ESC European Society of Cardiology

Cardiovascular Research (2019) **115**, 830–843
doi:10.1093/cvr/cvz032

SPOTLIGHT REVIEW

Completing the genetic spectrum influencing coronary artery disease: from germline to somatic variation

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Received 30 November 2018; revised 25 January 2019; editorial decision 29 January 2019; accepted 29 January 2019; online publish-ahead-of-print 21 February 2019

Abstract

Genetic and environmental factors influence the development of coronary artery disease (CAD). Genetic analyses of families and the population continue to yield important fundamental insights for CAD. For the past four decades, CAD human genetic research focused largely on the study of germline genetic variation in CAD and its risk factors. The first genes associated with CAD were discovered using basic Mendelian principles and pedigree analysis. Mapping of the human genome and advancement in sequencing technology sparked further discovery of novel genetic associations through exome sequencing and genome wide association analysis in increasingly larger populations. While prior work implicated *in situ* DNA damage as a feature of atherosclerosis, more recently, somatic mutagenesis in and clonal expansion of haematopoietic stem cells was found to influence risk of CAD. Mutations observed for this condition, termed clonal haematopoiesis of indeterminate potential, frequently occur within epigenetic regulator genes (e.g. *DNMT3A*, *TET2*, *ASXL1*, etc.), which are also implicated in leukaemogenesis. Hypercholesterolaemic mice with *Tet2* bone marrow deficiency are predisposed to the development of atherosclerosis that may be partly related to inflammatory cytokines. As the genetic basis of CAD expands from the germline to somatic genome, our fundamental understanding of CAD continues to evolve; these new discoveries represent new opportunities for risk prediction and prevention, and a new facet of cardio-oncology.

Keywords Genetics • Coronary artery disease • Clonal haematopoiesis of indeterminate potential • Somatic mutations

This article is part of the Spotlight Issue on Cardio-oncology.

1. Introduction

Cardiovascular disease is the leading cause of death in the world.[1,2] In addition to being the cause of death for over 3.8 million individuals in the member nations of the European Society of Cardiology each year, it accounts for a large share of each nation's health care system expenditure.[3] Much of the mortality and morbidity from cardiovascular disease stems from coronary artery disease (CAD). A deeper understanding of the causes of CAD is essential to guide effective prevention and treatment measures.

An individual's genes, environment, and chance dictate the development and severity of CAD and its risk factors.[4] The heritability, or trait variability explained by the sum of genetic differences, of CAD is estimated to be 40–60%.[5,6] Although the template for germline genomic variation for an individual is established at gametogenesis and fertilization, each mitosis thereafter is subject to the forces of mutagenesis by chance, or biological or environmental influences (*Figure 1*). In oncology, study of the acquired mutations has been central to understanding the mechanism of tumourigenesis, and although cardio-oncology has emerged as a field due to potential cardiovascular toxicities of oncologic therapies, recent discoveries in clonal haematopoiesis inform new appreciation of common features between cardiovascular diseases and cancer. In this review, we highlight genetic work performed over the past 40 years of largely germline genetic variation to now somatic genetic variation, and their risks for CAD.

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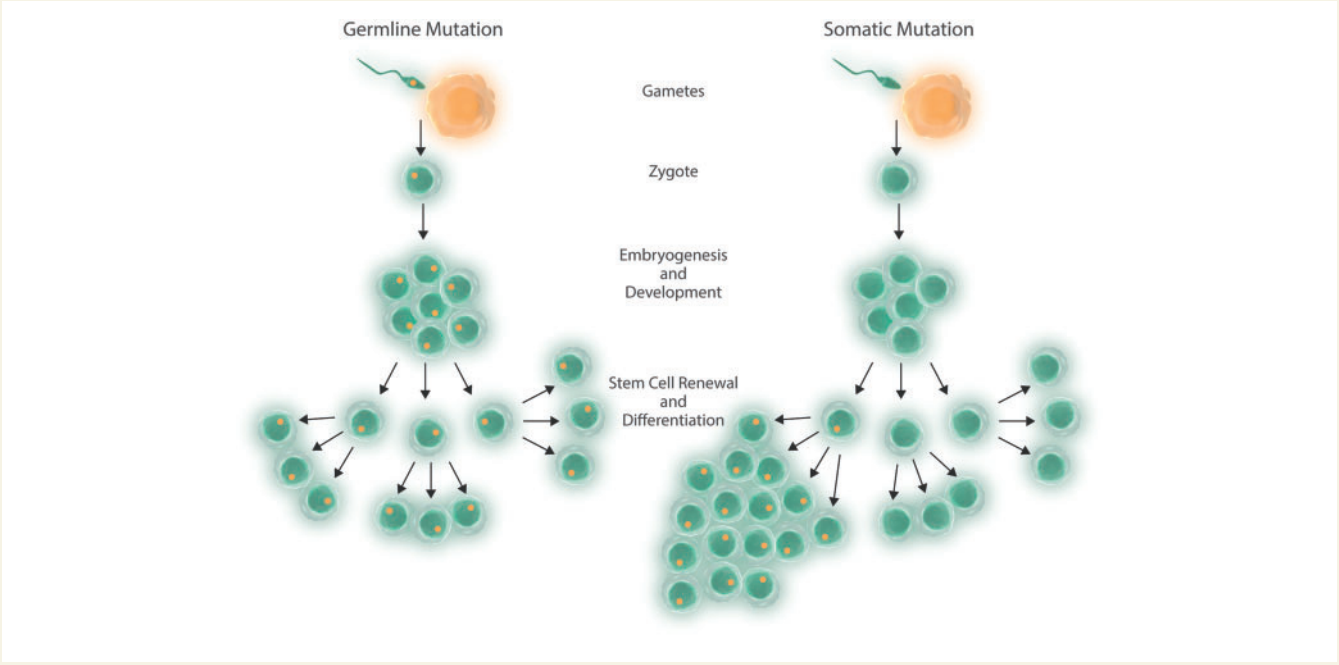

Figure 1 Germline and somatic mutagenesis. In germline mutagenesis, a mutation is transmitted from parent to offspring and all subsequent lineages carry it. In somatic mutagenesis, a mutation occurs in a stem cell or replicating differentiated cell leading to mosaicism, and potentially clonal advantage in replication and survival.

2. Germline variant discovery, Mendelian studies to exome sequencing

CAD often aggregates in families, particularly when it occurs earlier in life. Early human genetic studies of CAD analysed exceptional families displaying apparent Mendelian segregation of CAD using genetic linkage analysis (*Figure 2A*). In the first genetic analyses, families with co-segregation of severe hypercholesterolaemia and premature CAD were studied. In one such study, DNA sequence was deduced with cDNA from restriction enzyme digestion patterns and blot hybridization; a 5 kb deletion in the gene encoding for the low-density lipoprotein (LDL) receptor (i.e. *LDLR*) deficient in cell membrane adherence and LDL clearance was discovered.[7,8] Advancement in genetic mapping and development of Sanger sequencing technology led to further understanding of the sequence and variation in *LDLR* and other genes involved in syndromes of familial dyslipidaemia and myocardial infarction (MI) (*Figure 2B*).[9–11] In the study of families with apparent autosomal dominant severe hypercholesterolaemia but without *LDLR* mutations, mutations (later characterized to be gain-of-function) in the gene *PCSK9* were implicated through positional cloning and parametric linkage analyses.[12] At the population-level, loss-of-function mutations in *PCSK9* were associated with marked reductions in LDL cholesterol concentration and subsequent reduction in risk of CAD in both European and African Americans.[13]

Advances in next-generation sequencing technology now permit sequencing of the exome (i.e. the full protein-coding portion of the genome) or even the whole genome itself (*Figure 2C*). Whole exome analysis also facilities gene discovery within exceptional families. For example, exome sequencing of a family with apparent Mendelian combined hypolipidaemia identified causative nonsense mutations in *ANGPTL3*, an inhibitor of lipoprotein lipase and endothelial lipase.[14] Furthermore, advances now permit scalable application for gene discovery in large studies (e.g. case-control, cohort, etc). Exome sequencing of 9793 individuals of patients with early-onset MI and MI-free controls verified that variants in *LDLR* associated with 4.2–13-fold odds for MI, and showed that variants in *APOA5* associated with 2.2-fold odds of MI, each also with associations of hypercholesterolaemia and hypertriglyceridaemia, respectively.[15] A cohort study with exome sequences of 3734 individuals observed that loss-of-function mutations in *APOC3* were associated with lower levels of triglycerides and reduced risk of CAD.[16,17]

3. Germline variant discovery, the genome wide association study

While the analyses of rare alleles and their influences on cardiovascular traits can lead to broad insights about cardiovascular disease, a larger fraction of the heritability of common traits such as CAD appears to be explained by common genetic variation. Genome-wide association studies (GWAS) catalogue common coding and non-coding variation across the human genome through single nucleotide polymorphism (SNP) arrays and associate these variants with quantitative or discrete traits in population-based analyses (*Figure 2D*).

Over a decade ago, the first GWAS of CAD/MI showed that the top association was at the 9p21 locus, a genomic region not previously implicated in cardiovascular disease.[18–20] As GWA studies increase in size and ethnic diversity, novel loci continue to be discovered.[21–27] The most recent and largest meta-analyses identified 64 new loci implicated in

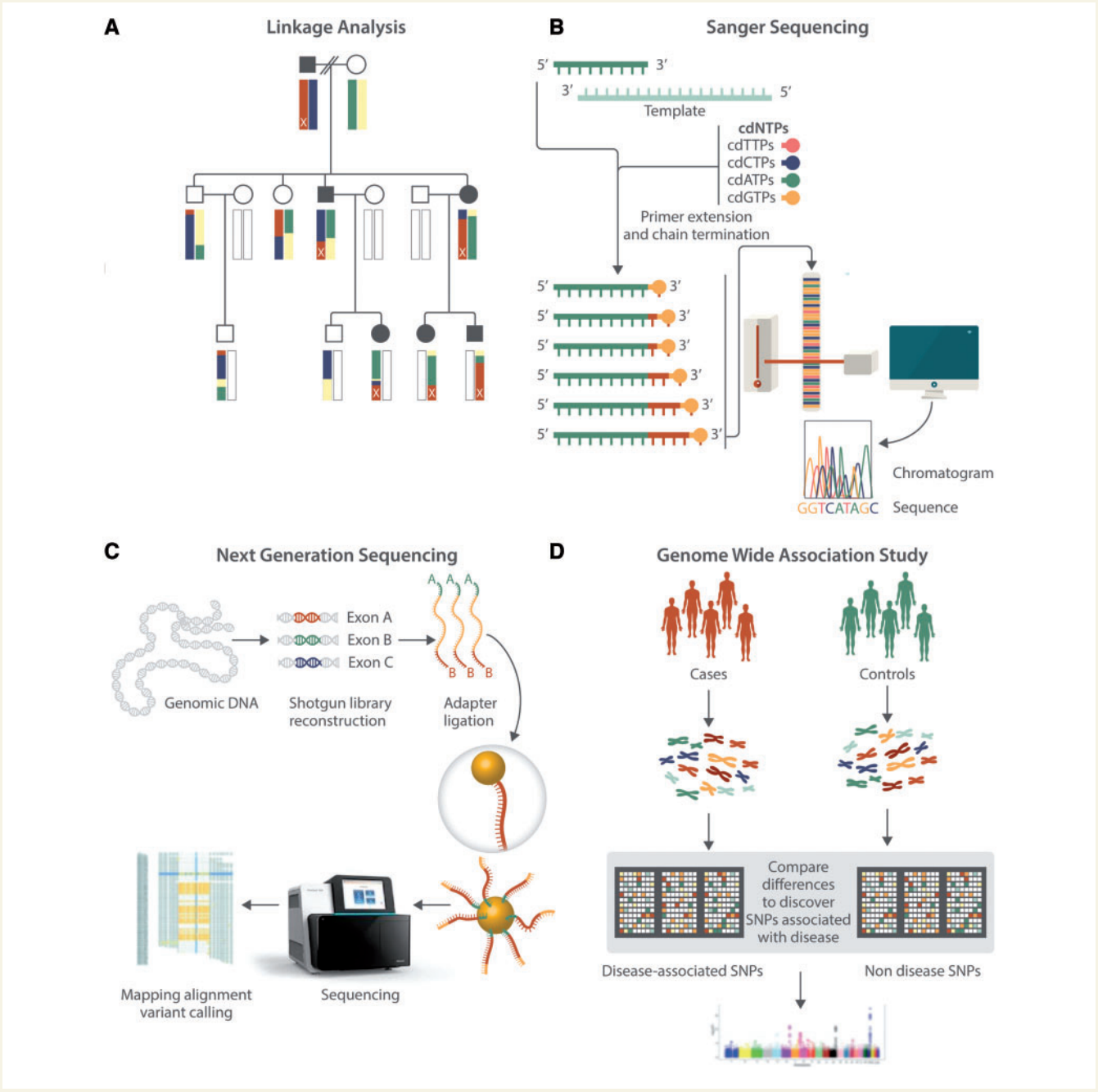

Figure 2 Advances in genetic analysis methodology. (*A*) Linkage analysis is a statistical method that tracks and correlates hereditary phenotype transmission with genetic loci relying on the consequences of genetic recombination between two autosomal chromosomes during meiosis. Finer resolution mapping is possible with larger pedigrees. This schematic depicts an autosomal dominant trait originating from the terminal chromosomal locus in the pedigree father (marked x) and being transmitted to a granddaughter via an affected son and to a grandson and granddaughter via an affected daughter. (*B*) Sanger sequencing is used to determine the DNA sequence for a locus. Repetitive polymerase chain replication is performed in which a constitutively replicated sequence fragment is randomly terminated with a labelled chain-terminating dideoxynucleotide. Several sequencing fragments of varying lengths and end nucleotides emerge and are separated based on length. The attached base for each length is imaged to yield a sequence of bases. (*C*) Next generation sequencing refers to a group of methods of large-scale, parallel sequencing using Sanger sequencing principles. This involves fragmenting genomic DNA, attaching adaptors to the newly formed DNA fragments, attaching these adaptors to a solid surface for sequencing, amplifying the fragmented DNA strands in parallel, enriching desired segments, sequencing in parallel using different methods, aligning the sequence reads that result from the pooled results to known genomic reference sequence, and examining the resulting DNA variants. (*D*) Genome wide association (GWA) involves using large genotyping arrays containing SNPs, each representative of a locus of bases that are generally inherited together due to linkage disequilibrium. DNA from cohorts of individuals affected and unaffected by a trait are genotyped with these arrays and the comparative differences in prevalence of certain mutations are used to statistically determine associations of polymorphisms at a certain locus with the trait of interest.

CAD, bringing estimated total to over 150 loci reaching genome wide significance.[24,28,29] Additionally, international collaborations identified numerous associations with risk factors implicated in CAD, notably in hypertension,[30,31] lipids,[32] and Type 2 diabetes.[33–35] At the individual-level, risk alleles can be summed as a weighted polygenic risk score to quantify genetic predisposition to CAD as well as its risk factors.[36–38]

Although associations are abundant, explaining the true disease mechanism of GWAS signals at regions without previously implicated Mendelian genes remains challenging. Disentangling the causal variant itself can be challenging as the lead significant variant with the strongest statistical signal is correlated with several other variants in linkage disequilibrium. Additionally, many of the implicated variants are in non-protein-coding regions, making hypothesis-driven candidate testing challenging. Musunuru *et al.*[39] reported one common, non-coding GWAS-implicated variant at the chromosome 1p13 locus to be involved in a novel regulatory pathway, where altered transcription factor binding leads to altered hepatic expression of *SORT1*. This gene is involved in pre-secretory degradation of very low-density lipoprotein (VLDL) cholesterol and leads to altered LDL cholesterol and VLDL cholesterol levels, thereby influencing risk for CAD.

4. *In situ* DNA damage and atherogenesis

Separate from the analysis of germline genetic variation, various investigational methods in model systems previously linked acquired damage to DNA and repair defects within endothelial cells and cholesterol plaques with atherogenesis.[40] The process of atherosclerosis is mediated by damage to endothelium, deposition of cholesterol, activation of inflammation and fibroproliferation.[41] Cholesterol bound to lipoprotein particles in the bloodstream attach to intima and aggregate to form the beginnings of fatty streaks. These particles undergo chemical modifications in the setting of oxidative stress.[42] Deletions or additions of whole or parts of chromosomes as well as loss-of-heterozygosity (LOH), strand breaks, base pair modification, and microsatellite instability (MSI) have been observed in the plaque environment. The phenomenon of vascular aging is thought to occur as a result of exposure to reactive oxygen species (ROS) and resultant genomic instability, which subsequently affects normal cellular function within atheroma.[43]

Oxidative damage to mitochondrial genes promotes the generation of ROS in vascular tissues and atherosclerotic lesions. Atherosclerotic plaques are enriched for markers of immunoreactivity against oxidized DNA with concomitant local up-regulation of DNA repair systems.[44] Mitochondrial DNA damage correlates with atherosclerotic extent in humans and in mice.[45,46] Furthermore, mitochondrial damage is widespread in cells involved in atherosclerosis and may promote lesion development.[47]

MSI and LOH are common genomic alterations observed in cellular nuclei within atherosclerotic plaques. Microsatellites are short, repeated sequences of DNA, and instability in their inheritance occurs when the number of repeats changes with erroneous replication. LOH is a phenomenon that occurs when a heterozygote loses a wild-type allele through mutagenesis or non-disjunction. Several human studies of atherosclerotic plaques showed greater burden of LOH and MSI in atheromatous plaques compared with unaffected vascular tissue within the same individual.[48] MSI is particularly enriched at proto-oncogene regulators and genes central to signal transduction in vascular repair and healing, implying a causal mechanism of disordered proliferation and transformation of disease-related smooth muscle cells with atherosclerosis.[49,50] Furthermore, such mutagenesis may facilitate selection since large clonal chromosomal abnormalities are more commonly observed in the smooth muscle cells from unstable atherosclerotic plaques than in stable plaques.[51] However, it remains unclear if observed plaque characteristic differences are causes or effects of the observed genetic instability.

Telomere dysfunction may also lead to genomic instability and to atherogenesis. Telomere shortening in blood leucocytes is associated with metabolic syndrome, diabetes mellitus, and CAD.[52–54] Within vascular smooth muscle cells, accelerated telomere attrition and replicative senescence is associated with upstream angiotensin II-induced ROS-mediated DNA damage.[55] Similarly, it remains unclear if this is a cause or consequence of atherogenesis. Nevertheless, statin use is correlated with accelerated DNA repair in vascular smooth muscle cells, reduced telomere shortening, and reduce vascular smooth muscle cell death.[56]

5. Somatic mutations in blood cells conferring clonal advantage

In addition to the passive deposition and transformation of cholesterol that occurs in the endothelium, monocytes play a central role in the formation of atheroma.[42] Somatic mutations can occur within the monocytes interacting with vascular tissues and may separately influence atherosclerosis.

Leucocytes are recruited to the intima through expression of extracellular proteins in the cell adhesion molecule, integrin, and selectin families. Monocytes subsequently accumulate lipids via scavenger receptors and subsequently transform into foam cells. These cells thereafter produce further inflammatory mediators as well as oxidant species that contribute to progression of atherosclerotic lesions. The vascular smooth muscle cells then rapidly multiply, produce extracellular matrix molecules, and foster calcification, leading to maturation and evolution of the atherosclerotic plaque.[42]

Haematopoietic stem cells continuously regenerate providing an ideal opportunity for selection and proliferation in the setting of mutagenesis (*Figure 1*). DNA from whole blood for germline DNA analysis is extracted from peripheral mononuclear cells; separate bioinformatics software can be used to distinguish the presence of acquired mutations of appreciable allele frequency (indicating clonal expansion).

Xie *et al.*[57] studied 2728 individuals with non-haematologic malignancy from The Cancer Genome Atlas and identified 77 somatic mutations in cancer genes in blood cells from whole exome sequencing. The majority of these mutations are commonly associated with haematologic malignancies, with nine loci that are recurrently mutated (*DNMT3A*, *TET2*, *JAK2*, *ASXL1*, *TP53*, *GNAS*, *PPM1D*, *BCORL1*, and *SF3B1*, *Figure 2*).

Further studies among individuals without prior malignancy demonstrated that clonal haematopoiesis with pre-malignant somatic mutations is associated with increased risk of haematologic cancer and death. From the analysis of 12 380 blood-derived whole exome sequences, Genovese *et al.*[58] identified 3111 somatic mutations in white blood cells based on the presence of unusual allelic fractions dispersed across the exome. There was similarly disproportionate enrichment of disruptive mutations in the genes implicated in haematologic malignancy, namely *DNMT3A*, *TET2*, and *ASLX1* (*Figure 3*). Prevalence was associated with age: 1% of individuals younger than age 50 were carriers and 10% of individuals older than age 65 were carriers. Furthermore, clonal haematopoiesis was associated with the development of haematologic

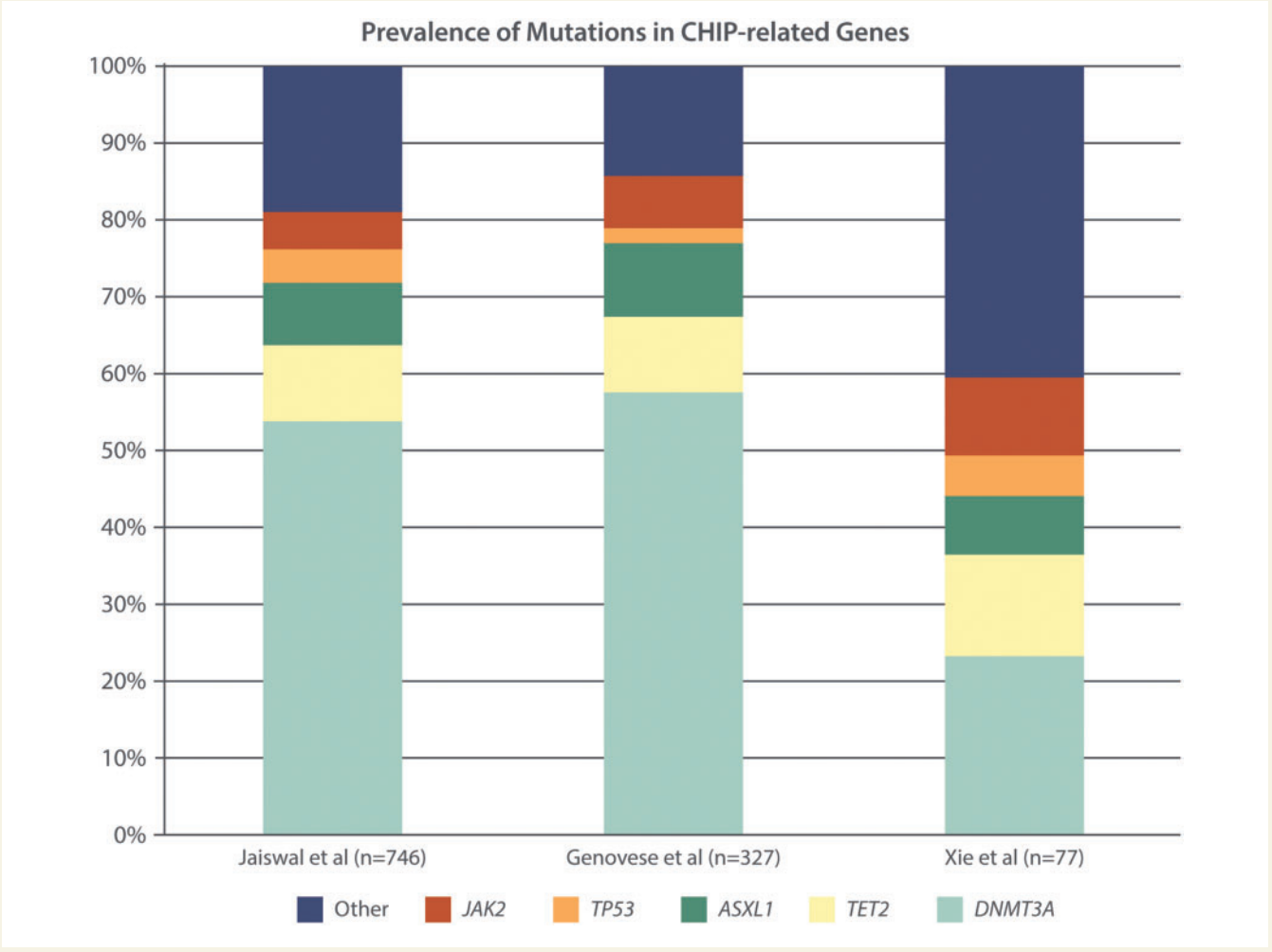

Figure 3 Prevalence of the most common mutations implicated in clonal haematopoiesis of indeterminate potential (CHIP) in three seminal studies.

malignancy, conferring a hazard ratio of 12.9 [95% confidence interval (CI) 5.8–28.7].[58]

Jaiswal *et al.* also report an age-related increase in observed frequency of clonal somatic mutations. In this blood-derived whole exome sequencing study of 17 182 individuals, they also reported that the majority of SNPs that were found in the same leukaemogenic genes (*DNMT3A*, *TET2*, and *ASLX1*, *Figure 3*). Not only was carrying these mutations associated with an increased risk of haematologic cancer but also all-cause mortality (hazard ratio 1.4, 95% CI 1.1–1.8). Exploratory analyses suggested an association with CAD (hazard ratio 2.0, 95% CI 1.2–3.4) and ischaemic stroke (hazard ratio 2.6, 95% CI 1.4–4.8) despite accounting for conventional risk factors.[59]

Notably, clonal haematopoiesis as detected by the presence of a pre-malignant mutation of variant allele fraction at least 2% in the absence of overt malignancy or other benign haematological conditions is termed 'clonal haematopoiesis of indeterminate potential' (CHIP).[60–62]

Recently, a whole genome sequencing study in Iceland demonstrated that clonal development has a heritable basis. In this study, a common 8-bp deletion in an intron of the telomerase reverse transcriptase gene (*TERT*) increases risk for developing clonal haematopoiesis, though the mechanism currently remains unclear.[63]

6. Clonal haematopoiesis of indeterminate potential and atherosclerosis

To examine the role of CHIP in CAD more closely, we exome sequenced 4726 participants with CAD and 3529 without known CAD, as defined by history of MI or revascularization from a total of four cohorts. As before, most mutations among those with CHIP were observed in *DNMT3A*, *TET2*, and *ASXL1*. CHIP carriers were found to have 1.9 times the risk of CAD when compared with non-carriers (95% CI 1.4–2.7; $P < 0.001$). Remarkably, despite being an age-associated condition, younger carriers were at 4.0 times the risk of early-onset MI when compared with non-carriers (95% CI 2.4–6.7; $P < 0.001$). Narrowing the pool of mutations to just *DNMT3A*, *TET2*, and *ASXL1* revealed that participants with these mutations had 1.7 to 2.0 times the risk of CAD compared with those without these mutations, and the p.V617F mutation in *JAK2* was noted to confer 12.1 times the risk. Similar results were found with early-onset MI. Furthermore, participants with CHIP mutations were noted to have 3.3 times higher amount of coronary artery calcification as non-carriers. Lastly, increased CHIP allele fraction, indicating a larger

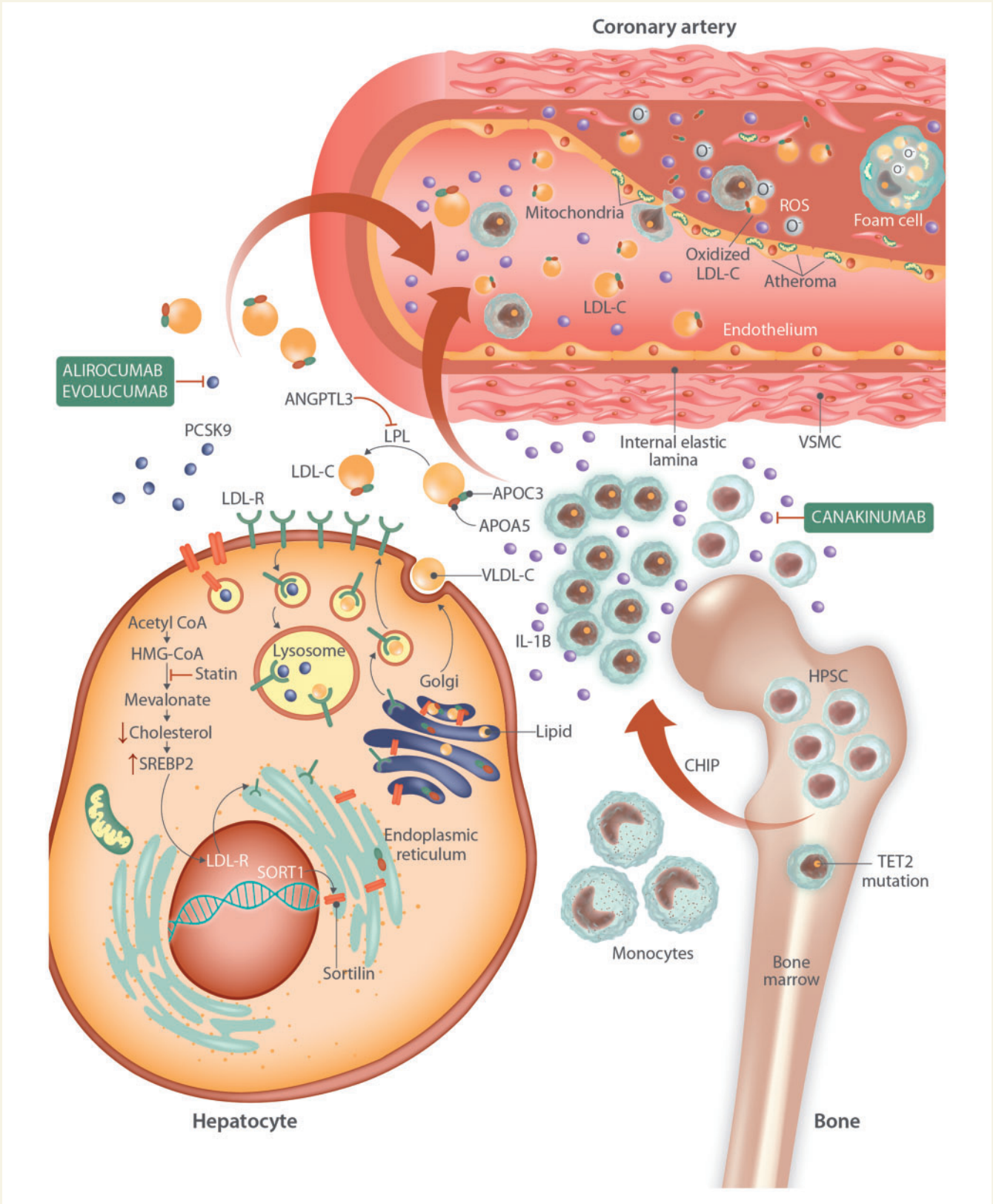

Figure 4 Interplay of well-known germline and somatic mutations in atherogenesis. In the liver, cholesterol biosynthesis begins with acetyl-CoA, and intracellular cholesterol levels are regulated with assistance the SREBP2 pathway. Statins interfere with cholesterol synthesis by inhibiting HMG-CoA reductase, leading to a drop in intracellular cholesterol levels, synthesis of more LDL receptors, and increased uptake of LDL cholesterol from the circulation. PCSK9 molecules help regulate the number of LDL receptors on the cell surface by aiding in their uptake from the cell membrane and transport to the lysosome for degradation. Evolocumab and alirocumab inhibit the activity of PCSK9, allowing more LDLR-mediated uptake of serum cholesterol. Sortilin expression, mediated by enhancers at the chromosome 1p13 locus, promotes release of mature VLDL particles into the circulation, PCSK9 secretion, and macrophage lipid accumulation leading to foam cell formation. In the circulation, various forms of lipoproteins combined with lipid molecules

clone size, correlated with greater effect size as well. Patients with CHIP variant allele fraction >10% had 2.2 times the risk of having CAD, compared with 1.4 times the risk seen in those with variant allele fraction of less than 10%.[64]

To further assess for causality, we investigated the role of *TET2*, a gene involved in DNA methylation and transcription regulation, further in a mouse model of hypercholesterolaemia and atherosclerosis. *Ldlr*-/- mice were irradiated and transplanted with bone marrows of *Tet2*-/- mice or *Tet2*+/+ mice. The hypercholesterolaemic mice with transplanted *Tet2* deficient bone marrow had twice the size of aortic root median atherosclerotic lesion size compared with hypercholesterolaemic mice transplanted with wild-type bone marrow. An intermediate phenotype was observed with *Tet2*+/− bone marrow *Ldlr*-/- mice.

To more specifically assess the consequence of knocking out *Tet2* on macrophage function, the above experiment was repeated with bone marrow from mice with just myeloid lineage *Tet2* knock out constructed using a Cre-Lox system; the lesion sizes in these mice were still observed to be 1.7 times as large as those of controls at 10 weeks. *Ex vivo* analysis showed that inflammatory cytokines and chemokines were up-regulated in *Tet2*-/- macrophages vs. *Tet2*+/+ macrophages. The human cytokine analogue of CXC (i.e. IL-8) was subsequently found to be higher in humans with *TET2*-associated CHIP compared with unaffected individuals.[64]

Fuster *et al.* created a model of competitive bone marrow transplantation with 10% *Tet2* deficient HSPCs and 90% *Tet2* wild type in place of irradiated bone marrows of *Ldlr*-/- mice. They demonstrated that the *Tet2* bone marrow deficiency had rapid clonal expansion without affecting cell counts, weight, spleen weight, glucose, or cholesterol levels. They further demonstrated that hypercholesterolaemic mice with *Tet2* bone marrow deficiency had larger aortic root plaque burden compared with hypercholesterolaemic mice with transplanted wild-type bone marrow. *In vitro* assays of macrophage activation showed significant alteration in inflammatory cytokine and chemokine expression between *Tet2*-/- and *Tet2*+/+ macrophages. Through various provocations, they showed that IL-1B may particularly link *Tet2* deficiency with atherosclerosis (*Figure 4*). Furthermore, inhibition of the NLRP3 inflammasome, an activator of IL-1B, mitigated atherosclerosis plaque size in the setting of *Tet2*-/- bone marrow in a murine model.[65]

7. Clonal haematopoiesis with chromosomal alterations

Apart from SNPs (mutations typically observed in CHIP) leading to cell's increased proliferation, age-related large clonal chromosomal alterations have also been observed in blood cells. Jacobs *et al.*[66] studied 31 717 cancer cases and 26 136 cancer-free controls from 13 GWAS and found significant aneuploidy or copy neutral LOH in a subset of clones. Like with CHIP, age remains the strongest predictor of mosaic chromosomal abnormalities. Frequency increased from 0.23% for cancer free individuals under age 50 years to 1.91% in individuals between age 75 and 79 years. Furthermore, these abnormalities were noted to be more common in those with solid tumours.[66]

Although external and stochastic factors may influence the development of somatic large chromosomal alterations, Loh *et al.*[67] recently demonstrated that germline genetic variation also plays a role. They studied 8342 mosaic chromosomal alterations ranging in size from 50 kb to 249 Mb in blood DNA from 151 202 individuals from UK Biobank. Using phase-based computational techniques, they identified three germline genomic loci (*MPL*, *TM2D3*/*TARSL2*, and *FRA10B*) associated with clonal chromosomal mosaicism in blood cells. They demonstrated that chromosomal alterations of trisomy 12, 13q, and +3/3q+ correlated strongly with incidence of CLL after 1 year; similarly 9p abnormalities were linked with risk of myeloproliferative neoplasms. Additionally, this condition was strongly linked with increased overall mortality.[67]

Despite these multiple associations of mosaic chromosomal abnormalities identified with cancers, less is known regarding associations with cardiovascular disease.[67] Bonnefond *et al.*[68] previously studied the role of large chromosomal clonal mosaic events and Type 2 diabetes. They examined chromosomal alterations in 7659 individuals, 2208 of which had diabetes, using DNA arrays and reported a significant association between the presence of alterations and diabetes (odds ratio 5.3, P 5.1 $\times$ 10^{-5}). In secondary analyses among diabetics, they noted that those with large chromosomal clonal mosaicism had a higher prevalence of microvascular or macrovascular complications compared with those without these alterations (19/26 vs. 810/2182, P 7.7 $\times$ 10^{-4}). It is currently unclear whether such clonal chromosomal abnormalities are the cause or consequence of Type 2 diabetes.[68]

8. Future directions

With ongoing evolution in our understanding of the complex genetic underpinnings of CAD, more questions and opportunities continue to arise. Just as understanding of principles of tumourigenesis have helped reveal novel mechanisms of atherogenesis, the discovery techniques and therapeutics from the genetic study of CAD can be applied more broadly to cardio-oncology.

8.1 Germline directions

8.1.1 Discovery

With advancement in technology and lower costs, large-scale genetic sequencing and genotyping is becoming more ubiquitous across research, clinical, and direct-to-consumer settings.[69] Large consortia

transport their contents throughout the body. APOA5 and APOC3 on VLDL particles participate in triglyceride metabolism and inhibit lipoprotein lipase (LPL), respectively. ANGPTL3 also inhibits LPL, leading to increased circulating levels of cholesterol and triglycerides, which are deposited in the endothelium of the vasculature. In the bone marrow, somatic mutations in *TET2* lead to hyperproliferative advantage for a subset of haematopoietic pluripotent stem cells (HPSCs), leading to clonal haematopoiesis of indeterminate potential (CHIP). The clonal monocytes that result from further replication produce an abundance of IL-1 beta, which promotes further inflammatory cascades and is in part inhibited by canakinumab. These macrophages adhere to the lipid-rich endothelium and traverse it. Within the vessel wall, mitochondrial dysfunction and generation of ROS leads to oxidation of LDL, generation of more inflammatory cytokines, and further damage to surrounding cells. Macrophages consume this oxidized LDL to become foam cells. Vascular smooth muscle cells (VSMCs) proliferate, damaged cells apoptose, and the atheroma continues to grow.

of researchers are comprehensively cataloguing the human germline genome and a multitude of somatic mutations responsible for causing cancer.[70–72] In pursuit of precision medicine, many national health systems and academic medical centres are amassing hundreds of thousands of samples of blood and tissue from patients.[73–76] With increasing sample sizes and increasing diversity, new genes and genetic variants related to cardiovascular disease and susceptibility to cardiovascular toxicities from oncologic therapies will be discovered.

8.1.2 Biological elucidation

GWAS already identified hundreds of new SNPs related to CAD and its risk factors in large populations. Most associated SNPs are non-coding (intergenic or intronic) and fall in regions of linkage disequilibrium extending tens of thousands of bases. These regions often contain many protein-coding genes. A major challenge at each GWAS locus is the identification of the culprit variant and gene as well as the mechanistic relationship between the two responsible for the associated outcome. Targeted sequencing has been applied with limited success in identifying novel loci within these regions.[77] Scalable, systematic approaches robust to diverse biological processes are urgently needed and require interdisciplinary collaboration.[78]

8.1.3 Risk prediction

With the ongoing accumulation of knowledge of SNP and disease association, polygenic risk scores for age-agnostic risk prediction are increasingly improving. Polygenic risk scores can be applied to identify individuals at future risk for virtually any heritable condition, including CAD, orthogonal to conventional approaches.[79] Similarly, variation in biomarkers and SNPs have been used to predict risk of anthracycline-induced cardiotoxicity risk, and this can further be expanded to other oncologic therapies involved in cardiovascular disease development.[80,81] With respect to CAD, genetic predisposition may also influence anticipated response to preventive strategies.[82] Continued study of the genetic basis of CAD in non-Europeans will improve polygenic risk prediction in these groups. Furthermore, prospective clinical trials testing the clinical efficacy of polygenic risk scoring are required.

8.2 Somatic directions

8.2.1 Discovery

By definition, CHIP requires the presence of leukaemogenic mutation in a previously implicated gene. However, many individuals have evidence of clonal haematopoiesis without driver mutations in known genes offering opportunities to discover newly implicated genes.[59] Additionally, while our understanding of the relationship between CHIP and CAD continues to progress, the clinical significance of clonal chromosomal mosaicism with respect to CAD is still less clear. Clonal selection and associated oncogenic somatic mutations were recently observed across tissue types in asymptomatic individuals[83] but whether this phenomenon is associated with additional risk for cardiovascular disease is currently not known. Furthermore, various chemotherapies, including platinum-based[84,85] and antimetabolite chemotherapies,[86] growth factor inhibitors,[87] and radiation therapy[88,89] are linked to increased incidence of vascular ischaemia but the contribution of DNA damage and mutagenesis is unknown.

8.2.2 Biological elucidation

Recent studies highlighted the role of *TET2* in atherosclerosis. However, the putative atherogenic mechanisms from other CHIP genes warrants further investigation. *DNMT3A*, a DNA methyltransferase and the most commonly mutated of the CHIP genes, is involved in *de novo* DNA methylation and epigenetic regulation in development, and mutations in the gene have been liked to acute myeloid leukaemia.[90,91] Inflammation-related pathways are implicated in *TET2* downstream signalling, however, it remains to be discovered if any other processes are found to contribute to CHIP-related atherogenesis.

Despite being a strong risk factor for age-related diseases, a minority of those with CHIP develops haematologic malignancy or CAD. Discovery and characterization of the predictors of CHIP-related clinical consequences may help clarify mechanistic relationships. For example, whole blood or single cell RNA-seq complemented by DNA-seq assessed longitudinally may help elucidate the biological processes linked to driver mutation onset, clonal expansion, and ultimate clinical outcome. Additional biochemical profiling may provide additional insights. To verify alterations are not reflective of reverse confounding, causal inference analyses using conventional and genetic epidemiology approaches and experimental studies in model systems will likely be necessary.

Furthermore, although we identify CHIP based on a set of commonly identified mutations in genes involved in haematologic malignancies, the clones that these mutated transcriptional regulators lead to have a host of passenger variants that also become clonal by proxy. Whether such passenger variants, independent from clone size, influence CAD risk is currently unknown.

Further knowledge of mechanistic insight of cytokines in CAD as seen in CHIP may also lead to broader connections of inflammation in atherosclerosis. This has the potential to further refine our understanding of the accelerated atherosclerosis in patients with human immunodeficiency virus (HIV) infection and autoimmune conditions such as psoriasis, where inflammation is presumed to be a unique driving force of atherosclerosis.

8.2.3 Risk prediction

It is now clear that the genetic basis for CAD extends from the germline to somatic genome. Currently, clinical genetic testing for CAD is largely restricted to gene panel testing for familial hypercholesterolaemia. Our work demonstrates that, at least among those with early-onset MI, the prevalence of CHIP and MI risk conferred is similar to that of familial hypercholesterolaemia.

Separately, array-based technologies are now implemented in the calculation of polygenic risk scoring to quantify heritable risk of CAD. However, this approach neglects the contribution of well-established Mendelian risk mutations and the newly-recognized influence of rare somatic clonal mutations. Whole genome sequencing offers the opportunity to most comprehensively quantify CAD genetic risk across germline and somatic variant classes. With technological advances and reductions in cost, implementation and interpretation of whole genome sequences for CAD risk will become increasingly feasible.

8.3 Therapeutics

8.3.1 Germline

The discovery of genes implicated in CAD has prompted the development of new therapeutic agents. For example, PCSK9 inhibitors reduce LDL cholesterol, and consequently, cardiovascular disease events.[92,93]

Agents targeting other recently discovered targets such as *ANGPTL3* are in active clinical trials.[94,95] Better mechanistic understanding of other germline targets may further identify orthogonal therapeutic strategies.

8.3.2 Somatic

Knowledge of CHIP carrier status may influence statin decisions as other similarly associated 'risk-enhancing' factors.[96] Whether those with CHIP have a different relative benefit from statins vs. those who do not requires further study.

Screening for genetic mutations in tumour samples is increasingly being used in oncology to more appropriately match patients to therapies and guide clinical trials.[97–99] With further knowledge of CHIP-related and other potential somatic mutations involved in atherosclerosis, a similar testing panel may help guide a personalized approach to managing a particular individual's CAD risk.

Vitamin C therapy was recently shown to mimic *TET2* restoration in otherwise deficient cells undergoing aberrant self-renewal via promoting DNA demethylation, differentiation, and cell death.[100] In CHIP clones where *TET2* is the driver, vitamin C therapy may also help curtail the implicated downstream cardiovascular pathology.

Since IL-1B is believed to be a key contributor to *TET2* deficiency-driven atherosclerosis, inhibition of IL-1B may be particularly efficacious for those with CHIP. In the CANTOS trial, canakinumab, an inhibitor of IL-1B, was shown to reduce recurrent cardiovascular risk among 10 061 patients with prior MI and elevated high-sensitivity C-reactive protein.[101] Preliminary analyses within the CANTOS trial suggests that individuals with CHIP and *TET2* mutations, experience a greater relative clinical benefit from canakinumab.[102]

Finally, genomic editing research in the past decade has led to significant breakthroughs leading to more efficient and accurate editing methods. Use of platforms such as CRISPR/cas9 paired with the appropriate delivery apparatus to implicated somatic cells may be curative in diseases stemming from somatic mutations, such as CHIP and atherosclerosis.[103]

9. Conclusions

Studying genetic variation and its association with CAD permits unbiased evaluation of CAD risk, development and progression, and treatment approaches. Germline associations identified through GWAS and next-generation sequencing continue to yield important insights. More recently, age-related clonal haematopoiesis with pre-leukaemic mutations was shown to influence CAD broadening the genetic basis of CAD to the somatic genome. Efforts to improve understanding of the genetic contribution to CAD can continue to advance our understanding of CAD.

Conflict of interest: P.N. reports grants from Amarin, Amgen, and Boston Scientific, and consulting income from Apple.

Funding

P.N. is supported by a grant from the National Heart, Lung, and Blood Institute (K08HL140203) and from a Massachusetts General Hospital Hassenfeld Scholar award.

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