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Clonal Hematopoiesis and Risk of Atherosclerotic Cardiovascular Disease

S. Jaiswal, P. Natarajan, A.J. Silver, C.J. Gibson, A.G. Bick, E. Shvartz, M. McConkey, N. Gupta, S. Gabriel, D. Ardissino, U. Baber, R. Mehran, V. Fuster, J. Danesh, P. Frossard, D. Saleheen, O. Melander, G.K. Sukhova, D. Neuberg, P. Libby, S. Kathiresan, and B.L. Ebert

ABSTRACT

BACKGROUND

Clonal hematopoiesis of indeterminate potential (CHIP), which is defined as the presence of an expanded somatic blood-cell clone in persons without other hematologic abnormalities, is common among older persons and is associated with an increased risk of hematologic cancer. We previously found preliminary evidence for an association between CHIP and atherosclerotic cardiovascular disease, but the nature of this association was unclear.

METHODS

We used whole-exome sequencing to detect the presence of CHIP in peripheral-blood cells and associated such presence with coronary heart disease using samples from four case–control studies that together enrolled 4726 participants with coronary heart disease and 3529 controls. To assess causality, we perturbed the function of *Tet2*, the second most commonly mutated gene linked to clonal hematopoiesis, in the hematopoietic cells of atherosclerosis-prone mice.

RESULTS

In nested case–control analyses from two prospective cohorts, carriers of CHIP had a risk of coronary heart disease that was 1.9 times as great as in noncarriers (95% confidence interval [CI], 1.4 to 2.7). In two retrospective case–control cohorts for the evaluation of early-onset myocardial infarction, participants with CHIP had a risk of myocardial infarction that was 4.0 times as great as in noncarriers (95% CI, 2.4 to 6.7). Mutations in *DNMT3A*, *TET2*, *ASXL1*, and *JAK2* were each individually associated with coronary heart disease. CHIP carriers with these mutations also had increased coronary-artery calcification, a marker of coronary atherosclerosis burden. Hypercholesterolemia-prone mice that were engrafted with bone marrow obtained from homozygous or heterozygous *Tet2* knockout mice had larger atherosclerotic lesions in the aortic root and aorta than did mice that had received control bone marrow. Analyses of macrophages from *Tet2* knockout mice showed elevated expression of several chemokine and cytokine genes that contribute to atherosclerosis.

CONCLUSIONS

The presence of CHIP in peripheral-blood cells was associated with nearly a doubling in the risk of coronary heart disease in humans and with accelerated atherosclerosis in mice. (Funded by the National Institutes of Health and others.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Address reprint requests to Dr. Ebert at the Department of Medicine, Division of Hematology, Brigham and Women's Hospital, 77 Avenue Louis Pasteur, HIM 423, Boston, MA 02115, or at bebert@partners.org; or to Dr. Kathiresan at the Center for Genomic Medicine, Massachusetts General Hospital, CPZN 5.830, 185 Cambridge St., Boston, MA 02114, or at sekar@broadinstitute.org.

Drs. Kathiresan and Ebert contributed equally to this article.

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Aging is associated with an increased incidence of cancer and cardiovascular disease. We and others recently used whole-exome sequencing data to identify a common, age-related disorder marked by an expansion of hematopoietic clones carrying recurrent somatic mutations, most frequently loss-of-function alleles in the genes *DNMT3A*, *TET2*, and *ASXL1*.[1-3] These mutations, which are also common in the myelodysplastic syndrome and acute myeloid leukemia,[4] provide a selective advantage to the hematopoietic stem cells in which they occur[5,6] and are detectable as clones in peripheral-blood samples because the mutated stem cells maintain the ability to differentiate into circulating granulocytes, monocytes, and lymphocytes.[7,8] Such clones rarely accumulate in persons younger than 40 years of age, but they become more common among older persons, with more than 10% of persons older than 70 years of age carrying such a mutation. Carriers of these mutations have 10 times the risk of a hematologic cancer as do those without such mutations. On the basis of these findings, we provisionally defined persons carrying such mutations in the absence of any other hematologic abnormalities as having clonal hematopoiesis of indeterminate potential (CHIP).[9]

Our exploratory analysis revealed that persons with CHIP are at increased risk for death from any cause and, surprisingly, for coronary heart disease.[1] Although traditional risk factors for coronary heart disease (e.g., hypercholesterolemia, type 2 diabetes, hypertension, and smoking) account for a large proportion of the risk, many persons with atherosclerosis or coronary heart disease do not have established risk factors,[10,11] which suggests that unknown factors may also contribute to atherosclerosis and its complications. In this study, we tested the hypothesis that CHIP contributes causally to atherosclerotic cardiovascular disease.

METHODS

STUDY SAMPLES

The primary samples that we used were obtained from four case–control studies that together enrolled 4726 participants with coronary heart disease and 3529 controls. The protocols for all four of these studies were approved by the ethics committees at all involved institutions; written informed consent was obtained from all the participants.

First, we used a modified nested case–control study design from two prospective cohort studies, BioImage and Malmö Diet and Cancer (MDC). BioImage was selected because of the enrichment of the cohort with older participants at high cardiovascular risk,[12] whereas MDC was selected because of its long follow-up period and extensive phenotypic data.[13] After the exclusion of participants with prevalent cardiovascular events, coronary heart disease was defined as a history of myocardial infarction or coronary revascularization after the time of DNA collection. Among these participants, we selected those with coronary heart disease who were matched with controls on the basis of age, sex, type 2 diabetes status, and smoking history without regard to follow-up time. The baseline characteristics of the participants in these two cohorts are shown in Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org.

We analyzed whole-exome sequencing data from two retrospective case–control studies, the Atherosclerosis, Thrombosis, and Vascular Biology Italian Study Group (ATVB)[14] and the Pakistan Risk of Myocardial Infarction Study (PROMIS),[15,16] to evaluate participants with early-onset (age, <50 years) myocardial infarction. In these studies, case participants consisted of those with early-onset myocardial infarction who were selected at the time of the index presentation to hospitals, and controls were persons from the same medical centers who did not have cardiovascular disease. In ATVB, case participants were 45 years of age or younger and were age-matched with controls, whereas in PROMIS we selected only case participants and controls who were from 40 to 50 years of age. The baseline characteristics of the participants in these two cohorts are shown in Table S2 in the Supplementary Appendix.

To understand which CHIP genes contributed to the risk of coronary heart disease, we performed a gene-level analysis on samples obtained from BioImage, MDC, and three prospective cohorts unselected for coronary events: the Jackson Heart Study (JHS), the Finland–United States Investigation of NIDDM [Non–Insulin-Dependent Diabetes Mellitus] Genetics (FUSION), and the Framingham Heart Study (FHS).[17] JHS and FUSION were part of our prior association study of CHIP with coronary heart disease,[1] whereas samples from FHS were newly analyzed for this study.

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WHOLE-EXOME SEQUENCING

DNA was obtained from individual studies, and further processing was performed at the Broad Institute of Harvard and the Massachusetts Institute of Technology. We performed whole-exome sequencing on blood samples obtained from 113 participants with coronary heart disease and 257 controls from BioImage and on samples obtained from 320 participants with coronary heart disease and 320 controls from MDC. Whole-exome sequencing in ATVB and PROMIS had been performed previously. We identified CHIP carriers on the basis of a prespecified list of variants in 74 genes known to be recurrently mutated in myeloid cancers (Table S3 in the Supplementary Appendix). Details regarding the sequencing procedures are provided in the Extended Methods section in the Supplementary Appendix.

ATHEROSCLEROSIS IN MICE

We transplanted atherosclerosis-prone mice that were bred to have a knockout mutation for the gene encoding low-density lipoprotein receptor (*Ldlr*) with bone marrow from *Tet2* control mice or heterozygous or homozygous knockout mice. The mice were started on a high-fat, high-cholesterol diet and then assessed for atherosclerosis at various time points. We performed gene-expression analysis on macrophages cultured from bone marrow obtained from *Tet2* control or knockout mice that had been exposed to vehicle or low-density lipoprotein (LDL).

STATISTICAL ANALYSIS

In the analysis of data from the BioImage and MDC studies, we used a Cox proportional-hazards model after adjustment for age, sex, type 2 diabetes status, total cholesterol, high-density lipoprotein (HDL) cholesterol, hypertension, and smoking status. In the analysis of data from ATVB and PROMIS, we analyzed whole-exome sequencing data using a logistic-regression model after adjustment for age, sex, type 2 diabetes status, and smoking status. Details regarding the statistical analysis are provided in the Extended Methods section in the Supplementary Appendix.

RESULTS

ASSOCIATION BETWEEN CHIP AND CORONARY HEART DISEASE

In the samples obtained from the BioImage and MDC studies, we found that the somatic mutations most commonly occurred in the genes *DNMT3A*, *TET2*, and *ASXL1* and that 72 of 77 participants (94%) with CHIP had a mutation in only a single driver gene[1,2] (Table S4 and Fig. S1 in the Supplementary Appendix).

The median age of the participants in BioImage at the time of DNA sample collection was 70 years, and the median follow-up time was 2.6 years. We found that 19 of 113 participants with coronary heart disease (17%) were CHIP carriers versus 25 of 257 controls (10%) (hazard ratio, 1.8; 95% confidence interval [CI], 1.1 to 2.9; P=0.03). The median age of the participants in MDC was 60 years at the time of DNA sample collection, and the median follow-up was 17.7 years. CHIP was identified in 21 of 320 participants with coronary heart disease (7%) versus in 12 of 320 controls (4%) (hazard ratio, 2.0; 95% CI, 1.2 to 3.1; P=0.003) (Fig. 1A, and Table S5 and Fig. S2 in the Supplementary Appendix). The combined analysis of the two cohorts in a fixed-effects meta-analysis showed that the CHIP carriers had a risk of incident coronary heart disease that was 1.9 times as great as in noncarriers (95% CI, 1.4 to 2.7; P<0.001).

ASSOCIATION BETWEEN CHIP AND EARLY-ONSET MYOCARDIAL INFARCTION

In both ATVB and PROMIS, participants with early-onset myocardial infarction had marked enrichment of CHIP, as compared with controls. In ATVB, 37 of 1753 participants with myocardial infarction (2%) were CHIP carriers versus 6 of 1583 controls (<1%) (odds ratio, 5.4; 95% CI, 2.3 to 13.0; P<0.001). In PROMIS, 52 of 2540 participants with myocardial infarction (2%) were CHIP carriers versus 13 of 1369 controls (1%) (odds ratio, 3.4; 95% CI, 1.8 to 6.5; P<0.001). A combined fixed-effects meta-analysis of these two cohorts showed that CHIP was associated with an odds ratio of 4.0 (95% CI, 2.4 to 6.7; P<0.001) for early-onset myocardial infarction (Fig. 1B).

ASSOCIATION BETWEEN RISK MUTATIONS AND CORONARY EVENTS

In samples obtained from BioImage, MDC, and the three prospective cohorts unselected for coronary events, we specifically tested for associations between coronary heart disease and mutations in *DNMT3A*, *TET2*, *ASXL1*, and *JAK2*. Participants with mutations in *DNMT3A*, *TET2*, and *ASXL1* had 1.7 to 2.0 times the risk of incident coronary heart disease as did those with no mutations,

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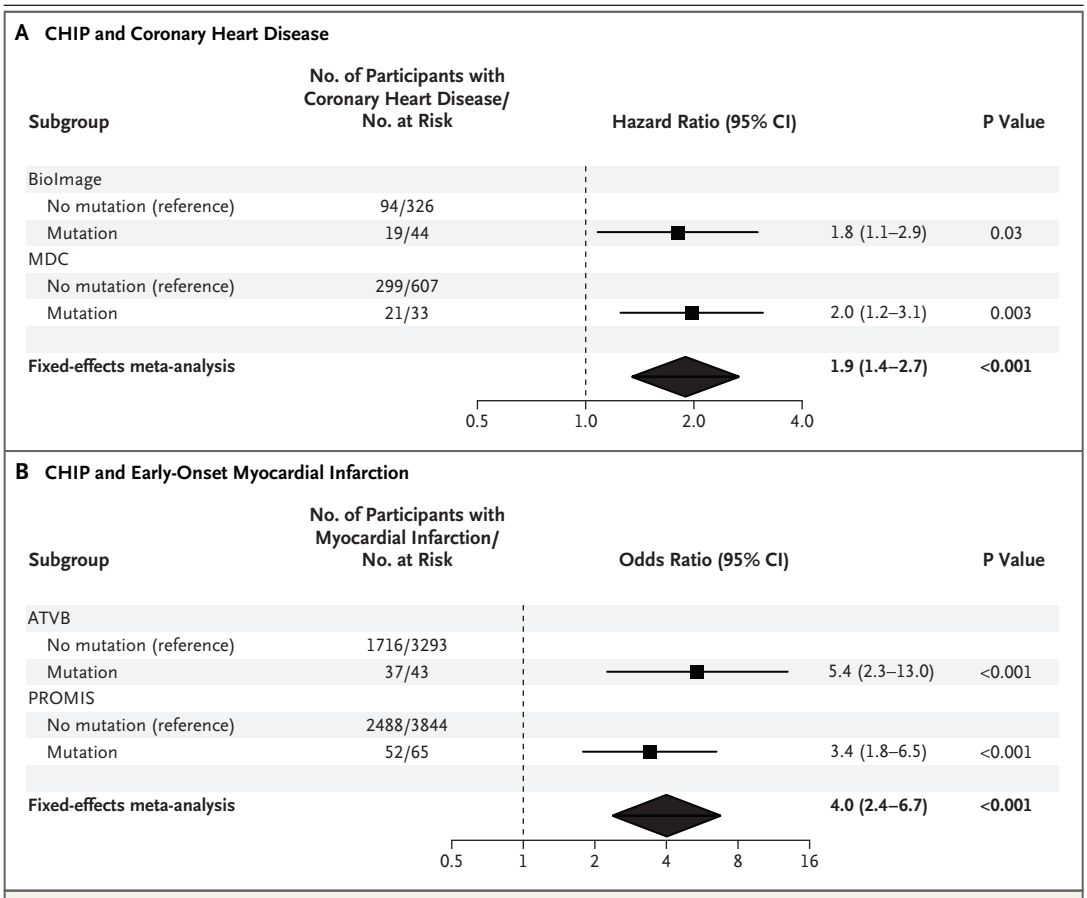

Figure 1. Association between Clonal Hematopoiesis of Indeterminate Potential (CHIP) and Coronary Heart Disease and Early-Onset Myocardial Infarction.

Panel A shows a forest plot of hazard ratios for the association between coronary heart disease and CHIP in the BioImage and Malmö Diet and Cancer (MDC) studies. Hazard ratios for coronary heart disease among study participants with CHIP mutations were obtained with the use of a Cox proportional-hazards model after adjustment for age, sex, type 2 diabetes status, total cholesterol, high-density lipoprotein (HDL) cholesterol, smoking status, and hypertension. Panel B shows a forest plot of odds ratios for the association between myocardial infarction and CHIP in the Atherosclerosis, Thrombosis, and Vascular Biology Italian Study Group (ATVB) and the Pakistan Risk of Myocardial Infarction Study (PROMIS). The odds ratios were obtained with the use of a logistic-regression model after adjustment for age, sex, type 2 diabetes status, and smoking status.

whereas the *JAK2* V617F mutation was associated with 12.1 times the risk (Fig. 2A).

Mutations in *TET2*, *JAK2*, and *ASXL1* also showed significant enrichment among participants with early-onset myocardial infarction in samples obtained from ATVB and PROMIS, with myocardial infarction identified in 12 of 13 participants with *TET2* mutations, in 8 of 8 participants with *ASXL1* mutations, and in 16 of 16 participants with *JAK2* mutations (Fig. 2B). *JAK2* V617F accounted for 19% of the total mutations among patients with myocardial infarction in ATVB and PROMIS but for only 4% in BioImage and MDC (Fig. 2C).

ASSOCIATION BETWEEN CHIP AND CORONARY-ARTERY CALCIFICATION

We hypothesized that an increased atherosclerosis burden drives the association between CHIP and coronary heart disease, rather than other factors that might cause myocardial infarction, such as increased thrombosis or vasospasm. Among the participants in the BioImage study, we assessed data on coronary-artery calcification, a noninvasive measure of atherosclerosis as detected on cardiac computed tomography. Among those without incident coronary heart disease, CHIP carriers had a median score for coronary-

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A CHIP and Coronary Heart Disease, According to Mutated Gene

Subgroup	No. of Participants with Myocardial Infarction/ No. at Risk	Hazard Ratio (95% CI)	P Value
No mutation			
BioImage	94/326		
MDC	299/607		
JHS/FUSION/FHS	169/3505		
DNMT3A			
BioImage	5/14	1.7 (0.7–4.1)	0.27
MDC	11/15	2.5 (1.4–4.7)	0.003
JHS/FUSION/FHS	8/99	1.1 (0.5–2.2)	0.90
Fixed-effects meta-analysis		**1.7 (1.1–2.6)**	**0.01**
TET2			
BioImage	3/7	1.6 (0.5–5.0)	0.46
MDC	2/6	0.8 (0.2–3.3)	0.76
JHS/FUSION/FHS	4/16	3.5 (1.3–9.6)	0.01
Fixed-effects meta-analysis		**1.9 (1.0–3.7)**	**0.06**
ASXL1			
BioImage	4/6	2.1 (0.7–5.8)	0.16
MDC	3/6	1.4 (0.5–4.6)	0.53
JHS/FUSION/FHS	2/10	2.8 (0.7–11.4)	0.15
Fixed-effects meta-analysis		**2.0 (1.0–3.9)**	**0.05**
JAK2			
BioImage	0/0		
MDC	2/2	10.0 (2.4–41.5)	0.001
JHS/FUSION/FHS	1/3	17.4 (2.4–127.6)	0.005
Fixed-effects meta-analysis		**12.0 (3.8–38.4)**	**<0.001**
Other			
BioImage	7/17	1.8 (0.8–3.9)	0.16
MDC	3/4	1.9 (0.6–6.0)	0.28
JHS/FUSION/FHS	6/35	3.0 (1.3–6.9)	0.009
Fixed-effects meta-analysis		**2.2 (1.3–3.7)**	**0.002**

0.5 1.0 2.0 4.0 8.0 16.0

B CHIP and Myocardial Infarction, According to Mutated Gene

ATVB and PROMIS	No. of Participants with Myocardial Infarction/ No. at Risk	Odds Ratio (95% CI)	P Value
DNMT3A	31/46	1.4 (0.7–2.8)	0.29
TET2	12/13	8.3 (1.2–357.5)	0.02
ASXL1	8/8	Undefined	0.02
JAK2	16/16	Undefined	<0.001
Other	20/22	6.9 (1.7–61.6)	0.001

C Myocardial Infarction and Coronary Heart Disease, According to Mutated Gene

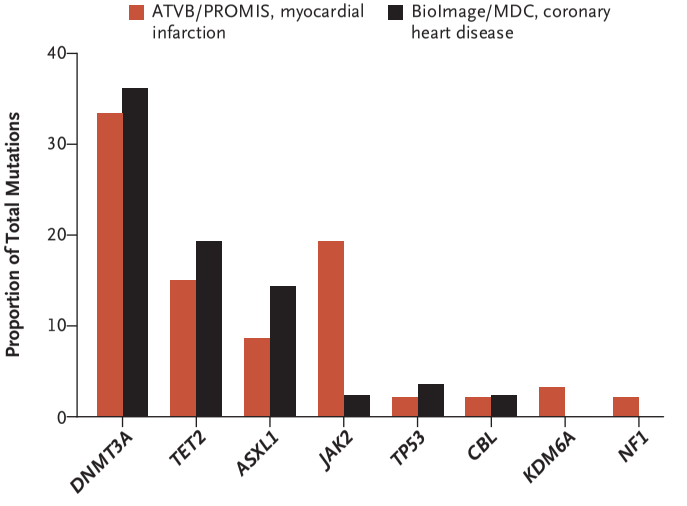

Figure 2. Association between Coronary Heart Disease and Early-Onset Myocardial Infarction among CHIP Carriers, According to Genetic Mutation.

Panel A shows a forest plot of hazard ratios for the risk of coronary heart disease in BioImage, MDC, and three prospective cohorts that were unselected for coronary events: the Jackson Heart Study (JHS), Finland–United States Investigation of NIDDM [Non–Insulin-Dependent Diabetes Mellitus] Genetics (FUSION), and the Framingham Heart Study (FHS), according to mutated gene. Hazard ratios for the listed mutations were obtained by a fixed-effects meta-analysis of Cox proportional-hazards models after adjustment for age, sex, type 2 diabetes status, total cholesterol, HDL cholesterol, triglycerides, smoking status, and hypertension. Panel B shows the risk of early-onset myocardial infarction among the participants in ATVB and PROMIS (combined analysis), according to the mutated gene. The odds ratios for myocardial infarction were obtained with the use of Fisher's exact test; P values were not adjusted for multiple hypothesis testing. Panel C shows the proportion of total mutations (according to gene) among participants with myocardial infarction in the ATVB and PROMIS studies, as compared with those with coronary heart disease in the BioImage and MDC studies.

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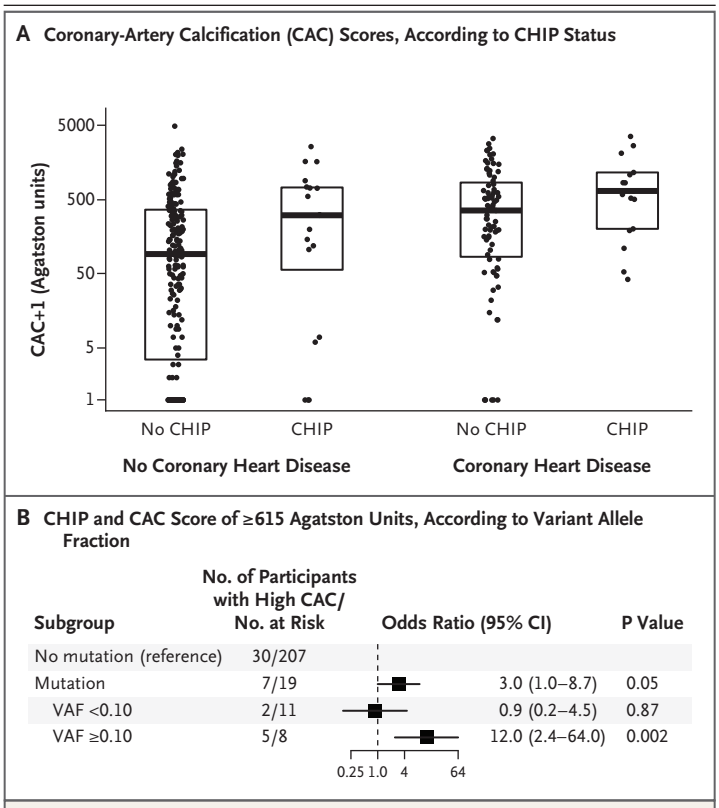

Figure 3. Association between CHIP and Increased Coronary-Artery Calcification.

Panel A shows coronary-artery calcification (CAC) scores in Agatston units among participants with CHIP and those without CHIP in the BioImage study, stratified according to the presence or absence of incident coronary heart disease. The horizontal line in each box indicates the median score, and the top and bottom of the boxes indicate the 75th and 25th percentiles, respectively. Among the participants with no coronary heart disease, the median scores were 92 for no CHIP and 306 for CHIP; among those with coronary heart disease, the median scores were 355 and 650, respectively. P=0.03 for the comparison between CHIP and no CHIP; the P value was obtained with the use of a linear-regression model of logarithm-transformed CAC plus 1, after adjustment for incident coronary heart disease status. Panel B shows a forest plot of odds ratios for the association between a CAC score of 615 or more and mutation status, stratified according to the variant allele fraction (VAF) among participants with incident coronary heart disease in BioImage. The odds ratios were obtained with the use of a logistic-regression model after adjustment for age, sex, type 2 diabetes status, total cholesterol, HDL cholesterol, smoking status, and hypertension. P=0.02 for heterogeneity between a VAF of less than 0.10 and a VAF of 0.10 or more.

artery calcification that was 3.3 times as high as that in noncarriers (306 versus 92 Agatston units); in those with incident coronary heart disease, the score was 1.8 times as high in CHIP carriers as in noncarriers (650 versus 355 Agatston units) (P=0.03 by linear regression after adjustment for incident coronary heart disease) (Fig. 3A).

A coronary-artery calcification score of 615 Agatston units or more has served as an empirical cutoff for identifying older persons at high risk for coronary events.[18] Among the participants without incident coronary heart disease, CHIP carriers were 3.0 times as likely to have a coronary-artery calcification score of 615 or more than were noncarriers (P=0.05 by logistic regression after adjustment for age, sex, type 2 diabetes status, total cholesterol, HDL cholesterol, hypertension, and smoking status) (Fig. 3B).

We had previously found that the presence of a CHIP clone with a variant allele fraction of at least 10% (which corresponds to ≥20% of nucleated blood cells harboring the mutation) was associated with a greater risk of a hematologic cancer than a CHIP clone below this size.[1] Therefore, we tested whether CHIP with a larger clone size was also associated with a greater burden of atherosclerosis. CHIP carriers without incident coronary heart disease but with a variant allele fraction of a least 10% had 12 times the risk of having a coronary-artery calcification score of 615 or more as did noncarriers (P=0.002 by logistic regression after adjustment), whereas participants with a variant allele fraction of less than 10% had no increased risk (P=0.02 for heterogeneity) (Fig. 3B, and Fig. S3 in the Supplementary Appendix).

We hypothesized that the participants with an increased proportion of mutated cells might also have a greater risk of incident coronary heart disease. In a meta-analysis of data from BioImage and the three prospective cohorts unselected for coronary events, we found that the risk of incident coronary heart disease was 2.2 times as great among the participants with a variant allele fraction of at least 10% as among those without mutations (P<0.001 by Cox proportional hazards after adjustment), whereas those with a variant allele fraction of less than 10% had a risk that was 1.4 times as great (P=0.16; P=0.24 for heterogeneity) (Fig. S4 in the Supplementary Appendix). In this analysis, data from MDC were not included because DNA in this cohort was obtained from granulocytes rather than whole blood, which would probably inflate the variant allele fraction.

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TET2 KNOCKOUT MICE AND ACCELERATED ATHEROSCLEROSIS

Having established a correlation between CHIP and coronary heart disease, we next sought to assess causality experimentally. We selected *Tet2* for further study because it is the second most commonly mutated gene in CHIP and has been associated with the risk of coronary heart disease regardless of age. In previous studies, hematopoietic stem cells from mice that are homozygous for loss of function of *Tet2* in all hematopoietic cells recapitulate the clonal advantage of mutated *TET2* hematopoietic cells seen in humans.[6]

We transplanted bone marrow from these mice and from control mice into irradiated, atherosclerosis-prone *Ldlr* knockout mice[19] and initiated a high-cholesterol diet after allowing time for hematopoietic reconstitution. As compared with mice that had received control bone marrow, mice that had received bone marrow from the *Tet2* knockout mice had a median lesion size in the aortic root that was 2.0 times as large after 5 weeks (P=0.02 by the Wilcoxon rank-sum test), that was 1.7 times as large after 9 weeks (P=0.01 by the Wilcoxon rank-sum test), and that was 1.4 times as large after 13 weeks (P=0.03 by Dunn's test) (Fig. 4A and 4B, and Fig. S5A and S5B in the Supplementary Appendix). By 17 weeks, the median lesion size in the descending aorta was 2.7 times as large in the mice that had received bone marrow from *Tet2* knockout mice as in those that had received control bone marrow (P=0.02 by Dunn's test) (Fig. 4C and 4D).

Most humans with *TET2*-associated CHIP have a mutation in only a single allele of the gene.[1,2] Therefore, we also tested the phenotype of *Tet2* haploinsufficiency. *Ldlr* knockout mice receiving bone marrow from mice that were heterozygous for the *Tet2* deletion had a median aortic-root lesion size that was 1.4 times as large as that in control mice after 13 weeks on the high-cholesterol diet (P=0.05 by Dunn's test) (Fig. S5A and S5B in the Supplementary Appendix); after 17 weeks, the median lesion size in the descending aorta was 2.7 times as large (P=0.03 by Dunn's test) (Fig. 4C and 4D).

Mice receiving bone marrow from *Tet2* knockout mice had normal levels of peripheral-blood white cells and a normal differential count during the study period. There also was no significant between-group difference in fasting serum lipid levels after 17 weeks on the high-cholesterol diet (Table S6 in the Supplementary Appendix).

LOSS OF *TET2* FUNCTION IN MYELOID CELLS

The earliest stages of atherosclerosis involve monocyte infiltration into vessel walls and differentiation into macrophages.[20] We hypothesized that *Tet2* loss alters the function of macrophages (and their precursor monocytes) in plaques to enhance atherosclerosis. We tested this hypothesis by generating mice that lacked *Tet2* in the majority of myeloid cells but not in other lineages; to create this murine line, we bred mice with loxP sites flanking exon 3 of *Tet2* with mice that express Cre recombinase from the *Lyz2* promoter.[21] In *Ldlr* knockout mice that had received bone marrow from these mice, the mean size of the aortic-root lesion was 1.7 times as large as in mice that had received control bone marrow after 10 weeks on the high-cholesterol diet (P=0.003 by the Wilcoxon rank-sum test) (Fig. S5C in the Supplementary Appendix).

Tet2 catalyzes DNA hydroxymethylation,[22] an epigenetic modification that can influence gene transcription. Therefore, we hypothesized that Tet2 modulated gene expression in macrophages exposed to excess cholesterol. We cultured bone marrow–derived macrophages from *Tet2* knockout or control mice, incubated them with either vehicle or a pathophysiologically relevant dose of native LDL (200 mg per deciliter),[23,24] and analyzed the transcriptome using messenger RNA sequencing. Gene set enrichment analysis revealed that the most significantly up-regulated functional class of genes in *Tet2* knockout macrophages contained cytokines or chemokines and receptors, whereas the most significantly suppressed class contained genes involved in lysosomal function. Gene class annotations were obtained from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Tables S7 and S8 and Fig. S6 in the Supplementary Appendix).

We focused on the set of 217 genes that showed differential regulation by both loss of *Tet2* and by LDL treatment. Transcript levels in *Cxcl1*, *Cxcl2*, *Cxcl3*, *Pf4*, *Il1b*, and *Il6* were among the most highly induced in *Tet2* knockout macrophages in this set (Fig. S7A and S7B in the Supplementary Appendix). *Cxcl1*, *Cxcl2*, *Cxcl3*, and *Pf4* belong to a single C-X-C motif (CXC) chemo-

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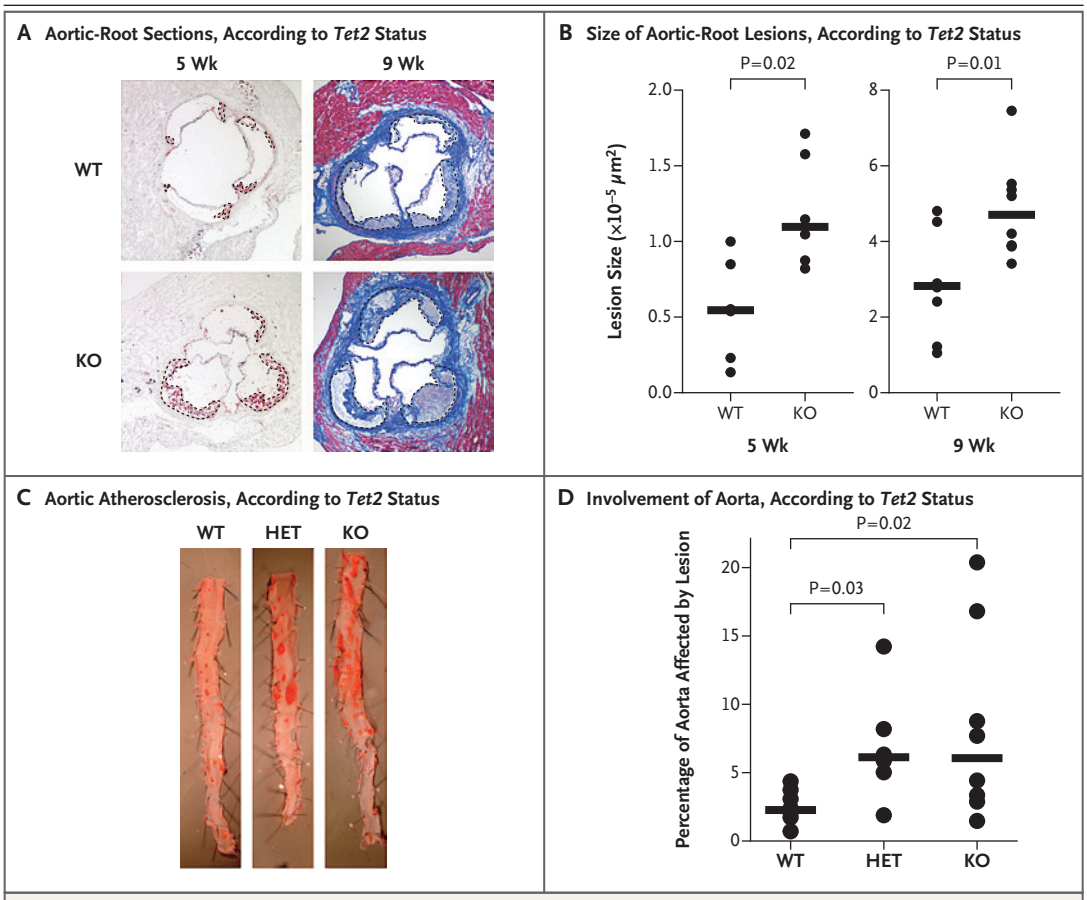

Figure 4. Loss of *Tet2* in Hematopoietic Cells and Atherosclerosis in a Murine Model.

Shown are the effects of the transplantation of bone marrow into female, atherosclerosis-prone *Ldlr* knockout mice, according to whether the donor mice had wild-type (WT) *Tet2*, heterozygous (HET) *Tet2*, or knockout (KO) *Tet2*. Deletions in *Tet2* were obtained by using Cre recombinase expressed from the Vav1 promoter. Panel A shows aortic-root sections obtained from mice that had received transplants from WT or KO *Tet2* mice after the mice had received a high-cholesterol diet for 5 weeks and 9 weeks (oil red O staining at 5 weeks and Masson's trichrome staining at 9 weeks). The dashed lines indicate the lesion areas. Panel B shows the quantification of aortic-root lesions in mice that had received transplants from WT or KO *Tet2* mice at 5 weeks and 9 weeks. P values were obtained with the use of the Wilcoxon rank-sum test. Panel C shows lesions in the descending aorta that were stained with oil red O at 17 weeks in mice that had received transplants from WT, HET, or KO *Tet2* mice. The amount of red dye that is visible indicates the degree of atherosclerosis, according to *Tet2* status. Panel D shows the quantification of lesions in the descending aorta at 17 weeks, according to *Tet2* status. P values were obtained with the use of Dunn's Kruskal–Wallis test for multiple comparisons and the Benjamini–Hochberg correction. In Panels B and D, the black horizontal lines represent the median values.

kine gene cluster, whereas *Il1b* and *Il6* are classic proinflammatory cytokine genes. *Tet2* knockout macrophages also secreted more of these proteins in vitro in response to LDL loading or endotoxin exposure than did control macrophages. Although either LDL or endotoxin strongly induced the CXC chemokines, endotoxin but not LDL caused robust secretion of interleukin-1b and interleukin-6 (Fig. S7C in the Supplementary Appendix).

To assess the in vivo importance of these observations, we measured CXC chemokine levels in the transplanted mice after they had been on an atherogenic diet for 13 to 17 weeks. We found that the levels of Cxcl1, Cxcl2, Cxcl3, Pf4, and Ppbp were 2 to 4 times as high in the serum of mice that had received bone marrow from *Tet2* knockout mice as in mice that had received control bone marrow, whereas those that had received bone marrow from mice that were heterozygous

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for *Tet2* deletion showed intermediate levels (Fig. S7D in the Supplementary Appendix).

We also sought evidence of increased inflammation in other tissues. In mice that had received *Tet2* knockout bone marrow, there was development of prominent xanthomas in the spleen and middle ear, marked foam-cell accumulation and glomerulosclerosis in the kidney, and large inflammatory infiltrates in the liver and lung (Fig. S8A and S8B in the Supplementary Appendix).

Because we found increased levels of CXC chemokines in the serum of mice that had received bone marrow from *Tet2* knockout mice, we tested for an analogous increase in humans with *TET2* clonal hematopoiesis. The prototypical CXC chemokine in humans is interleukin-8, which mice lack. Plasma levels of interleukin-8 were available from 2689 controls (age range, 40 to 82 years) in the PROMIS study. The 12 participants with *TET2* mutations in this cohort had significantly higher circulating interleukin-8 levels than did those without the mutations (median level, 50 vs. 21 ng per milliliter; P=0.02 by the Wilcoxon rank-sum test on log-transformed values) (Fig. S8C in the Supplementary Appendix).

DISCUSSION

In four distinct studies involving human participants, we found that somatic mutations leading to CHIP had significant associations with the risk of coronary heart disease or early-onset myocardial infarction. In a murine model of atherosclerosis, the loss of *Tet2* function in hematopoietic cells accelerated atherogenesis.

These results support several conclusions. First, the relationship between CHIP and coronary heart disease appears to be a causal one. Experimental manipulation of one of the genes that is most frequently mutated in CHIP — *Tet2* — worsened atherosclerosis in mice. In humans, coronary events increased in relation to clone size, and there was also a dose–response relationship between clone size and atherosclerosis on imaging.

Second, mutations in multiple CHIP-associated genes were linked to coronary heart disease. We suggest that these mutations may increase the risk of coronary events owing to altered transcriptional output of macrophages. These cells mediate many inflammatory responses and prominently populate atherosclerotic plaques.[25,26] In support of this model, we found that loss of *Tet2* augmented the expression of inflammatory chemokines in macrophages that were exposed to native LDL, an effect that is similar to that in *Tet2*-deficient macrophages that were exposed to bacterial endotoxin.[27]

Previous studies have shown that CXC chemokine interaction with the receptor CXCR2 can mediate firm monocyte adhesion to inflamed endothelium[28,29] and that this interaction promotes atherogenesis.[30,31] We propose that a major consequence of TET2 deficiency in tissue macrophages is the enhanced recruitment of monocytes and other blood cells to peripheral sites, including the arterial intima, because of elevated expression of CXC chemokines. Several organs of mice lacking hematopoietic *Tet2* harbored large leukocyte aggregates, and *TET2* mutations in humans were associated with increased plasma levels of interleukin-8. A recent study also identified augmented *Il1b* and inflammasome activation as a major mediator of atherosclerosis in mice with hematopoietic *Tet2* deficiency.[32] It is unclear which particular inflammatory mediators predominate in driving atherosclerosis associated with *TET2* deficiency and whether mutations in *DNMT3A*, *ASXL1*, and *JAK2* also influence the risk of coronary events by means of increased inflammation, issues that invite further experimentation.

An alternative explanation for the observed association is that CHIP-associated mutations provide a proliferative advantage to hematopoietic progenitors and a consequent increase in circulating myeloid cells. Elevations in levels of peripheral-blood granulocytes and monocytes have been linked to coronary outcomes in the general population,[33] whereas persons with *JAK2*-mutated myeloproliferative neoplasms are at increased risk for venous and coronary thrombosis, according to the degree of leukocytosis.[34,35] However, nearly all persons with clonal hematopoiesis caused by mutations in *DNMT3A*, *TET2*, and *ASXL1* have a normal white-cell count and differential,[1,36] and *JAK2* mutations account for only a small percentage of CHIP. Furthermore, mice that lacked hematopoietic *Tet2* had normal blood counts in our study and in previous studies.[32,37] Nonetheless, we cannot fully rule out a role for leukocytosis in human coronary heart disease, since CHIP could lead to a myeloproliferative state over several years.

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In conclusion, our data support the hypothesis that somatic mutations in hematopoietic cells contribute to the development of human atherosclerosis. We propose that clonal hematopoiesis may be a modifiable risk factor, perhaps through the use of cholesterol-lowering medications or targeting of specific inflammatory pathways.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

APPENDIX

The authors' full names and academic degrees are as follows: Siddhartha Jaiswal, M.D., Ph.D., Pradeep Natarajan, M.D., Alexander J. Silver, B.A., Christopher J. Gibson, M.D., Alexander G. Bick, M.D., Ph.D., Eugenia Shvartz, B.S., Marie McConkey, Ph.D., Namrata Gupta, Ph.D., Stacey Gabriel, Ph.D., Diego Ardissino, M.D., Usman Baber, M.D., Roxana Mehran, M.D., Valentin Fuster, M.D., Ph.D., John Danesh, F.R.C.P., Philippe Frossard, Ph.D., Danish Saleheen, M.B., B.S., Ph.D., Olle Melander, M.D., Ph.D., Galina K. Sukhova, Ph.D., Donna Neuberg, Sc.D., Peter Libby, M.D., Sekar Kathiresan, M.D., and Benjamin L. Ebert, M.D., Ph.D.

The authors' affiliations are as follows: the Department of Medicine, Division of Hematology, Brigham and Women's Hospital (S.J., A.J.S., M.M.) and Harvard Medical School (B.L.E.), the Department of Medicine, Division of Cardiovascular Medicine, Brigham and Women's Hospital (E.S.) and Harvard Medical School (G.K.S., P.L.), the Department of Pathology (S.J.) and the Center for Genomic Medicine (P.N., S.K.), Massachusetts General Hospital, the Department of Medicine, Division of Cardiology, and Cardiovascular Research Center (P.N., S.K.), and the Department of Medicine (A.G.B.), Massachusetts General Hospital and Harvard Medical School, and the Departments of Medical Oncology (C.J.G.) and Biostatistics and Computational Biology (D.N.), Dana–Farber Cancer Institute, Boston, and the Program in Medical and Population Genetics, Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge (P.N., A.G.B., N.G., S.G., S.K.) — all in Massachusetts; the Department of Cardiology, University Hospital, Parma, Italy (D.A.); the Department of Medicine, Division of Cardiology, Mt. Sinai School of Medicine, New York (U.B., R.M., V.F.); Centro Nacional de Investigaciones Cardiovasculares Carlos III, Madrid (V.F.); Medical Research Council–British Heart Foundation Cardiovascular Epidemiology Unit and National Institute for Health Research Blood and Transplant Research Unit in Donor Health and Genomics, Department of Public Health and Primary Care, and the British Heart Foundation, Cambridge Centre of Excellence, Department of Medicine, University of Cambridge, Cambridge (J.D.), and the Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton (J.D.) — both in the United Kingdom; the Center for Non-Communicable Diseases, Karachi, Pakistan (P.F., D.S.); the Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia (D.S.); and the Department of Clinical Sciences Malmö, Lund University, Lund, Sweden (O.M.).

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