subject and their correlation with effectiveness of treatment and minimization of undesired side effects such as liver function and muscle pain.

[00120] Provided herein are sensitive and specific methods to detect and closely monitor somatic mutations associated with disease, particularly a cardiometabolic disease and a hematological cancer. The companion diagnostic methods provided herein are based on the finding that clonal hematopoiesis due to somatic mutation is a common finding in the elderly, and most frequently involves *DNMT3A*, *TET2*, and *ASXL1*. This clinical entity, CHIP, is associated with increased risk of developing hematological malignancy, minimal changes in blood counts, increased overall mortality, and increased risk of cardiometabolic disease. In addition, the companion diagnostic method provided herein also is based on the finding that detecting mutations in *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *JAK2* and *SF3B1* specifically provides superior prognostic and treatment selection information as compared to other markers involved

in cardiometabolic disease and a hematological cancer. In exemplary methods provided herein, the diagnostic and prognostic methods are companion methods to therapy with any treatment described herein.

[00121] In one embodiment, the companion diagnostic is used to monitor clonality of somatic mutations in a patient. In another embodiment, clonality is determined. Not being bound by a theory, the size of a clone harboring a somatic mutation, as described herein, can be used to monitor the effectiveness of a treatment.

[00122] In one embodiment, a patient is treated with a cholesterol lowering drug if a mutation in *TET2*, *DNMT3A*, and/or *JAK2* is observed. Not being bound by a theory, *TET2*, *DNMT3A*, and/or *JAK2* mutations result in a deficit of reverse cholesterol transport in macrophages and treatment with a cholesterol lowering drug described herein can ameliorate this deficit. In another embodiment, a patient is treated with an anti-inflammatory drug described herein, if a mutation in *TET2*, *DNMT3A*, and/or *JAK2* is observed. Not being bound by a theory, *TET2*, *DNMT3A*, and/or *JAK2* mutations result in a protracted inflammatory phenotype in macrophages and treatment with an anti-inflammatory drug described herein can ameliorate this phenotype.

[00123] The present invention also relates to identifying molecules, advantageously small molecules or biologics, that may be involved in inhibiting one or more of the mutations in one or more genes selected from the group consisting of *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *JAK2* and *SF3B1*. The invention contemplates screening libraries of small molecules or biologics to identify compounds involved in suppressing or inhibiting expression of somatic mutations or alter the cells phenotypically so that the cells with mutations behave more normally in one or more of *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *JAK2* and *SF3B1*.

[00124] High-throughput screening (HTS) is contemplated for identifying small molecules or biologics involved in suppressing or inhibiting expression of somatic mutations in one or more of *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *JAK2* and *SF3B1*. The flexibility of the process has allowed numerous and disparate areas of biology to engage with an equally diverse palate of chemistry (see, e.g., Inglese et al., Nature Chemical Biology 3, 438 - 441 (2007)). Diverse sets of chemical libraries, containing more than 200,000 unique small molecules, as well as natural product libraries, can be screened. This includes, for example, the Prestwick library (1,120 chemicals) of off-patent compounds selected for structural diversity, collective coverage of multiple therapeutic areas, and known safety and bioavailability in humans, as well as the NINDS Custom

Collection 2 consisting of a 1,040 compound-library of mostly FDA-approved drugs (see, e.g., US Patent No. 8,557,746) are also contemplated.

[00125] The NIH's Molecular Libraries Probe Production Centers Network (MLPCN) offers access to thousands of small molecules – chemical compounds that can be used as tools to probe basic biology and advance our understanding of disease. Small molecules can help researchers understand the intricacies of a biological pathway or be starting points for novel therapeutics. The Broad Institute's Probe Development Center (BIPDeC) is part of the MLPCN and offers access to a growing library of over 330,000 compounds for large scale screening and medicinal chemistry. Any of these compounds may be utilized for screening compounds involved in suppressing or inhibiting expression of somatic mutations in one or more of *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *JAK2* and *SF3B1*.

[00126] The phrase "therapeutically effective amount" as used herein refers to a nontoxic but sufficient amount of a drug, agent, or compound to provide a desired therapeutic effect.

[00127] As used herein "patient" refers to any human being receiving or who may receive medical treatment.

[00128] A "polymorphic site" refers to a polynucleotide that differs from another polynucleotide by one or more single nucleotide changes.

[00129] A "somatic mutation" refers to a change in the genetic structure that is not inherited from a parent, and also not passed to offspring.

[00130] Therapy or treatment according to the invention may be performed alone or in conjunction with another therapy, and may be provided at home, the doctor's office, a clinic, a hospital's outpatient department, or a hospital. Treatment generally begins at a hospital so that the doctor can observe the therapy's effects closely and make any adjustments that are needed. The duration of the therapy depends on the age and condition of the patient, the stage of the a cardiovascular disease, and how the patient responds to the treatment. Additionally, a person having a greater risk of developing a cardiovascular disease (e.g., a person who is genetically predisposed) may receive prophylactic treatment to inhibit or delay symptoms of the disease.

[00131] The medicaments of the invention are prepared in a manner known to those skilled in the art, for example, by means of conventional dissolving, lyophilizing, mixing, granulating or confectioning processes. Methods well known in the art for making formulations are found, for example, in Remington: The Science and Practice of Pharmacy, 20th ed., ed. A. R. Gennaro,

2000, Lippincott Williams & Wilkins, Philadelphia, and Encyclopedia of Pharmaceutical Technology, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York.

[00132] Administration of medicaments of the invention may be by any suitable means that results in a compound concentration that is effective for treating or inhibiting (e.g., by delaying) the development of a cardiovascular disease. The compound is admixed with a suitable carrier substance, e.g., a pharmaceutically acceptable excipient that preserves the therapeutic properties of the compound with which it is administered. One exemplary pharmaceutically acceptable excipient is physiological saline. The suitable carrier substance is generally present in an amount of 1-95% by weight of the total weight of the medicament. The medicament may be provided in a dosage form that is suitable for oral, rectal, intravenous, intramuscular, subcutaneous, inhalation, nasal, topical or transdermal, vaginal, or ophthalmic administration. Thus, the medicament may be in form of, e.g., tablets, capsules, pills, powders, granulates, suspensions, emulsions, solutions, gels including hydrogels, pastes, ointments, creams, plasters, drenches, delivery devices, suppositories, enemas, injectables, implants, sprays, or aerosols.

[00133] In order to determine the genotype of a patient according to the methods of the present invention, it may be necessary to obtain a sample of genomic DNA from that patient. That sample of genomic DNA may be obtained from a sample of tissue or cells taken from that patient.

[00134] The tissue sample may comprise but is not limited to hair (including roots), skin, buccal swabs, blood, or saliva. The tissue sample may be marked with an identifying number or other indicia that relates the sample to the individual patient from which the sample was taken. The identity of the sample advantageously remains constant throughout the methods of the invention thereby guaranteeing the integrity and continuity of the sample during extraction and analysis. Alternatively, the indicia may be changed in a regular fashion that ensures that the data, and any other associated data, can be related back to the patient from whom the data was obtained. The amount/size of sample required is known to those skilled in the art.

[00135] Generally, the tissue sample may be placed in a container that is labeled using a numbering system bearing a code corresponding to the patient. Accordingly, the genotype of a particular patient is easily traceable.

[00136] In one embodiment of the invention, a sampling device and/or container may be supplied to the physician. The sampling device advantageously takes a consistent and

reproducible sample from individual patients while simultaneously avoiding any cross-contamination of tissue. Accordingly, the size and volume of sample tissues derived from individual patients would be consistent.

[00137] According to the present invention, a sample of DNA is obtained from the tissue sample of the patient of interest. Whatever source of cells or tissue is used, a sufficient amount of cells must be obtained to provide a sufficient amount of DNA for analysis. This amount will be known or readily determinable by those skilled in the art.

[00138] DNA is isolated from the tissue/cells by techniques known to those skilled in the art (see, e.g., U.S. Pat. Nos. 6,548,256 and 5,989,431, Hirota et al., Jinrui Idengaku Zasshi. September 1989; 34(3):217-23 and John et al., Nucleic Acids Res. Jan. 25. 1991;19(2):408; the disclosures of which are incorporated by reference in their entireties). For example, high molecular weight DNA may be purified from cells or tissue using proteinase K extraction and ethanol precipitation. DNA may be extracted from a patient specimen using any other suitable methods known in the art.

[00139] It is an object of the present invention to determine the genotype of a given patient of interest by analyzing the DNA from the patent, in order to identify a patient carrying specific somatic mutations of the invention that are associated with developing a cardiovascular disease. In particular, the kit may have primers or other DNA markers for identifying particular mutations such as, but not limited to, one or more genes selected from the group consisting of *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *JAK2* and *SF3B1*.

[00140] There are many methods known in the art for determining the genotype of a patient and for identifying or analyzing whether a given DNA sample contains a particular somatic mutation. Any method for determining genotype can be used for determining genotypes in the present invention. Such methods include, but are not limited to, amplimer sequencing, DNA sequencing, fluorescence spectroscopy, fluorescence resonance energy transfer (or "FRET")-based hybridization analysis, high throughput screening, mass spectroscopy, nucleic acid hybridization, polymerase chain reaction (PCR), RFLP analysis and size chromatography (e.g., capillary or gel chromatography), all of which are well known to one of skill in the art.

[00141] The methods of the present invention, such as whole exome sequencing and targeted amplicon sequencing, have commercial applications in diagnostic kits for the detection of the somatic mutations in patients. A test kit according to the invention may comprise any of the

materials necessary for whole exome sequencing and targeted amplicon sequencing, for example, according to the invention. In a particular advantageous embodiment, a companion diagnostic for the present invention may comprise testing for any of the genes in disclosed herein. The kit further comprises additional means, such as reagents, for detecting or measuring the sequences of the present invention, and also ideally a positive and negative control.

[00142] The present invention further encompasses probes according to the present invention that are immobilized on a solid or flexible support, such as paper, nylon or other type of membrane, filter, chip, glass slide, microchips, microbeads, or any other such matrix, all of which are within the scope of this invention. The probe of this form is now called a "DNA chip". These DNA chips can be used for analyzing the somatic mutations of the present invention. The present invention further encompasses arrays or microarrays of nucleic acid molecules that are based on one or more of the sequences described herein. As used herein "arrays" or "microarrays" refers to an array of distinct polynucleotides or oligonucleotides synthesized on a solid or flexible support, such as paper, nylon or other type of membrane, filter, chip, glass slide, or any other suitable solid support. In one embodiment, the microarray is prepared and used according to the methods and devices described in U.S. Pat. Nos. 5,446,603; 5,545,531; 5,807,522; 5,837,832; 5,874,219; 6,114,122; 6,238,910; 6,365,418; 6,410,229; 6,420,114; 6,432,696; 6,475,808 and 6,489,159 and PCT Publication No. WO 01/45843 A2, the disclosures of which are incorporated by reference in their entireties.

[00143] For the purposes of the present invention, sequence identity or homology is determined by comparing the sequences when aligned so as to maximize overlap and identity while minimizing sequence gaps. In particular, sequence identity may be determined using any of a number of mathematical algorithms. A nonlimiting example of a mathematical algorithm used for comparison of two sequences is the algorithm of Karlin & Altschul, Proc. Natl. Acad. Sci. USA 1990;87: 2264-2268, modified as in Karlin & Altschul, Proc. Natl. Acad. Sci. USA 1993;90: 5873-5877.

[00144] Another example of a mathematical algorithm used for comparison of sequences is the algorithm of Myers & Miller, CABIOS 1988;4: 11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. Yet another

useful algorithm for identifying regions of local sequence similarity and alignment is the FASTA algorithm as described in Pearson & Lipman, Proc. Natl. Acad. Sci. USA 1988;85: 2444-2448.

[00145] Advantageous for use according to the present invention is the WU-BLAST (Washington University BLAST) version 2.0 software. WU-BLAST version 2.0 executable programs for several UNIX platforms can be downloaded from the FTP site for Blast at the Washington University in St. Louis website. This program is based on WU-BLAST version 1.4, which in turn is based on the public domain NCBI-BLAST version 1.4 (Altschul & Gish, 1996, Local alignment statistics, Doolittle ed., Methods in Enzymology 266: 460-480; Altschul et al., Journal of Molecular Biology 1990;215: 403-410; Gish & States, 1993;Nature Genetics 3: 266-272; Karlin & Altschul, 1993;Proc. Natl. Acad. Sci. USA 90: 5873-5877; all of which are incorporated by reference herein).

[00146] In all search programs in the suite the gapped alignment routines are integral to the database search itself. Gapping can be turned off if desired. The default penalty (Q) for a gap of length one is Q=9 for proteins and BLASTP, and Q=10 for BLASTN, but may be changed to any integer. The default per-residue penalty for extending a gap (R) is R=2 for proteins and BLASTP, and R=10 for BLASTN, but may be changed to any integer. Any combination of values for Q and R can be used in order to align sequences so as to maximize overlap and identity while minimizing sequence gaps. The default amino acid comparison matrix is BLOSUM62, but other amino acid comparison matrices such as PAM can be utilized.

[00147] Alternatively or additionally, the term "homology" or "identity", for instance, with respect to a nucleotide or amino acid sequence, can indicate a quantitative measure of homology between two sequences. The percent sequence homology can be calculated as $(N_{ref}-N_{dif})^*$ 100/- N_{ref} , wherein N_{dif} is the total number of non-identical residues in the two sequences when aligned and wherein N_{ref} is the number of residues in one of the sequences. Hence, the DNA sequence AGTCAGTC will have a sequence identity of 75% with the sequence AATCAATC (N N_{ref} =8; N N_{dif} =2). "Homology" or "identity" can refer to the number of positions with identical nucleotides or amino acids divided by the number of nucleotides or amino acids in the shorter of the two sequences wherein alignment of the two sequences can be determined in accordance with the Wilbur and Lipman algorithm (Wilbur & Lipman, Proc Natl Acad Sci USA 1983;80:726, incorporated herein by reference), for instance, using a window size of 20 nucleotides, a word length of 4 nucleotides, and a gap penalty of 4, and computer-assisted analysis and interpretation

of the sequence data including alignment can be conveniently performed using commercially available programs (e.g., Intelligenetics.TM. Suite, Intelligenetics Inc. CA). When RNA sequences are said to be similar, or have a degree of sequence identity or homology with DNA sequences, thymidine (T) in the DNA sequence is considered equal to uracil (U) in the RNA sequence. Thus, RNA sequences are within the scope of the invention and can be derived from DNA sequences, by thymidine (T) in the DNA sequence being considered equal to uracil (U) in RNA sequences. Without undue experimentation, the skilled artisan can consult with many other programs or references for determining percent homology.

[00148] The invention further encompasses kits useful for screening nucleic acids isolated from one or more patients for any of the somatic mutations described herein and instructions for using the oligonucleotide to detect variation in the nucleotide corresponding to one or more of the somatic mutations, such as but not limited to, one or more genes selected from the group consisting of DNMT3A, TET2, ASXL1, TP53, JAK2 and SF3B1, of the isolated nucleic acid.

[00149] Another aspect of the invention is a method of screening patients to determine those patients more likely to develop a cardiovascular disease comprising the steps of obtaining a sample of genetic material from a patient; and assaying for the presence of a genotype in the patient which is associated with developing cardiovascular diseases, any of the herein disclosed somatic mutations.

[00150] In other embodiments of this invention, the step of assaying is selected from the group consisting of: restriction fragment length polymorphism (RFLP) analysis, minisequencing, MALD-TOF, SINE, heteroduplex analysis, single strand conformational polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE).

[00151] The present invention also encompasses a transgenic mouse which may express one or more of the herein disclosed somatic mutations. Methods for making a transgenic mouse are well known to one of skill in the art, see e.g., U.S. Patent Nos. 7,709,695; 7,667,090; 7,655,700; 7,626,076; 7,566,812; 7,544,855; 7,538,258; 7,495,147; 7,479,579; 7,449,615; 7,432,414; 7,393,994; 7,371,920; 7,358,416; 7,276,644; 7,265,259; 7,220,892; 7,214,850; 7,186,882; 7,119,249; 7,112,715; 7,098,376; 7,045,678; 7,038,105; 6,750,375; 6,717,031; 6,710,226; 6,689,937; 6,657,104; 6,649,811; 6,613,958; 6,610,905; 6,593,512; 6,576,812; 6,531,645; 6,515,197; 6,452,065; 6,372,958; 6,372,957; 6,369,295; 6,323,391; 6,323,390; 6,316,693;

6,313,373; 6,300,540; 6,255,555; 6,245,963; 6,215,040; 6,211,428; 6,201,166; 6,187,992; 6,184,435; 6,175,057; 6,156,727; 6,137,029; 6,127,598; 6,037,521; 6,025,539; 6,002,067; 5,981,829; 5,936,138; 5,917,124; 5,907,078; 5,894,078; 5,850,004; 5,850,001; 5,847,257; 5,837,875; 5,824,840; 5,824,838; 5,814,716; 5,811,633; 5,723,719; 5,720,936; 5,688,692; 5,631,407; 5,620,881; 5,574,206 and 5,569,827. The transgenic mouse may be utilized to mimic cardiosvascular disease conditions and may be useful to test novel treatments of cardiovascular disease in a mouse model.

[00152] Although the present invention and its advantages have been described in detail, it should be understood that various changes, substitutions and alterations can be made herein without departing from the spirit and scope of the invention as defined in the appended claims.

[00153] The present invention will be further illustrated in the following Examples which are given for illustration purposes only and are not intended to limit the invention in any way.

Examples

Example 1: Methods

[00154] Sample ascertainment. Subjects were ascertained from 22 population-based cohorts in 3 consortia (see Supplementary Table S1). These studies were performed using protocols approved by the ethics committees of all involved institutions, as well as with informed consent from all participants. Samples with missing age (116 subjects) or cell lines as the source of DNA (492 subjects) were excluded.

[00155] Whole Exome Sequencing and Targeted Amplicon Sequencing . DNA was obtained from individual cohorts and further processed at the Broad Institute of MIT and Harvard. Briefly, genomic DNA was subject to hybrid capture, sequencing, and alignment using the Broad Genomics Platform and Picard pipeline. BAM files were analyzed for SNVs using MuTect with OxoG filtering and indels using Indelocator (Cibulskis K et al. Nature biotechnology 2013;31:213-9, Costello M et al. Nucleic acids research 2013;41:e67). A clinically validated, targeted amplicon assay was used for sequencing of 95 genes in select samples.

Variant calling

[00156] Applicants defined a list of pathogenic variants reported in the literature and/or the Catalog of Somatic Mutations in Cancer (COSMIC, http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/) in human hematologic malignancies

from 160 genes (see Supplementary Table S3). As a negative control, Applicants also searched for variants that were recurrently seen in non-hematologic malignancies (see Supplementary Table S5) (Lawrence MS et al. Nature 2014;505:495-501).

Statistics

[00157] All statistical analysis was performed using R (http://www.r-project.org/).

Example 2: Results

[00158] Identification of candidate somatic mutations. To investigate the extent of clonal hematopoiesis with somatic mutation, Applicants analyzed whole exome sequencing data from peripheral blood cell DNA of 17,182 individuals who were selected without regard to hematological characteristics. Of these, 15,801 were cases and controls ascertained from 22 cohorts for type 2 diabetes (T2D) association studies, and the remaining 1,381 were additional, previously un-sequenced individuals from the Jackson Heart Study, a population-based cohort (Supplementary Table S1). The median age of individuals was 58 (range 19-108), 8,741 were women, and 7,860 had T2D.

[00159] The identification of somatic driver mutations in cancer has come largely from studies that have compared differences in DNA sequence between tumor and normal tissue from the same individual. Once mutations are identified, investigators may genotype samples for these somatic variants without relying on a matched normal tissue. Because Applicants had DNA from only one source (blood), Applicants limited Applicants' examination to variants previously described in the literature for 160 recurrently mutated candidate genes in myeloid and lymphoid malignancies (Supplementary Table S2). Potential false-positives were removed by utilizing variant-calling algorithms with filters for known artifacts such as strand-bias and clustered reads, as well as additional filtering for rare error modes using a panel of normal (Cibulskis K et al. Nature biotechnology 2013;31:213-9). The lower limit of detection for variants depended on the depth of coverage. The median average sequencing depth over exons from the 160 genes was 84X, and ranged from 13 to 144. At a sequencing depth of 84X, the limit of detection for SNVs was at a variant allele fraction (VAF) of 0.035; for indels, the limit was 0.07.

[00160] With this approach, Applicants identified a total of 805 candidate somatic variants (hereafter referred to as mutations) from 746 individuals in 73 genes (Supplementary Table S3).

As a negative control, Applicants searched for previously described, cancer-associated variants in 40 non-hematologic genes (Supplementary Table S4) (Lawrence MS et al. Nature 2014;505:495-501) and found only 10 such variants in these genes. Below, Applicants show that the frequency of apparent mutations is exceedingly low in young people, and rises with age. These internal controls indicate that the rate of false discovery due to technical artifacts is low. Applicants also verified a subset of the variants using amplicon-based, targeted sequencing; 18/18 variants were confirmed with a correlation coefficient of 0.97 for the VAF between the two methods (Figure 14A).

The frequency of clonal somatic mutation increases with age. Hematological [00161] malignancies, as well as other cancers and pre-malignant states, increase in frequency with age. Mutations were very rare in samples collected before the age of 40, but rose in frequency with each decade of life thereafter (Figure 1). Mutations in genes implicated in hematological malignancies were found in 5.6% (95% CI 5.0-6.3%) of individuals age 60-69, 9.5% (95% CI 8.4-10.8%) of individuals age 70-79, 11.7% (95% CI 8.6-15.7%) of individuals age 80-89, and 18.4 % (95% CI 12.1-27.0%) of individuals older than 90. These rates greatly exceed the hematologic incidence of clinically diagnosed malignancy in the general population (Surveillance, Epidemiology, and End Results (SEER) Program Populations (1969-2012) (http://www.seer.cancer.gov/popdata). National Cancer Institute, DCCPS, Surveillance Research Program, Surveillance Systems Branch. 2014).

[00162] Though Applicants searched for mutations in genes implicated in multiple hematologic malignancies, Applicants primarily identified genes that were most frequently mutated in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). The most commonly mutated gene was *DNMT3A* (403 variants, Figure 2A, Figure 6), followed by *TET2* (72 variants) and *ASXL1* (62 variants). Of note, only exon 3 of *TET2* was baited by exon capture (corresponding to ~50% of the coding region), and the portion of exon 12 of *ASXL1* that accounts for ~50% of the mutations in this gene had poor coverage depth. Thus, mutations in *TET2* and *ASXL1* are likely underrepresented in this study. Other frequently mutated genes included *TP53* (33 variants), *JAK2* (31 variants), and *SF3B1* (27 variants).

[00163] In sequencing studies of MDS and AML, most patients have mutations in 2 or more driver genes (the median number of recurrently mutated genes in *de novo* AML patients is five (Cancer Genome Atlas Research N. Genomic and epigenomic landscapes of adult de novo acute

myeloid leukemia. The New England journal of medicine 2013;368:2059-74)). In this study, Applicants found that 693 of 746 individuals with a detectable mutation had only 1 mutation in the set of genes Applicants examined, consistent with the hypothesis that these subjects had premalignant clones harboring only the initiating lesion (Figure 2B, Figure 7A-B).

[00164] The most common base pair change in the somatic variants was cytosine to thymine (C-T) transition (Figure 2C), which is considered to be a somatic mutational signature of aging (Welch JS et al. Cell 2012;150:264-78, Alexandrov LB et al. Nature 2013;500:415-21). The median VAF for the identified mutations was 0.09, (Figure 2D), suggesting that they are present in only a subset of blood cells, and supports their somatic, rather than germline, origin.

[00165] Somatic mutations persist over time. Blood cell DNA obtained 4 to 8 years after the initial DNA collection was available for targeted sequencing in 13 subjects with 17 somatic mutations (4 subjects had 2 mutations). In all cases, the mutations detected at the earlier time point were still present at the later time point. For 10 mutations, the VAF stayed the same or slightly decreased, and for 7 mutations, the VAF clearly increased. New mutations were detected in 2 subjects, but no subjects were diagnosed with hematological malignancies (Figure 14B).

[00166] Risk factors associated with somatic mutation. To understand risk factors that contributed to having a detectable mutation, Applicants performed a multivariable logistic regression that included age, sex, T2D status, and ancestry as covariates (Supplementary Tables S6-7 and Figure 8). As expected, age was the largest contributor to risk of having a mutation. MDS is characterized by a slight male preponderance. In those age 60 or older, men had an increased likelihood of having a detectable mutation compared to women (OR 1.3, 95% CI 1.1-1.5, p=0.005 by logistic regression). Hispanics and Native Americans are reported to have significantly lower incidence of MDS than other groups in the United States (Rollison DE et al. Blood 2008;112:45-52). Applicants found that Hispanics had a lower risk of having a mutation relative to those of European ancestry, whereas other groups were not significantly different (Supplementary Table S6 and Figure 8). Of the genes Applicants queried, the spectrum of mutations did not differ between ancestry groups (Figure 9).

[00167] Association of somatic mutation with risk of hematologic malignancy. Pre-malignant states such as MGUS, MBL, and others are associated with an increased risk of subsequently developing a malignancy. Of the cohorts that contributed data to the study, two had longitudinal

follow-up information on cancer that arose subsequent to DNA collection (JHS and MEC). Together, these comprised 3,342 subjects, including 134 (4%) in whom Applicants detected somatic mutations in the blood. In a median follow-up period of 95 months, 16 hematological malignancies were reported, of which 5 (31%) were in the group that had detectable mutations (Supplementary Table S11).

[00168] In a fixed-effects meta-analysis of the 2 cohorts adjusted for age, sex, and T2D, hematological malignancies were 11-fold more common in individuals with a detectable clone (95% CI 3.9-33), a difference that was highly significant (p<0.001). In individuals with a VAF greater than 0.10 (indicating a higher proportion of cells in the blood carrying the mutation), the risk of developing a diagnosed hematological malignancy was nearly 50-fold (HR 49, 95% CI 21-120, p<0.001) (Figure 3A). Consistent with this finding, individuals with a mutation who went on to be diagnosed with a hematological malignancy had a significantly higher mean VAF at the time of blood collection than those who did not (25.2% vs 12.0%, p=0.003 by Wilcoxon rank sum test, Figure 3C).

[00169] While individuals with detectable mutations had a markedly increased risk of developing hematological malignancy, the absolute risk remained small; overall, approximately 4% of individuals with a clone developed a hematological malignancy during the study period (Figure 3B). This translates to a risk of developing hematological malignancy of $\sim 0.5\%$ per year overall, and $\sim 1\%$ per year in those with VAF>0.10.

[00170] Blood cell indices of individuals with somatic mutations. Somatic mutations found in MDS and AML lead to abnormal differentiation, ineffective hematopoiesis, and cytopenias. Blood count data was available on 3,107 individuals from 5 cohorts (JHS, UA non-diabetic controls, Botnia, Malmö-sib, Helsinki-sib), including 139 subjects with a detectable mutation. When looking at individuals with single mutations (*ASXL1*, *DNMT3A*, *JAK2*, *SF3B1*, and *TET2*) or mutations in more than 1 gene versus those with no mutations, Applicants found no significant differences in mean white blood cell count, hemoglobin, platelet count, or white blood cell differential after accounting for age and sex (Figure 10). The only statistically significant difference in blood cell indices was an increase in red blood cell distribution width (RDW), a parameter describing the variation in size of red blood cells (13.8% vs 13.4%, p=0.002 by Wilcoxon rank-sum test, Supplementary Table S8). This finding suggests that those carrying

somatic mutations might have perturbations in hematopoiesis similar to those seen in MDS, even in the absence of cytopenias.

[00171] Applicants also asked whether the presence of a mutation was associated with increased likelihood of having abnormally low blood counts (Supplementary Table S9). Most of those with a mutation had no cytopenias, nor did they have a higher rate of any single cytopenia than those without mutations. A small fraction of subjects had multiple cytopenias, and these were enriched in the fraction with mutations (OR 3.0, p=0.037 by Fisher's exact test). Furthermore, among anemic subjects, those with mutations had a higher fraction of unexplained anemias than those without mutations (Supplementary Table S10).

[00172] Association of somatic clones with overall survival. Applicants next assessed whether the presence of a somatic mutation had an effect on overall survival based on available data from 5,132 individuals in 7 cohorts (Figure 4) with a median follow-up period of 96 months. In a model adjusted for age, sex, and T2D, carrying a mutation was associated with increased all-cause mortality (HR 1.4, p=0.018 by fixed-effects meta-analysis with beta-coefficients derived from Cox proportional hazards models for individual cohorts, Figure 4A). Kaplan-Meier survival analysis within subjects 70 or older showed an increased risk of death in those with a mutation (p=0.002 by rank-sum test, Figure 4B and Figure 11). Death from hematologic neoplasms alone could not account for the observed increase in mortality, as only 1 individual with a mutation died from hematological malignancy. When Applicants performed a cause-specific mortality analysis, Applicants found that those with mutations had a higher risk of death due to cardiovascular causes, but not cancer (Figure 15).

[00173] Because Applicants found that the presence of a somatic mutation was significantly associated with higher RDW, Applicants also examined whether harboring mutations was synergistic with elevated RDW for risk of death. High RDW has been associated with increased all-cause mortality in the aging and critically ill population (Patel KV et al. Archives of internal medicine 2009;169:515-23, Perlstein TS et al. Archives of internal medicine 2009;169:588-94, Bazick HS et al. Critical care medicine 2011;39:1913-21), but the mechanism behind this association is uncertain. Information on RDW was available on 2,409 subjects in 2 cohorts. In an analysis adjusted for age, sex, and T2D status, Applicants found that having a mutation in conjunction with an RDW≥14.5% (the upper limit of normal) was associated with a marked increase in the risk of death compared to those without mutations and normal RDW (HR 3.7,

p<0.001, by fixed-effects meta-analysis with beta-coefficients estimated from Cox models for the two cohorts). In contrast, those with no mutation and high RDW had a more modest increase in mortality (Figure 4C, Figure 12).

[00174] Association of somatic clones with cardiometabolic disease. A recent paper reported that large, somatic chromosomal alterations in peripheral blood cells were associated with having T2D (Bonnefond A et al. Nature genetics 2013;45:1040-3). Applicants also found that somatic mutations in genes known to cause hematologic malignancies were significantly associated with increased risk of T2D, even after adjustment for potential confounding variables (OR 1.3, p<0.001, Figures 11, 12). Those with T2D were slightly more likely to have mutations than those without T2D at each age group (Figure 8).

[00175] Cardiovascular disease is the leading cause of death worldwide. Given the association of somatic mutations with all-cause mortality beyond that explicable by hematologic malignancy and T2D, Applicants performed association analyses from two cohorts comprising 3,353 subjects with available data on coronary heart disease (CHD) and ischemic stroke (IS). After excluding those with prevalent events, Applicants found that those carrying a mutation had increased cumulative incidence of both CHD and IS (Figure 5A and 5B). In multivariable analyses that included age, sex, T2D, systolic blood pressure, and body mass index as covariates, the hazard ratio of incident CHD and IS was 2.0 (95% CI 1.2-3.5, P=0.015) and 2.6 (95% CI 1.3-4.8, P=0.003) in the individuals carrying a somatic mutation as compared to those without (Figure 5C and 5D, Figure 8).

[00176] For a subset of individuals, the traditional risk factors of smoking, total cholesterol, and high-density lipoprotein were also available; the presence of a somatic mutation remained significantly associated with incident CHD and IS even in the presence of these risk factors, and the risk was even greater in those with VAF≥0.10 (Supplementary Table S12). Elevated RDW and high-sensitivity C-reactive protein (hsCRP) have also been associated with adverse cardiac outcomes (Tonelli M et al. Circulation 2008;117:163-8, Ridker PM et al. The New England journal of medicine 2002;347:1557-65), possibly reflecting an underlying inflammatory cause. In a multivariable analysis of 1,795 subjects from JHS, those with a mutation and RDW≥14.5% had a markedly increased risk of incident CHD, and this effect was independent of hsCRP (Supplementary Table S13).

[00177] Further validation was performed showing the relationship between clonal hematopoesis and risk for cardiovascular disease by analyzing two additional cohort studies. The BioImage Study is a study of the characteristics of subclinical cardiovascular disease, as measured by imaging modalities, unsupervised circulating biomarker measurements, and risk factors that predict progression to overt clinical cardiovascular disease, in a diverse, population-based sample of 7,300 men (aged 55-80) and women (aged 60-80). The socio-demographics of the study population aims to mirror the US population as a whole with approximately 69% of the cohort will be white, 12% African-American, 13% Hispanic, 4% Asian, predominantly of Chinese descent and 2% other (U.S. Census Bureau: 2000). The Malmö Diet and Cancer study is a 10-year prospective case-control study in 45-64-year-old men and women (n = 53,000) living in a city with 230,000 inhabitants.

[00178] Participants from the nested case-control studies that passed sample quality control (contamination, prevalent cardiovascular disease, germline Ti/Tv, germline total variants / depth, germline F inbreeding coefficient) are displayed (Table 1). Participants were matched by age, sex, diabetes status, and smoking status. LDL cholesterol and total cholesterol are adjusted accounting for statin medications as previously described. Individuals that carried at least one mutation in a gene conferring risk of clonal expansion with a variant allele fraction > 0.10 are defined as carrying a somatic clone.

[00179] Table 1. Baseline characteristics of study participants

	Bio]	[mage	Malmo Die	t & Cancer
	Cases	Controls	Cases	Controls
	N = 150	N = 344	N = 536	N = 531
Age, y	70.2 (5.8)	70.3 (5.9)	59.9 (5.4)	59.9 (5.4)
Female	60 (42.9%)	136 (39.5%)	233 (43.5%)	233 (43.9%)
Total cholesterol, mg/dL	208.8 (38.1)	214.0 (37.5)	271.2 (173.1)	252.7 (133.8)
LDL cholesterol, mg/dL	127.4 (31.4)	122.9 (32.8)	186.9 (117.1)	172.0 (92.5)
HDL cholesterol, mg/dL	51.0 (14.7)	53.1 (15.1)	49.1 (13.0)	51.8 (13.7)
Triglycerides, mg/dL	181.6 (94.1)	169.6 (93.6)	127.4 (57.4)	123.7 (58.9)
Diabetes mellitus type 2	36 (24.0%)	89 (25.9%)	82 (15.3%)	80 (15.1%)
Hypertension	130 (86.7%)	256 (74.4%)	421 (78.5%)	354 (66.7%)
Smoker	24 (16.0%)	55 (16.0%)	167 (31.2%)	166 (31.3%)
BMI	27.8 (4.7)	27.4 (4.7)	26.5 (4.0)	26.2 (4.0)
Somatic clone carrier	24 (16.0%)	34 (9.9%)	31 (5.8%)	22 (4.1%)

[00180] Applicants show an increased risk of cardiovascular events from carrying a somatic clonal mutation in both studies (Figure 18). Furthermore, Applicants show that an increase in

coronary arterial calcification quantity is associated with somatic clonal mutation carrier status (Figure 19).

Example 3: Discussion

[00181] Applicants find that somatic mutations leading to clonal outgrowth of hematopoietic cells are frequent in the general population. This entity, which Applicants term clonal hematopoiesis with indeterminate potential (CHIP), is present in over 10% of individuals over 70, making it one of the most common known pre-malignant lesions. The exact prevalence of CHIP is dependent on how cancer-causing mutations are defined and on the sensitivity of the technique used to detect mutations, and thus may substantially exceed this estimate. Unlike other pre-malignant lesions, CHIP appears to involve a substantial proportion of the affected tissue in most individuals; based on the proportion of alleles with the somatic mutation, Applicants find that a median of 18% of peripheral blood leukocytes are part of the abnormal clone. CHIP also persists over time; in all tested cases, the mutations were still present after 4 to 8 years.

[00182] The genes most commonly mutated in CHIP are *DNMT3A*, *TET2*, and *ASXL1*. This is consistent with previous studies that have found *DNMT3A* and *TET2* mutations to be frequent and early events in AML and MDS (Jan M et al. Science translational medicine 2012;4:149ra18, Shlush LI et al. Nature 2014;506:328-33, Papaemmanuil E et al. The New England journal of medicine 2011;365:1384-95, Welch JS et al. Cell 2012;150:264-78). Murine models of *DNMT3A* or *TET2* loss-of-function demonstrate that mutant HSCs have altered methylation patterns at pluripotency genes and a competitive advantage compared to wild-type HSCs, but mice rarely develop frank malignancy, and then only after long latency (Jeong M et al. Nature genetics 2014;46:17-23, Koh KP et al. Cell stem cell 2011;8:200-13, Challen GA et al. Nature genetics 2012;44:23-31, Moran-Crusio K et al. Cancer cell 2011;20:11-24). Similarly, Applicants' data show that humans with CHIP can live for many years without developing hematological malignancies, though they do have increased risk relative to those without mutations.

[00183] Certain genes commonly mutated in AML and MDS are absent or very rare in this study. Their rarity likely indicates that they are cooperating rather than initiating mutations. While mutations in genes specific for lymphoid malignancies were rarely detected, it is

important to note that *TET2* and *DNMT3A* are frequently mutated in some lymphoid malignancies, and the initiating event for such tumors may occur in a HSC (Neumann M et al. Blood 2013;121:4749-52, Quivoron C et al. Cancer cell 2011;20:25-38, Odejide O et al. Blood 2014;123:1293-6, Asmar F et al. Haematologica 2013;98:1912-20, Couronne L et al. The New England journal of medicine 2012;366:95-6). While it is most likely that these mutations occur in a HSC, it also possible that they occur in committed myeloid progenitors or mature lymphoid cells that have acquired long-term self-renewal capacity.

[00184] The use of somatic mutations to aid in the diagnosis of patients with clinical MDS is becoming widespread. Applicants' data demonstrate that the majority of individuals with clonal mutations in peripheral blood do not have MDS or another hematological malignancy, nor do the majority develop a clinically diagnosed malignancy in the near term. At this time, it would be premature to genetically screen healthy individuals for the presence of a somatic clone, as the positive predictive value for current or future malignancy is low. Further studies are needed to definitively assess whether the detection of a mutation in conjunction with blood count abnormalities is sufficient to make a presumptive diagnosis of MDS or another hematological malignancy.

[00185] Perhaps the most surprising finding in Applicants' study is the lower overall rate of survival in those with clones as compared to those without. This effect is much larger than can be explained by hematological malignancies alone, is synergistic with high RDW (which could be a marker of perturbation of hematopoiesis due to the clone), and may be related to the increased risk of incident CHD and IS in those with clones. The association of somatic mutations with non-hematological disease may be due to confounding by variables that are currently unknown, or may simply represent a shared consequence of the underlying process of aging. Alternatively, it may represent an underlying shared pathophysiology of seemingly unrelated disorders. For example, cells of the monocyte/macrophage lineage are considered important mediators of atherosclerosis and type 2 diabetes (Libby P. 2002;420:868-74, Olefsky JM, Glass CK. Annual review of physiology 2010;72:219-46). Applicants propose that one possible explanation for these findings is that somatic mutations that lead to clonal hematopoiesis cause functional abnormalities in differentiated blood cells that modulate the risk of cardiometabolic disease.

[00186] In summary, Applicants find that clonal hematopoiesis due to somatic mutation is a common finding in the elderly, and most frequently involves *DNMT3A*, *TET2*, and *ASXL1*. This clinical entity, CHIP, is associated with increased risk of developing hematological malignancy, minimal changes in blood counts, increased overall mortality, and increased risk of cardiometabolic disease.

Example 4: Supplemental Methods

[00187] Sample ascertainment and cohort descriptions. Subjects were ascertained as cases and controls for T2D from 22 cohorts in 3 consortia (Supplementary Table 1). Details on sample ascertainment for cases and controls from the most of the GoT2D (8 cohorts, 2,376 subjects) and SIGMA (4 cohorts, 3,435 subjects) consortia have been previously described (Flannick J et al. Nature genetics 2013;45:1380-5, Consortium STD et al. Nature 2014;506:97-101). The other ascertained individuals were part of T2D-GENES (9,990 subjects), a consortium comprised of 10 population-based cohorts. Details on sample ascertainment can be found in Supplementary Table 1. The remaining 1,381 individuals were additional subjects in the Jackson Heart Study (JHS), a large population-based cohort of African-Americans in Jackson, Mississippi, who had given consent for genetic testing but were not in any previously sequenced cohorts (Sempos CT et al. The American journal of the medical sciences 1999;317:142-6.). Including the 1,027 subjects from JHS enrolled through T2D-GENES (513 with T2D), a total of 2,408 subjects from JHS were in this study. Since 3,400 subjects in JHS were consented for genetic studies, ~70% of consented subjects from JHS are represented in this study, with a modest overall enrichment for T2D.

[00188] Subjects for which age was not available (116 subjects) or with cell lines as the source DNA (492 subjects) were excluded, including all subjects from the Wellcome Trust Case Control Consortium (WTCCC).

[00189] Vital status for Finland-United States Investigation of NIDDM Genetics Study (FUSION), The Botnia Study (Botnia), Helsinki Siblings with Diabetes cohort (Helsinki_sib), and Scania Diabetes Register (Diabetes_reg) was ascertained from the Finnish or Swedish Hospital Death Registries. Subjects from Botnia, Helsinki_sib, and Diabetes_reg were pooled for survival analysis. Vital status for subjects from the Multiethnic Cohort (MEC) was ascertained from Center for Medicare Services (CMS) data. Vital status for subjects from the JHS was

ascertained from vital records and annual follow-up interviews. For individuals lost to follow-up, if there was no death certificate, the individual was assumed to be alive. Vital status for non-diabetic Ashkenazis in the Longevity Genes Project (LGP) was ascertained from hospital death records and annual follow-up interviews.

[00190] Malignancy information for MEC was ascertained through linkage of the MEC with cancer registries of California and Hawaii. Malignancy information for JHS was ascertained from annual interview. Malignancy information for some subjects from FUSION and Botnia was available from the Finnish Hospital Discharge Register and Death Register, but not included because it was deemed to have significant ascertainment bias.

[00191] Standard blood cell indices (white blood cell count, hemoglobin, hematocrit, platelet count, and white blood cell differential) were available for most (but not all) subjects from JHS, LGP, Botnia, Malmo-sib, and Helsinki-sib. Information on red blood cell distribution width (RDW) was available on most subjects in JHS and LGP.

[00192] Data on cardiovascular outcomes for JHS was obtained from annual patient interview and adjudicated from hospital records. Data on cardiovascular outcomes for FUSION was obtained from Finnish Hospital Discharge and Death Registries. Coronary heart disease (CHD) included fatal and non-fatal myocardial infarctions as well as coronary revascularization procedures. For CHD analysis, those with prior CHD events were excluded. For ischemic stroke analysis, those with prior ischemic stroke were excluded. Lab data (blood pressure, body mass index, serum high density lipoprotein, serum total cholesterol, and high-sensitivity C-reactive protein) was obtained at the same time as blood collection for DNA.

[00193] Exome sequencing. DNA was obtained from individual cohorts and further processed at the Broad Institute of MIT and Harvard. DNA libraries were bar coded using the Illumina index read strategy, exon capture was performed using Agilent Sure-Select Human All Exon v2.0, and sequencing was performed by Illumina HiSeq2000. Sequence data were aligned by the Picard (http://picard.sourceforge.net) pipeline using reference genome hg19 with the BWA algorithm (Li H, Durbin R. Bioinformatics 2009;25:1754-60) and processed with the Genome Analysis Toolkit (GATK) to recalibrate base-quality scores and perform local realignment around known insertions and deletions (indels) (DePristo MA et al. Nature genetics 2011;43:491-8.). BAM files were then analyzed for single nucleotide variants using MuTect (http://www.broadinstitute.org/cancer/cga/mutect) with Oxo-G filtering

(http://www.broadinstitute.org/cancer/cga/dtoxog) and for indels using Indelocator (http://www.broadinstitute.org/cancer/cga/indelocator), followed by annotation using Oncotator (http://www.broadinstitute.org/cancer/cga/oncotator/) (Cibulskis K et al. Nature biotechnology 2013;31:213-9). All MuTect and Indelocator analyses were performed using the Firehose pipeline (http://www.broadinstitute.org/cancer/cga/Firehose) at the Broad Institute.

Variant calling. Cancer genome studies typically compare sequence from tumor and [00194] germline DNA, and define somatic mutations as the sequence variants present in tumor but not germline DNA. To circumvent the lack of matched tissue in this study, Applicants defined a list of pathogenic variants reported in the literature and/or the Catalog of Somatic Mutations in http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/) Cancer (COSMIC, in human hematologic malignancies from 160 genes (see Supplementary Table S2). **Applicants** specifically excluded genes known to be involved in hematologic malignancy that had a relatively high frequency of heterozygous loss-of-function germline mutations in the population (NF1, SH2B3, BRCA1/2). Identical frameshift variants that were seen 3 or more times from the same ancestry group were also excluded, unless such variants were previously reported as somatic. Frameshift and nonsense mutations were further excluded if they occurred in the first or last 10% of the gene open reading frame (Ng PC et al. PLoS genetics 2008;4:e1000160), unless mutations in those regions had been previously reported, (e.g. DNMT3A (Walter MJ et al. Leukemia 2011;25:1153-8)). Applicants used minimum variant read counts of 3 for MuTect and 6 for Indelocator. Python (http://www.python.org) scripts were used to parse mutation annotation format (MAF) files produced by MuTect and Indelocator for variants of interest.

[00195] To further confirm that Applicants were detecting bona fide somatic mutations, Applicants examined variants in 40 driver genes involved in non-hematologic malignancies (Lawrence MS et al. Nature 2014;505:495-501). Applicants hypothesized that very few variants would be detected from these genes if Applicants' methodology had high specificity for real mutations. Using the same calling approach as with hematologic genes (Supplementary Table S4), Applicants detected only 10 variants that were the same as those mutated in non-hematologic cancers, and most of these appeared to be rare germline polymorphisms as evidenced by allele fraction (Supplementary Table S5).

[00196] Targeted re-sequencing. Validation of variants discovered by whole exome sequencing was done with "Rapid Heme Panel" (RHP), a Laboratory Developed Test designed

and validated at a CLIA-certified lab (Center for Advanced Molecular Diagnostics, Brigham and Women's Hospital). RHP uses TruSeq Custom Amplicon Kit (Illumina, Inc. San Diego, CA, USA) and contains 95 genes (50 for AML/MDS, 8 for MPN, 27 for ALL, and 10 others). For oncogenes, known mutation hotspots are targeted; and for tumor suppressor genes the entire coding sequence is analyzed. The average amplicon size is 250-bp and about 50% of the regions are covered on both strands. Library preparation was according to manufacturer's instruction and sequencing was 150 bp paired-end reads with MiSeq v2.2 chemistry. Raw data was analyzed with Illumina on-board Real-Time-Analysis (RTA v.2.4.60.8) software and MiSeq Reporter. The VCF files were filtered with a cutoff for any nucleotide position with 10 or more variant reads or with 5-9 variant reads (if allele frequency >33%) as well as a Q score greater than 30 and germline single nucleotide polymorphisms were removed by comparison to dbSNP database (NCBI Human Build 141). The filtered variant lists were manually reviewed and BAM file examined in Integrated Genome Viewer (IGV, Broad Institute).

[00197] For 13 subjects from JHS, DNA obtained from a peripheral blood sample collected 4 to 8 years after the original DNA was available for analysis. RHP was used as described above to assess VAF of the previously detected mutations at the second time point, and to assess for the acquisition of new mutations.

[00198] Genes: ABL1, ASXL1, ATM, BCL11B, BCOR, BCORL1, BRAF, BRCC3, CALR, CBL, CBLB, CD79B, CEBPA, CNOT3, CREBBP, CRLF2, CSF1R, CSF3R, CTCF, CTNNB1, CUX1, CXCR4, DMNT3A, DNMT3B, EED, EGFR, EP300, ETV6, EZH2, FANCL, FBXW7, FLT3, GATA1, GATA2, GATA3, GNAS, GNB1, IDH1, IDH2, IKZF1, IKZF2, IKZF3, IL7R, JAK1, JAK2, JAK3, KIT, KRAS, LUC7L2, MAP2K1, MEF2B, MPL, MYD88, NOTCH1, NOTCH2, NOTCH3, NPM1, NRAS, NT5C2, PAX5, PDGFRA, PDS5B, PHF6, PIGA, PIK3CA, PIM1, PRPF40B, PRPF8, PTEN, PTPN11, RAD21, RET, RIT1, RPL10, RUNX1, SETBP1, SETD2, SF1, SF3A1, SF3B1, SH2B3, SMC1A, SMC3, SRSF2, STAG2, STAT3, TET2, TLR2, TP53, U2AF1, U2AF2, WHSC1, WT1, XPO1, ZRSR2.

[00199] Statistics and analysis plan. All statistical analyses were performed using R. Cox proportional hazards and Kaplan-Meier analysis was performed using the **survival** package (http://cran.r-project.org/web/packages/survival/index.html). Competing risks regression (CRR) was used to estimate hazard ratios for developing hematologic malignancy with death as the competing risk (Fine JP and Gray, RJ. Journal of the American Statistical Association

1999;94:496-509). CRR and cumulative incidence analysis was performed using the **cmprsk** package (http://cran.r-project.org/web/packages/cmprsk/index.html). Fixed-effects meta-analysis using beta-coefficients for risk estimates from individual cohorts was used to provide summary hazard ratios across heterogenous cohorts. Meta-analysis was performed using the **meta** package (http://cran.r-project.org/web/packages/meta/index.html).

[00200] Analyses for factors associated with clonal hematopoiesis were performed using logistic regression with the pre-determined variables age, sex, type 2 diabetes status, and ancestry.

[00201] Primary outcomes for clinical associations with clonal hematopoiesis were predetermined to be all-cause mortality and hematologic malignancy, using Cox proportional hazards models and CRR, respectively. Association of mutations with blood counts was also a primary analysis. For hematologic malignancy outcomes, only events incident to the time of DNA collection were considered. Red cell distribution width was also included as a variable in the survival analysis because of its association with mutations and previous reports of its association with increased mortality.

[00202] An analysis of cause specific mortality revealed an increase in cardiovascular deaths. For this reason, Applicants examined incident events of coronary heart disease and ischemic stroke using CRR and the pre-determined variables age, sex, type 2 diabetes status, body mass index, and systolic blood pressure. For some subjects, data was also available on smoking status, total cholesterol, and high-density lipoprotein. This was examined as a subgroup analysis in a separate regression (see Supplementary Tables S12-13).

[00203] For some analyses, a cutoff of VAF at 0.10 was used. This value was chosen because it was close to the median VAF in the dataset and is roughly the lower limit of detection using Sanger DNA sequencing, and was thus used to designate large versus small clone size.

Example 5: Supplemental Tables

Supplementary Table S1 Cohort descriptions and baseline characteristics

TOTAL	Vo T2D	T203 .	136 (100000	Câncat internation available	Colort descriptions and sample ascertamment	Saferences
Female (number with clone) 4. Mean age (SD)		7861(278) 3845(173) 59(10) 29(5.6)	18(2) 3(0)			
Population-based	Nec 1739	720				
		589(25) 401(28) 58(21) 34(6,4)	18(1) 8(0)	Sorukeal, makignacy, téosel cosets, cerdowascular events	The audison Heart Study or a projection-bound colors of \$,356. African-Armadosin Heart is believed, filtrativities are served as a modern installation continued to commercial, filtrativities are served filtrativities and commercial policy continued as a part of the 46th accordance field in Commercial (4710), or reconduly familiar part of the 46th accordance field in the continued (4710), or reconduly familiar part of the 25th accordance filtrativities are installated, participated as ever continued to telephone to capable information professional installation, decident in the continued are telephone for capable information and forestimate states, and observe additional continual formation. Quantifiers also made are required and professional continual formations. Quantifiers also made are required as a continued as a continued and observe and determined and continued accordance and determined and continued accordance and determined and continued and determined and det	Wilson, 1/3, exist. (2005). "Boudy design for general methylic to the Jack con Reset Dudy." Ethnicky and Olsewer SS 50-37. Taylor, M. A. (2005). "The Back con Near Concept for Observation," Ethnicky and Disease SS 10-37. Fuges, S. E. et al., (2005). "Bertholog African-Anemarcan Research Participation in the Jack control Reservation." Ethnicky and Disease Starty. Ethnicky and Disease SS 10-29, Fuges, S. E. et al., (2005). "Ethnicky and Disease SS 10-29, Fuges, S. E. et al., (2005).
GeT20	%a 320	720				
Bobsia (The Botnia Skudy), Diabetes_reg (Scania Diabetes Registry), Heisinki-sib (Heisinki sibilings with diabetes cohort), Malmö-sib (sibilings in Malmö, Sweden) Oserali (norder with done) Female (norder with done) Mean 884 (SD) Mean 884 (SD)	(3)261 (91)28	211(5) 57(10)		Sonaireal, blood mounts, candiovescular even	The Bookin Project was stanted by 1990 to study risk factors for T2D in visitors finitend and southern Sweeze and knowles 11,000 people from 1900 Foodbas. The Bookin Disbetos Registro stanters over 7000 related patients records from the other Sweeze to believe, Sweeze as from 1996. The weighting of the protesses come from the other of blooks, and they socious for asset 1996 of all violating patients on the registro. Destin information was obtained from the Franch of Coadrich Beeth and Hopping Stockings Registro. Individual covers stellar at concentration for 1996 the Patients of Sweeze Stockings Registro. Beeth individual covers stellar at concentrage to a 1996 the Patients of Sweeze Stockings Registro. Beeth individual covers stellar at concentrage to 1996 the Patients of Sweeze Stockings of the Patients (Sweeze Sweeze source component as the 1996 defeated between of before the Patients Patients (Sweeze Sweeze Sweeze Sweeze Sweeze 1996 of the Patients (Sweeze Sweeze Swee	
FUSION (Finland-United States Investigation of NIDDM Genetics Study) Overall (number with stone) Female (number with stone) Mean age (SD) Mean BM: (SD)	215(11) 53(7.2) 28(3.9)	201(10) 58(8.0) 31(5.5)		Survival, blood counts, cardiovascular exem	The Prolomotion had between to recognitions of \$100,064 Generators (FUSODA) crossly its invegenment offerm to Selectify generics understood being prolomous for your 2 Calabrases (TOTA) or that in inquart the variationable of TOTA-valued quantitative tradits. Moreovers of the variational of TOTA-valued quantitative tradits. Moreovers of the variative control of TOTA-valued quantitative tradits. Moreovers or the value selection of the variative control of the Value of	Value, T., et al. (1298), "Obspoling genes for VIDEAL Congr. of the Endon-Circuse Shape investigation of MODAL Service (2000)." Study "Diabetes Care 2001; 549-559, Dozzi, L. et al. A. genome-wide accordation study of trave 2 orbitation in Prince detects enuitying properties of the Congr. Science 218(5503); 1344- 1345 (2007).
KORA Oversit (number with close) Female (number with close) Nason age (50) Mean BMI (50)	91(11) 58(3) 70(5.6)	97(6) 49(3) 61(8.1)			PCRA it a regional versionin production for population based divides, subsequent follow-up studies and family studies, established in 1996. Cause and controls make all of bennan streetty. Care where rearrishes my self-inject of 170% in a persion experies in which was violated by a quantification maked to the breating physician analysis by medical chart review. Controls were incondisionly as delined by self-injects.	Wichreson, N.E., et al. (2005). YIOPA-gen- resonce to population general, production as bread operation of disease previously." Gesundhatawezen 67 Suppl 1, 528-50.
MPP (Malmo Preventive Project) Overall (number with clone) Fennale (number with dane) Mean age (50) Mean 684 (50)	97(7) 68(5.2)	51(5) 47(6.3)			The MFS was stated in the ward 1970s or a convening survey to the model-leged population of fallers), the third leged of Sweden, Subjects from in Native's and residence of the city were mixted for a clinical submitted, questionastic and blood sangling. In \$12,445 oral and 10,500 women perhapsited during the period 1994-1992. Care and controls were celested or for Stotia.	Bengland, G., et al. (2000). "Congressin autoase of the Matria primeralise projects marticipy and conformations marbidity." I laters (Next 247(1)) 59-29
STF (UKT2D Consortium, Contro Overoit (number with close) Female (number with zione) Meen age (30) Mean BM((50)	319(14) 255(11) 51(16)	*			Non-disharbs cannotes selemed from the Tuber Lik Study. A robin pair was cancered for selection if there was no rescribed from history of dishares, neither from masseum accorded at impeliate glocose selemen, there was exhibite generated to make and general day, and no evidence of elementaries. From each dispelhing progress, the bast control from execution of calculations of frashing process lever to BMI across all readings.	Societies (A., et al. 1993). "The UP Antot Tube. Segister (TerroLOS Resource)," Tube has more Gener 196 (): 164-169.
SIGMA	No 130	130				
Female (number with clane) Mean age (50)	445(24) 225(13) 58(7-2) 27(4.5)	498(27) 269(14) 68(7.3) 30(5.7)		Survival, metignatory, cardiovectular events	The MED consists of 255,251 man and women in trevial and Los Angeles. Those self- beerfolded as before some seed for segmenting to a prior steed, Services 1993 and 1995, solids terminant 45 and 75 years old were enrolled. Emparted cohort numbers was identified monthly Regardment of Mohor Medice thereof forest forest file, some registerion of file and Health Case Financing Administration data files. Services 1995 and 1990, Who disperiences were solidated from 17,100 MED participants. Controll were finigened, creations to seed on sey, which, and are a carry into the colors (Syster age ground and particle of Mohor). Medice, South or Leonard Alexands. Personal to the colors the service produce of Mohor Control Alexands Mexico, South or Leonard Alexands. Personal to the colors the service personal agestions that have been extendibled by state statute in Manachi and Conference.	characterístics. Asn J Epicennisk 132: 346-357.
MexB1 (UNAM/INCMNSZ Diabetes Study) Overall (nomber with close)	543(7)	\$50(30)			Cases were instructed as the outgestern districtes after the December of Encountribley and Democratical Section of the Control of Section Sec	3/9994-720 Consortham, et al. (2011). "Sequence versions in NCOSK12 are a standard risk floridar-for region 2 discretes to Substance." Nations SIS(7486): 97-991.

Female (number with clone) Mean age (\$0) Mean (\$4) (\$5)	336(8) 55(9,4) 28(3,9)	328(13) 55(13) 28(4,4)	empropers (from color-mantes) and objects reading for intention to medical solor for explanation between five color-mantes) and objects reading for intention to medical solor for explanation beddes from some conditioned make exclusions ordered. (Solor-Solor-Mantes) (Solor-Solor-Mantes) (Solor-Solor-Mantes) (Solor-Solor-Mantes) (Solor-Solor-Mantes) (Solor-Solor-Mantes) (Solor-Mantes) (Solor-Man
MexB2 (Diabetes in Mexico Study) Duerali (number with clone) Female (number with clone) Mean age (SD) Mean (RM (SD)	177(3) 134(3) 56(9.9) 28(4.6)	393(12) 275(9) 57(32) 28(5.5)	Individuals were contribution to a terriary level institutions (MSS) and SSSTE) to reced to 1997.4 TSC Consortium, et al. (2014) Trapients Mesons Day, unradiate treatily, subjects older than 45 years and with facing government of the Consortium, et al. (2014) Trapients before 2015 report of 2015 and 2015 report of
Mex83 (Mexico City Diabetes Study) Decreii (number with clone) Female (number with clone) Mean age [50] Mean BMI (50)	550(23) 364(24) 62(7 6) 29(4.9)	205(8) 184(2) 64(7.5) 30(5.5)	The Mesons City Distillates Study is a population based prospective intestigation. AS 35-39 years of against a root open preparative increase residing in the citizut sets (low shocked in SECTIVE Consortium, et al. (2014). "Dequested years of against an original variation existing in the citizut sets (low shocked in SECTIVE Consortium, et al. (2014). "Dequested years of against a consortium stands with a storic population of 55.000 visionations." IN SECTIVE Consortium, et al. (2014). "Dequested years of 57.500 visionations and SECTIVE Consortium, et al. (2014). "Dequested years of 57.500 visionations, et al. (2014). "Depued year
T20-GENES	No T2D	T2D	
AW (Wake Forest School of Medi Overall (number with clone) Female (number with clone) Mean age (SD) (Mean BM (SD)		y) 529(48) 317(28) 64(9.2) 29(6.6)	Custor are serforage mail disebation with richastic rephrepathy, recovered from disebatic strict. Factors, N.D. et al. A genome-wide accordation with age of areas 255. Custods rever recordate from contractify and internal medicine choice. Search for type 2 disebation game in African and tree or custom diseases of disebation or serial disease. Americans, PloS One 7 e25002, (2011)
EX (Korea Association Research P Overall (number with clone) Female (number with clone) Mean age (50) Mean 8981 (50)	554(33)	524(20) 287(4) 54(7.5) 25(3.3)	Casas ordered for age of onces of T20, 190 years. Participants with early areas: T20 and the charge of the participant with early areas: T20 and the charge of the participant with early areas: T20 and the charge of the participant with early areas: T20 and the charge of the participant with early areas: T20 and the participant early areas: T20 and talk early areas: T20 and
Study and Singapore Prospective Study Program Overall (number with clone) Female (number with clone) 94ean age (50) 54ean 88h (50)	592(25) 563(11) 58(7.0) 23(5.4)	478(24) 250(11) 58(9.3) 26(5.5)	Cases were obdazily accertained T2D from printery care circus, behinduish with early age of San, X. et al. Transfersibility of type 2 debtariss diagnosis and with a early need for the great reschible, with T2D were preferentiatly selected. Controls were referred as fasting board glascre of monoly, as certains instrumed debtaries observed and mannishabetic medication. Diden controls preferentially selected. (2011)
HA (Hispanics from San Antonic Family Heart Study, San Antonic Family Diabetes/ Galiblatider Study, Veter ans Administration Genetic Epidemiology Study, and the Investigation of Mephropathy and Diabetes Study family component) Overall (number with clone) Female (number with clone) Mean age (SD) Mean BMI (SD)	112(6) 62(4) 44(10) 80(5.5)	142(3) 50(3) 51(22) 53(6.3)	Data ware drawn from hour separate hands studied and mark the following criteries: PUX 2002 Microek, IX. D. et al. Carretic and environmental criterion, WHO 1999 ordering, or physicisms reported disanguar with correct medical binary. Controls defined by not being fasting glocope <126 mg/du at each risk and mobilistics of global Medical Americans. The lies absolute Family disbusce medication. Heart Study. Carretines 94, 1255–126 (1999), Hourt, IX. J. at al. Genome-white Nature and Americans and Laborate selection of the part being analyses of the part being fasting glocope. According to San Americans 34, 1255–1266 (1998), Hourt, IX. J. at al. Genome-white Nature analyses of the part being analyses of the part of the
HS (Hispanics in Starr County, Ter Overall Interber with clone) Female (number with clone) Mean age (5D) Mean BMI (SD)	(as) 704(8) 506(3) 53(8.9) 59(6.2)	751(82) 449(15) 56(12) 52(6.4)	Cases defined by facing goodse 2.145 mg/dt on mose titien 1.compoon or serive ported priorition-diagronest discretes with current medical disregor. In intracross where cases were to serve to the conformation of the horizontal distriction. Control ascertained from spidemiologically represented compile of individuals in Stem County, Triviation demonstrated and conformation of district serveds. Detailed and segmental compile of individuals in Stem County, Triviation demonstrated and conformation of district serveds. Detailed and segmental representation of district serveds. Detailed and segmental representation of district serveds. Detailed and segmentation of district serveds. Detailed and segment from segmentation of district serveds. Detailed and segment from segmentation of district served segment segments. Detailed and segment from segments of district segments. Detailed and segment segments of district segments. Detailed and segment segments of district segments. Detailed and segments are segments. Det

St (tondon Life Sciences Propulation Study (UK Indian Asians)) Versil (number with clone) Female (number with clone) Mean see (SD) Mean BMI (SD)	85(4) 83(9-2)	531(18) 75(2) 58(5 6) 17(2 8)		A capitation-based orders study of trades Adams Fring in West London, UK with shift grandparents born on the violes subcontinent. Prevaient TED before daily membrane approximation displayed of statemes on interstinent, with place of displayed after the ED statement, with place of displayed after the ED statement, with place and violence after the ED statement, with place of displayed at the trade of the ED statement, and fracting postering places of 5 months? Common displayed at the provided at the provided at the place of displayed, the participation of displayed, the participation of displayed at the place of the place	1172 (2009); Chambers, 2.C. et al. Common
55 (Singapore Indian Eye Study (Singapore Indians)) Overall (number with done) Female (number with done) Mean age (SD) Mean BM (SD)	238(5) 56(28)	565(81) 250(14) 81(9.7) 27(5.5)		Cases selected for which Cha.Shi or personal history of dispetes with age at diagnosts smokable. Perimentally exicuted cases with at head one first degree practice with TOD. Control selected for Hold Chair, no present history of diabetes, and not resing anticlases or exiduation. Older amounts preferentially selected.	Ser, C. et al. Transferentiaty of type 2 disperses implement text or motivarishing coborts from locohours Asia. Plad Genat. 7(4), e2002265 (2011)
UA (Ashkenazis) Overall (number with close) Fetosile (number with close) Mean age (SD) Mean AMI (SD)	195(20) 79(13)	505(46) 242(18) 68(8,8) 27(8,2)	Survival, bland counts	aon. Feeder account perculated. TSD cares were severated from two segments DNA enfections. Li Generate violen, effected disting-leak indeeps crossy (Ferminist et al. Disbasses 2003) on 1, Soudy to deteratione genetic rest for disbastic compositions (Section et al. PLCS One 2003). Controls were severated for fronting blood groups -7 monethy, so personal indictory of disparties, and the arms dispared resolvations. Controls included elidativity-950 years) includes to who were part of the Longevity Genes Propect.	conserved pathway for exceptional longevity in numate. PLoS Biol. 8(4), e133 (2006); Atamon, G.
UM (Metabolic Syndrome in Mer Overall (number with close) Fentale (number with close) Mean age (30) Mean BMI (3D)	500(9)	487(24) - 50(6.7) 51(5.1)		delected from the population register of the town of Euoplo, Eastern Finland, and examined in 2005-2010. The aim of the study is to investigate	Dancasona, A. et al. Dranger in basilità sendrochy and proble release in relation to glycenus and poscoo traleranse in 5,454 Friends men. Urabetes 58, 3229—5023 (2006)

Supplementary Table S2 List of hematopoletic genes and variants queried

Gene name	Reported mutations used for varient calling	Accession	Number of
			variants found
ARIDIA	Premieronity/morphesiae, AUGA, MS728	RW_000015	8
ASXL1	Fremeshift/horsense in extra 11-12	NM_015538	82
BCL10	France/off/ponsense/cplice-offe	MM2_008972	9
BCL11B	Frameshift/nonsense/splice-site. A3601, C432Y, H445Y, R447H, G452K, H478Y, G5980, L627O, G647R	RM_138578	3
8C16	Bach, Y1114, F3614, S3506, X585w. <u>1</u> 675x	866_001330945	0
BCOR	Frameshift/aunsense/uplice-site	NM_001125885	4
BCORL1 BIRC3	Frances/Afficience/septice-rate	NR 071946	2
BRAF	Frameshift/horsense/splice-site expn 2 25544, 25545, 25544, 25545, 255444, 255444, 255444, 255444, 25544, 255444, 255444, 25544	NM_182962	1
O COC	7930, 00900, 1970, 1970, 1970, 1974, ASSIG VISION (ASSIG VISION VISION VISION VISION ASSIG ASSIG RESIGN ASSIG VISION VISION ASSIGNMENT ASSIGNME		4
	WONG 1835G 1605F, 1605N, GRORE, GRORA, GRORA, HRORE, HRORE, GRISH, WILEP, 1615F, 1615N, 1616W	NM_004353	
BRCC3	Frameshift/monsense/splice-site	RM_024852	ű
87G1	\$35, H26, P56, P56, \$311, 3324, \$250, \$279, \$390, 1315, \$386, \$370, \$385, \$400, \$480, \$480, \$480, \$580, \$590, 1340, \$1150, \$1170, \$1180,	MM_001233	8
BTG2 CARD11	A457, A45E END, 4:125, 3106D 71000 F150 R176W, K181N, M183L, K215M, O100N, L181L, M199M944994, K1447, S150P, S150P, L181P, V056P	NM_005765	0
CANDII	25386, 7555* 75579, YSSIH, MX658, LSETY, LSETY, L441Y, 84180, R455W, F455X, EGGK	N02_032435	4
CSE	RISG finger missense ti 351-421	NM_005288	12
C818	RING Riger maseriae p 875-412	NM_170662	8
ECND3	FramesNft/aansense/jglite-site, 72114, 72121, V215G	NM_001760	0
C258	Francesistf, inconsense project-site, 92120, 92120	800_001779	3
C070	LSDR, G684, F1868	NM_001252	9
C079A	deli91 235, deli70-125, deli91-236	NNS_501788	ø
CD798	VSA, D89G, Y92F, D193G, 0194G, V196C, Y197C, V197O, V197H, T207ft, V207 [©] , V212A, del195_197, del196_215, del193_215, del205_225	NMA_909626	1
CDKNZA CDKNZB	Francis (h) kosence (spice-site	N98_000077	9 0
CEBPA	Frameshift/honzenze/splice-tite Frameshift/honzenze/splice-tite	NM_094958	9
CHD2		NM_004864	9
CNOTS	HS20_F1146L_11270F Protestiff/matiente_E20A_RS7W_RS7Q_ETOX	MM_001271 MM 014818	3
CREBBP	Frameshtf/nonsense/sobce-ste, 01435c, 81446c, R1446c, R1446c, F1459c, F1476R, Y1481M, M1467f, W1502c, Y1503D, Y1503H, Y1503F,		ō
	5168034:	NM_094880	
CRLFI	FESC	NNS_022548	8
CSF1R	L301F, (3012, 78691, 7989N, 7989F, 7969H, 7969D	NMS_005211	0
CSF3R	ISISA INISI bunang CN1/91	207502_508	8
CTCF	Frameshift/honsense, R377C, R377H, P378A, P378L	MM_006565	0
COXI	Promestic Stories and a service of the service of t	NW_181562	2
D853	RZ76K, R2761, R3781, D506Y, R526C, R526H, R5342, R524H, P5360. R760Y, R780T, R780H, R7800, D486H, P486X, D488N, D477R, R467Q, M682R, R589C, R5140	NM_001956 NM_014958	9
AETMIND	Framestift/monsense/spiice-site_PROTS, FRATE, RESEN, RESEN		403
	DSITP, YSSQ, GS43A, GS43A, GS43C, LS47H. LS47F, LS47F, MS48Y, MS48X, MS48X, GS50R, WFS1R, WS51G, WS81C, G649Y, G646E, L653X, LB62F, V657A, V657A, R659H, Y680C, R756W, R758W, R658H, G685E, G685A, G689R, G699R, G699R, G699R, G70C, P70CR, P70CA, P70C		
	68698, 68894, M8894, 38818, 88814, 88814, 88814, 88814, 88816, 38888, 48898, 18918, 19414, F9441, F9461, A910A	NN4_022552	
EBF1	Carriero (f. Societice, M.14, 31'6, 1544, 3143Y, 31715, 3135F, N.1378, S.135T, S.135Y, K.1913	959_324957	9
EED	Fremeth/fronsense/splice-site, 1240C, 1383M	NM_009797	9
EFY6		NM 001428 NM 001587	1
EZH2	Frameshift increase/splice-site Nomentiff increase/splice-site 08.08, N1005, F1450, F1450, F1450, G1550, E1640, RXCCQ, X153E, F2444, RXSSQ, H2500, N4085, F457Q, RSSLH, TSSD, H252E, K4610, K4611H, K4612, K4612, K4612, K4613, N5564, W2740, M5770, M5770, M6780, M678H, M6852, M6871, M6880, RSSLH, TSSD, H252E, K4610, K4611H, K4612, K4612, K4612, K4613, M6854, W27400, M6770, M6770, M6780, M678H, M6852, M6851, M6871, M6880, RSSLH, TSSD, H252E, K46110, K4611H, K4612, K4612, K4612, K4613, M6854, W27400, M6770, M6770, M6780, M6780, M6852, M6857, M6880, RSSLH, TSSD, H252E, K46110, K4611H, K4612, K4612, K4612, K4613, M6854, W27400, M6770, M6770, M6780, M6780, M6852, M6857, M6880, RSSLH, TSSD, H252E, K46110, K4611H, K4612, K4		2
EZR	NASSE, HANDY, SARIVE, TODIX, TODIX, TODIX, ETZCX, ETHON CASSM	NM_001208297	8
FAM46C	Parago (filosogo asigo asigo	NN 017798	1
FAS	Frameshift/noncense/splice-site	NR4_000043	9
FBXO11	France nulti (nonze nije) sprine - site	958_3031350374	8
FBXW7	Frameshift/honsense/splice-site, E744, G1G1V, F286L, B465H, BSGSC, G567E, R116SQ	MM_085652	1
FLT3	VST8A VS98A VS961 F5941, MT271 F1590-34161	NM_004319	1
FOXP1	Frame:htt/consense/spice-site	NM_082682	1
FYN	1174F, 81761, Y501H	X802XCX8X	9
			6
GATA1	Framesinft/nonsense/spice-site	NA-002049	
	Frameshift/framsense/spilce-site Frameshift/framsense/spilce-site	NM_002049 NM_001145881	8
GATA1	Frencesh®yososeoseyspice-sise R2000, R517+, R5157, R5109, R5262, E3219, E3219, E3219, E3219, E3219, E3219, E320, E3311, E3209, E		0
GATA1 SATA2 GATA3 GNA13	rmeneshfyncoseosekpide-sse, 62990, 6317h, 43187, 43109, 43100, 23100, 231F, 1325-13219, 13297, 83200, 89611, 13597, 43717 83540, 83580	558_901145551	0
GATA1 GATA2 GATA3 GNA13 GNAS	Premerbiff/Generose/op/Ge-drs, R2800, R327H, A3187, A3180, A3160, 23186, 1321F, 1321F, 1321F, 1321F, 1321F, 13287, R3200, RX611, 1358V, A3777 83860, F8880 Framerbiff/Introense/spiice-dre ZNF-domain, R276W, R2780, N288T, 1543V, 84F, G879, 888F, M888, R1348, Y148F, 1332F, E3670, C188H, R284H, E273K, V323G, V363G, 1373F R261(844)S, R2G1(844)C, R2G1(844)H, R2G1(844)K, G217(876)H, G227(876)H, G227(876)K, G227(876)H,	950,001145681 851_001002295 851_006972 851_016592	0 0 8
GATA1 GATA2 GATA3 GNA13 GNAS GNR1	Premierbiff/Conserves/Optionate R2880, NS27H, AS187, AS189, AS180, E318E, E32F, E32F, E32F, E32F, E3287, NS300, RX61, E35W, AS777, R8880, R3887 R8880, R8880 Frameshiff/housense/Spiice afte ZNF domain, R278W, R2780, N2887, E348W, B41, G479, S488, M284, E3248, Y4887, E328, E3670, C389H, R284H, E3278, V3280, V3620, E3797 R501,84418, R201,844W, R201,844W, R201,844W, D327(870)M, G227(870)M, G227(870)M, G227(870)M, R574(1017)M, R570, R57M, R57E, R577, R677, R677, R670, R59W	504_001145581 504_001052295 504_008573 504_016592 504_083074	0 8 8 8
GATA1 GATA2 GATA3 GNA13 GNAS	Premerbiff/Generose/op/Ge-drs, R2800, R327H, A3187, A3180, A3160, 23186, 1321F, 1321F, 1321F, 1321F, 1321F, 13287, R3200, RX611, 1358V, A3777 83860, F8880 Framerbiff/Introense/spiice-dre ZNF-domain, R276W, R2780, N288T, 1543V, 84F, G879, 888F, M888, R1348, Y148F, 1332F, E3670, C188H, R284H, E273K, V323G, V363G, 1373F R261(844)S, R2G1(844)C, R2G1(844)H, R2G1(844)K, G217(876)H, G227(876)H, G227(876)K, G227(876)H,	950,001145681 851_001002295 851_006972 851_016592	0 0 8

HIST1H1D	Fremeshift/monsense	F6M_005320
HIST1H1E	8.58T, R18TV, P1985, K202E, K2059	NM_005328
HIST1H3B	A485, S87N, 587T	NM_693587
HLA-A	Frameshift/nomense, G1240, A1641	NM_XQ119
1D3	Fromeshift/honsense/splice-site, S898, V55G, R568, 164E, S65R, 174V, 1808, M96R.	6M_002167
10H1		
	R1300, R1316, R1314, R1311, R1312, R131V, V178	NM_005898
IDH2	R140W, R140D, R140D, R140G, R172W, R172G, R172K, R172T, R172M, R172N, R172S	NM_002158
XBKB	82736	MM_DXG-525
IKZF1	Framestift/toosense	NS4_008080
{XZF2	nament/namens	NM_016353
IKZF3		
	frameshift/nonsense	NM_012481
ILTR	exon 8 systems insertion	NW_000185
ints12	M445i	NS4_020895
1884	925, \$187, 1639, 1439, Q608, G604	MM_002450
IRFS	Frameshilt/nonsense from c 377-426, 9341, 955A, 730A, X10SE.	NM_002153
JAK1	T478A, 7470S, M623A, A664D, (M55F, 8724H, 8754C), T763M, (789F	NM_0X3227
JAK2	NS335, NS337, NS335, HS38R, XS39E, NS39L, NS40T, IS40T, V617F, R683S, R633G, del/ms537-539L, del/ms539-539L, del/ms540-540MK,	······································
JARZ	del/ms540-544k/k, del/ms541-543k, del542-543, del543-544, ms11546-547	NS4_004972
JAKS		
	MS137 MS11/ 46720, 45727, 4573V R6570, V715/ V715A	888_300225
JARID2	Firemeshift/nonsense/spilice-site	NM_004978
KDM6A	Processiff (scores e) (pilce-site, pol4)8	NM_SSI343
KIT	htsp3, vs55A, vs56D, vs56G, vs58, vs60D, vs60A, vs60G, vs60C, dw56O, e562K, deis79, P627L, P627L, P634V, K643E, K642C, V654A, V654	ī,
	H697Y, H697D, 5761D, Y807R. D816K, G816Y, D816F, OS16I, D816Y, D816H, delf51-539	NM_990222
Kihi6	F461, LSSY, T646, T641, L65Y, L65Y, L65Y, L65Y, L95Y, L95Y, L95Y, L95Y	864_120448
KRAS	G120, G124, G125, G120, G130, G130, G137, G138, G138, G138, G159, G136, T581, G600, G604, G609, G616, G616, G618, G611, G6116, G616,	
	K117E, K137N, A186T, A146P, A146N	NM_033363
LEF1	France: Not (consense	NA 016269
LBRK2		
	E155N, IS435	NM_130576
LTB	Enables in the Constance	NM_002042
LUC7L2	FrantesNitt/noncense/splice-site	NM_015019
MALT1	(96aF, 17505	MM_006765
MAP2K1	F58L, 056P, K577, K578, K57E, K0986, C3218, N122D, F134Q	/eM_002755
MAPSK14	45303	NM 008954
MED12		
	£33%, £389, \$44%, \$559, \$520H, £3234F	NM_005120
MEF2B	(190165)45/100106106/508/04-046, (SF, 1578, 1529), EF78, (SSIX, NC17, 2534, 2534	NM_001145795
MLL	Frameshift/nonsense	NR4_005958
MILL2	Framework/Increase	WM_003488
MPL	55055, 8505N, 8505C, 1510F, delsts, W515A, W515A, W515K, W515K, W515L, A519T, A519V, Y581D, W515-519KT	/eM_005878
MXRA5		NN4_015429
MYD88	Premishit (increase) colice size	
	V217F, 3213C, M240T, 5251N, P166, L273P	NM_081172567
NOTCH1	Francisid/Mosense	NW_017617
NOTCHZ	Frameshift/nonsense	NR4_024468
NFM1	Frameworth p. w/200fs (Insertion at 1,056_200,200_851,852_061,062_204)	944_002520
NRAS	9128, G128, G120, G120, G120, G127, G120, G120, G120, G120, G120, G120, G130, G130, G130, G130, G130, G130, G130, G130, G300, G600,	
	Q52R, G512, Q51K, Q51F, Q51H, G51Q	NM_002524
F2RYS	MS28, YS20, 8861, 03448, MS84, ACRET, N2545, FESSI, MESON	585_178128
PAPDS	Frameshift/nonsense	NM_081040808
PAX5		
**********	Pravies inflynories regisphore ite, 300 R, PSCR.	N/A_216734
PD55B	Frameshift/noncense/splice-site, R1299G	NM_015052
PD5S2	Frameshift/horsense	MM_026567
PHF6	Frameshift/nonsense/splice-sits, A400, M125t, S246Y, F2661, R274G, C297Y, H302Y, H329L	NM_001015977
PRK3CA	548Y, 1545G, GACCTO, LACESP, MADMO, DADMSH, HADMSR	NW_006218
POT1	Frameshift/nonsense/splice-site before OB domain (c.374), Mhz., Y36N, K90E, Q89R, Y323K, H366L, G372N, C593W	NM_015450
POUZAF1	F276	N/A_008236
POU2F2	T2234, T2235, T2393, R282H, T3071, G392F	
PROMI		NM_302698
	Framesinft/morsense/solice-site	WM_001298
	Prameshift/nonsense/splice-site, P15H, M5SI, P405L, P562S,	NM_001031E98
PRPF40B		
PRPF8	W1507	NN6_006445
		NM_00 4 445
PRPF8	M1807) Frameshift/honsense/splice-sits, D24G, F479, F56V, 157W, H61R, K56N, Y68H, C71Y, F61C, Y68C, D819, D91Y, D81E, H93Y, H83D, H93D, N94R, P951, 15017, C165F, C165S, D167Y, 1111Y, H123Y, C114S, C114S, K115E, A126D, K128N, R150G, R150D, R150D, H25D, H25V, H25K, C196R,	NW_00#4*X
PRPF8	M1807 Frameskrithonsense/spice-ske, D14G, F479, F56V, 157W, H61P, 856N, Y56H, C71Y, F61C, Y68C, D819, D82Y, D82E, H63Y, H63O, H63O, N84E, P86L, 1501T, C16SF, C105S, C167V, 1112V, H122V, C114R, C114K, K115E, A126O, K116N, R150G, R15OL, R15OL, B121D, H25V, H25C, C156F, C156F, K144Q, A151T, D153Y, G153N, Y155H, Y155C, R158N, R158N, R151E, R36CE, G165R, G165E, S170N, S170C, R37NC, Y174D, Y177Y, H38KY,	W. 1084 X
Prpfs Pten	M1807) Frameshift/honsense/splice-sits, D24G, F479, F56V, 157W, H61R, K56N, Y68H, C71Y, F61C, Y68C, D819, D91Y, D81E, H93Y, H83D, H93D, N94R, P951, 15017, C165F, C165S, D167Y, 1111Y, H123Y, C114S, C114S, K115E, A126D, K128N, R150G, R150D, R150D, H25D, H25V, H25K, C196R,	NN_000514
PRPF8	M1807 Frameskrithonsense/spice-ske, D14G, F479, F56V, 157W, H61P, 856N, Y56H, C71Y, F61C, Y68C, D819, D82Y, D82E, H63Y, H63O, H63O, N84E, P86L, 1501T, C16SF, C105S, C167V, 1112V, H122V, C114R, C114K, K115E, A126O, K116N, R150G, R15OL, R15OL, B121D, H25V, H25C, C156F, C156F, K144Q, A151T, D153Y, G153N, Y155H, Y155C, R158N, R158N, R151E, R36CE, G165R, G165E, S170N, S170C, R37NC, Y174D, Y177Y, H38KY,	
Prpfs Pten	MO1807: Frameshit/hordense/sphc=588, D14G, F479, F5GV, 157W, HGIR, 85GN, YSSH, C71Y, FGIC, YGBC, D21G, D51Y, D31E, H63Y, H83C, H63C,	NM_000514
Prefs Pten Pteni	M018071 Frameshift/nonsense/sphc=sits, D14G, F479, F50V, 157W, H61P, K56N, Y68H, C71Y, F61C, Y68C, D819, D81V, D81E, H63V, H63D, H63D, N64H, P86L, H30T, C105F, C105S, D107V, L111Y, H123Y, C114R, C124K, K115E, A126D, K128N, R150G, R150L, H30V, H315D, H31V, H125V, C156F, K144Q, A151T, D153Y, G153N, Y155N, Y155C, R156N, R159S, R161N, P161T, G165R, G165E, S170N, S170T, R175C, Y174O, Y177C, H196V, R124W, G151C, D181Y, F871S, D126G abovi 3 frameshift/nonsense, Q85	NM_000514
Prefs Pten Pteni	### ##################################	NN5 000514 NM_000827 NM_002824
PRPFS PTEN PTPN1 PTPN11 PACC1	M1807 Frameshit/honsense/spice-sits, D14G, F479, F50V, 157W, H61P, 1650N, V58H, C71Y, F61C, V68C, D819, D52Y, D92E, H69Y, H69D, H69D, N69D, N69D	NM_003514 NM_002837 NM_002834 NM_08555
PRPFS PTEN PTPN1 PTPN11 PACC11 RBBP4	MISSON Frameshit/horsense/sphc=sks, D14G, F479, F56V, 157W, H61F, 856N, V58H, C71Y, F61C, V68C, D813, D52Y, D81E, H63Y, H63D,	NM_000514 NM_000827 NM_002834 NM_005635 NM_005630
PRPFS PTEN PTPN1 PTPN11 RAGG1 RBBP4 RHOA	M1807 Frameshit/honsense/spice-sits, D14G, F479, F50V, 157W, H61P, 1650N, V58H, C71Y, F61C, V68C, D819, D52Y, D92E, H69Y, H69D, H69D, N69D, N69D	NN5_000514 NN5_000827 NM_002824 NM_006385 NM_005550 NM_005580
PRPFS PTEN PTPN1 PTPN11 RACC1 RBBP4 RHOA RIT1	MISSON Frameshit/horsense/sphc=sks, D14G, F479, F56V, 157W, H61F, 856N, V58H, C71Y, F61C, V68C, D813, D52Y, D81E, H63Y, H63D,	NM_000514 NM_000827 NM_002834 NM_005635 NM_005630
PRPFS PTEN PTPN1 PTPN11 RAGG1 RBBP4 RHOA	N18071 Frameshit/Honsense/spice-sits, D140, F479, F50V, 157W, H61P, R50N, Y68H, C717, F61C, Y68C, D819, D511V, D918, H93Y, H93D, H93D, N93D, N94P, P95U, 1917, C165F, C195S, C167V, 1111V, H129V, C194R, D124K, K175S, A126D, K189N, R189D, R190D, R190D, R19D, R1	NN5_000514 NN5_000827 NM_002824 NM_006385 NM_005550 NM_005580
PRPFS PTEN PTEN PTEN1 PTEN1 RAGGI RAGGI REBE4 RHOA RITI	### ##################################	NM 000514 NM 002827 NM 002834 NM 002835 NM 00585 NM 005550 SM 001884 NM 00812
PRPFS PTEN PTEN1 PTEN11 PAGG1 RBBP4 RHOA RNT1 RPLS	### ##################################	NM 000514 NM_002827 NM_002834 NM_006585 NM_005650 984_003884 NM_006612 NM_006612 NM_006623 NM_000623
PRPFS PTEN PTPN1 PTPN11 RAGG1 RBBP4 RHOA RUT1 RPL10 RPL15 RPS15	### ##################################	NM_000514 NM_000817 NM_002834 NM_005835 NM_005850 SM_001884 NM_006812 NM_006813 NM_000563
PRPFS PTEN PTEN1 PTEN11 PAGG1 RBBP4 RHOA RVII RPLS	### ##################################	NM 000514 NM_002827 NM_002834 NM_006585 NM_005650 984_003884 NM_006612 NM_006612 NM_006623 NM_000623

SETBP1	D366N, D865T, S869N, G379S, IS73T, D380N, D890G	NM_015558	3
SETD2	Noment #/nomente, 91190M	NM (024258	. 4
SETOB1	Framerbift/honsense, K7355	NM_001145415	1
SF1	Fremesh ff/norsense/spine-site TASAM TATAA, 7470, ADS05	MM_XX4630	3
SF3A1	Frameshift/manuenta/splice-site_AS75, M1171, K186T	MM 005877	1
5F3B1	03477, F35740 83573, 53974, 55321, 76275, R625, 36275, 766575, H5325, K6360, K6665, K6665, K7675, K7755, K7755, K7765, K7		27
	A7NAP, DTS1G, ETGSK	NM_012438	
SFRS2	Y44H, P95H, P95L, P95L, P95R, P95K, P307H, P95f;	NM_003015	11
SGK1	Francis (Francisco segripare este	WW_001143676	0
SMC1A	N190T, R586W, M88SEV, R807H, R109CH, R109CC	NM_006806	1
SAMC3	Processity operation RIFFE (23675, 72729, 85719, REG19, GERIC	\$81_005445	1
SOCS1	Fromeshift/nonsense/sp8ce-site_R48W	NM_603745	- 3
SPRY4	1112N, G117R	NM_0XQ127496	8
STAG1	Frameshift/honsense/splice-site, H1085Y	NM_005882	3
STAG2	Prairiego Atrogonie os atrogonie os te	883-8082-884	1
STATS	M2G6F, G61SF, Y64GF, N642H, N647F, D661N, D661H, G661Y, D661Y	NM_139278	4
STATSA	NSCON	NW_0X6152	9
STAT5B	6542H, 7865F	6M_012449	9
STATE	N4179 N4175 D419H D419G D4199 N421S N450T N450S	885_001278081	0
SUZ12	Frameshift, nonzenze	MM_015855	1
SWAP70	Rivines/Mytomerse/spilices/se	NM_015055	3
TBLIXRI	Fremeshift/inonsense/splice-site	NM_024685	1
TCF3	NSSIR VSSTE VSSTG, (SALE DSSIR DSSIR MSTOR	8M_005250	, s
TET1	Frameshiftynoncense/splice-cite, V328F, R3297V, R18560, V3230M,	NM_086825	1
TET2	Frameshit/nonserve/spice-one 5352F, 63155 (346F, 5460F, 5666C, F9415 (3135Y)	NM_001127268	32
TMEMBOA	Fremeshift/nonsense	NM_018247	9
TNF	5479, H521, P585	MM_000594	Ø
TNFAIPS	Frantzskiftynancense, 0.117V, M478i, P574L	MM_006298	3
TNFRSF14	Prameshib nonzenzejspice zite 1200, 1250, 0400, 0400, 0400P	KM 003823	2
TP53	Promesont/honosense/pulce-site, 1465, G1056, G1056, G1056, G1066, G1066, R1106,	NM_001228112	33
TRAFE	Provestiff province	5/NS_345725	Q
2WYT	8515, DX48	NM_010264	Ð
LIZAFI	514C S24F S24F R25L F156H F156C C157F C157F	NAX 308758	S
U2AF2	N38W, G148L M144E, 1167V, G149E	MM_007279	Q
UBR5	Framesi Milynor sense/Spinor-alte exon 58	54M_015900	Q.
WT1	Frameshift/nonsense/splice-site	NM_024426	5
X8P1	CIST, POINE	NAM 000079589	v
XPO1	5571A, 8571R	NM 003400	0
ZNF471	CASS PAGE NATE	586 520835	
ZRSR2	Frameshith/nonsense, R126P, E133G, C181F, H191Y, 1202N, F239V, F239Y, N251Y, C230P, C302R, C326R, H330R, N381K	NM: 005084	3
Total		-	805

Supplementary Table S3 Called somatic variants in 160 hematopoeitic genes

Contract	Christia	i Strict	Warrison.	Participation of the Control of the	Spiritorii:	Charlest Charlest	Specially.	agenda Alektu	Association	Wardens	Machine Mach	president RS
consiste.	000000	is face discount	Allen		(coccusts	0.0000000000000000000000000000000000000	STATE OF			Receipts Receipts		es.
30000	307	20020000	360	8.	ý	Piccolerous, Studiation	2.00	43320001	886,386830	Securities Securities	26	80 60 6660
2003	200	22000203.0	288	8	Ş	Secondon, Materia	2.116.85	43010001	886 (0.588)	8,300,500	11	23 /5483
4116	28	80008323	388	Ç	¥	November Absorber		51239034	986,003336	1600000	3,5	28 958503
ASSEC:	X	80008333	386	<u> </u>	¥ ¥	Novana America		£1539033	886 058338	0.538400	- 38	36,796636
9200.0 9200.0	38	\$\$5553358 38553338	598°	0	3	Nonecole, Monthly Nonecole, Monthly		6.12330000 6.123300000	986_003308 986_003308	0.000000		33.7 N68333
A0015	30	330003360	588	2	*	Statements, Marketton		2.00000A	866,033550	228833	- 8	30 (0000
2000	30	9200020505	38	8.	8	Scorpioros, Michellico		±3.2502594	886 (335)	22,747,533	28	88 80980
43333	\$27	0350005500	2000	83	ž.	Standarde, Stateballia		2000000	886 223555	553,5558	25	228 500028
ADD	\$33	935355555	2000	83	ž.	Storosesse, Stobeton		20.00000	986 1233550	555555553	6	10112 CH 156
2200.7	300	200000	5002	Ç		Sharrie, (SSS), (Self	V58558	e triffication	5985, 2233 285	\$15	38	68 1653567
9200.3	38	85003368	598	<u> ç</u>	8	Named Working		e.13800039	986, 223638	0.003.000	- 4	78 (22,500)
A200.0	38	85025028 85025502	558 558	\$	25	Nicherton, Maracher Nicherton, Maracher		e.1383008 e.1383008	588,023503 588,023503	9.235527	\$3 -4	28 345563 29 346643
ADD	\$37	230203200	280	8	8	Storoisence, Stratetico		1343301	888,288888	222878357	- 2	2802 (580252)
ADD	227	22002203	389	8	Ý	Storolenge, Stocketton		2333907	886 (034333)	22.3.22869	28	202 90000
53333	20	220722500	2889	82	Y	Storobosce, Situateliana		x25000000	2005/200 2888	0380800	3	22% 16235566
53333	.22	20002560	28%	79		55555K 365K 666	\$13834B	22245, 515000400	2006 5224552	5233	<i>10</i> 2	3337 16000006
53333	220	2002200	56%	79		55,000 30 St. 1866	X23348	22245 3350000	224 325334	2000	20	302 1033300
A200.G	200	200000000	5568	×	Ŷ	Stoneous, Matation	with 154	e.25690007	5005_0005000	0.003020	35	200-503525
A2003.	385	33003563	5558	8	*	Nonseous, Mutation	A 58659	0.3880, 2888,66004	CONT. POSSESSOR	9.000000	25	554 503999
Section 2	866	versions	i, w	.~		and a series of the series of	303.00	Caraci Transpage	and leavester.	vissesine	**	200 3012000
50000	233	2000/2000	\$85.	824		S20000 38585 3000	68459kg	20860_068664	N56: 235/236	228	*2	2000 50000000
45863	35	20022300	388	0	1	Noneacue Ministina		5288863	886,088338	0.538538	83	166 5658388
								£33000 5000 50004				
2332A	25	83008302	380	~	(0.4)4(h)	transi shit, isa	82865W	ASASS	986,5888398	25,535	5 8	93.1688336
ASN(3	30	2000000	588	Ř	Ŷ	Stoneous Mutation	A STATE OF	0.3088059	566 (036680)	0.708660	55	35,903990
S55.00	-0.0	Season	388		SAACACS	Name of Street	Acass	400000,00000000000000000000000000000000	THE ASSESS		***	200-200127
86003 86003	300	340000000	588	8	20000000000	Propose (Staff) (Inc.) Processerve (Staffetton	20000	4040° 6373300°	585,035500	400 1999033	50 50	20 50 200 500 00 500
ASSES	200	83000388	300	8		Nonzense Ministro		222880-4	886_035500 886_055506	2000	- 1	95,729,250
COSCA	33	2000000	384	- 2	3.	Sogregi (State Sec	K8808	81231_3703988	886,000000	0.50	28	60.28636
4886	N	32023346	360	Ĉ.	3 .	Name of Assetts		6.1231, 3273299A	884,028308	0.303995	38	550 7550,000
4116	388	35003350	390	¢	3.	Nonema Abanin	40,000	5.1282XX4	884,028308	0.58128	302	30.0000
0,00024	389	833253363	583	•	Ť	State State State	\$4500000	e1986 (988) est	300,023320	8,38	203	58 855354
				RADIAGOS				437400 JULES				
	222		2020	(4)(4)(6)(6				000000000000000000000000000000000000000				
ADU	\$27	93503960	OS.	2002000	•	State State See	200000	ASSESSED	886,535333	939	8	37 58058
				90000000				640000000000004 84000000000000004				
9200.3	38	822003438	590	00000		Frence, Stoth, Sec	2,852,966	27002 0 492	598,028808	8,28	28	68 (8800)
9200.3	,30	59969998	986		*	Precise (2020), 300	400000	6.2303(_33077866)	986, 223390	8,28	- 55	28 1623997
9000 G	389	2222238	586.		8	Precie (2000), Son	p. 000000000	6.23335_23378665	588,223530	8038	8	28/85/30/37
62223	\$27	22666665	368	-	8	556559, 3555, 355	258825	23/328, 222/5/6/2	886 594559	239	•	25 (25)
ACCES!	\$22	22662525	368		8	556,3692,36965	253825	23/228, 222/39/62	886 724583	232	8	20,600,000
23333	.58	236202365	303	e-	8:	in flet, seed	20000	23/228_222/26/2	1006 223,550	283	53	24 107,000
23333	50	22002999	2889	8	3	Storosocce, Stinteline		x2000004	886,555556	20202325		90, 192,923,35
9200.0	38	333253332	5990	<u> </u>	0	Surgery Mounts	**************************************	CX07X39	986,023608	0.2083308	- * 5	28 1603403
A200.G A200.G	389	55555555 55555555	5568 5568	ŝ	*	Stongering Matarian Stongering Matarian		e.2007/0007 e.2007/0007	586_013503 586_013503	90,2866007	- 8	89 99988 89 99888
A200.G	30	22/22/23/60	250	À	:	Science, Mills, Mel	p.25008%	4360/600.5	5085_0235500	0.00	- 2	28/2026/26
2003	385	25000000	283	45		965, 1816, 4040°	p.4073066	0.00000000	586 (03550)	0.34	22	32 900088
53333	200	REFERENCE	2000	80	Y	Standardon Schabeline	2000000	2008000x	2003/00/2008	28	Ý	28688a RK
6000	330	22002000	388	85	Y	Standarde Styleton	80000	2233304	886, 585,336	0.00003	39	68.98800
ASSES	225	820033398	388	0	3	Nonecose Ministry		2533803	886,000000	2002	•	38.7853385
ASSES	20	80002012	389	0	0	National Africance		500000	200,000	33		88.382838
8200 S. 8200 S.	30	25000000	588	X X	7	Stockeron Modation Stockeron Modation		6,00000009 6,00000009	588 (028888) 686 (028688)	2005 2008(200,0)	29	87 503533 35 Nexes
ADMS.	30	34000360	588	Ř.	*	Scottween, Modation	~53550*	6,040,070,07	5005_0175500 5005_0175500	9.1139800	3	40 50006 45 50006
A2013	30	2200200	200	8	\$	Secretary Marketon		4,0400001	886 (337533)	2,2000	36	86 801380
SSSA	32	83003300	865	Y.	•		212000	22-933965	886 (0883)8	222	30	333 165800
2382A	30	80053000	388	ÿ	8	Name and Attribution	\$10000 F	2.26655665	856,005008	0.000000	9	1600 1600,000
1111	38	\$55683255	N4	-	3 .	Strategy (State, State	40000	62286_2333999A	986,525336	8,83	38	68 166664
ANG:	N	80008088	300	*	<i>t</i>	Street, Absorbs	V 8300.0	2.0000000	986,00000	89,6	Ŕ	60,760394
2000	685	3,400,400,4	1000	07000		Suppose Subsection	A2460	7.5000 (10000046).	6900 0000000	3.04	38	ga janeces
86005 86005	300 300	92009880	083. 083.	8778	-	Hell, Hell, gemech Hell, Hell, gemech	p.(2500) p.(2000)	(A 0.000(0.000)	886_005500 886_005500	2.0	12	50 634666 50 634665
Appli	30	23002554	083.	<u> </u>	,	960, 1972, security	20000000	2,30235(56);	886 (33253)	232	11	83 (63,0838)
4 2523	\$27	5555556	200	8	×	Storoseros Storietico		28120004	NSC 23/2233	55555555	- 3	228 9036800
ANK:	N	83008300	399	¢	Y	Norvenie Absorbe		5.555553	886,028808	1110000	Ŕ	160000
X2538	38.	\$866307	3568	¢	¥	Missione, Stateston	48300	6.2300004	98222,388	6.4(W)/68	\$8	28 88828
80008	Ř	3033500035	986		Ý.	Preme State State	4833039	24008-8000sC	586,0003,0086		8	\$200,000,4400
00000	Ř	200000000	565	Q2	•	Secret State Sec	VILLER	e3335 \$2359953	596, 2003, 22500		\$3	308 18690
80000	8	200022964	283	Š	Š.	556056 (\$950) (\$66	g32889	28866888 222 223 223 223 223 223 223 223 223 223	888,3883,3888		- 32	36 (6868)
8008 8008G	8	100000000	39S	8	8	Statistic (SNSS) (SNS Statistics (SNSSSS)	0.90000	5875040 5875040	860,000,000 860,000,000	20,350,785 20,350,785	<u> </u>	35 (63658)
800000	.8	1000A0020		8	Y	Storosope Storeboo		2388804	2002,000	2000000	- (38 988553
00000	43	\$222555003		•	8	Prieste, (2000), Stal	V83005	e.627, 6200x60	588, 2200002	803	3	363 3633338
9890	3	340900000			3	Middlesse Strategy		6.17598754	5985_2005522	9.238523	23	98 888937
8888	2	340993400	5568	ŝ	ŷ	Militarios Aparellos	p. 8000000	c.3488856	5085_0005000	6,239,278	35	355 5533555
65553	2	326223625		Š	9	Stoneous Materiae		e2805547	588_225522	9.249.502	53	300 500066
88000	3	32635200		Š.	\$	Stockwood Moration		6,080/5/7	5885 (0008500	2500000	ý	28.900988
88833	.8	256995834		35	Σ	Storozope Stobelins		2000000	850,0000000	20207403	- 6	200 500,000
888000 888000	.8	254505834 264505668		8	<u> </u>	Norozone Michelios Norozone Michelios		2000000 2000000	NSR_20/92001 NGC 05/30000	0.5366663 0.646	*	38 558388 368 56838
88603 88603	3	324332333		8	7	Spirite Site	20000	\$4000 a	886,004300 886,004300	202232	33	67000 000 69000 000
CARREST A	3	2328346	5568	8	R	Minimum Minimum:		e.138/2507	586,00066	200000	- 8	28 86822
581	88	158168835		Ÿ	8	Missione Missions		e33437-0	585 (3855)	0.00000	2	2000000
5381	88	128368852		100	â	Missense Misselfor		440000A	585 (395555)	6.7683	23	32 9038800
5381	88	17/1/19/1/52		8	Ç.	Missione Mission:		e,2,5400000	200, 300,00	3335833	Š	32.95333
CNS.	3.5	3,055,886004		4.	43	Missense Moteston		52584665	886,008(388)	0.092500		78 168458
183	3.3	3,000,000,000	2000	*	€:	Missense, Medeston	50.05500000	22240400	986,009,000	220000	*	68,792,593

Sierre Minister	Shroo	Sauce produce	Windood Maria	Naturalis State	Variant Albeir	Various Charlesanthus	Processos.	elistik etmogra	Acception	Windows artists	Processor 3	teterance to
******	********		13000	***************************************			Second to			(mathin		monet
2223	53	2009-0020	389	⊗	A	Minerio, Matrito		x 52233384	588,000,000	0.328800		233 553553
08.	38 38	1202-90000	588	8	A A	Minister Minister Minister Minister	0/0998A	21221224 21221224	500 (300 (300 500 (300 (300	0.079563		500 500 500 505 500 500
C85.	25	2235985,828	390	8	4	Ricerce Potestro	2000000	6.234000A	2015/10	0.04000	- 8	\$20,760,800,8
esse.	3.3	200040020	5955.	\$	ý	Streetie, Streetin	5/374550	970789333	985,3383389	32,550	. 3	23/3 52/2025
323	53; 53;	2502402349	5885	\$ \$	<u> </u>	Michaeler Michaeler	603000	x 5280064	3886_00000000	0.0000000		288 588880
C258	2.8	12/0/2000	290.	8	A	Minimore, Minimizer Minimore, Minimizer	(90096a 20000a	20000004 20000004	586_300300 586_300330	0.08980		20,765,600,
5000	3.5	8800088	2800	8	<	Stickense, Stuberon	\$ 81.068	esessesc	966,000000	0.000000		\$4 (300)50
536353	3.2	54660000	210.	Š.	ÿ	Minerie, Minerie	11.50	Killitz	859492,380	2.2855		284 (2000)
C000000	38	82500000 80000000	585.	*		Signature (States) according	60000000000000000000000000000000000000	\$640000000 \$4460000000000000000000000000	596_3065300	2020 2020		2001 1602-040 1400 16002-00
C66989	38	200,000	580 580	*	3	Strantag (Strift) (Stall Strantag (Strift) (Stri	0.000000 0.40000	7.53.0 3300,000 2.7020000	20 2000	888		302 300000
2255	3.0	32(2)20	388		©	doese, \$600, in	0000ss	22000000,0000000	(2019), 226	6.63		\$80 300074
5555500	3.2	2002003	2000	Ś	'n	STORY STREET		23288237	2000000	2200000		258 (2008)
5255	58	2000000000	(865 (888)		- S	Nanagana Shahilata Nanagana Shahatan	6/2000000 	X255 X50000	5886 (00063300)	2020		2002 M005000
2002 2003	3	302747844	200	<u> </u>	4	Stockering Madeline		2.753566-7 2.66333564	584,385550 866,280550	0.119808		370 M0000 137 M10886
22/83	ý	300807531	2000	8	*	2006-206	248343	2,35577, 300004	986, 181550	8.422342	. 4	39 30230
\$85658	X	200000228	368		Š.	tracrie strift, in	60000	2,000,000,000	585,565,285	900	3.8	\$500 300,7500
				CACCIACCAS					***			****
200003 50000034	3	25305050 25457250	283	80846 4.	-	Spring Site France State Set	2,60000	c.1897_perioe c.27974e87	986 (00096) 986 (00960)	8(5) 8(6)		92 58860 180 68860
22 (1990)	3	33487180	380	À	•	Street, State, Sec	\$13000	42759460	025500, 026	6.68		\$82,805606
200000	33	3364833338.	3300.	©.	A	SECONOMICAL SECONDO	\$12000.	82778302F	2887,002,005	0.388300	2.5	30, 1600000
6375382	ž	2545.75.75	288	8	4	Missione Mission	1611103	6233352	886,000860	0.08700		68 2655584
68668758 88758863	3	25457525	288	8	4	Milyaeroe, Mutation		63753555 63753655	882,000980 882,00980	5.056550 5.0555555		20 50000
2858675A	3	33487128	288	43	3	Misseriae Misselfon	\$23,000 \$20,000	6235303	386,30350	2.00000		80 605400
28/20/8/03	30	225525252	289	8	A	Misserie Matelio:	98888.9	X3788008	3885,0050550	0.00000		82 (82.523)
08683384	*	2865728	2502	800		Scarce, Stall, Sec	2000000	62305 23080600		8.8		28 20323
58660154 58660154	3	256577355 256577355	286	٠.	<u>4</u>	Anama Sittle inc. Arguerna, Arutation	20000	200000 0000000 2000000A	966, 000960 966, 000960	0.00 X-X00000		550 60054 66 56 666
20000 SA	3	338833333	350	•	÷	50000000000000000000000000000000000000	\$136,636,6	20000 <u>2000000</u>	200 (100,520)	0.000000		80 (40) 660) 87 (40) 8800
68,69855	ž	28457538	898	\$	Ý	Ministrae, Ministra		c.2855000-A	New (0000000)	2/2009		95 100000
6868888	X	255577525	286	-	4	Acesse, Stattly, feet	Mating	CONTRACTORIES	888,000880	888		88 998339
5888835A	3	28457243	2865	<u>0</u>	3	Athonorus Atobetion Athonorus Atobetion	2000000 2000000	0.0005004	965 (00960 986 (00960	0.000000		36 (6560)
C9865534	2	28492245	580	8	*	Mingray, Mingray	\$1000000	6-200000-A	886 (333283)	0.000000	29	06:190000
45756653	ż	28400040	3500	į:	Ý	Ministrae, Ministra	7433BA	6.389988.5	3695 (0)000000	0.28520	28	985 NO 4254
68756882	X	28657553	288	8	¥.	Misserce Moteston	2000000	4,000,000,0	888,000880	\$1,565t,655	23	28 20003
5888875A	3	35657243 35657243	200	6	3	Attioumne_Attiobation Attioumne_Attiobation	p.348555 p.348555	0.0095004 0.0095004	986,000960	0.000000	23	54 (600)364
4000000	2	28492242	580	8	÷	Mineral Marketon	240000	6.000000A	884 (000000)	0.00000		303884300
686889	8	28400040	\$500	£:	Ÿ	Minima Moterin	Willell	6.289988.5	3695_0200002	(2/3/30)		68 (503.00)
68759862	ž	25557050	2500	ε.	33	Missesse, Moteston	Soppos	e,38998000°	888_000860	2,239900		52,990553
60000000 ACCOMOS	3	35855,5783	2885	<u> </u>	3	Milatoria, Mutation	200000	4/03/6/04	986,000883	0.080780		50 (4004) 50 (4004)
400000	2	28452242 28452242	390	8	<u>%</u>	Minerou Minerios Minerou Minerios		200480030 20048003A	584 (000000 884 (000000	0.00000		467 145(2000)
6000000	ż	28607092	\$88	ė:	Š	Minima Molecia		6.2009.000.4	3000 (0000000	0.332399		98 2003 283
5005535		35683383	588.	E.	à	Militarian Militarian		6,0099004	986 (033,863	0.000000		22 94633
AC 200003	2	2848/2342 2848/2342	588°	£ £	<u>⊗</u>	Missense Misseller		505686050 505686054	566 (2002)20 566 (2002)20	0.38888		70 M0047
(18)	ż	25452042	380	0.	9	Mineral Material		2.284982.A	385 (002000)	0.22220		35 1853750
46116662		28600363	288	£:	Š	Minima Motation		6.3886888	300 (000000	0.80000		28, 200,652
ACORES	30	286883383	2300	X.	3	Mineral Material	430000	20086002	288,202,980	0.000803		28,100,00
200200000 40026600	8	2848/2249 2848/2249	588	<u> </u>	<u>%</u>	Minister Minister Minister Minister	6/8000000 published	x 284862×0 x 284862×4	5655 0000000 5655 0000000	0.380600		36 1663336 36 166343
(500)	÷	28400040	388	<u> </u>	9	Minimus Materian	143335.02	8.0000000	\$86 (000800)	0.03000		32 1600600
4000000	ž	288553383	288	€:	À	White Wolseiler		6,3899935,4	388 (320863	0.538389		92,9924392
26,03603	3	28882283	3300.	Ś.	*	Mineral Matella	pc3000000	x2008005x	598,000000	0.088803		48 500000
ACCIA603 ACCIA603	- S	28482243 28482243	580	<u> </u>	8	Mineral Materia.		20048000A 20048000A	\$86 000080 \$86 000080	0.08690		88 1658800 86 165805
55,995,59	÷	25400040	388	- 8	8	Minerae Moteston		6.000000A	SSS (000000)	0.0888		48 (88340)
AZ 178203	ŝ	33487283	5885	₹	3	Sticence, SticeSco		20088003	986, (00.853)	2,277533	36	30 (41.00)
26,006,003	20	28887288	389	Ŕ	*	Microscop Materior		x255886554	28/62/02/3885	0.038803		87 588282
4004603 4004603	2	28452242	586°	\$ \$	4	Mineros, Minerios.		2.2848034 2.2848034	566 (000000) 566 (000000)	0.039900		30.1663400 30.1663400
2008/CSA	3	32687243	2000	₹	Â	Africance, Michelian	* 84868	4/03/900/4	36,00,960	0.00000		33 68600
AC198883	ž.	33987283	5995	ζ.	\$	Statements, Stateston	estation.	20080004	9262, 2020	2,388,334	3,2	30 (2053)
20020036	30	28888888	389	ŝ.	3	Sticosome Statistics		x 200880056	3880,000,000	0.000000		20.600400
ACCOMOS 42/25/00/02	8	28487242	588	8	* *	Ministracy Materials Ministracy Materials	96588836 26588835	20048004 20048004	5885_0000850 5885_0003850	0.000800	5 28 6 6	65 565655 65 565655
20000 NA	3	39887283	590	3	3	Stignerne, Studeston	\$ 84805	2,00000004 2,0000004	26,000	0.302300		25 (42)(4)
AC 111103	š	339887283	5959.	Š.	W.	Miceria, Michillo	\$2,000000	220000000	1881.00, 300	230000	9.	30 (20)(30)
200200000	20	288882880	399	ŝ.	3	Microscop, Microscop	pc8000000	x 200880056	3880,000,000	0.088580		86 settent
ACCORSOS ACCOSODOS	3	28487242	588	8 0	* *	Mineros Materios Mineros Materios	6.535555 2.555555	2.00480014 2.0048004	5885_0000850 8685_000850	0.533504	8 8	30,980,980
20000 SA	3	336837383	586	0	*	Michaeline, Michaeline	\$10000	5/30/9003.	365,00,250	0.332463		A0 (608000)
AC 19863	š	33883383	2992	Š.	·ý	Micheline, Michellin	200002	100000000000000000000000000000000000000	990,000000	220000	8 8	35 (50,000)
50000,00	8	226823383	399.	*	A	Miconic Malako:		X 200996250.	286 00,000	0.00000		37 50000
\$200000000 000000000	3	25667252	288	8	*	Missesse Materian		6,0099003 6,0099033	888, 000880 888, 000880	3,000		07 500000 07 5000000
88758983 88758983	3	35657243 35657243	280	G	*	Michaeroe, Musellion Michaeroe, Michaelion		6/06/90013	986,000960	9,362363		80 30 8880 80 30 8880
20000000		28887283	289	ŝ	ý	SERVER SERVERS		20099007	300,000,000	0.338890		32 5233838
637565632	ž	28697383	598	8	4	Minerae Monerico		12300355	2007/2003	0.508583		88 555548
4275983	3	25657250 25657250	288	8	*	Allignamore (Alludration		6,0868603 6,0868603	988, 0009800 988, 0009800	0.000000		58 565537 30 655530
20000 SA		33487243	286	8	*	Michael Michaelon		C3088C27	2007.00	0.000000		30 (00000
2888938	2	28492045	880	8	- K	Ministry, Ministry	Willia.	5.584465.5	888 3000800	0.089960		28.5605.65
6868882	ž	28497793	588	**	4	Rhinera Rousin	WHICH.	1,639,000.5	888_000860	0.008000	38	38 5054050
4275943 4275943	3	25457243	288	8	4	Minana Massia	5.49000.	6,0000003 6,0000003	8882_000880 889_000860	0.0000000 0.000000		50 50000 65 50 665
200000000	3	39487243	280	8	*	Militariae Mutetion Militariae Mutetion	5/8880X	67699003 67699003	986,000,986	8.338386 8.338386		30 30 350 30 30 40
28888538		28402045	988	8	š	Minerae Moteston		7.639486.5	886,000000	0.084558		58 168818
4275060	ž	25457545	588	ši.	4	Minerie Miterio	\$15000.	7,6399,865	888_3333863	3023	. 28	50,999,00
	- 100	23/25/25/25	222	83	4	Militarios Mutation		0.00000000	985 333983	5,5005334		35,946933
42759903 42759903		39887383	280	- 67	4	within would be		6,000,000	200 100	0.000000		30 (00000)

		wa .	~		78 A 8	AY A A	T 01	mar A	~ ×	en / a	22 A 3 A 4	.00
	Z.	Secretaries Select	gaine.	Balleton Alleste	and the same	Constitution	Constants Constants	च्छिमिते कोस्तरकुरः	A Company of the Company			district fits
		A	1,000							direction.	stacking consent	
	3	326832562	2002	6	Ŕ	Missense Michigan		c284624.	886,325333	42	3	43, 85,534
1177335	7	52425345	380	8	A	Schooling Schooling	¥ 300000	4.538882.52	222227,388	0.072300	33	34 95/392
2002/2005 2003/2006	3	35653583	3865	8 6	8	ostrocki, omedik	\$ 2000 E	0.0000000 0.0000000	88 (3333)	8,035,639		87 1680307 72 88718
2028/02	3	325832353	200	6	š .	Microsop, Microsophics Microsop, Microsophics	71116a 71116a	52344525	886,000000 886,000000	0.39639	- 52	43 8505
25555335	3	25857385	380	8	A	School Schools	2.5835.5	4.2882835	888,500000	0/88628	3	48 550000
08800M	7	52425348	388	ì	Ñ	scheens, presents:	\$2000.	4,533355.05	88,53335	62,66663	38:	44 363334
20000000	3	25555555	2002	ŗ	S.	Microst Memor	V500000	55000000	555 200020	8085289	Ř	63 (80000)
2002/2006 2002/2006	2	354557583 354577383	300.	<u>C</u> &	*	Afternoon Michigan Propose (NA) (Na)	2.00000 2.00000	5.2000.00T 5.2000.000T	886,3003507 886,300350	8/08/1830	32	48 880003 43 880008
2223555		200200300	2882	8	3	Stronger Months		4.283882.9C	888 622553	90,000,000	3	88 3655965
2020/2020	3	33655555	353	· ·	ÿ	Tourney Staffy See	2468865	1,0000 20000000	5656 (200222)	937	8	228 862383
20020000	3	23655565	5867	Ø.	S	Microscy Microscop	75000in	97/23/22/42.	886 200220	493065.0	\$3	25 6500000
2005000	3	334533555	2000	8	8	Missesse Micheles	NATHIA.	\$25,555.00.	886, 2072027 300, 200, 200, 200, 200, 200, 200, 200,	8638933	8.	323 889088
255525055 255526255	72	25,8565327 25,8566323	365. 365.	86 S	•	Process (\$685, Deli	2 (8000)	c 2000, 200006677 c 20000666	986 000020 986 000020	8.6	<u> </u>	50 500000
20080276	3	33655633	283	600		States State Sai	HILLIA	0.3838,23826667	8686 (200200)	5.38	53	88 880753
							,	0.0000 2000 00000				
5555555	7	25800000	388.	5253953		Stococo (\$685) (\$46	2,00003	000	888 (02030)	8333	32	57 8657338
22,000	3	225,500,000	903	•	X.	Process (\$680, 500)	2000	c 2008, 2000005	885 885,885	8.58	32	32.2823928
20000000	3	25658665	283	<u>8</u>	7	States State See	62000A	V.2600 Proper	5555_0070077 6664_00700077	0.000	- 58	32 863000
20000000	2	52530000	388	7	\$	Spilling Silver Spilling Silver	5,623-5	c.2000, pplice c.2000, pplice	886,000000 886,020000	0.020520	- 8	25, 286522.
288882	2	35838383	2882	Ť	<u>;</u>	Spiller, 1866	5-628-2	s 2823, seesse	NR (023353	9983986	*	52 2533536
32236962	.2	22629598	3888	Ÿ	€,	Notice Site	2/60000	5,3575 yakes	988, 2002200	0.8825	Ŕ	985 65503630.
2009020	.3	32000000	2002	ŗ	€.	Mille Me	9,9883-8	62886 38866	5555,2002,200	0.00000	8	28 883330
22223	2	35000000	3385	Š	31	Alle Alle	20023003	c2898_scales	886 (05520)	30558288	8	22 255502
25555238 25556224	3	325000000	3885	ç	5.	Specific State	5000000 - 50000	2.2822, 350000 - 2.6822.00	887,500350 887,600350	30.0000007 A.50	8	25 585,7080 40 522000
92296902 92296902	.ž	25605000	2002	å å	8	Microscop Microsipe.	5.20000 5.200000 5.2000000	6/28/28/09F 6/28/88/09K	5656_0002000 5656_0002000	9.38	- 8	25 5552005 43 6552005
2822228	2	3000000000	288.	\$		Scoops, 2005, Self	p.C28889	.2352266C	NR (02000)	98.5	327	AS 8500000
20000000	2	3596533055	3005	Š	N.	Mineral States	2000003	C2002854	886 (02330)	8098969	- 3	AC 500555
92296992	.2	25665555	3669	٤	Ä	sittenth, oronth	V90360y	X222323A	995,200250	0.20000	, N	83 859833
0000000	.8	328055522	202	8.		260, 2002, 2006	\$2200k	X22289941	335,202202	828	52	265 5590000
26222263 26222263	2	358856355	2005	5	8.	Stituence Michaeles	2.0000.0	\$200206C	880 322322 880 322322	500000000 500000000	- 8	023 880038 AC 4605565
ACCOMMO ACCOMMO	3	288683233. 288683233	3885	<u>s</u>	<u> </u>	Aprilia Sible Michaelia Adabatica	\$150,358 \$160,358	2.2002_spiles 500000996	894,000000	\$2887379 \$33927	.38	302 8830580
42736962	.8	25603328	3665	8.	8.	Microso Manthia	\$100000	200000040	595, SCCSSC	9200000	\$	200 900,000
202228	3	324825383	2005	Ċ	5.	Managa Matatha		6233325ah	885,6225525	\$333000	3	88 880720
2002200	7	354855585	496.	C	7	edistate senativ	\$2888.g	6205209A	886 (32522)	2000000	8	323 883923
20020000	2	356653355	3365	C	3,	stituiste, paraettis	\$22258g	2,200,2009	886/2002/20	2002020	8	200 800000
62596935	.2	28668365	388	8	3	Sticense Manthia	£23755	<.200305A	886,000550	9288688	8	80 552330
00000000 80000000	3	32485583	3887	8	8	Statement, Martin	Property.	2232230A 46222325	888,000000 888,000000	2500000 C0200000	¥3.	228 553552
SMOOTH	13	358855355	SW	8	8	Succession, Statistical Succession, Statistical Statistics		620000000	886 325223	2032339	43	273 883928
42796662	ž	28468332	380	*	k	situally second		x200300A	886,500550	5.088235	32	334 5553833
02550935	ž	52863355	389	ŵ.	k	Although Michigan	\$1833.55	K2053074	884,000450	696232	3.6	330 800530
SYSSERS	>	23823352	200.	Ç	8	Administration paragraphics	255554	46252555	886,322333	288833	3	353 86 558
2035238	3	324855183	265	6	8	Scottering, Statethin		4e055555	886,335355	490000	\$3	353 833550
05206923 05206932	\$ 5	28466382	200	8	<u>&</u>	Messense Massens	£30308	C2032265A	888, 620580 2007, 2007, 2007	8238383 8238383		36 500000
2886228	3	324523355	286.	8	Ĉ	Microsop, Microsopo Microsop, Microsopo	2.022330	52338053	886,000000	8344683	3	382 8855288
28228863	3	33483488	288:	A	Š	Ottomen, Micheles	247733	62338795	886,6323533	282836	ŝ	328 8834789
4579,6467	3	25463347	280	Ţ.	A	Menores, Marrier		4.33MSKN7	886,533,553	4/9/33/22	3	35 59565
0520000	.3	58465336	362	Ę	*	Statte, Statt, Sat	H100003	2 200200 00	886,600,600	6658	÷	237 80222
202200	3	32463533	383	88	v	Towns, 2009, Tale	#12005.4	5,533,73,666	886 33,5255	939	38	26. 35300
282528	3	32483033	200	7	C	Misery Measur	5/402020A	6.2333.KvG	886,8228833	86686	\$8	388 85983
00000000 00000000	2	25469328 25469328	383	8	8	Street, Sitt, Sat Street, Street, c	\$18780% \$18783%	0.00000000 0.0000000000000000000000000	88,50000 88,50000 88,50000	6/57 6/588278	38	38 56687
2823/22/8	:2	32483238	2005	8	6	Missone, Micretox	\$22223	928882×3	886,300000	0.247939	28	73 5550550
200200	3	32463529	2005	Á	G.	Missess Meaning	58885.9	C233802FC	888 (200222)	8,080,83	8	223 8550030
2572337	3	253653338	380	À	%	scheene, scheens	X 23328	4.2068750	6255557888	6063903	36	893, 900033
1525333	3	28463330	388	à	3	estracts pressing	£23300	4.25633.798	525555 388	9753555	ě	82,92,600,00
2002/2004 2003/2002	3	2545230(30 2545230(31	283	8	2	Mineral Michigan	200000 2000000	6.02222548 7.000000000	886,2003523 664,0003000	8389839	33	28 883252
20000000	3	32463233	250	8	7	Tourne, Tode, Tod Misserve, Michelox		C75555296	886,207807 886,207807	8635386	8	20, 96200
25525555	3	25465332	3300	S S	3.	Street, Market		4.23383554	525555 888	66382338	35	25, 20, 20, 20, 20, 20, 20, 20, 20, 20, 20
								4.5552 XSS356632	*******			
SKERNE	3	3556555566	583	828	**	de Staates Stel	p.20%2006	ż.	5656 (200022)	9.38	<u> </u>	262 662263
2000000		33483533	2000	6	0	Mississ Mississ		6.2538093	886,3073537	25855	- 8	96. 8\$6.558
25752820 25752820	2	25.4602330 25.4602330	385. 3889	A .	*	Steame State Date	\$100000 \$100000	c 201906667 c 201907562	520050 988 520050 988	9953963	35	588 5652555 588 5652558
20000000	3	32003332	2005	8	7	Macone Michigan	Process.	575586249 575586249	888 (000000)	8:38838	83	223 8852080
	3	336537/39	2007	8	Č.	Moreon Micreton		6.32388546	886 (20022)	8,083883	3	228 885388
20000000	3	25,600,000	385.	Š.	•	Process (NOS), Dell	x 223589	c.23553666C	888 (023352)	2003	¥	33. <i>2000</i> 22
2222552	3	22463343	368.	Ş.	•	State (1997), See	\$122,255	c 225336660	88 83333	038	ž.	386 265626
20030000	3	52000000	3887	û.	C.	Manager Michigan	62000	62000000	5555 (\$772\$37)	20000	35	23 96003.
3886276	3	324632543	383	8	**	Some Staff Sou	V15000	5.550090.	886,3003000	838	- 8	3556 896365
25555555	2	7538652466	2987	980	38.	Actionace Statement	x 27998	4.2048, 3283833333	2000 (0223/52	0.002338	36	30.38030
20000000	.3	526022562	3887	8	2	Missone Maneson		63288656	5555 (20022)	500000	2.	228 385228
9236402	3	52002535	386		535	States Staff, See	683336		5555_00000000	929	23	72 868923
25222255	2	326665300	3000	ð	ś.		\$1525555	6.2000000	880,000,000	2 52230	383	52 702652
25253353	3	2000,000	3889	à	- N	estados escessos		4.200000M	NR (02005)	20000000	32	322, 2865.53
98296862 98296862	3.	25005505	388	8.	<u>0</u>	Microson, Microsica Microson, Microsica	\$4575380 \$4575380	602388948 602388948	666 000000	9,000,00	\$: 5:	23 599865
20000000	15	324600300	2002	<u>C</u>	5		p. 675000	0.00000000 e.20000000	888_0000000 888_0000000	0.000000 0.0000000	18	88 830088 88 830088
53555555 23555555	2	3206202560	3565	\$	2 2'	Sillianesco, Silvitatilia. Sillianesco, Silvitatilia.	p.279899 p.279899	c 20070566	886/025200	90300000	5%	82 880000 89 8000000
	.8	25605500	3887	8.	0	Microssy Microston	\$20000	K\$28000.60	5656, 0002000	9.000000	<u></u>	352 822958
02296962	3	52005200	360	Ĉ	3	Mineray Member	5000000	0.0000000	3656 (0000000)	9.2507253	55.	59 522353
			200.	Ø	35.	sittatica, assentita	28888.g	8200000	880,5523533	50000000	8	NS 182808
00000000 00000000 00000000	2	35883583				Acres AND AN	169759.4	230000000	886 (825)503	8338	ž.	200 8800
00000700 0000700 0000700 0000700	2	326662333	228.	\$	*	Steene, 5885, Set			****			
0886736 0886738 0886738 0886738 0886738	2 2 2	354555387 354555387	5565 5565	E		ochiecolik, evenedik	22797C	52000000	886,022550	20062825	S	302 83300.
28886738 28886738 28886738 28886738 28886738	2 2 3	32400000 32400320 32400320	\$265 \$560° \$460°	\$ \$	ŝ	otherica streethic scittistic gammide	\$183,000; \$183,000;	7.0000000 7.0000000	595,000000	9,2005.83	×	32 9853853
2888/0124 2888/0128 2888/0128 2888/0128 2888/0124 2888/0124	3 3	256833287 256833287 25683387	205. 5567 2667 2667	E		Minerae Minerica Minerae Minerica Minerae Minerica	\$187585C \$187586C \$187588C	6.0000000 6.00000000 6.00000000	666_002052 566_002052	95-20075-83 75933555:22		20 58032 20 58032 20 58032
28886738 28886738 28886738 28886738 28886738	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	32400000 32400320 32400320	\$265 \$560° \$460°	\$ \$ \$	št št	otherica streethic scittistic gammide	p.875860 p.875880 p.875880 p.975888	7.0000000 7.0000000	595,000000	9,2005.83	ž Ž	32 9853853

Quecie:	φî	, Short	Vertune	Reference	Victori	The character	Protein	all the sharing	Accession	Vindont	Market Sele	www.gp
000000		s foregrafies	gara.	Allindo	ARRES	Charifferenties	Charage			*****		e.
2829632	2	5554555550	2550.	ç	₹	Manager Shrishing	\$150000.	32300000	886 823552	2,0003	COLOR COLO	30.555930.
2038022	ž	230002289	2000	Ÿ	4	ethics/X, exectly	200000	0.2320000	988, 222522	2,020	13	77 (502700
S886538	3	200000000	2000	7	<u> </u>	Massac Adminis		2338886 - NSSSS	688C_0000000	8.00000	25	80 88728 90 68099
528385529 528385555	3	20000000	5997	ž.	<u>6</u>	Sticonous Sticotion Sticonous Sticotion		0.2000/00/0 0.2000/00/0	5000000 3000 5000000 3000	0.006520	.8	32, 90,998
9570465)	3846349	360	7	0	Stimmer, Michigan	\$199960	39999353	\$2000,388	8,084688	*	42.7653402
25234352	3	33463345	882	7	8	Stierres Micheles		3/6/2005	22222788	8,888888	3	98 883938
2889620	5	52:402:055 52:405:005	200.	\$	t	Sifeculation States Six Services		22000000 22000000	886,302500 886,302500	8385855 8385855	5.	200 2000000
2038238	\$	25800000	2009	8	:	Massey Streets		3489000 B	884,002343	83.	-8	33 5055350
383838	2	529050500	365		ð.	States (100), Sec.	5/500096	3.2336_23373e07	886,500,000	2000	8	89 952355
(388623)	3	200000000	3000	ķ	\$	Monne Maines	\$15,555.	0.20000550 0.2000_2000566007	3656_000500	0.0000000	.8	28 (4000)
200200	\$	25000000	665	ARG		\$1,578mm, 390	2.756, 20080	4.5	686,000000	6.05	9	334 55668
288823	.3	35365330	5950	Ņ	\$	School Sections	2077003	0.2338337.5	5555 (257522	5/3503/35	Ÿ	50, 76600
9579A855	- 5	35465300	283	386		30,50000,388	p.700, 70000	- 0.2005/20050007 - 0.2005/20050007	528453,388	838		055 166625
		· · · · · · · · · · · · · · · · · · ·		.90		03/230314/3003	Acces Contraction	52883, 288360CT	44,332.00		<u> </u>	
2889628	2	55405546	555	880	•	30_\$100000_2 36 5	400000000000000000000000000000000000000		996 503500	\$255.	58	52 853000
20022233	2.	25/8022599	385	55556		Segme, SSS, Del	267895	- 6.2536(_2)55466607 - 707	866,000000	8.88	-8	38 58923
28/8/278	2	32/05/200	2505	8	7	Mississe Mississive		31.038863.E	886, 500,000	0.000,530,5	-8	25 65000
59865.59	Ş	300000000	5000	ę	3	ottome kentos		46000000	886,000500	0.00033003	8	20, (60,000)
2825228	3	30000000	2002	3	*	ethics M. perestle	-	53386557 535000500	688,0000000 698,0000000	0.000000	8	84 80008
52538502	.3	20000000	5967 5967	8	ŝ	School Medical	\$120,000 \$120,000	6.23885253	5227552,2696	0.000000	- 8	55, 565,50000 35, 565,50000
9220092)	3540334	200	ů.	8	School Assessed	\$1000000	620000Y	88(30250)	223	-\$:	70 30000
0000000	3	886830	\$67	*	0	Minney Mentler	2:37200	62383036	44 C3332	8,088608	*	77 169333
2009000	2	52,405336	200	<u> </u>	.X.	Minister States	\$10000000 \$100000000	22000000 22000000	966 S25500	2023,000	- 8	52 MSSSS
888838	\$	\$50000000	2000	© C	3	4,014.00; 3490	28365	< 3378, applies	888,000000	0.383436	- 8	35 60068
223562	\$	52,052,050	300	8	7	1017 or 100	0.859/5	3 23 25 34 500	86,00000	2525555	-8	33 859630
65598888	3	20000000	5969	ž	3	Spiriter Sitter Spiriter Sitter	2,652,60 2,656,5	c.2522_epitos	5656_000500	5.0203696 6.0203696	.8 8	38,555333
5950559 55596538	3	20400000	S967	- C	5	Spice (30e	0.60000 0.60000	c.2020, spiles	\$220503_9896 \$220503_9898	0.000000	- 8	97 (85386) 97 (87788)
5555555	.2	55465540	366	3	ÿ	20000 2000 20000 2000	01403943	4,2528,55000	5886 (220)5523	6.006004	-8	305.965385
222332	λ	55,605,555	355	ž.	*	Steloe Xibe	cce8945	:c2235_xq55co	886 ECSEC	0.000,7000	52	200 500000
9829882 8839882	2	20000000	300	6	γ γ	Spire Silve	4.000000 - 2000000	6.2555; 55656 	886 222322 No. 202000	5354533	- 4:	46 95555
28/36/22	2	52865553	2007	3	•	Ministra (Ostables Sturra (SM) (Sat	6.000000 6.000000	0.2006006 0.2006000	MR 20200	6,000,000	\$3. -8:	255 5452256
22,000,00	2	55465555	2565	*	.8.	Minera Abitables	498253	222500N	996 ESSEE	0.00000000	3,5	366 250336
2000.27	ž.	25802M63	(80)	8	5	March Streets	5/42/90	23480v6	886,002003	0.000000	8	333 84688
2822528 65228263	3	20000000	5000	8	₹ .c.	Minoria, provide contracta, provide	p.07580 p.07580	0.23882200	888, 000000 888, 000000	0.000000		300 00000000 NO 60000000
5986559	3	20000000	5000	X	7.	Ottownie Kladelos	200000	4.2322234	988, 223552	0.225500	13.	228 (6262)
5555485	3	55353825	887	ð	F	Science, Middles	\$10700K	53533558	886 333823	8,642,683	*	248 166003
5520462 2620462	.2	20400000	3967	8	8	Steeress Michaeles	\$126625 \$126625	02688365 0268696	\$22055,988 U22055,988	0.000000	- 8	88. 8683787 48. 388883
255955	÷	85466533	887	8	0	Stimers, Micather	4002080	5333356	885 (22352)	8.340833	- 3.	333 853386
2003220	3	22,6525.55	350	G	.A.	Misself Spirite	\$2000Y	3.538863.52	888,0002220	0000000	8	535 5636537
2000020	2	52,000,00	350	3	2	Marieta Shitathic	7 7 7	2200805K	886 203520	8.888888	53.	59, 595033
888828 8638883	3	20000000	2002	8	3	Stane, Suff, Del Massey, Stanese	250000	<.2000000000000000000000000000000000000	994,020002 994,000000	0.058394	-8 -8	392 686 686
28886259	.2	200000000	3987	8	Ś	Adiomous Microphon	x27000	0.3000030	5227552 2665	0.3380020	28	67 8650000
62203662	3	550000000	5000	č	3	Statement Nicotton	\$100000	0.00000000	3856_2227522	3,277,6	18	87 88878
25234502 25234502	3	20400000	S67	<u> :</u>	P.	Steeres Meditor		6.3000000 6.3000000	528555,2888	0.000003	20	58 88390 53 85390
2889828	2	528655255	300		7	Manage Shitake		2.2005036	528755,388 528555,388	0.000000	5.7	28,85550
283626	5	35803386	286	2	×	Monteste generals		6.20050NF	886,30250	80278	-88	338 888332
2000EW	ş	220000000	200	Y	\$	301(3), 38%	0.828/2	3W5,8p200	686,022747	0.293302	28	55 65565
20096236 652528263	3	200000000	380	7	8	Settler (200) Settler (200)	0,608/5 0,602/56	3.2005_aprice 5.2005_aprice	5000,0000000 5000,0000000	6.739003	- 8 13	28 800000 29 8000000
9986559	3	5000000	2000	×	Š.	20000 2000 20000 2000	080765	2000 Stone	986,222522	0.203,083	- 8	20, 920000
25233352	.}	35858555	\$982	ą.	۶	Spirit Sta	3.43394	6,3002,50006	2555555,3455	6.8886683	3	43 989996
08880039 08880039	3	20000000	3967 3967	ž	Š.	Microscop Microscop Microscop Microscop		0.00000007 0.00000007	5985_000500 5985_000500	6.353835	.8	30 966635 44 6663655
2554460	3	3040960	200	3	ž.	Sheeres, Michigan		6764678	\$25555,228	8.334288	- 4	52 762840 98 9000930
22289552	.2	35363655	\$627	રે	S	Schooling Michaelton	\$150000 p	5.NSSSSM	2255252 3496	5.385X33		48 888338
920000	2	525626360	2000	E.	3	Missesse Shitelitic	20000000	x 20000000	300,00000	600000000	S.	25, 859655
S886528 C880636	3	52469600	285	<u>u</u>	ž.	Schweres Michelles Topony Selff, So.	\$20000 \$60000	6.2007.30006660 6.2007.30006660	888,002350 888,002325	8.888888	5. 58	35-500000
5856528 69865-58	2	52900000	200	٤	Ť	Missesse States		3.38756.60	986 200000	9352	- 80	35,09886
8259863	:	200000000	2000	8	3	Ottownia, Named St.		5.30PM698	998,020302	0.238383	8	55 35550000
5886528 5886528	\$	200000000	2002	8	7	Massay Manths		4-03PEG-2	888,0000000 884,000000	0.000000	8	40 887888 50 6608000
52538555	3	204000046	S987 S987	<u>र</u>	3	Stitution Microston Stitution, Microston	\$28555555 \$28555555	6.0000004 6.0000004	5227552,2696	8.338353 8.338533	.8	20 5656200 20 5656200
2000000	3	23002345	25%	Š.	Ÿ	SSIBDLE, ASSEMBLE	44000000	5.20000M	300,002000	8,867886	*	45 99885
25,54460	3	52405245	286	Ÿ.	Ř.	Strange, Strange	2.0000	C1988575	44 33323	8.533	*	22.7655702
282528	2	200600000	2000	8	8.	Minera Strains Entre Site	6760205.	ic 00000000 ic 00000, pasico	NR 30250	SC2360558	52	25 542020 25 5420200
2002277	\$	MOUNCE	2000	6	3	1,01000,1000	0.655%)	3342,38000	886,020747	8,0835	-8:	80 0M367
383838	\$	22000003	350	8	À	Monteney Mighton		× 88480×7	886, 202222	0.03/653.	8	83 838883
200000000	3	20002000	583	8	3	States State Col.	69/000.g	6.38.286860 - 5885554	3656_000000	9.32	-8	82 8887888 80 888888
59554555 59556559	3	25455393	387	*	8	Schedule, parameter Schedule, proportie		c.38838564 c.38838557	886,0000000 886,0000000	8:333888	34	48 35550W
957928	3	936930	3987	Ÿ.	ŝ	Simonny Statemen	233300	4,3583036	5250555,3895	8,080333	Ŋ	23 868600
2829993	2	52465595	2000	2	.X.	Montes generally	100000g	2388889	M6 25552	0000000	<u>\$.</u>	22 75555
2022/09/2 2022/2023	2	22/60/28/2	200	8	A.	Stranger Stranger		%3783037 %3782733	888, 002000 888, 002000	8,049,004 3,650,003	88:	48 5000000 48 5000000
2000226	2	22602283	350	3	.e. .8.	Measuring Measures Measuring Measures		2 2500000	985 22522	0.000000	<u>\$</u>	28 752555
145.00	3	2002003	388	8	ŝ	Sonomer, Maratico	28340	0.3390334	986,020002	6.0003357	8	462 355323553
	3.3	250077234	505	8	3	Science Sett Set	20220	0.03840000 0.00000000	888, 522743 868, 522743	6.50 6.50 6.50 6.50	28 53.	47 (40000) 28 54000
		528655353	2000	S .	- X	Montesty Montesty Microscopy Nicolates	500000000 50000000	0.03833390 9.03863390	888, 000000 888, 000000	6.325265 6.325265	- 23. N	20 000000
20035278	2	2000022220					Accessory.					
5555555 56555555	3	200607100 200607400	2000	X	£	2010/2010	0.82845	1991 Septime	686 222322	0.2003920	8	40.80000
25238622 25238623	3	328C28C5 88F28F25	5060 5067	3	Ř	Aprilan, Sibe Spilan, Sibe	3.48845	5392,5983	\$22755,249	6,0653	Ą.	46,748,430
28986238 28986238	3 3	2000,000	5000			Aprilia julia	5.48845 5.48845					

(Comme	Ohmon	(Short)	Stanfords	Station contract	"Checkment	Charling	The folia	at 1980 a face and	Abnounthur	Markosti	Theires Station	essere (B)
OCCUPANT.		Security Sec.	3900 3900	Alieda Alieda	All some	Checolination	Change	ASSESS ASSESSED	Accountage	adiobs		desire (g)
	Ų.			*		**************************************	227.00	- 200000-0		- Startite	स्कृतको स्कृतको	*
20000000	2	258653030	389	2.	7	Misserie, servette eduscid, servette		26/20055.5 26/20055.2	\$86,00050 \$86,00050 \$86,00050	0.1588.05 0.1588.05	32	400 MESSESS
200525200	ŝ	38868838	200	8.	è	Mayore Streets	255223	4.300005-0	886,322552	25833333	Š	28.768865
2000000	2	38863888	3000	2.	3	sidnsiid, someelik		2554552	88,8288	0.200092		46 868660
9000000	,	528628625	3000	8	8	Mhone hizate shash pandis	\$5500	5000000 50000000	280,00000 280,000000 280,000000	9.339997	39	12, 888832 52, 8888323
2000020	3	22802923	883		0	States Soft for	\$100400	<.0000,0000000	48 SEE SEE	600	- 6	352 353635
2000000	λ	33863986	3939	\$1	2.	Microscopy Microschio	\$688836	2,38 200 000	286,55225	0.00000	\$	72 8623682
2000/000	<u>, </u>	25603669	3889	<u>e</u>	2	Missey Mineton	\$184885 \$184885	C0000000	886,602555 886,602555	288845 202	- 250	45, M366 58, M3666
20000000	3	28465925	2000	8	5	Microsco, Microsco Microsco, Michiglio	\$10000000	7.2000.0000	885 SSSS	5.253325	33	48 88223
33338833	λ	33853885	3932	\$	3	Stateston, Statether	\$3200°	8/39/98/3	88,85200	6,200000	300	20,000,00
95598603	3	358653836	200	<u>8</u>	ń.	Arama Statt Sar Monamor Manatha	\$200000 2 000000	0,00000000 0,00000000	986 (02200) 986 (02200)	5000 6000000	- 22 - 4	78. 803357 85. 865870
20000000	2	35665983	383	S.	*	Atama Staff, Sign	\$100000 \$1000000	C.0000000	88,5325	9.00	- Y	322 8233552
2000000	ð	32443333	2000	8	-r;	Montenes Metablic	2.32394	4,000,000,000,000	386 355525	2458342	3	28, 26,252
(368,663)	2	258653055	300	T.	r.	Sec. (468), 666	62230	2000 000000000000000000000000000000000	28,5225	6.32	,	45.89.03
00000000	2	528055055	3000	8	8	Minerce Advantos Sciences Strations	\$100000 \$100000	CARROLLES	22555,949 72555,949	9.595339 2.553399	*	49, 50,000
SHEES 14	>	(84055)	58.	ĊΧ	w	Danie Soft Se	\$5000	4.650 6500000	28433,88	6.03	*	48.169683
2000/00/00	2	\$26623022 \$2663423	3889	7	8.	Spilite Site Spilite Site	0.0056 0.00546	COSSE AND CONTRACTOR	286.00300 286.00300	2238653 2002203	52 3	46 MASS
00000000	2	52005523	2005	÷	2	Salta Sta	esecono esecciona	5,5552,55555	88 52552	9.3620253	- 2	25.555000
20000000	2	35365533	300	C	3	Solice; Site	0.6009-2	2,0000,00000	28,53225	9/3625/35	4	55 555000
23080036	2	233605328	300		7	Pagag 1836 (a)	280386	7.2245. 22459X9	88 33332	8.33	80	30 555550
000000000 0000000000	2	23682360	383	<u>.</u>	8	Microspes, Michigan Propos, Statis, inc.	\$000000	5,5525,552595C	88,30200 88,30200	0.000000	52	500 868000 500 868000
20000000	ž	32665300	365	2.		248500 Staff See	\$2000	C35224865	28,5025	23	8	25 85588
20000000	2	27/6522305	283	:	•	Passas Static Sas	68886.6	. 5697566K	28, 55,555	868	83	225, 96,900
000000000 000000000	2	256500000	392	2	7	Ricory Statistics	\$200000 \$200000	2,3698660 2,3698666	988, 300000 988, 300000	988888	500	520 880080 800 880080
22886236	2	22222555	2000	Š	8.	Selle Sie	eneggig.	5.3522_cp868	88 2222	2.00	35	30 55553
28655286	ş	35688500	383	3		S0000 Sto	9,808.0	5.2557; 55168	4866, 00000000	500	Ş	30 98000
COMMODIA CAMADIA	2 2	22600000	383.	2	<u> </u>	Spilling Silve Process, State, Sali	2,61505	5.3620 <u>.36600</u> 5.26840003	886,000000 886,000000	0.000900	8	35, 94,000
220000000	2	2336000000	283	\$	•	200000, 20000, 200	20000	2,35533665	88 83255	2.00		222 252000
2877082	3	546990.	3867	3	.¢.	Antonio Abronio	V. (186)	5.3855455	486,000000	868	8	34 965553
G8885238	2	23500055	3987	S	. <u>A</u> .	Secretor Streeten	200100	5,55588007	886 922222	0.336553	- 88	25 950200
0888508 0888008	2	256555555	3987	8	- R	3000,500 3000,500	0.800-0 0.800-0	5.3838) satta 5.3838) satta	886 000000 886 000000	0.00000		44 800007 30 8600060
2000000	2	52809000	3000	\$:	7	2000,000	e168348	College March	46 2000	940000	ů.	222 525503
2000000	3	25000000	3987 3988	\$	3	Spilite Site	49653-5	5,3830; 5 665 8	88 0000	92585559	8	525 96300
G98600300	2	255000000	3967	8	A.	Stroken Alimeter	0.000045 p.660007	2,05000 (6000) 2,05000000	886, 5000000 886, 5000000	2238455	- X - XR	225 990222 255 990202
22228822	ž	25502550	283	2.	•	Action State Six	\$19,000	1.3533395	28,33255	9.33	32	355, 552,0000
CAMPUA	3	224620000	383.	8	.3.	Process, 2005, 346	64004	2.2333490	486,000000	555555 5555555	3 8	222 20122
000000000 0000000000000000000000000000	7	33489986	383	86	.0.	Sameron, Shrinitan Paran, Shift, Shi	59884 58884	5,0000000 5,00000,0000000000	886 000000 886 000000	938		252 95399
		,,,,,,,,,,,	1000				*******	7.2335.733909000			- 777	
2886228	\$	38683486	283	8877	*,	2000 M. 30	2000	87	886,000000	868	58	255 95500
2000228	•2	32688628	38%	×		Storae, 3005, 946	2000000	5,1333, 333 00000 87	966, 9223522	\$3.63	ε	3307 500000
ALC: COL	· _	parcoucto.	5565	8603853535			,coor	P. 2383 TSSSPANCE	0.0, 1440.0			(C) .Q(C)
2000000	3	326699902	388,	5353		Stories, 2005, (56	2000000	AGMAGMATERY.	866,020302	\$35	38	288-860258
00000000 00000000	3 7	324888638	3987	8	<u>र</u> ८	Shire Ste Mineral Mathier	0.600643 p.540360	5.0200 ppt0p 6.02000000	888,000000 888,000000	0.0000000 0.007000		27 36666 330 36968
SSAME	3	226888553	3967	8	₹	Stienne Months		2833333	886 000000	2:393633	83	500 80028
20092000	\$	38488643	388	8	A.	oristed summe	2011/1/A/A	c-22000000	88,22332	239700	32	30,98886
20000000	2 2	30000000	3867	8 8	3	Account Market		6.22009366	886, 0000000 0000, 0000000	0.080000 0.0008000	\$ \$2	200 80000
SOBSCON	v	30600065	3865.		,	orisitetti juuniili	X00000.	C-2223, 2233-0000	355,222352	300,98000	30	255 855000
2889938	3	3555555	288.	23835	45	350000(3005),566	990000	28	885,000300	833	183	85.8558838
2655980 2655980	3	30888808	388	88 8:	š	Parts, 388, 58	2000000 2000000	6.2239(_2239999CC 6.2239539T	886,000300 886,000300	898	8	200 000000 200 000000
20000000	3	300000000	366	8	- X	Stonesson, Morabico Stonesson, Morabico	20,0000	5175080000 6175080000	988,020302	2,02888	33	356 5663330 356 5663330
380000000	3	32483548	383	•	3	Province (2018), 500	HEEPING.	23337 2333975	885 925552	868	88	33. 33mm
20000000 20000000	3 -	32489800	388		<u>(8)</u>	States, 3000, 500	p.C0095 p.43096	200600000000000000000000000000000000000	886,020302 886,000220	8638	20	203 900000 200 900000
2889238	3	30888618	388.	8		Process (2005), See Storon, 2005, See	2000000	c.2556665	886,0000000 886,0000000	200	- 385 - 53	505 90599
20000000	\$	2502016	383	8	•	Partie 1885, 188	D11634	23/88/96.	88 (0000)	868	83	500 99000
2009238 2009238	3	2000000	383.	8. 8		Storce (200), Del Porce (200), Del	900000g 900000g	6.20000600 6.20004600	886,000000 886,000000	0.08 0.33	38	534 9054900 535 9054900
2869538	2	20609650	388	€.	-5	Station 1886	iditor.	c.228590900 c.2205_345600	986 227322	00000000 000000000	- S	50.0000
088603X	Ş.	200000	366	À	<	Sapil Re	0.650-0	C-25500 (3 99104	986 555555	3233494	352	20.8000
86556662	3	25555555	366	č.	3.	Miller Miller	X8863	c.2237_35556	9858, 222352	0.550000	3	79: 889993
20000000	2	30809908	366	জ জ	8	States Mile Moreovery Moreover	2000/3 2000/3	c.1000_598888 c.20058660	988,020802 988,020802	0.5900000	\$ &	20, 88,00000
X559802	3	304609920	360	8	ś	staces gamen.	2000	5.35355K)	896,020352	2,38838	3	52 885 5868
2888838	2	25555555	200	ξ. 3	3	Marche Streetsx	2020000	4.3307856	886 333552	2525555	ž	22 20 20 20 20
20050000	3	35665565	302	ड क	4.	Minore, Middiss Minore, Mindiss		c.20000.wc c.20000.cc	\$86,000300 526000,000	20000000	3	22.768255
2869238	2	32693658	300	8	Š.	viitielle jamanii.	910000°	4.3000000	956,000352	25952550	8	49 90000
20052234	ð	35555555	900	8	Á	Science States	\$10,000%	c.30000007	332(22)322	6,8898933	8	85.36050
2000000X 20000000	2	5242,639	388.	8	.52.	Storce (SSS) (SS)	2020300	296000000 extens 2000	955 22252 955 22252	683	35	20:30:000
2882538	3	5265205	888. 388.	8.	8	\$50000 (500) (500)	2000-0 20002000	c.00005.apploa c.200056607	986 (333,865 255,333 (859)	6.40 888	8	28 8653887 28663388 386
2000020	3	2868888	200	C.	8	Managers, Metachie	2.03300	<394954	2000,000000	5,428945	У	25,286552
20002200	2	30000000	388	<u>e</u>	7	Standard Market		c 2007 (2007 Processors)	886 (SS) 888	00000000 00000000	ý.	22.00000
20000000	3	28826888	380	₹.	2.	Montenes, Medicine Montenes, Montenes		4880894 (306/3808082)	886 333353 886 333353	6.0666.00 6.0666.00	- 82	523 P\$55500 92 P\$55000
20000000	>	22223333	1000	è	5	Sympton Statester	\$3035c	(30)3/4	224(3),209	5/08/3307	*	325.88938
2000000	2	3000000	286	ζ.	2.	Nominal States of	20202327°	4383334	586 555555	0.000000	ž	326.16(3300)
20000000	2 2	30600000	386	र र	3	Minera Streets		<.22220-y	988,2225522 888,2225522	0002000 0000000	- % - &	88 865238 88 8652388
2889938	2	32632865	366	8.	-\$	Macocce, Stateston		585235/4,	355,200302	823	.23	30, 866030
20000234	3	3843503	2000	Č.	5	Mayong Myzetex	\$2000	4352009	386,33253	0.6435594	3	22, 70,752802
2009238	3	3883888	200	8	8	Marca March		4.8888248	886 (83338) 886 (83338)	0.552800 6.6603000	35.	\$50, 655000 40, 36550844
Costo (c)	2	000 childs.	000	49	**	Missene hizzetex	Sections.	e debugging	-endictors sad	0.0000005	*	AC-1500000

	Shoot	a Shipok	Noticet	Stationseco:	(Section)	Nectical .	Produkto	of0866 ofmogra	Jaconsolese	(Mechanical)	Vincilaret Staffe	E4468 12-
CARCOLOR	Secretary.	e georgeogra	Spine-		Alberto	ConsultingCont	Stereo			militaire.		
2835538	ÿ	32502590	5882	8	6	Microscop, Shibables	4-X000.	x328093	3656_0007500	2.082562	*************************************	(99, 952)(955)
0086036	2	2000,000	\$87	S.	ź	Missey March	24550	5,4335048	286 (33382)	0.0368	30	37 3407030
0000000	2	20420438	360	8 4	8	Ministry property Williams Michille	8.80090 x.80090	5/86880/6 6/8680/6	222025,289 222035,389	224463	- 3	35 566333 37 666833
200000	È	3345538	367	S.	٨	Missey, Markey	5,63393	5.433532	32433,249	0.532588	38	70 140330
25586250 25586250	-2	25000000	200,	8	A	Minerios Michelos Minerios Michelos	x 20000	4,8890/7 4,8890/7	88,30200 88,30200	0.088803		384 8883258 320 8883259
288882	3	25635696	200	6	8	Minaria Manther	\$ RING	0878047	88 (33332)	6238862	35	45.0000
2000000	2	2255255	200	6	Ř	Simonny Rhining	X 255500	4,8780.47	-888 852335	27,228,552	\$:	63 863605
0888538 0888538	3	28670036 28670036	5000	6	8	Statemente, Marietania Statemente, Marietania		0300007 0300007	988,9203522 988,922352	9,0333333	.23 .5	25.7 (2007)
20082238	3	200000000	5002	8	6	Statutes, Stateton	6.X020°	5038880pt	5555 237553	9,000000	€.	32, 25,25,25
2888238	3	20000000	2000	6	8	Statemy Statems:		0900007 0300007	686,0000000 686,0000000	0.0000004	12	200 200 200
28552238	3	20500035	5902	\$	á	Resease, Michelia	57X255	2625250c	3656 227522	9,000,00		98 2652590
28355238	ş	590050303	5000	8	8	Action of the Security		088004	986,000000	2,023,559	×	552,852,000
20000220	3	234522232	\$967 \$967	S S	7	Managery Magazine Managery Magazine		5/6888036 5/8888868	\$225552 (327552)	23,00028 2,68	8	20 2452530
00000000	ž	30455500	2007	Ą.	8	Statistical Versities.	2.838333	0.0000005	36 3550	0.0004000	5	32 88988
2008020	5	33473536 35433558	367	£.	\$ 2	Microgram, Microgram Microgram, Adultablica		2:4037 x0 2:2030 x6.	285 233 338 285 233 338	0.000000	S -5	36 900000
\$3886036	2	20000000	200.	5	2	Street, Abstables		5.0000000 5.0000000	88,3255	00000000	- 8	32 98998
2000000	2	20030000	2000	Ç	3	Sixones States		522225A	-RR (00000)	2728855	335	33 665538
3939930	- 3	20030000	200	C .	3	Stockers Address: Stocker (558), 544	\$ 300000.	C385576 C3855865	988,000000 988,000000	2039333	- 88	500 6600000 60 680700
282682	3	22626266	200	č	à	Account States		C386665	48,00000	0.000007	36	35 985400
2839228	3	2000000	5989	A C	8 7	35000 300 20000 200	0.00000 0.00000	20000 Japobse	9858_0000000	9,098983	3	27 599992
20080008	3	2560368	3987	8	\$ 5	Space She	0.6560 0.6560	0.0000 persons 0.0000 persons	\$220000,888	9,000,000	20	25 560,0560
2885538	ÿ	23/0/2008	5882	£.	á	Stekte, Ste	0.00000	x8802_396000	3836,755,255	8,000,0	Ř	599888 65.
900000	ž 3	35479855 35479880	383 283		ζ	Strange, SARE, Jose Strange, SARE, Sare	p.400045 A 200045	2.805, 800 basi 2.823, 833 basis	528555,389 528555,389	\$43 868	. S	52 MASASS 200000 000
20000000		2005,007.5	288	80		200000 (2000) (200 200000 (2000) (200	p.200000	2.200; 2.0900000; c.20000000	386 303503	5758	87	969 56000000
2000000	ž	33455576	367	r).	ĸ	Women's Motation	5469Mc	6.883037	22223,329	2,943,233	38	37 1403537
08886038 08886038	~	20062/2020 20062/2020	550) 550s	3 2	8	Strocores StateStro Scene State De	200000	6,735,6900 6,735,6900	88,80000 88,80000	3032503 3034	35.	520 680000 90 680000
3878485	3	2562,8262	50.	6		Process State See	3.2386c	0.0000000	48,00000	0.00	*	283 864246
20000000	3	5565,5595	335.	7	•	Parene S188, 196	3:5363.6	0,300,0000	88,30000	5,50	ÿ.	25. 85522
2009/20X	3	25073560	5000 500	C	8 3X	Stationne, Khaletten Statio, State, Six	\$20000°	03203888 03200 320000688	988,000000 988,000000	9.285953	8	200 (88000)
2886238	3	5262,6363	395		A	Processe State See	25552.x	0.300, 300003	88 33233	6,85	- 6	23.5 (65.59)
0888008 0888008	3	2200,000	265	7		(200706_0388_038	4.020000	00000000 0000000	955,020502	9,59	- 1	500 800000
2888238	3	2007/0003	360	\$ \$	á	Postor State Series	\$1830396 \$1830396	3: 823) 58433	988 (352852) 988 (352852)	0.609609	33	35 5655000
353/35	322	43255000	380		8	States (2005, 5to)	488844	x3200_22000000	355,265,028	202	,22	253 199396
2352	2 25	10000000	366	5	7	Statement Statement	p./20000 p.2002005	20000000 5-2000000	3656_3003887 3656_3003233067	2082093	7	380 565500
53555	7	3662/3566	383	\$	2	States, State, See	900000	2.3395,33975525	300,300200007 300,300200007	3/38 3/38	- 8	537 688858
386665	3	33655300	303		€	Starry, 1885, Inc.	24350	5.533 _{1,} 523480	SSE 352238	8-38	80	33,8866.0
555565	28	360000000	366	8.	,9 (S)	Spitting 1996 Stillnerster, Killandster	2,93324	6.005/jq666 6.0000702	388,3533352 388,3533353	2000000	<u> </u>	50 600000 30 600000
20000	3	25035030	895		7	Promo SME for	200000	0.0000, 0000000	888 (00008)	0.00	ÿ	238 863236
28385	205	22553222	2000	Č.	8	Schools Seconds:	X 200603	63330006	286 000000	2.5655578	55.	35 653606
0898 0898	30	200866602	2000	C	8	Minator, Modelica Minator, Modelica	\$200000 \$200000	02000000 02000000	966,000000 966,000000	9,03893	. 20	10 150000 10 1500000
2893	255									WHITE !		
2000	307	255555523	5000	\$	6	science some	\$2000G	882822EE	388 200392	9,000007	20	50, 1000050
2893		25000033	389	Ç	š	Sticence, Michiller	\$2000K	C3815/2NK	966 000000 966 000000	9,000007 9,000007	\$	28, 855,250
6866	255								388 200392	9,000007		
33.83	300	22554623 22656823 22656823 22656823	3867 3867 3867	© © ©	& & & &	Sheene Shinking Sheene Chinking Sheene Shinking Sheene Kiraking	2.38685 2.38685 2.38685 2.48640	**************************************	566 (20092) 566 (20092) 566 (20092) 566 (20092)	0.000007 0.000000 0.0000000 0.000 0.000	\$ \$ \$ \$	26 9850388 27 148508 26 148608 26 148508
206000	38 38	27984423 57484423 57484423 57484423 1767337	360 360 360 360 360 360	8 8 8 8	& & & & &	Street, Matter Street, Gardin Street, Gardin Street, Gardin Street, Gardin	5.785855 5.785655 6.785655 5.485650 5.655786	(2003)69W (2003)69W (2003)69W (2003)69W (2003)69W	566 200002 566 200002 566 200002 566 200002 566 200002 566 200002	0.000007 0.001007 0.0010090 0.0010090 0.0010090 0.0010090	\$ \$ \$ \$ \$	25 950000 25 100000 26 100000 26 100000
8988 8888	300	57666823 57666823 57666823 57666823 5767377 5767377 5767378	3867 3867 3867	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	6 6 6 6 6 9	Sheene Shinking Sheene Chinking Sheene Shinking Sheene Kiraking	2-38685 2-38685 2-38685 2-38665	0.000.00 AN 0.000.00 AN 0.000.00 AN 0.000.00 AN 0.000.00 AN 0.000.00 AN 0.000.00 AN 0.000.00 AN 0.000.00 AN 0.000.00 AN	566 (20092) 566 (20092) 566 (20092) 566 (20092) 566 (20092) 566 (20092) 566 (20092) 566 (20092) 566 (20092)	0.000007 0.000000 0.0000000 0.000 0.000	\$ \$ \$ \$ \$ \$	26 9850388 27 148508 26 148608 26 148508
0888. 0886.	38 88 8	57666623 57666623 57666623 57666623 5767337 5767335 5767338	598 597 597 597 597 597 597	2 2 2 2 2 3 4 5	6 6 6 6 8 8	Missaure Nobelica Streets George Georg	\$36665 \$36665 \$36665 \$46665 \$46576 \$46576 \$46576 \$46576	C00000000 C00000000 C00000000 C0000000 C000000	999, 200000 999, 200000 999, 200000 999, 200000 999, 200000 999, 200000 999, 200000 999, 200000 999, 200000 999, 200000	0.000007 0.000007 0.000000 0.000000 0.000000 0.000000 0.000000	\$ \$ \$ \$ \$ \$ \$ \$	26 860000 27 58600 28 58600 28 58600 28 58750 20 58750 21 580000 47 580000 50 58000
33:63	38 88 8	57666823 57666823 57666823 57666823 5767377 5767377 5767378	2007 2007 2007 2007 2007 2007 2007	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	6 6 6 6 6 9	Missaue Modellos Missaue Modellos Missaue Modellos Missaue Modellos Missaue Modello Missaue Modello Missaue Modello	\$100000 \$200000 \$200000 \$200000 \$200000 \$200000 \$200000 \$2000000 \$200000 \$200000	0.000.00 AN 0.000.00 AN 0.000.00 AN 0.000.00 AN 0.000.00 AN 0.000.00 AN 0.000.00 AN 0.000.00 AN 0.000.00 AN 0.000.00 AN	566 (20092) 566 (20092) 566 (20092) 566 (20092) 566 (20092) 566 (20092) 566 (20092) 566 (20092) 566 (20092)	5.000007 5.023891 5.054588 5.05 5.052586 5.055588 5.055588 5.055588	\$ \$ \$ \$ \$ \$	36 850388 37 58568 38 50566 38 50588 38 50588 32 65088 32 65088
28/85, 28/85, 28/85, 28/85,	20 20 2 2 2 3 3 3 3	\$2656603 \$2656603 \$2656603 \$2656603 \$265502 \$367502 \$367502 \$367502 \$367502 \$367502 \$367502 \$367502 \$367502 \$367502 \$367502	\$60 \$60 \$60 \$60 \$60 \$60 \$60 \$60 \$60 \$60	4 4 4 5 5 8 8	6 6 6 6 8 8 8 8		\$388885 \$38885 \$38886 \$3886 \$3886 \$38886 \$3886 \$38886 \$38886 \$38886 \$38886 \$38886 \$38886 \$38886 \$38886 \$38886 \$38886 \$38886 \$38886 \$38886 \$38886 \$38886 \$38886 \$38886 \$3886 \$3886 \$388	03000000000000000000000000000000000000	666, 200002 666,	5.088807 9.023861 0.084886 5.08 0.08228 0.088800 0.0862888 0.086288	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	38 800088 37 58608 38 80088 30 80088 30 80088 30 80088 31 80088 51 80088 52 80088 53 80088 53 80088 53 80088
0880. 0880. 0880. 0880. 0880.	28 28 2 3 3 3 3	\$265,000 \$2,000 \$	988 982 982 982 982 982 988 988 988 988	7 7 7 8 8	6 6 8 8 8 8 8 2 8		2,35000 2,35000 2,4500	12000000000000000000000000000000000000	566, 030002 566, 030002 666,	0.000007 0.023951 0.074550 0.07450 0.07450 0.074550 0.074550 0.07450 0.07450 0.07450 0.07450 0.07450 0.074550 0.07450 0.07	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	38 3853388 37 385638 38 385638 38 385538 37 385338 37 385338 38 38533 38 38 38 38 38 38 38 38 38 38 38 38 38 3
28/85, 28/85, 28/85, 28/85,	30 3 3 3 3 3 3 3 3	\$2656603 \$2656603 \$2656603 \$2656603 \$265502 \$367502 \$367502 \$367502 \$367502 \$367502 \$367502 \$367502 \$367502 \$367502 \$367502	\$60 \$60 \$60 \$60 \$60 \$60 \$60 \$60 \$60 \$60	4 4 4 5 5 5 5 6 8	6 6 6 6 8 8 8 8		\$2000 \$2000	\$250.00% \$25	696 200002 996 200002 996 200002 996 200002 996 200002 996 200002 996 200002 996 200002 996 200002 997 200002	5.088807 9.023861 0.084886 5.08 0.08228 0.088800 0.0862888 0.086288	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	38 800088 37 58608 38 80088 30 80088 30 80088 30 80088 31 80088 51 80088 52 80088 53 80088 53 80088 53 80088
0980 0980 0980 0980 0980 0880 0880 0880	30 3 3 3 3 3 3 3 3 3 3 3 3	02888603 52888623 52888623 52888623 52886023 5287022 5287022 5287028 5287028 5287028 5287028 5287028 5287028 5287028 5287028 5287028 5287028 5287028	366 367 367 367 367 366 366 366 366 366	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	6 6 6 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8		\$2000000000000000000000000000000000000	- (2000/2006) - (2000/2006)	666 02002 566 02002	5.0000007 5.0000007 5.0000000 5.0000000 5.0000000 5.0000000 5.0000000 5.0000000 5.0000000 5.0000000 5.0000000 5.0000000 5.0000000 5.0000000 5.0000000 5.0000000 5.0000000	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	38 9503386 37 365686 38 365686 38 365686 38 365686 48 365686 48 365686 38 366686 38 366686 38 366686 38 366686 38 366686 38 366686 38 366686 38 366686 38 366686 38 366686
08/80, 08/80, 08/80, 08/80, 08/80, 08/80, 08/80, 08/80,	30 3 3 3 3 3 3 3 3 3 3 3	20888823 23888223 23888223 23888223 2388222 2388222 2388222 2388223 23882 2388	388 382 382 382 382 382 382 382 382 382	4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8		\$2000000000000000000000000000000000000	2,000,000 2,000,000	696 200002 996 200002 996 200002 996 200002 996 200002 996 200002 996 200002 996 200002 996 200002 997 200002	5.000007 5.000007 5.000000 5.000000 5.000000 5.000000 5.000000 5.000000 5.000000 5.000000 5.000000 5.000000 5.000000 5.0000000 5.0000000 5.0000000	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	28 8203386 27 195686 28 193886 29 193886 20 193886 23 193886 23 193886 23 193886 23 193886 23 193886 23 193886 23 193886 24 193886 25 193886 27 193886 28 193886 29 193886 20 19
2883 2883 2883 2883 2883 2883 2883 2883	30 3 3 3 3 3 3 3 3 3 3 3 3	200000033 200000033 20000003 20000003 20000003 20000003 20000003 2000003 2000003 2000003 2000003 2000003 2000003 2000003 20000003 2000003 2000003 2000003 2000003 2000003 2000003 2000003 20000003 2000003 2000003 2000003 2000003 2000003 2000003 2000003 20000003 2000003 2000003 2000003 2000003 2000003 2000003 2000003 2000003 2000003 2000003 20000003 20000003 20000003 200000003 200000000	366 367 367 367 367 366 366 366 366 366	2 2 2 2 2 2 2 2 2 2 2 2 3 3 3 3 3 3 3 3	6 6 6 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8		4.35665 4.35665 4.35665 4.35665 4.45765 4.4	-2002-000 -2000-	555 022532 555 022532 555 023532 555 02352 555 02352	9.000007 9.000007 9.0000000 9.0000000 9.0000000 9.0000000 9.0000000 9.0000000 9.0000000 9.0000000 9.0000000 9.0000000 9.00000000	5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	38 852538 87 36538 88 36538 30 36138 31 36138 32 36138 33 36138 33 36138 34 36138 35 3638 37 3638 37 3638 38 36 36 38
28823 28823 28823 28823 28823 28823 28823 28823 28823 28823 28823 28823 28823 28823 28823 28823	288 288 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3	\$2556623 \$2556623 \$2556623 \$2556623 \$367537 \$362532 \$36253 \$	2006 2007 2007 	4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	8 6 6 6 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8		\$250000 \$25000 \$250000 \$2500000 \$2500000 \$2500000 \$250000 \$250000 \$250000 \$2500000 \$250000 \$250000 \$250000 \$250000	-0.000.00 Mills (1990)	595 200022 595 200022	9.000007 9.000007 9.000	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	38 820388 37 145088 38 145085 39 145086 30 145083 30 145086 31 145086 31 145086 32 145086 32 145086 33 14508 33 14508 34
28823 20923	\$20 \$2 \$2 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3	\$70000035 \$7000003 \$7000003 \$7000003 \$7000003 \$7000003 \$7000003 \$7000000 \$7000000 \$7000000 \$7000000 \$7000000 \$7000000 \$700000000	2000 2000 2000 2000 2000 2000 2000 200	2 2 2 2 2 2 2 2 2 2 2 2 3 3 3 3 3 3 3 3	8 6 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8		\$-25000000000000000000000000000000000000	- 2000-000 - 2000-000	555 202322 555 202322 555 202322 555 202322 555 202322 555 202322 555 202322 555 202322 555 202322 555 202322 555 202322 555 202222	0.000007 0.000007 0.000000 0.000000 0.000000 0.000000 0.000000	5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	38 952338 37 34538 38 34538 38 34538 39 34538 30 34
2002 2002 2002 2002 2002 2002 2002 200	\$30 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3	\$7555623 \$7555623 \$7555623 \$7555623 \$7555623 \$757528 \$	200 200	8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	8 6 6 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8		\$2000000000000000000000000000000000000	-2000-00 M 2000-00 M 2000-	988 200922 \$64 (20092) \$65 (20092)	0.000007 0.000007 0.000000 0.000000 0.000000 0.000000 0.000000	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	38 952338 37 145538 38 145538 38 145538 39 145538 30 145538 30 145538 31 145538 32 145538 33 145538 34 145538 34 145538 35 145538 36 145538 37 145538 38 145538 38 145538 39 145538 30 145538 30 145538 31 145538 32 145538 33 145538 34 145538 35 145538 36 145538 37 145538 38 145538 38 145538 39 145538 30 145538
20023 20023	288 28 2 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3	20000023 52000023 52000023 52000023 52000023 52000023 5200023 5200023 5200023 5200023 5200023 5200023 5200023	100 100	8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	8 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6		\$2000000000000000000000000000000000000	- 1,000,000 Associated	955 202022 555 202022	0.000007 0.000000 0.0000000 0.0000000 0.0000000 0.000000	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	38 852338 37 30538 38 32538 38 32538 38 32538 38 32538 39 32538 30 32538 31 32538 32 32538 33 32538 34 32538 35 32538 36 32538 37 32538 38 3253 38 3253 38 3253 38 3253 38 3253 39 3253 30 30 3253 30 30 30 30 30 30 30 30 30 30 30 30 30 3
2002 2002 2002 2002 2002 2002 2002 200	\$30 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3	\$7555623 \$7555623 \$7555623 \$7555623 \$7555623 \$757528 \$	200 200	8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	8 6 6 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8		\$2000000000000000000000000000000000000	-2000-00 M 2000-00 M 2000-	955 200022 955 200022	0.000007 0.000007 0.000000 0.000000 0.000000 0.000000 0.000000	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	38 952338 37 145538 38 145538 38 145538 39 145538 30 145538 30 145538 31 145538 32 145538 33 145538 34 145538 34 145538 35 145538 36 145538 37 145538 38 145538 38 145538 39 145538 30 145538 30 145538 31 145538 32 145538 33 145538 34 145538 35 145538 36 145538 37 145538 38 145538 38 145538 39 145538 30 145538
2882 2885	\$30 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3	\$2550623 \$2550623 \$2550623 \$2550623 \$2550623 \$257022 \$257022 \$257028 \$	500 500 500 500 500 500 500 500 500 500	4 4 4 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8		\$2000000000000000000000000000000000000	- 1200.00 As 1200.00 A	999 (2009)2 999 (2	0.000001 0.000001 0.000000 0.0000000 0.0000000000 0.0000000000	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	38 382338 37 362382 38 362382 38 362383 32 362383 33 362383 34 362383 35 362383 36 362383 37 362383 38 362383 38 362383 39 362383 30 362383 30 362383 31 362383 32 362383 33 362383 34 362383 35 362383 36 362383 37 362383 38 362383 38 362383 39 362383 30 36238 30 362383 30 362383 3
20023 2	288 28 2 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3	02050623	500 500 500 500 500 500 500 500 500 500	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8		\$2000000000000000000000000000000000000	\$200.00 to \$20	956 202022 856 202022 856 202022 856 202022 856 202022 856 202022 856 202022 856 202022 856 202022 856 202022 857 202022 858 202022	0.0000001 0.00000001 0.0000000000000000	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	38 952338 37 102538 38 102538 39 102538 30 102538 31 102538 32 102538 33 102538 34 102538 35 102538 36 102538 37 102538 38 102538 38 102538 39 102538 30 102538
2882 2885	\$30 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3 \$3	\$2550623 \$2550623 \$2550623 \$2550623 \$2550623 \$257022 \$257022 \$257028 \$	500 500 500 500 500 500 500 500 500 500	4 4 4 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8		\$2000000000000000000000000000000000000	- 1200.00 As 1200.00 A	999 (2009)2 999 (2	0.000001 0.000001 0.000000 0.0000000 0.0000000000 0.0000000000	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	38 382338 37 362382 38 362382 38 362383 32 362383 33 362383 34 362383 35 362383 36 362383 37 362383 38 362383 38 362383 39 362383 30 362383 30 362383 31 362383 32 362383 33 362383 34 362383 35 362383 36 362383 37 362383 38 362383 38 362383 39 362383 30 36238 30 362383 30 362383 3
2882 2883	500 500 500 500 500 500 500 500 500 500	02506023 52606023 52606023 52606023 5260502	500 500 500 500 500 500 500 500 500 500	8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	6 6 6 6 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8		- 2000000 - 20000000 - 20000000 - 20000000 - 20000000 - 20000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 20000000 - 20000000000	1,000,000 to 1,00	956 202022 856 202022 856 202022 856 202022 856 202022 856 202022 856 202022 856 202022 857 202022 858 202022	0.0000001 0.000000000000000000000000000	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	38 852338 37 10538 38 10538 38 10538 39 10538 30 10538 30 10538 30 10538 31 10538 32 10538 33 10538 34 10538 35 10538 36 10538 37 10538 38 10538 39 10538 30 10
28822 28823 28	200 200 200 200 200 200 200 200 200 200	20060623 20060623 20060623 20060623 20070623 2007062 2	500 500 500 500 500 500 500 500 500 500	2	8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8		- 2000000 - 20000000 - 20000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 20000000000	- \$200.00 As 1200.00 A	999 (2009)2 999 (2	0.000001 0.000001 0.0000000 0.00000000000000000000000	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	38 852338 37 105383 38 105383 39 105383 30 105383 30 105383 31 105383 32 105383 33 105383 34 105383 35 105383 36 105383 37 105383 38 105383 39 105383 30 105383
2882 2882 2883	500 500 500 500 500 500 500 500 500 500	\$2550623 \$2550623 \$2550623 \$2550623 \$257029 \$257029 \$257029 \$257028 \$2	500 500 500 500 500 500 500 500 500 500	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8		- 2000000 20000000 20000000 20000000 20000000 20000000 20000000 20000000 20000000 20000000 20000000 200000000	\$200.00 As	999 NESSES 999 SESSES	0.000001 0.000001 0.00000000000000000000000000000000000	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	38 852338 37 10538 38 10538 38 10538 39 10538 30 10538 30 10538 30 10538 31 10538 32 10538 33 10538 34 10538 35 10538 36 10538 37 10538 38 10538 39 10538 30 10
3883 3883	200 200 200 200 200 200 200 200 200 200	02050623	100 100	8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8		\$2000000000000000000000000000000000000	\$250.00 M \$250.0	956 202022 857 202022 858 202022	0.000001 0.000001 0.00000000000000000000000000000000000	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	38 852338 37 102538 38 102538 39 102538 30 102538 31 102538 32 102538 33 102538 34 102538 35 102538 36 102538 37 102538 38 102538 39 102538 30 102538 30 102538 30 102538 31 102538 32 102538 33 102538 34 102538 35 102538 36 102538 37 102538 38 102538 39 102538 30 102538
2882 2882 2883	200 200 200 200 200 200 200 200 200 200	\$2550623 \$2550623 \$2550623 \$2550623 \$257029 \$257029 \$257029 \$257028 \$2	500 500 500 500 500 500 500 500 500 500	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8		\$2000000000000000000000000000000000000	\$200.00 As	999 NESSES 999 SESSES	0.000001 0.000001 0.00000000000000000000000000000000000	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	38 852338 37 105382 38 105382 38 105383 32 105383 33 105383 33 105383 34 105383 35 105383 36 105383 37 105383 38 105383 38 105383 39 105383 30 105383 30 105383 31 105383 32 105383 33 105383 34 105383 35 105383 36 105383 37 105383 38 105383 38 105383 39 105383 30 105383
3888 3920 3920 3920 3920 3920 3920 3920 3920	6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6	\$25500000000000000000000000000000000000	500 500 500 500 500 500 500 500 500 500	8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8			- 2000000000000000000000000000000000000	1,000,000	999 (2002) 999 (2002)		\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	38 852338 37 105383 38 105383 38 105383 38 105383 38 105383 38 105383 39 105383 30 105383
Sept.	6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6	00000000000000000000000000000000000000	500 500 500 500 500 500 500 500 500 500	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	6 6 6 6 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8		- 2000000 - 20000000 - 20000000 - 20000000 - 2000000 - 20000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 20000000 - 20000000 - 20000000 - 20000000 - 20000000 - 20000000 - 200000000 - 200000000 - 20000000000	- \$200.00 As 1200.00 A	999 (2009) 999 (2009)	0.000001 0.000001 0.00000000000000000000000000000000000	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	38 852338 37 105383 38 105383 39 105383 30 10538 30 10538 3
2888 2888	6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6	00000000000000000000000000000000000000	500 500 500 500 500 500 500 500 500 500	8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8			- 2000000 - 20000000 - 20000000 - 20000000 - 2000000 - 20000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 20000000 - 20000000 - 20000000 - 20000000 - 20000000 - 20000000 - 200000000 - 200000000 - 20000000000	1,000,000	999 (2002) 999 (2002)		\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	38 852338 37 105383 38 105383 38 105383 38 105383 38 105383 38 105383 39 105383 30 105383
3888 3980 3980 3980 3980 3980 3980 3980	200 200 200 200 200 200 200 200 200 200	02566623 52666623 52666623 52666623 52666623 5267623	300 300 300 300 300 300 300 300	8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8			- 2000000 - 20000000 - 20000000 - 20000000 - 2000000 - 20000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 2000000 - 20000000 - 20000000 - 20000000 - 20000000 - 20000000000	\$1,000,000	999 (12992) 999 (1	0.000001 0.000001 0.00000000000000000000000000000000000	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	38 852338 37 105383 38 105383 38 105383 39 105383 30 105383 30 105383 31 105383 32 105383 33 105383 34 105383 35 105383 36 105383 37 105383 38 105383 39 105383 39 105383 39 105383 30 105383

*Gento	Shron	Stiggt	Vaccional	Shelincegon	Markers':	Variant	Předálački	xCFF0X-xberqpo	Acceptation	Mindaget	Wardoork Black	erence IP
SPECIAL		problem	2004			Variant Constitution	Specifics			addition.	ellecto elle	
5860	2	20000000	3987	¢.	ż	Silvenous Michaelee	2:33233	9,20000000	888, 308872	CONTRACTOR	K CONTRACT	30 8000390
58Q	Ą.	5000000	3867	2	Ϋ́	Schools, Michiga	\$100.000	0.58800000	866,000000	5320000	48	82 (80)(80
380	3:	8928339	387	*	2.	Stimes, Michiga	3:300000	2,000,000	886 (00805)	868898	- 8	34 90003
380	<u>8</u>	2022222	388	8	*	Misserre Statebio:	p.4653272 p.4653272	3:300007.	3550_3055972 3550_3055972	9337538	137	258, 868778 58, 888828
3863	3	6666555	4909	S	3	vinestit, proutti		2-2 000 63-3	288,388233	200003	3.	45 1693007
3860	3	20000220	2000	8	3	Managa Madan		2388832	2656 (2006)222 2656 (2006)22	0.000000	3	252 8852288
5900 5900	3:	2005020	2000	8	-(-	foliosopo Micheleo foliosopo Micheleo	\$745032 \$765032	7/2000055 2/2000055	38,33837	2002000	38 4	50, 9502658 50, 9502658
3883	2	20000000	3987	&	9	Adicional Michelon	\$7,552.55	9,000,000,0	886,008077	0553500	8	300 100000
3863 3862	3	5005000 5005000	3887	<u>8</u>	3	Accessed Micheles	\$188335 \$188335	4,3868695 4,3868695	886,008872	20055005 64658600	38	589 MARKE
380	*	8878770	387	v.	7	Schoolse, Michiller Schoolse, Michiller	31,985,535	36368655	886 (00807)	326325	26.	34 803373
5883	2.	20000000	3997	Ø.	75	solution Michigan	5: 252,222	9,0888655	886,008077	8633528	8	558 565358
580 580	\$	\$6000000 \$6000000	366	\$ \$	7	Minera, Middlin Minera, Middlin	\$150,000 \$150,000	C3889594.	866,006970	0000000 0000000	- S - S	38 3533557 386 345363
380	8	20333325	200	ß	3	reference Statement		2.588883.3	886,988632	0.458/655	32	22 96200
3880	Ř	2000000	200	25	ž	Silvente, Stockhice	9/98/252	× 2000/24.	986,968655	0:200929	8	365 (65365).
380	3	5000000	200	8	₹ ₹	Allowing Alexander	\$55390g	5.588633 5.588633	986,908600 986,908600	0.005000		70 N6030
3883	55	25583855	3999	€.	3	educated secretific	250053	4.2532354	386,000035	37,22,358	52	222 822500
346500	8	52885538	38 3	٧	4	500,5000,5000	999603	5,500,000,000	286,000653	2235	Ś.	40,75000
\$28600 \$28600	X.	R80502200	392	₹	35	Standarden Schrieber: Standar Statis Stati	\$1255000 \$125000	0.0000AT 0.0000AE	986 (020080 986 (020080	2000 2000 2000	\$8. \$	25 993865
935	3	50096395	366	15	×	Schoolse, Michillica	\$200000	0.0000000	866,000000	0.333000	38	30 (0300)
25,855	5	382555550	397	7	8	Minness, Michalico	2:2000	430000	446 73246	8383738	8	35 600,000
52560: 52560:	33 22	200528095. 2005280000	389) 386)	8	8	Selectora Selectorio	p.85888 p.855888	50533035 6353366	936 5225202 936 5225202	32505 9380333	35	68 (600000) 68 (600000)
K083	-83	523M582	2005	€	.4.	Managa Middia Managa Jawana	905330 905330	23658E	200,000,000	8/333		39 6503690 25 866035
10033	3	230002000	388	6	•	Sec. (862, prees)	25000	8.8834960	203503	6.5	38	286 855398
55,6235.2	7 7	239300000	2000	Ç.	3.	Succession Shebbler		4.20000NF . 9000N, A	280,028009	2.5(20056)	2. KB	55, 850005
5663C5 5663C	32	280920301	303		- S	Same Sufficient	5:383986 5:459055	<.2000.3003866 <.2000.3003866	986,828698 886,963882	222222 252		200 860000 8000000 860
33500		00000000	-my	`	-19	200000,0000,000	(0.003303	100000000000000000000000000000000000000	300, 300,000	****		300, 404,0300
N832	25	300000000	383		8	States, 3005, inc	3.232236	4,000,000,000,000		678	38	860 80080
8833 8833	32	9000000	398	\$ \$	Ň	Manager Marketon Nacce 2001 Set	2-30000	200000000 200000000	988 903882 988 908822	0.00	- 3	255 562755 255 568755
2000		www.c	****	.4	•		yourox	0.5028_223306607	.00,000		- 4	CAL ARTES
28%;	3	43834983	388.	0.00		by Storens Sec	940000000	2	886,988330	2:22	ĕ	2002 500 20020
585) 585000	3	202222300 202223300	3868	3	र १	Manera Shishin	5050000 500000	5/500000/4 5/5000000	986,000075 986,00007587	9.552555	55	38 863833
345566	3	28355863	300	7	č	congestit, provided	5/3739	28380XC	286,202602036	233000	30	48 80000
9000000	3	222000000	200	E.	.N.	Married Married		5.6388379.	2000 3000000	2/26/82	Ş	48.8833338
901010 901010	3	\$50865835 \$58655865	333	8	.8.	Supported States of	2000000	c.0000000 c.0000007	986,027867 986,028828	232 234334	- 8	275 M660 255 M66223
2002000	ž.	2303033333	3987	Ť	7	Steroomer Steresco	X 200000	c.30004	886 02860	2000000	- 3	222,522,522
50000	\$.		3999	ĭ	S	educide journalie		4.3836XC	385,385538	0.502933	35.	5258 8538536
7676 7676	ž.	33050000 33050000	3987	*	0	Administration (Allegandria)	2000	080866 080866	884,000038	0.000332 0.000332	- 8 83	200 (2000) (6922)3 450
8888	3	332555555	3883	8	7	Minera Statelia	278805.	2.53865-5	386, 300,003	9:393395	20	235 852235
1000	3	332528098		ž.	S.	Sillabeth, Assentitio		C300008	986/360008	9,000,000,0	28	200 (6300)67
90000	3:	3570356065 3570356066	300	8	<u> </u>	Street Streets	p.65538 p.855386	5.00766C	560(30000) 260(3000)	9.53	32	43 566635
8583	8	10000000		8	3	Science, SSSC, Oct.		4.838697	380,3030,000	2353705	33	55, 94,000
1666	3	520,000	300	8	A	vincett, graetti	2,000	4/2003	280,3300,00	6,9333	333	SC 750559
2000000	8.	200002000	383 383	•	<u> </u>	Danne State dec	X22230	4.5382 5388945.	286,000,000	23	58	.80 245000
2000000 2000000	0. 0.	200000000 300000000	380		6	Statuse Staff, Sec Status, Staff, Sta	2/2/2/2/25 2/2/2/2/25	6,3500_35000ec 6,6500_35000ec	986_983558 966_983568	6.25	<u> </u>	30 88030
2000000	33	5000(3000)	3987	₹	Ť	Maragere States		4,333355	886 (0033) (008	82.28	53	0000000 46.
558666	33	352888033	350	à	8	Managa Middhe	2,787.7	00000	836,003038	9,000,000	35	35 6503030
2755633 2755633	33	\$52888.073 152888.083	366	8	8	Minera Mitalia Morro Standa	2,9995.c	0.000000 0.000000	886_000008 886_000888	8,000,004 8,000,004	- 88 28	30.386333
0.660000	3	22385285	355.	3	*	States (2005), 103	3053222 3053222	2.0038_2035666	866 3000357	10:0	20.	38 8030883
48003	8	55:23:05555	-2009	3	.4.	Symmes, Michigan	20,000	6.3834CX3	280,000,00	650030	,	28 85555
0.60003	3	3528855888	338	8 8		Section (2005) (Self.	A6000A	5 5392 ,530156605 AGMCMCC	2000,2000222	8:33	8	777 5600077
	•,•		-5.00	7/80/08/2007	:		20000000	2,385,235,000,746		0.05		
48000	8		488	80	*	Coppe, Still, Sel	4866024	SCENTA	288,000384	833	32	48 800000
200000	N N	233825858 233825858	3982	₹ 8	35	Silvenos Midallos Sociocos Silvinios	300000 200000	2/22/2005 2/2003/55	200000000000000000000000000000000000000	0.0000000	- 58 -40	227 8222380 242238 722
253	2.	322624286	383		Ř	Speak 1885, in	3/3/2000	0.0000,3078027	86(3680)	645	3	30 80003
25552	87	3343935	3997	₹	S.	School Meadle	5:83555	5,0000000	886 000000	0.35555	75.	32-800323
35,252	3	400000000	355	`	8.	States SSS in	\$2000000	200000_7086660 200000_600786607	386,288238	929	à	537 557200
32753	3	42300230	388	75787		Creme (\$68) (546)	98663386	998 2007/2010/2010	856,856330	200	38	288.8682288
353,83	3	40,555,000	388.		8.	20000072007700	\$45500000	78355 2552865	986/55555	9.33	8	5353 885355
507000 5070000	3	68358235 150835381	3000 3000	3	<u>-</u> 8- ₹	Symmes, Milation Source, SSM, Jos	2.858555 2.858555	6.8834CX7 v.1020, 12236647	385,328338 386,328338	2,342,857	32 8	78 567 (02) 196 567 (03)
553	- 55.	000000000000000000000000000000000000000	383	•	8	State State See	6000000 60000000	5.5585 5355940, 5.5885 5386690,	380,000000	833	- 8	35, 999000
583	55	68556533	2000	ë		Spille, Side	4.8345	8110g 700cs	38/38/88	3338303	5.	53 753555
50355	25	300,53552	2000	· ·	ê	Storag State, inc	2.2300	c386,3868cc	886,363877	355	2	265 84286
59336 393393	3	388238623	399	<u> </u>	7	Administra Michigan Managara Madabistra	30 00 00 00 00 00 00 00 00 00 00 00 00 0	C2002004	886 000400 886 000400	0.0000000	- 2	32 (48003)
83355	î	34658423	397	7	Ĉ	School South	\$959KL	(238868)	886,000403	6.233333	38	46613 45
07050	2	388388838		7	ξ	Missione Shiration		X20006-8	2000 200000	83	ž	83 8660000
32353 32353	3	200200000		7	<u>v</u>	Manuse, Middler Manuse, Samuel		22000-0 22000-0	258,232633 258,232632	0.2300000	- 32	40 (600)000 40 (600)004
97883	2	2000000		7	रे	Marco Stranger		2.33880-02 2.32880-02	200,000000 200,000000	0.346983	25	20, 16,030
35386	3	20020000		7	š.	estatistik generaliti:	200000	0.20000000	380,000,000	20025000	8	23 823863
50003 50005	3	20000000		7	<u>\$</u>	Officers Maries		6.3388.95 - 200003.40	288,028833 200 200000	2.622888 x.oore	320	26 262325
503305 503305	3	2000000000		Ť	6	Sittleman Michaelen Sitteman Michaelen		0.000000.cc 0.000000.cc	986_033653 986_033653	52,552,5558 52,552,5558	<u>8</u>	26 9696055 30 9655555
85.553	Ţ	590,500.54	2000	Ÿ	ζ.	votessiid, joveestiid	233333	5.20802.vc	386,533863	3,533,550	ý.	49.85233
50354 50354	3	3000000000 30000000000	3997	₹ ₹	0	Stinenay Midatice	2.300000 2.300000	<20000000 - 200000000	884 (000400 884 (000400	0.000048 0.000048	\$ 22	22 83863 20829 755
33333	2	3000000000		<u>1</u>	Ř N	Administry Michigan Managery Michigan		4,3000000C	866 0000000 866 0000000	0.000000	- 22 8	307 (000000)
38335	>	300555700	386	₹	8	Stimus, Months	23000	C/88000C	46/33433	0433343.	3	35 853665
35,283	2	3362362359	300	S	8.	vibrield, provide	200000	2.59993-5.	2536,252,353	932233	ŝ	28 8833348

Section Sect	-Seese	Sheer	Strait;	Verient	Substance Silveb	Visited:	Verland Constitution	Posteiro Circosco	ogostic kirigo	Vacconsolium	Nation 3	todaut Reik	centre 45
Section Sect	News I		Brouggest	<u>office</u>	Allerton .	All the	(Character out to a constitution of the consti	Closespo			adiate s	Beder stied	*
1885 1886	38/3384	\$	\$655555	283.	4	g.	Minney Middles		5.53M65.0	886,80380		\$3	45.895888
18 18 18 18 18 18 18 18	44.0	3			8							33	
1985 1986 1986 1986 2		3		1,5 111	· ·								
1965 1966		3			χ					886,352832		3	
Prop. 1		2				Y-1							
1985 1987		_			`		Micesse, Michiga	2000000	0.000000000	888,000403			
1985 1987				1									
Property Property	69850	82	260353889	2007	€:	34.	Missessy Misselex	3292		886,000,000	0.33383		23,8892333
Property Property	92352 000000												
1985 19			260003000				Milanora, Silvinibro	400000					
1985 1987	\$69.50 \$2.800												
1985 1987				,									
Section Company Comp	3353				40		Marries Michiga	\$1 99 33	23800 A		2,783526		25,886,286
Section Column													
Section Color				.,									
Section Company													
Transport Tran				4.4					********		10 10 44 11 10		
1977 2					,		restants, summerifi-	2070°					
Section Column	· ·	_		.,									
Company													
Section Sect	2283.3	33	40000000	2002	Ķ.	Q.	Stitutelli, parecettic	9000SS	0.0822880.0	2000000	9333339	353	20.2000000
Security Security	8980	37	33555465	302	85	*	Process, Soft, Del	M222345	e-2546; 334789490	886,505355	888	y	87 (4563)
Process	380000	3	SUSSIE	(88)			Account, Maratics		4-2033/23/3	986 50000	3034965		80 68290
Process 1985					<u>«</u>								
Proceedings	35525		\$2855,53953	335.			20000 SSSS SSS	A2000A	4,350,6866.	806,000,000,000	9.23	33	500 56000000
Property		2											
Proceedings						x .		aciden			8.33		
1975 S. 1975					92		NO ASS years	\$2585W		886,000,000,000			
Property Property	440.00				*	•							
Processor Proc					ŝ.		Process (2005) (Set	50 323 555		96 6033339			
Processor Proc					2	-							
Second Color Seco									6.2280008				
Process 1985	2533	_											
Process Proc		_		0.40									
	33555				S.								
					8.	•							
Property		\$.				3			6.388365/7				
Property	2220		000000000	202.		eene	Decree Orde to	A-110-000-0		2002-02000000000	0.03		ON MARKE
State					8							32	
1975												83	
1975 \$ 20010000 809 7 \$ 200100000 200100000 200100000 2001000000 2001000000 2001000000 2001000000 2001000000 2001000000 2001000000 2001000000 2001000000 2001000000 2001000000 2001000000 2001000000 2001000000 2001000000 2001000000 2001000000 2001000000 20010000000 2001000000 20010000000 20010000000000					•							3	
Proceedings Proceeding Proceeding Proceeding Proceding Proceeding Proceding Proceding	25555	À	10000000	200			Successory Maderials	400000V	C15995555	46 30,000		4.	25.855.000
Section Sect													
2007 2				100.		•					446141661	- 33	
The color of the													
Total	3.4.4.4.1		200020000					4/3/200 ₄	5				
Process Proc	7.757.57			77.707		**					27.57	7777	
Section Sect	777.50		,			v							
Process Security												· ·	
Process State Process State Process State State	1.11	<u> </u>			38							700	
								313773°		965 363 23320			
Process Proc	(5255)	Š.	2832538	283	Y		Station State State	(0.5%)(30)	x23333843	505,000,000	888	- 0	38 88793
Text													
Company Comp	2535	\$	20020202	283	ţ.	*	Mr. 1995, people	(2000 88)	0.007778900	44 35 533	9.29	38.	43.853393
	3503	2	3X(23,X)E	90.	3	v	Presse Sirkt Dec	2016H		985 3635 <u>3339</u>	838	36	83 58553
Text		5.	202327289	233	8888		Statemer, States, State	30.577338c		886 00000000	8,88	:8	352 SB355
Text		_			£.								
					<u> </u>	•							
	3533	S.	30X4257433	886	e.		Freezin, Staff, 302	2000000	6.2852, 38268cs	586,500,000	8,38	3.\$	59 55553
Text Street Str													
2007 2	2533	8.	20033000	583	.		State growth	940000cg	638505500 x	886 300 527500	\$1.58	8.3	20.8852803
	35525		200523000	3000			Shorency Scholetin	9/3000bs	4.33888.74	806,000,000,000	87555285		80 8600000
	55/2/8	s	283,250000	,902.	3)	**	10000 (1000) (1000)	5000/3500		900,580333330	858	38	550000 eq.
Temporal		_			v				C200				
Text S	2555									886,000,000,000 886,000,000,000			
2003 \$	252.5		X632333	333	40	•	States, State, Sar	#200	× 2226 , 273546637	24 30 8 23	9.22		
TTT: d DESCRIPTION A Access Soft for p-PMHM CANNEL (SOFT) (SOFT) (SOFT) (SOFT) (SOFT) (SOFT) COST DESCRIPTION DESCRIPTION COST DESCRIPTION							distinct summed.	2000m	4.38380/3	986 96353339	200000		35 550035
2000 d 2000 000 - A 2000 000 00 0 A 2000 000 0 A 2000 000					•								
[27] 6 NO.13630 90, 4 4 Acresse/Principle (21)55,4 NO.2012128 (26)857 3 TS PR. PR.	3533	Š.	2X32338	883		A.	started periods	24/22/98	828887388878	505,365,27230	8238	328	68.822292
	253.5	*	200320300	283.	4	3	Microspesse, Microspess	1030000	6.36 28 00-7	88 35555 <u>58</u>	6/288925	3	326 298 303

Constant Constant	Chapter Chapter	Starti profities	Kinchnob Rigger	Redecemes Albeig	Herdend Addies	Vertect Countration	Studente Charge	49808-Manager	Accession	Variant electron	Warrings affects compared	Bodinesson SD edicin count
1000	-5.	33535333	2002	C	?	Monteoux Monteon	$\nabla GOOO_{2}$	3.3000007	985, 36353,5390	@ N2855 N		
9992	.2-	300350003	382		-50	sol, Stoll, secoels	3888860g	Section 3000 Section 19	595,000,00000	9:30	s 8	200,000,000
322	4	300030000000000000000000000000000000000	502	C		1603 Hell 160000	0000002	0.000000000000000000000000000000000000	984,000022000	9.23	5 8	334 (2000)
2222	3	200220226	283	577		Process Staff, Saf	2/20025b	c.3332 3323abec7	5656 (300) 5270 538	2000		98 88300
2222	A.	300355000	283	77		Specie State, See	200230200	4.7338; 33336677	5656 (300)(270)(0	9.33	: 8	355.6636867
1553	S	200200000	383.	·5		Shanner, Sabah, Sadi	d00000000	2,2356, 235526677	2006-2007-2008	833	33	25 2500000
1553	A	SNEEDES	283	80	No.	States, 5585, 566	4.753.188e	2.2392; 239246AC	885,000,000,000	8.88		
5553	-5.	30000300	595	12	3	Teacon (1887), 160	600000000	20000000000000000000000000000000000000	988 968535399	GN	23	20,00000
1553	-5	353553355		a	.X.	Manager, Soldablica		8869	888,388335388	(6.3.3559)		
9992	- 2:	300332300	3000	8	ŝ	Spirital State	0.80%	0.3870, eggione	996,0000022000	8.3	5 29	60.600860
983	5.	8665688		6	A.	letter liter	0.8365	3477, againe	886 000000000	0.053000		
200000	\$	5200550055		Š	Ť	Simpore Statelia		4.7307.57	5655-3000200	63625		
250000	35	3.885558885	5999	\$	T.	coldatable, econocide		0.560000	5955, 2000,200	8:38:323		
968.66	48	120/00/2009		8	3	ecitatule, canacció		SMMG.	985,900090	9.223939		
25.53.6.55		2002003	2005	*	0	40,939	3,963.5	c-179, spiles	886,308823	8.339583		
200000		3893333	283	8		90000 300K 506	2012/2012/201	0.377,0007	886 30863	2.33		
1983	37	282868	388	- 0	8	Manual Statement		6.0000007	N66 (00000000000	(0.087293)		
1585	-37	2022000	265	8	8	Silvenes Matables		2.0000000	886 300 000 000	8.823883		
2000	53	22023000	300	C	3			4626833	888 86333833	(0.000000)		
1983	57	520,0000	2005		.8.	Missione Shristico Missione Shristico		2000000	886 (8633)8333	8833333		
9990		20000000		Ø.	3						_	
7953 7953	23		2000	0		attainin Attainis		0845898	886,000,000,00	9.3233380		
	83	355556	4900	7	5	Missey Streets		30M9E	886 368304830	60003330		200 000000000
3333	33	5655538	2007	8	ÿ	Minoray Mineton		4,757,757.5	886 300 5200 52	5735533		
3353	.22	5555575	2887	S.	3	citainité pocesité.		0.3353866	996,300,000,00	9.288230		
1525	37	32332386	28.	8	8	schoold, periodice		678369Y	886,983535353	8,033,033		
1984	3.7	3533559	200.	\$	Ç	solution Assertable	\$2000	6.0380.00	886 365 355 35	6/26/33		4. 444 4.1
1990	<u> </u>	53055535	3995	7	₹.	velletels, proceeds	VS555555	2000 E	306 3055226552	(659)350)		52, 90,6663
55%	3.7	5205555	2505	7	0	Silvense, Michilles	20000000	Sept. 1885	200 300000000	0.000,000		32, 25,050
5555	83	32,3856	4800	٨	Ą.	reignatel, provided	2/02/09	0.8387NE	880,36603860	6.253555	: 5	37 80 80
50000	83	52,523,222	5905	7	ζ.	originals, proceeds	2000000	3036523	985 503528352	9,000		5296866 55.
3353	37	25528558	5999	\$5	ŝ	Althouse Micheles	22,652.00	c.N62000	3935, 2005, 2005, 2	9,00066	5 8	37.888888
5555	53	3558656	2002	©.	4.	etimistik gorastib	200000	SHIN	88,363,2832	0.323343	. 33	300 6850056
2223	377	25333535	25%	8	3.	School Metables	2020200	2278208	886 300333033	0.087933		36.88835
2000	2.7	20230500	3987	8	7	sotroción Micheles		2.558556	5656 (2005)220552	9.288883		55,560,0006
2200	57	2000000	2562	8	5"	Manager Alabables		V-\$28525V	3656, 3603 333 333	3356238		
1383	37	2828833	285	ě.	9	Stances Midatles		2.2383-35	886 300 300 300	233463		
79933	83	3338833	2805	C	3	Managa Shipping		4920303	888 063038303	60000000		
25/23	52	2222863	200	č	3	Missesse Statestics		3.8888.5	866 56332833	2000000		
5553	337	3008600	2007	8	\$	Officeron, Nationals		0888885	998, 968528852	0.223322		
7955	53	353886	2000	C	3	Western Meaters		2000000	886 968 988 9	0.000000		201 2001 9701
7000	83	50,000	2975	Č	3	Winners Modelin		490000	88,3630830	6.38383		
23825 2385	.27	2000000	388	1	8			2.39600cG	566 300520052	0.000033		
7900 7900	33	2000000	2002	-	<u>.</u>	Selection Selection		6.0000000 6.0000000		6.233522		
,						Obsessed Accounts			66,3602002			
1250	-37	2020033	393		0	Property States are not	200000	2,37% 3770007	886 900130013	839		
2555	.23	25236558	2007	8	Š.	Actionsous describes		0.000007	5656 (300) 5200 52	9.308083		
55%	57	5000000	2994	<u> </u>	.N.	Statestell, passentite		2.000057	886 96552655	0.0000000		
1520	57	3235588	28.	ŵ.	8	School Assemble		4.200007	996 903536353	(28)(39)38		
1000	83	55,500	985	41	Ψ.	States (201), 202	45556	3.232696C	986 363338333	600		
227.2	37	323832	200.	a		Stepher (1887) (Set	40000	(0.238696)	366, 563326332	8.83		
D2ACC	23	86236255	2002	C	4	etinistik gamistik		0.80700A	886,000,000	9.553995		
22.5	23	44245265	4900	C	3	Marin Marin	V 15259.	S 482,438-97	886 300.548	0.670	. 22	40 686668
(245)	35	8003808	3987	\$i	Ň	Schools Micheles	255333	0.3000000	886,300,388	9.33983		- 67 S803860
2285	.33.	383333838	5887	85	Ś	Addressed Attachestes		4,366235	5555,0000000	9.3006.20		
33433	32	885225588	28.	٠,٠	¢	School Assessed	20000	SASSAS	855 300055	22222		23 820000
33552	3	35555500	200	5%	Č	Steeres Metables		c.0739x0	20226,328	839		
		23838383	350	Ċ	3	Monteness Modernion		×38880×7	965 560000	6883333		3355515
200000	8.											

Supplementary Table S4 List of non-hematopoietic genes and variants queried

Gene пате	Reported mutations used for variant calling	Accession	Number of
			variants found
ACVRES	Francisco Control (Control Control Con	300,300,000	
AXT1	\$38,338,638,6568	388, 088388	0
890 <u>C</u>	Proceedings research COS (COS) Radiographics (COS)	386,360,3275.0	8
AP082	51976, \$1898, 618800	5855 (6800000)	3
48905AP35	Francis (SA)	350 (66430);	8
ARISI2	Proceedingster, 350.045, 353.7401, 833.975, 833.060, 830.053	985, 332842	8
27/53	200, 2001	300,000000	8
RZM	Proceedings:	980,080	۵
BAPS	Franch (Screense 12) (gr. 12) (gr. WK) (22) (33)	300 3000	8
CASPS	Fraceoistic	588 550 580	2
OD683	Annahilia Chin Chin, Wan Nas	300 300000	
CD8/8/3/E	Proceeditty:	388, 388366	5
306%24		300,300000	Š
	No. of the Control of		
CHO1	882,030000	889-362,555	Ø
GH04	EMACE POLICY SELECTION AND AN ALL AND	886,083283	ž.
CTSSES.	\$2009, \$2004, \$2000, \$2000, \$2000, \$2000, \$2000, \$2000, \$2000, \$2000, \$2000, \$2000, \$2000, \$2000, \$2000, \$2000	8882,000,9996	a
ESFR	The state of the s	365 308355	×.
	(A1804 (A1804, 1864))		
E8/8/85	\$2500, \$2500, 85000, \$7500, \$7500, \$76000, \$76000, \$7500, \$7775, \$8600, \$2500, \$6000, \$50400, \$10440,	2602 (2004)	0
68883	BOX (108, 11984 (100), 2011) (100), 6108 (610), 1064 (100, 610)	966 (855.002)	₹
EZRI	Proceedablyhis	5655, 5455,5555	0
PSFRE	5.00(1.00) (1.00) (100) (100) (100)	386,300362	8
GPS2	Promotifying	200 30000	2
HRAS	900A, 6000, 6000, 600A, 600A, 600A, 600A, 600A, 600A	888, 278788	0
9286C	\$0000013550, \$23\$, \$23\$, \$35\$2, \$55\$2, \$55\$3, \$23\$\$, \$23\$3	200 (2007)	\$
Keapi	STAND, STAND, PETENG TERMEN, KERMEN, KERMEN, KERMEN, KETTEN, KERMEN, KERMEN, KERMEN, GERTEN, GARTEN, G	9886_0552988	8
R44F2N4	Franchistania (150 NOV 106 DE TESTE DE	366 300000	č.
884P3011	France (68%) (515) (68.515) (69.51) (50.51) (50.61)	966 09865	Σ
357028	CARE CARE CARE CARE CARE CARE CARE CARE	350, 386530	\$
RPENIE	8000, 8000, 1200, 6000, 6000, 6000, 6000, 6000, 6000, 600, 6000, 6	3887,0000386	ż
PBRM1	Executação	386 333 365	ž
Price	RESE, ROSE, ROSE, ROSE, ROSE, ROSE, CHEN, CHEN, CHEN, CHEN, CHEN, CHEN, CHEN, RESER, RESER, CHEN, CHES, RETER, RET	.ass/3000338	Z
PIXERI	Through Through (* 17 17 18 17 18 17 18 17 18 17 18 17 18 17 18 18 18 18 18 18 18 18 18 18 18 18 18	WK 52153	<u> </u>
24243	Entrantification, CCCA, WEIGER (COD, ECOD), MINO, PRINCE	2825 (200, 200)	Ø
R81	Franch Consum 600m	2000,0000000	8
SEFT12	Chebral Nobbes (Ottos Nobbes Nobbes, Nobbes	3882, 5445605	<u></u>
SAKADA	Exemple 10% to the 1050, 1050, 1050, 1050, 1050, 1050, 1050, 1050, 1050, 1050, 1050, 1050, 1050, 1050	300 (306) 500	ž.
SAKKRCAK	PUR, PIN, RISKI, #4661, Y0000, TWO, FRISK, PRIKE, PRIKE, SPRIKE, SILVES, SILVE	886 (300000)	2
SPOP		986 (855)(55)	
579616	Francis/Manuscript state	5550, \$6005555	6
1894 <u>.</u>	Property Communication (Co. 1970), 1979, 1989, 1	800 X8253	
Total			86

Supplementary Table S5 Called variants in non-hematopoeitic genes

Gene	Clare	Start	Yarasıd	Referen	Variant	Various Classification	Protes	<5984A	Accession	Variant	Varient	Reference 10
257 XX	() () () ()	BORDONA	Type	80E	AWERE		Change	Charge		edale.	olicie:	zářele:
	222E									Startion	STATE.	පහසාව
\$9C	5	112175232	DEE	ಸ್ಥಾಸ್ಥ್	-	Frame_Shift_Del	p.31307%	c.3921_39	.1884_ 0 01127511	6.28	29	24 993442
EASPS	2	200149654	E8E.	¢	-	Frame_Shift_Del	p.N39565	2 9150eX	88M_683355	0.53	24	29 %S125
0.8220	3	302149654	D82	C	-	Frame_Shift_Del	p.530865	7.933ae0	XXX_033355	9,47	25	209 %7578
CASPS	2	202149901	28%	C	Ŧ	Namence Mutation	p.Q335*	c.2265007	NW 03355	0.387755	19	30 %16144
೭೭೮೯೪	2.	202149901	38%	C.	Ŧ	Nancense_Mutation	p. 03329*	c 31850/7	884_083355	0.385714	27	43 64304
GPS2	37	7217689	2//2	G	S	Nanzenze Mutation	p. Q00*	c.2380\/T	WW 004459	0.098381	13	130 613023
384F383	5.	56152535	28%	6	S	Nonzense Mutation	p.\$9337°	c.5919A	NW 805923	0.272234	49	67 3664000
XPE2X2.	2	173293309	21/2	G	A	Missense_Mutation	p.4824V	7.371E\T	3/5/2 (2006)164	8.069767	3	40 3/14589
99K381	5	67589168	28/26	E	C	Nancerce_Mutation	p. 2386°	c.21560-7	NM_181523	0.107143	3	25 %11720
988.	3	10191614	25/25	e.	Ŧ	Nonzense Mutation	p./2223**	± 60 7€∞₹	884 000551	0.333333	13	26 (613940)

Supplementary Table S6 Logistic regression for factors associated with clonality

Logistic regression was performed using the variables age (as a continuous variable), ancestry, sex, T2D, and age/sex interaction. Other interaction terms were modeled, but none were significant. Proportion of variance explained is derived by analysis of variance (ANOVA) for the generalized linear model, and is equal to deviance for the variable divided by residual deviance for the null model.

	Beta coefficient	OR(95 CI)	p-value	Variance explained
Age	0.07	1.08(1.07-1.09)	<0.001	0.06
European (referent)				0.003
African-American	0.11	1.12(0.9-1.39)	0.3	
East-Asian	0.02	1.02(0.79-1.31)	0.86	
Hispanic	-0.39	0.68(0.55-0.83)	< 0.001	
South Asian	-0.2	0.82(0.63-1.05)	0.125	
No T2D (referent)				0.002
Has T2D	0.26	1.3(1.12-1.51)	<0.001	
Male (referent)				0.001
Female	0.83		0.066	
Age:Female	-0.02	0.98(0.97-1)	0.023	0.001

Supplementary Table S7 Logistic regression for factors associated with clonality by ancestry group

Logistic regression was performed using the variables age (as a continuous variable), sex, T2D, and age/sex interaction for each ancestry group. Proportion of variance explained is derived by analysis of variance (ANOVA) for the generalized linear model, and is equal to deviance for the variable divided by residual deviance for the null model.

African	A	
Annean	-W(3)	erwan

	Beta coefficient	OR(95 CI)	p-value	Variance explained
Age	0.07	1.08(1.05-1.1)	< 0.001	80.0
Female	0.37	1.45(0.21-10.2)	0.7	0.002
Has T2D	0.24	1.27(0.9-1.79)	0.18	0.002
Age:Sex	-0.01	0.99(0.96-1.02)	0.52	0.0003

East Asian

	Beta coefficient	OR(95 CI)	p-value	Variance explained
Age	0.1	1.11(1.06-1.16)	< 0.001	0.03
Female	1.3	3.65(0.08-172)	0.51	0.003
Has T2D	-0.12	0.89(0.57-1.38)	0.61	0.0003
Age:Sex	-0.03	0.97(0.92-1.03)	0.385	0.0006

European

	Beta coefficient	OR(95 CI)	p-value	Variance explained
Age	0.07	1.07(1.06-1.09)	<0.001	0.05
Female	1.73	5.62(1.26-25)	0.023	0.001
Has T2D	0.31	1.36(1.04-1.79)	0.026	6.003
Age:Sex	-0.03	0.97(0.95-0.99)	0.013	0.0033

Hispanic

	Beta coefficient	OR(95 CI)	p-value	Variance explained
Age	0.08	1.08(1.06-1.11)	<0.001	0.08
Female	0.69	2(0.27-15.6)	0.5	0.00001
Has T2D	0.22	1.24(0.9-1.72)	0.18	0.001
Age:Sex	-0.01	0.99(0.96-1.02)	0.485	0.0003

South Asian

	Beta coefficient	OR(95 CI)	p-value	Variance explained
Age	0.08	1.08(1.05-1.11)	< 0.001	0.07
Female	-0.82	0.44(0.01-12.3)	0.64	0.0001
Has T2D	0.49	1.63(1.04-2.56)	0.033	0.007
Age:Sex	0.01	1.01(0.96-1.07)	0.677	0.0002

Supplementary Table S8 Logistic regression for factors associated with RDW≥14.5%

Individuals were from Jackson Heart Study or UA control cohort.

Covariate	OR (95% CI)	p-value
Age 60-69	1.3(0.9-1.8)	0.17
Age 70-79	1.8(1.2-2.7)	0.002
Age 80-89	2.7(1.3-5.2)	0.005
Age >90	1.7(0.9-3.1)	0.09
Female	1.1(0.8-1.5)	0.76
Has T2D	0.9(0.7-1.2)	0.45
DNMT3a mutation	1.9(1-3.6)	0.048
WBC	1.1(1.0-1.1)	0.092
Hemoglobin	0.7(0.7-0.8)	< 0.001
Platelet count	1.0(1.0-1.0)	0.65

Supplementary Table S9 Association of cytopenias with clonality

Individuals were classified as having cytopenia as defined in Methods. Statistical comparisons were performed using Fisher's exact test. WBC - white blood cell count, Hgb - hemoglobin, Plt - platelet count. Individuals were from Jackson Heart Study, Longevity Genes Project, Botnia, Helsinki-sib, and Malmo-sib. Of the 5 individuals with multiple cytopenias and clonal mutations, 2 had 2 detectable mutations, suggesting that these might be undiagnosed cases of MDS.

	Clone	No Clone	
Low WBC	8	59	65
Normal WBC	139	2628	2747
	125	2687	

OR 2.2(0.8-5.2), p=0.066

	Clone	Na Clane	
Low Hgb	30	616	646
Normal Hgb	109	2350	2459
	139	2966	

OR 1.0(0.7-1.6), p=0.83°

	Clone	No Clone	
Low Pit	- \$	1837	333
Normal Pit	132	2789	2921
	336	2896	

OR 0.8(0.2-2.1), p=0.82

I	Clone	No Clone	
Any cytopenia	38	745	780
No cytopenia	104	2224	2328
	138	3969	

OR 1.0(0.6-1.5), p=1

	Clone	N	o Clone	
2+ cytopenias		8	3.7	42
1 or 0 cytopenias		134	2932	3966
		139	2969	

OR 3.0(0.9-7.7), p=0.037

Lower limit of normal for blood counts were defined as follows:
White blood cell count:
African-Americans 3.0x10°/L, white 4.0x10°/L

Hemoglobin: African-American women 11.2g/dL, African-American men 12.5 g/dL, white women 11.9 g/dL, white men 13.4 g/dL

Platelet count: 150x10⁹L

Supplementary Table S10 Association of clonality with known versus unknown causes of anemia

During clinical evaluation, most patients with anemia can be found to have an attributable cause. Using subjects from the Jackson Heart Study, Applicants assessed whether the anemia was attributable to iron deficiency, anemia of chronic inflammation, or renal insuffuciency. Individuals with clonality were less likely to have anemia attributable to one of these causes.

Iron deficiency anemia/microcytic anemia

- -mean corpuscular volume<80 fL
- -ferritin<20 ng/mL
- -ferritin 20-100 ng/mL WITH EITHER total iron binding capacity >370 mcg/dL OR serum iron <50 mcg/dL OR iron saturation< 20%

Anemia of chronic disease

- -serum iron <65 mcg/dL WITH total iron binding capacity <250 mcg/dL
- -ferritin >350 ng/mL for males
- -ferritin >300 ng/mL for females

Renal insufficiency

-estimated glomerular filtration rate <30 mL/min/1.73m²

	Clone	No Clone	
Known cause	2	360	362
Unknown cause	7	151	158
	9	511	

OR 0.1(0-0.6), p=0.004

Supplementary Table S11 Details on subjects that developed hematologic malignancies

Age at sampling	zizgwysia	modeo	hetes/bujkA	iatency (years)	Mutation on WES (VAF)	Mutations on RHP (VAF)	W8C	HGB	PLT	Death	Cause of death
32	Cancer of Spleen.	52 3	No	5	3482 p.V617F (0.23)	NA	7.8	11	247	Yes	CARCINOMIA Unispecipied Site
54.	LEUSERNA (prior NHL) .	A)	No	7	45% 1 1 p.D 616 f: (D 25)	ASXL1 p.D&16fs (2.18)	3.5	12.9	123	No	
52	ENSPHOMA :	. \$3	No	2	00:00734 p.90828 (0.39)	SA	14.3 (51.3% iymahacytex) /	21	248	Titles	
šS	DESCS, large intentine -	MEC	Yes	5	TET2 p.C1135Y {8.25};45X11 p.19196 {8.21}	Tet2 p.C11357 (0.35); Tet2 p. G1192V (0.30); ASN11 p.19196; (0.25)				No	
33	\$2D5-RAE8	MEC	Yes	7	ASXL1 p.75146 (0.24)	ASXL1 p.T514fs (0.30)/TET2 p.1815fs (0.15)				Уeз	Myzloid teutrzmia
5 0	AMOHRMYS	26.	No	2	None	52	6.3	222	220	Yes	EUG EMORABIBANOS AMORANIS IZT
48	LYMPHOMA	AS	%a	3	None	88	4.8	3.4.3	225	₹es	RENAL FAILURE
57	LYMPHOMA	34	ña:	7	None	None	7.4	33.7	250	Yes	HYPORIO RESPIRATOR FARIURE
€3	Lebrenna	A.S	8ia	9	Sisse	84	2.8	3.5.8	158	3%e	
51	8630D	A3	No	8	Same	28	5.7	13.7	325	No.	
5 7	LEUNERMA	53	No	Ž	None	None				Na	
54	MULTIPLE MYELOMA	.43	Siz	9	None.	SA	4.4	11.4	258	7ves	
59	EYMPHOMA	<u>,8,5</u>	No.	8	Nose	5/A		23.2	195	We	
61	ACUTE MYELGID LEUKEMIA	24.	No	10	None	None		21.7	158	Yes	rigusym studa Ameriusz
53	LEBREMMA	24,	కిస్తు	10	None	562	4.8	22	258	We	
58	Multiple myeloma	MEC	Yes.	8	Nene	52				Wes	

Prevalent Cases											
Age at sampling	Siagnosis	Cohort	Adjudicated	Time (years prior)	Mutation on WES (VAF)	Mutations on RHP (VAF)	WEC	∺GB	PLT	Death	Cause of death
5 \$	LEBRENNA	Aì	%a:	7	Sizne	84	3.5	12.9	129	Nο	
48	EXMPHOMA	AS	కిశ్వ	3	State	84	4.8	14.3	225	%es	RENAL FARLISE
76	lessenna.	At	Sec.	17	Sizore	84		11.4	253	No	
53	NHL organizyrx	3358	Yes	24	tione	8/4					
54	NHE (subsequent ESCREMIA) see above)	.\$ 3	No	7	AGNE1 p. D6168: (0.23)	ASNI1 p. D616% (0.15)	3.5	129	189	?des	

Mile-Non-Hoogshin's lymphome. DEBCE-diffuse large B-cell lymhome, MIS-RACE-myelodycplastic syndrome, refractory aremia with excess blants

 $\label{eq:definition} Al = \operatorname{lackson} \operatorname{HeartStudy}, \, MEC = \operatorname{thutb-esthetic cohort}, \, \operatorname{Hispanics in Los Argeles}$

WES-whole exome sequencing, RMP=Rapid Herne Panel targeted re-sequencing, X&F=xariant skiele fraction

WSC=white blood cell count (x 10°9 cells/1), MGE=themoglobin (g/61), PCT=platelet count (x10°9cells/1)

NGANOC evaluation

a. This is thely uple conceptly secondary to a 1482-mutated myeloproliferative neoplasm.

is This Netly represents a therapy related ANE/MOX, as ASKS mutations have never been described in lymphoid malignancies

 $c. 2000734 \ mutations have been found in peripheral 7-self lymphomas and early 7-progenitor 444.\\$

of This is the second highest absolute lymphocyte count in the cohort of 1.428 subjects who had a WBO differential. The subject with the highest absolute lymphocyte count also had a DRMT34 mutation.

e TETZ has been reported to be mutated in 0-12% of DLBCL, and the musations are reported to be found in hemstopoletic stem cells

Supplementary Table S12 Risks associated with developing incident coronary heart disease and ischemic stroke using traditional risk factors and clonality.

Hazard ratios were estimated using competing risks regression with death as the competing risk. P-values are derived from the Fine-Gray test. Individuals with prior coronary heart disease (CHD) were excluded for CHD analysis, and individuals with prior ischemic stroke were excluded for stroke analysis. Models shown in A or B are from the same population and differ by having covariates either removed or added. A) Coronary heart disease, B) ischemic stroke. Individuals were from Jackson Heart Study and FUSION.

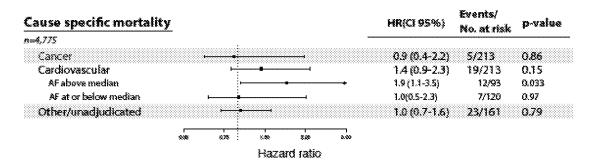
n=3,286	Model 1		Model	2	Model 3			
Covariate	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value		
log(Age)	5.3(2.1-12.5)	<0.001	4.7(1.9-11)	45.05t	4.5(1.5-11)	200.00		
Has T2D	3.3(2.1-5.3)	KG.601	3.4(2.1-5.4)	<0.001	3.5(2.2-5.5)	200.8×		
Female	6.7(6.5-1.1)	9.13	9.7(0.5-1.1)	0.13	0.7(5.5-1.1)	8.13		
HDL<35	1.1(0.5-2.1)	0.77	1,1(0,6-2,1)	5.81	1.1(0.5-2.1)	0.78		
mg/dŁ	1.150.5-2.15	G.73	2,150,6-2,15	0.01	1.1(0.5-2.1)	10.542		
HDL>60	es motor as a resi	27.479	e min a a mi	10. EVC	sambara at	6:25		
mg/dL	0.7(0.4-1.2)	9.18	9.7(9.4-1.3)	0.25	8.7(0.4-1.3)	0.2%		
TC >240	2.1(1.3-3.2)	⊲0.001	I(1.3-3.1)	~30.501	2(1.3-3.1)	<0.003.		
mg/dL	2.1(1.3-3.2)	CONOT	5(7.3-3.7)	~33.00C	377.2-2.73	SO(2022)		
Former or								
current	1.5(1.1-2.5)	0.024	1.5(1-2.4)	0.035	1.6(3.1-2.5)	0.02		
smoker								
Hypertension	1.8(1-2.5)	0.66	1.4(0.5-2.3)	0.15	1.4(5.9-2.3)	6.15		
stage #-NV	1.0(1-1.5)	5.06	2.498(3-2.5)	U.43	1.4(0.2-2.3)	5.22		
BMI>25	1.2(0.5-2.5)	6.55	2.4(5.6-2.8)	0.43	1.3(9.6-2.8)	2.42		
Clone			23(1.1-4.8)	0.026				
present			5.3(4.5→.0)	0.000				
VAF-0.10					1.4(0.5-4)	8.55		
VAF-0.39	1				4.4(1.9-16.5)	<0.003		
Processio Log-Sibelibo		- 6 81		-655		-55		
Pseudo kiekkood n	sko test	203 හා 9 ක්		309 an 19 df		113 ox 11 d		

n=2,420	Model 1		Model	2	Model 3			
Covariate	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value		
log(Age)	14.5(4.8-44.7)	49.001	13.3(4.3-40)	<0.00J	13.2(4.3-40)	<0.301		
Has T2D	2.9(1.7-5)	<0.001	2.9(2.7-5)	-03.091	3(1.7-5.2)	<0.501		
Female	0.8(0.5-1.3)	5. 24	0.9(0.5-1.4)	0.53	0.9(0.5-1.4)	0.55		
HDL<35	a mission make	2.53	a sin s s si	0.45	1 3(3 T 3 E)	2.25		
mg/dL	1.2(5.6-2.4)	0.52	1.3(0.7-2.5)	9,45	1.3(2.7-2.5)	9.45		
HDL>60	1(0.6-1.9)	0.5%	1 (2) (C 1)	0.84	1.1(0.6-2)	9.86		
mg/dL	25000-2.55	V:58	1.1(0.8-2)	10. 25	7: Tites 32-T3	0.00		
TC>240	a also e a al	0.29	a win n n ah	9.31	1 2 (2 2 2 3 1)	5.29		
mg/dL	1.3(6.8-2.1)	W28	1.3(0.8-2.1)	9.31	1.3(0.8-2.1)	0.29		
Former or								
current	1.8(1.3-2.9)	8.814	1.8(1.1-7.9)	0.016	1.8(1.1-2.9)	0.914		
smoker								
Hypertension	1.8(1-3.1)	0.037	1,7(0,5-2,9)	9.677	1.7(1-2.9)	9.074		
stage II-IV	4.9(2-5.2)	0.037	2.3(6.3-2.3)	WON)	2.7(2-2.5)	2.034		
BM(>25	1.5(0.6-3.5)	0.35	1.6(0.7-4)	છ.ક	1.5(0.7-3.9)	©.3		
Clone present			2.2(1.1-4.8)	0.029				
VAF<0.10	ţ				1.8(5.7-4.6)	0.2		
VAF:x0.10	5				3.1(1.2-8.4)	0.925		
Pseudu ing-likelihov	od:	-4 % 4		-452		-48		
Pseudo likelihood ra	rida ised	79 on 9 of		విడే ఇం కేను జగ్		33,5 ao 11.3		

Supplementary Table S13 Risks associated with developing incident coronary heart disease using traditional risk factors as well as hsCRP, RDW, and clonality.

Hazard ratios were estimated using competing risks regression with death as the competing risk. P-values are derived from the Fine-Gray test. Individuals with prior coronary heart disease (CHD) were excluded for CHD analysis. Models shown in A or B are from the same population and differ by having covariates either removed or added. All individuals were from Jackson Heart Study.

n=1,795	Model 1		Model 2		Model 3	
Covariate	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
log(Age)	3.3(0.6-17)	0.17	2.7(0.5-13)	0.23	2.8{0.6-14}	0.21
Has T2D	2.9{1.4-5.9}	9.884	3(1.5-6)	0.002	3(1.6-5.8)	<0.001
Female	8.7{0.3-1.3}	0.21	0.7(0.3-1.3)	0.21	0.5(0.3-1.2)	0.13
HDL<35 mg/dL	1.5(0.5-4.5)	0.38	1.8(0.6-5.1)	0.29		
HDL≥60 mg/dL	1{0.5-2.1}	0.99	1(0.5-2)	0.9		
TC≥200mg/dL	2.2(1.1-4.1)	0.021	2.3(1.2-4.6)	0.015	2.3(1.2-4.5)	0.013
Former or current smoker	2(1.1-3.7)	0.027	2.1(1.1-3.9)	0.021	2(1.1-3.8)	0.024
SBP≥160 mm Hg	2.4(1.2-4.7)	9.011	2.2(1.1-4.4)	0.023	2.2(1.1-4.3)	0.03
hsCRP > 1.0 mg/L	1.1(0.5-2.5)	0.76	1{0.4-2.3}	0.99		
No clone, RDW≥14.5%			2.3{1.2-4.6}	0.017	2.2(1.1-4.4)	0.62
Clone, RDW<14.5%			0.9(0.1-7.4)	0.95	1.7(0,4-7.7)	8,46
Clone, RDW≥14.5%			9.8(2-48.3)	0.005	9.3(2-43)	0.005
Pseudo Log-likelihood		-273		-268		-278
Pseudo likelihood ratio tes	at.	39.1 on 9 of		49.0 on 12 of		48.0 cm 9 df



* * *

[00204] Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the above paragraphs is not to be limited to

particular details set forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope of the present invention.

WHAT IS CLAIMED IS:

1. A method for identifying and selecting a subject with increased risk of developing a cardiometabolic disease and optionally a hematological cancer, comprising the steps of:

- (a) sequencing at least part of a genome comprising one or more genes selected from the group consisting of *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *JAK2* and *SF3B1* of one or more cells in a blood sample of the subject,
- (b) identifying from said sequencing one or more mutations in one or more genes selected from the group consisting of *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *JAK2* and *SF3B1*, wherein presence of said mutation(s) indicates an increased risk of developing a cardiometabolic disease and optionally a hematological cancer.
- 2. The method according to claim 1, wherein the presence of said mutation(s) also indicates an increase in red blood cell distribution width (RDW).
- 3. The method according to claim 1, wherein the cardiometabolic disease is atherosclerosis, coronary heart disease (CHD) or ischemic stroke (IS).
- 4. The method according to claim 1, wherein the hematological cancer is a leukemia, a lymphoma, a myeloma or a blood syndrome.
- 5. The method according to claim 4, wherein the leukemia is acute myeloid leukemia (AML) or chronic myelogenous leukemia (CML).
- 6. The method according to claim 4, wherein the blood syndrome is myelodysplastic syndrome (MDS).
- 7. The method according to claim 1, wherein the one more cells in the blood sample are hematopoietic stem cells (HSCs), committed myeloid progenitor cells having long term self-renewal capacity or mature lymphoid cells having long term self-renewal capacity.
 - 8. The method according to claim 1, wherein the part of the genome is an exome.
- 9. The method according to claim 1, wherein the sequencing is whole exome sequencing (WES).
 - 10. The method according to claim 1, wherein the subject is a human.

11. The method according to claim 10, wherein the human also exhibits one or more risk factors of being a smoker, having a high level of total cholesterol or having high level of high-density lipoprotein (HDL).

- 12. A method for identifying and selecting a subject with an increased risk of developing a cardiometabolic disease and optionally a hematological cancer and providing a personalized medicine method, said method comprising the steps of
- (a) sequencing at least part of a genome comprising one or more genes selected from the group consisting of *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *JAK2* and *SF3B1* of one or more cells in a blood sample of the subject,
- (b) identifying from said sequencing one or more mutations in one or more genes selected from the group consisting of *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *JAK2* and *SF3B1*, wherein presence of said mutation(s) indicates an increased risk of developing a cardiometabolic disease and optionally a hematological cancer, and
- (c) initiating a treatment or monitoring regimen to suppress said mutation(s) in the subject, thereby decreasing risk of developing a cardiometabolic disease and optionally a hematological cancer.
- 13. The method according to claim 12, wherein the presence of said mutation(s) also indicates an increase in red blood cell distribution width (RDW).
- 14. The method according to claim 12, wherein the cardiometabolic disease is coronary heart disease (CHD) or ischemic stroke (IS).
- 15. The method according to claim 12, wherein the hematological cancer is a leukemia, a lymphoma, a myeloma or a blood syndrome.
- 16. The method according to claim 15, wherein the leukemia is acute myeloid leukemia (AML) or chronic myelogenous leukemia (CML).
- 17. The method according to claim 15, wherein the blood syndrome is myelodysplastic syndrome (MDS).
- 18. The method according to claim 12, wherein the one more cells in the blood sample are hematopoietic stem cells (HSCs), committed myeloid progenitor cells having long term self-renewal capacity or mature lymphoid cells having long term self-renewal capacity.

WO 2016/086197 PCT/US2015/062787

19. The method according to claim 12, wherein macrophages with said mutation(s) are treated with an agent to increase reverse cholesterol transport, reduce inflammation, or both.

- 20. The method according to claim 12, wherein the part of the genome is an exome.
- 21. The method according to claim 12, wherein the sequencing is whole exome sequencing (WES).
 - 22. The method according to claim 12, wherein the subject is a human.
- 23. The method according to claim 22, wherein the human also exhibits one or more risk factors of being a smoker, having a high level of total cholesterol or having high level of high-density lipoprotein (HDL).
- 24. A method according to any one of claims 1-23, wherein the one or more mutations are frameshift mutations, nonsense mutations, missense mutations or splice-site variant mutations.
- 25. A method according to any one of claims 1-24, wherein the mutation in *DNMT3A* is a mutation in exons 7 to 23.
- A method according to any one of claims 1-24, wherein the mutation in DNMT3A 26. is a mutation selected from the group consisting of P307S, P307R, R326H, R326L, R326C, R326S, R366P, R366H, R366G, A368T, F414L, F414S, F414C, C497Y, Q527H, Q527P, Y533C, G543A, G543S, G543C, L547H, L547P, L547F, M548I, M548K, G550R, W581R, W581G, W581C, G646V, G646E, L653W, L653F, V657A, V657M, R659H, Y660C, R676W, R676Q, G685R, G685E, G685A, D686Y, D686G, G699R, G699S, G699D, P700S, P700R, P700Q, D702N, D702Y, V704M, V704G, I705F, I705T, I705S, C710S, S714C, N717S, N717I, P718L, R720H, R720G, Y724C, R729Q, R729W, R729G, F731L, F732del, F732S, F732L, F734L, F734C, Y735C, Y735N, Y735S, R736H, R736C, R736P, L737H, L737V, L737F, L737R, A741V, R749C, R749L, F751L, F752del, F752C, F752L, F752I, F752V, L754R, L754H, F755S, F755I, F755L, M761I, M761V, G762C, S770W, S770P, R771Q, F772I, F772V, L773R, E774K, E774D, D781G, R792H, G796D, G796V, N797Y, N797H, P799R, P799H, R803S, P804S, P804L, S828N, K829R, Q842E, P849L, D857N, W860R, F868S, G869S, G869V, M880V, S881R, S881I, R882H, R882P, R882C, R882G, Q886R, G890D, L901R, L901H, P904L, F909C and A910P.

WO 2016/086197 PCT/US2015/062787

27. A method according to any one of claims 1-24, wherein the mutation in *TET2* is a mutation selected from the group consisting of S282F, N312S, L346P, S460F, D666G, P941S, and C1135Y.

- 28. A method according to any one of claims 1-24, wherein the mutation in *ASXL1* is a mutation in exon 11-12.
- 29. A method according to any one of claims 1-24, wherein the mutation in *TP523* is a mutation selected from the group consisting of S46F, G105C, G105R, G105D, G108S, G108C, R110L, R110C, T118A, T118R, T118I, L130V, L130F, K132Q, K132E, K132W, K132R, K132M, K132N, C135W, C135S, C135F, C135G, Q136K, Q136E, Q136P, Q136R, Q136L, Q136H, A138P, A138V, A138A, A138T, T140I, C141R, C141G, C141A, C141Y, C141S, C141F, C141W, V143M, V143A, V143E, L145Q, L145R, P151T, P151A, P151S, P151H, P152S, P152R, P152L, T155P, R158H, R158L, A159V, A159P, A159S, A159D, A161T, A161D, Y163N, Y163H, Y163D, Y163S, Y163C, K164E, K164M, K164N, K164P, H168Y, H168P, H168R, H168L, H168Q, M169I, M169T, M169V, T170M, E171K, E171Q, E171G, E171A, E171V and E171D.
- 30. A method according to any one of claims 1-24, wherein the mutation in *JAK2* is a mutation selected from the group consisting of N533D, N533Y, N533S, H538R, K539E, K539L, I540T, I540V, V617F, R683S, R683G, del/ins537---539L, del/ins538---539L, del/ins540---543MK, del/ins540---543MK, del/ins540---544MK, del/ins541- -543K, del542---543, del543---544 and ins11546---547.
- 31. A method according to any one of claims 1-24, wherein the mutation in *SF3B1* is a mutation selected from the group consisting of G347V, R387W, R387Q, E592K, E622D, Y623C, R625L, R625C, H662Q, H662D, K666N, K666T, K666E, K666R, K700E, V701F, A708T, G740R, G740E, A744P, D781G and E783K.

Figure 1



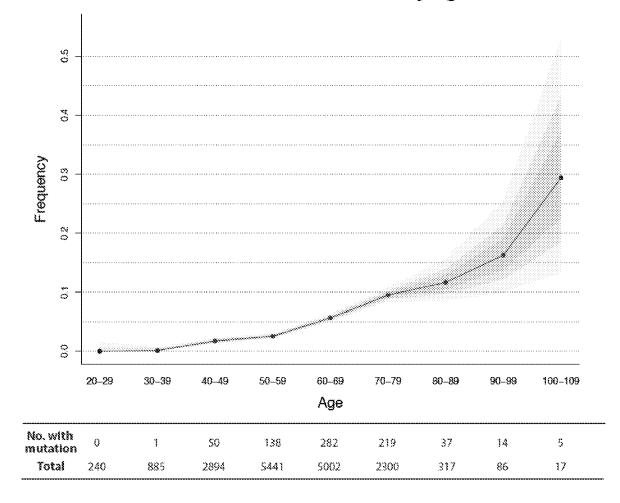
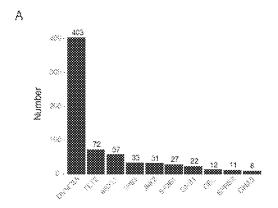
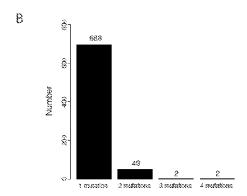
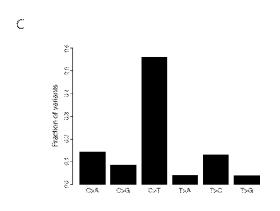


Figure 2







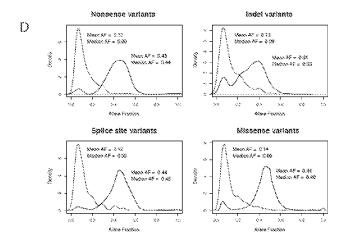
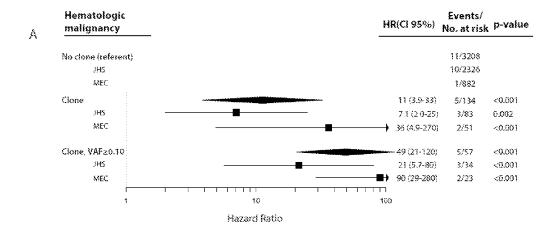
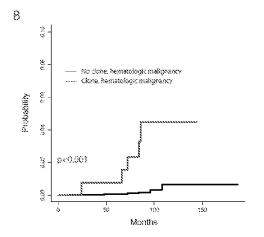
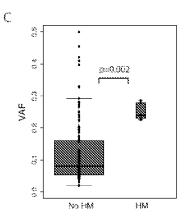


Figure 3



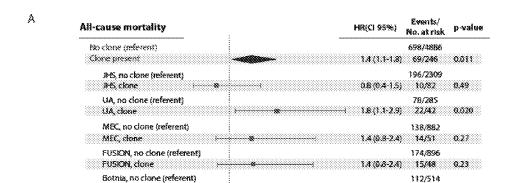


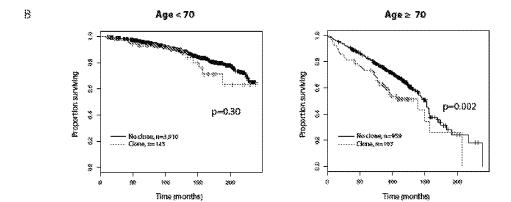


Botnia, clone

ø

1.4 (0.7-3.0) 8/23 0.35





Hazard Ratio

2.0

3.0

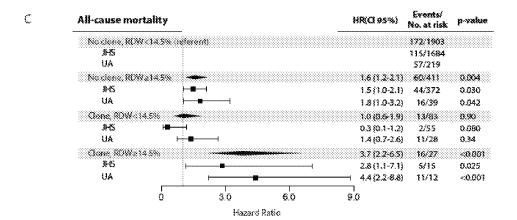
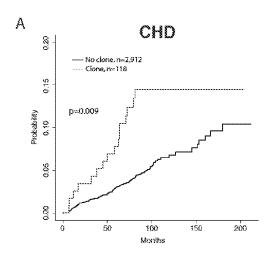
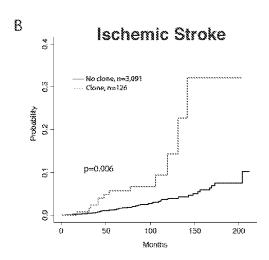
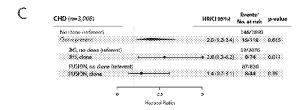


Figure 5







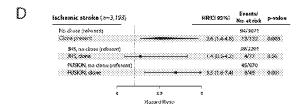


Figure 6

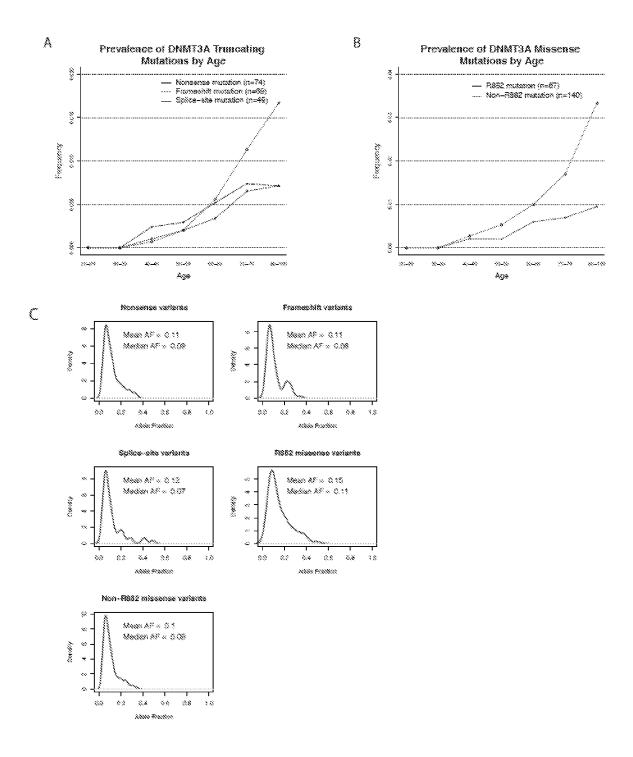
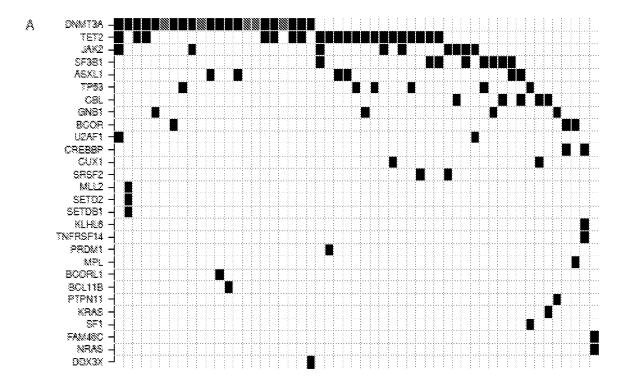


Figure 7





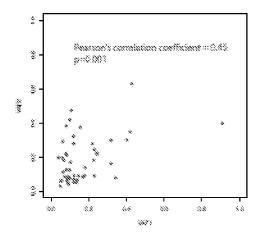
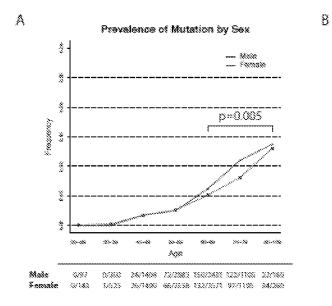
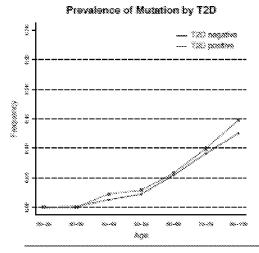


Figure 8





 T2D meg
 COTT
 CAST
 CD/1668
 COTT
 CD/1668
 CD/1678
 CD/1678

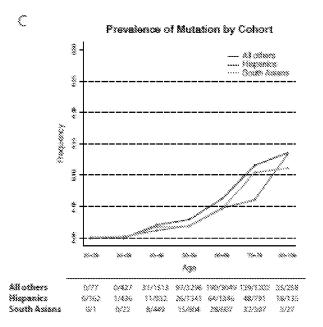
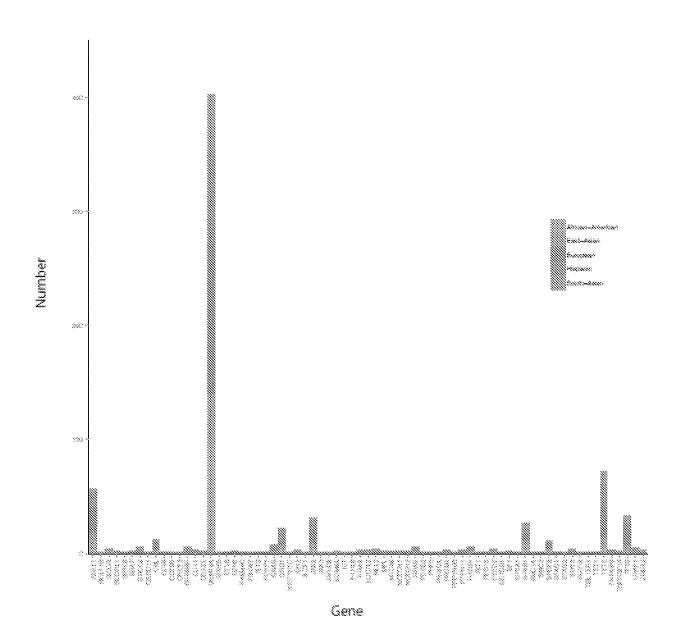


Figure 9



PCT/US2015/062787

Figure 10

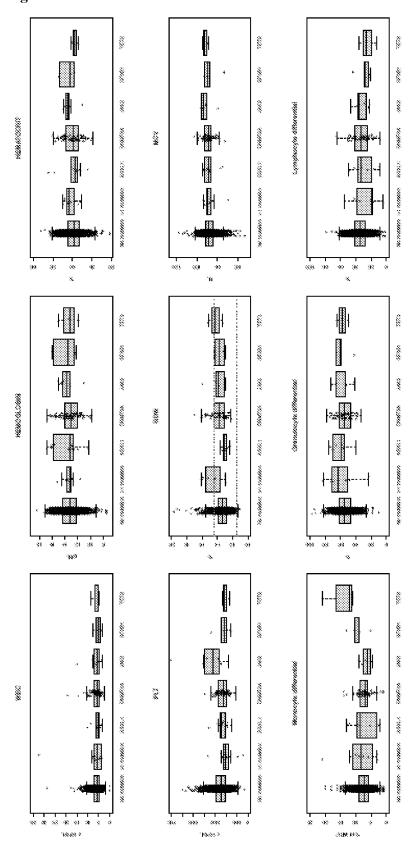


Figure 11

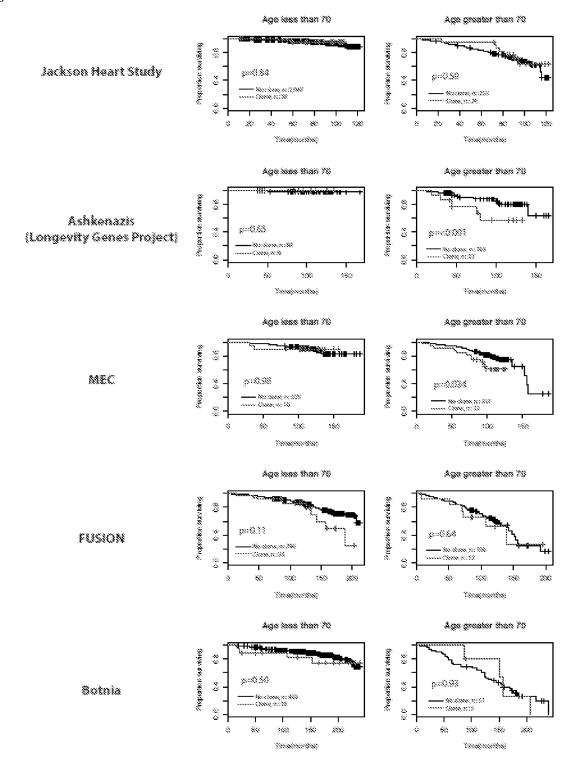


Figure 12

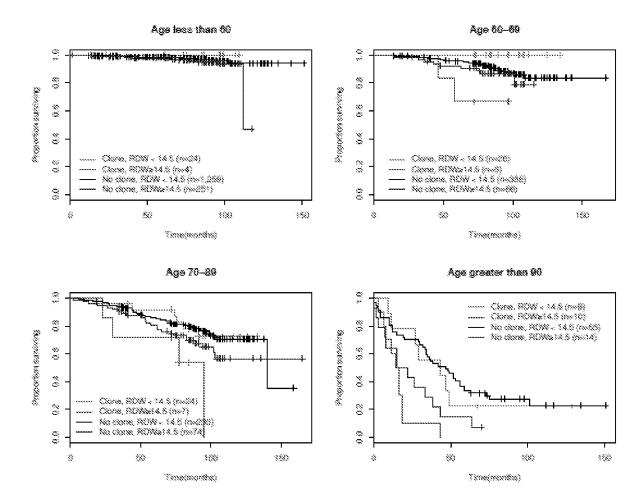
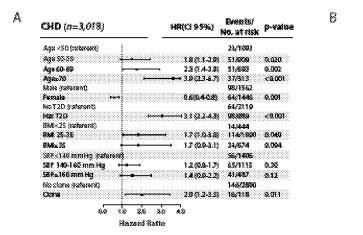


Figure 13



ischemic Stroke (n=3,193)	HR(C) 99%.)	Brents/ No. at risk	p-vatue
Age < 90 (veteront)		12/1116	
8/32 50/50	1.5 (0.5-2.1)	18/967	0.86
Arga 63-60	22(1.1-4.3)	38,6754	0.524
Agau/70 -	 4.8 (25.8%	32/354	O20201
Mas (referent)		50/1671	
Female (#)	2892-120	46/1522	0.33
No Tably eferanti		38/3217	
Hzs T30	25 (1.6-3.8)	58,975	<0.001
2989 (25 (Nafarent)		6/468	
6MI 25-35		88/2012	0.662
BM16:35	20 (1.1-7.6)	22/713	0.007
SEPKI 40 mmHq (material)		35/1400	
SEP 140-160 mm Hq	1.2 (1.1-3.2)	40/1180	0.032
S8Pull@mmHq	23 (1.24.1)	33/534	0.005
No ciona ()e%rant(84/3068	
Oxe :	24 (1.3-44)	127125	0.005
\$0 1.8 \$0 48	\$0		
Hazard Fatto			

Figure 14

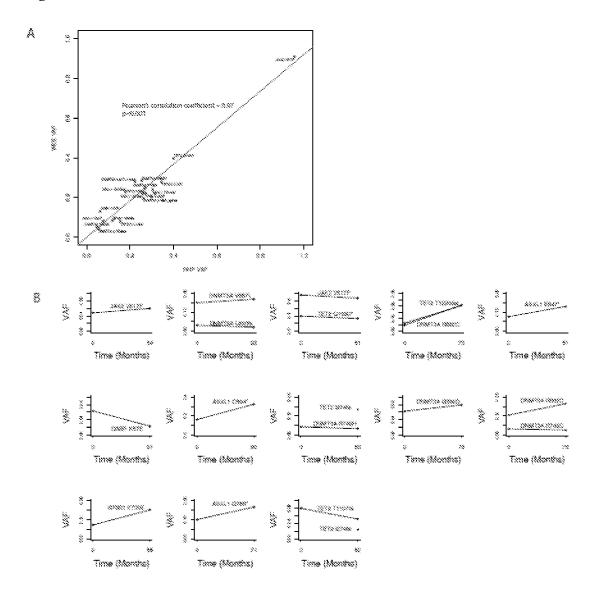


Figure 15

Cause specific mortality	<u>y</u>					HR(CI 95%)	Events/ No. at risk	p-value
n=4,775					•			
Cancer		·				0.9 (0.4-2.2)	5/213	0.86
Cardiovascular		÷				1.4 (0.9-2.3)	19/213	0.15
AF above median		٠				1.9 (1.1-3.5)	12/93	0.033
AF at or below median						1.0(0.5-2.3)	7/120	0.97
Other/unadjudicated						1.0 (0.7-1.6)	23/161	0.79
	\$20	47.70	1:50	2.55	9.98			
			Haza	ud ratio				

Figure 16

8.3 (2.0.74) 		A Chandi hemanopoleciis and Mi		makakon/ Wo. m rluk		60	Com	Confide Cases (F-2008) (F-440)	CONTROL CONTROL (F-4405)
### ##################################	•			2/0/2		₽ P	CAMPE	% . %	8
### ASX1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0		# 17/8, 1/1, 1/4)	83(20,4	427.0	(C)			63	\$100 \$200
		APP in the Will what so southwest in			000000000000000000000000000000000000000		ASS.	0	460 400
######################################		A (V) (A) (A) (A)	2		\$ 5 5		THE S		183
* 3nosther 1 11/2000 20 11/4-631 37/11/75 0.001		F (\$100.0), p. 20.00, 42.45 (100.00)			300000000000000000000000000000000000000		3.53.2 cm	186 186 186	
1.87.300 		P310p4K, A8, 415.45		2377420	0.20		aranthe.	2000 2	¥ W
		PACONIS, No. 46-50 (milesen) PROMIS, NR. 46-50	20(1463	27.175 27.175	8				È

Figure 17

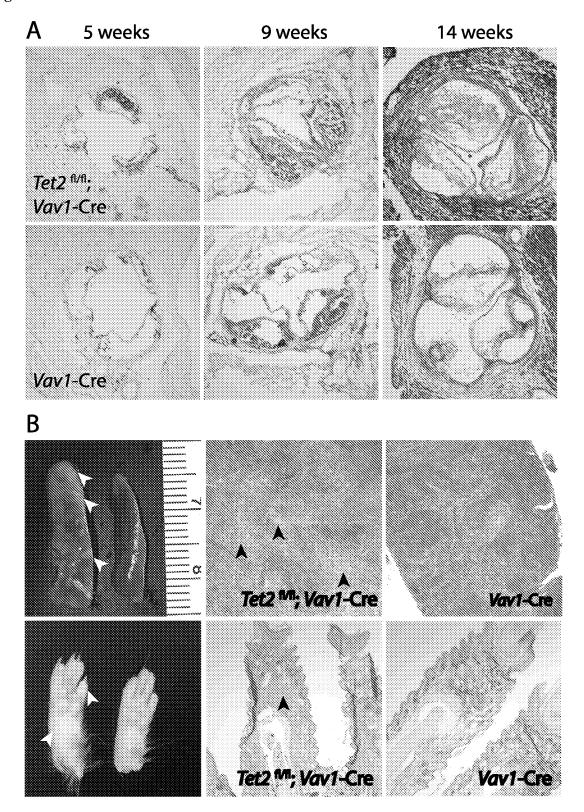


Figure 18

Cohort		HR [95% CI]	P-value
Biolmage	-	1.46 [0.93, 2.31]	0.10
Malmo Diet & Cancer		1.60 [1.11, 2.32]	0.01
Summary		1.55 [1.16, 2.06]	0.003
	0.75 1.0 1.5 2.0 2.5 Hazard Ratio		

Figure 19

Carrier		Relative CAC		CAC Median
Status		[95% CI]	P-value	[IQR]
Non-Carrier		1	•••••	154 [14, 535]
Carrier	_ _	2.60 [1.23, 5.47]	0.01	310 [91, 864]
	0.75 2.0 4.0 Relative CAC			

INTERNATIONAL SEARCH REPORT

International application No PCT/US2015/062787

a. classification of subject matter INV. C12Q1/68

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols) C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, FSTA, WPI Data

C. DOCUM	ENTS CONSIDERED TO BE RELEVANT	
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AXEL MUENDLEIN ET AL: "Occurrence of the JAK2 V617F mutation in patients with peripheral arterial disease", AMERICAN JOURNAL OF HEMATOLOGY, vol. 90, no. 1, 26 October 2014 (2014-10-26), pages E17-E21, XP055246813, US ISSN: 0361-8609, DOI: 10.1002/ajh.23874	1-25
Υ	abstract; p. e17, para. "introduction"; p. e18, right-hand col., 4th para.	26
Υ	US 2010/197518 A1 (XU HUICHUN [US] ET AL) 5 August 2010 (2010-08-05) para. 6, 18, 77, 122; p. 16, table 3	26

X Further documents are listed in the continuation of Box C.	X See patent family annex.
* Special categories of cited documents :	"T" later document published after the international filing date or priority
"A" document defining the general state of the art which is not considered to be of particular relevance	date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other	step when the document is taken alone
special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is
"O" document referring to an oral disclosure, use, exhibition or other means	combined with one or more other such documents, such combination being obvious to a person skilled in the art
"P" document published prior to the international filing date but later than the priority date claimed	"&" document member of the same patent family
Date of the actual completion of the international search	Date of mailing of the international search report
3 February 2016	04/05/2016
Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Ripaud, Leslie

1

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/062787

C(Continua	tion). DOCUMENTS CONSIDERED TO BE RELEVANT	PC1/032013/002/07
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	SIDDHARTHA JAISWAL ET AL: "Age-Related Clonal Hematopoiesis Associated with Adverse Outcomes", NEW ENGLAND JOURNAL OF MEDICINE, vol. 371, no. 26, 26 November 2014 (2014-11-26), pages 2488-2498, XP055246853, US ISSN: 0028-4793, DOI: 10.1056/NEJMoa1408617 the whole document -& SIDDHARTHA JAISWAL ET AL: "Age-Related Clonal Hematopoiesis Associated with Adverse Outcomes - Online supplementary appendix", NEW ENGLAND JOURNAL OF MEDICINE, vol. 371, no. 26, 26 November 2014 (2014-11-26), pages 2488-2498, XP055246869, US ISSN: 0028-4793, DOI: 10.1056/NEJMoa1408617	1-26
A	AMÉLIE BONNEFOND ET AL: "Association between large detectable clonal mosaicism and type 2 diabetes with vascular complications", NATURE GENETICS., vol. 45, no. 9, 14 July 2013 (2013-07-14), pages 1040-1043, XP055246874, NEW YORK, US ISSN: 1061-4036, DOI: 10.1038/ng.2700 cited in the application the whole document	1-26
Α	MINGCHAO XIE ET AL: "Age-related mutations associated with clonal hematopoietic expansion and malignancies", NATURE MEDICINE., vol. 20, no. 12, 19 October 2014 (2014-10-19), pages 1472-1478, XP055246879, US ISSN: 1078-8956, DOI: 10.1038/nm.3733 the whole document	1-26
A	JP 2005 151854 A (JAPAN SCIENCE & TECH AGENCY) 16 June 2005 (2005-06-16) the whole document	1-26

1

International application No. PCT/US2015/062787

INTERNATIONAL SEARCH REPORT

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)
This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)
This International Searching Authority found multiple inventions in this international application, as follows:
see additional sheet
As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. X No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 25, 26(completely); 1-24(partially)
The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee. The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 25, 26(completely); 1-24(partially)

concerns a method of identifying a subject with increased risk of developing a cardiometabolic disease and optionally a hematological cancer comprising sequencing at least part of a gene from blood cells and identifying the presence of at least one mutation in said gene, wherein said gene is DNMT3A.

2. claims: 27(completely); 1-24(partially)

idem invention 1, wherein said gene is TET2.

3. claims: 28(completely); 1-24(partially)

idem invention 1, wherein said gene is ASXL1.

4. claims: 29(completely); 1-24(partially)

idem invention 1, wherein said gene is TP53.

5. claims: 1-24, 30(all partially)

idem invention 1, wherein said gene is JAK2 and the mutation is N533D, N533Y or N533S.

6. claims: 1-24, 30(all partially)

idem invention 1, wherein said gene is JAK2 and the mutation is H538R

7. claims: 1-24, 30(all partially)

idem invention 1, wherein said gene is JAK2 and the mutation is K539E or K539L

8. claims: 1-24, 30(all partially)

idem invention 1, wherein said gene is JAK2 and the mutation is I540T or I540V

9. claims: 1-24, 30(all partially)

idem invention 1, wherein said gene is JAK2 and the mutation

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

is V617F

10. claims: 1-24, 30(all partially)

idem invention 1, wherein said gene is JAK2 and the mutation is R683S or R683G $\,$

11. claims: 1-24, 30(all partially)

idem invention 1, wherein said gene is JAK2 and the mutation is del/ins537---539L or del/ins538---539L

12. claims: 1-24, 30(all partially)

idem invention 1, wherein said gene is JAK2 and the mutation is del/ins540---543MK, del/ins540---544MK, del/ins541---543K, del542---543 or del543---544

13. claims: 1-24, 30(all partially)

idem invention 1, wherein said gene is JAK2 and the mutation is ins11546---547

14. claims: 31(completely); 1-24(partially)

idem invention 1, wherein said gene is SF3B1.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2015/062787

Patent document cited in search report		Publication date		Patent family member(s)	Publication date
US 2010197518	A1	05-08-2010	CA	2685382 A1	13-11-2008
			CN	101688245 A	31-03-2010
			EP	2152907 A1	17-02-2010
			EP	2311981 A1	20-04-2011
			HK	1156368 A1	31-07-2015
			US	2010197518 A1	05-08-2010
			WO	2008137465 A1	13-11-2008
JP 2005151854	Α	16-06-2005	NONE	: :	