Genetic loci associated with coronary artery disease harbor evidence of selection and antagonistic pleiotropy

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Abstract

Traditional genome-wide scans for positive selection have mainly uncovered selective sweeps associated with monogenic traits. While selection on quantitative traits is much more common, very few signals have been detected because of their polygenic nature. We searched for positive selection signals underlying coronary artery disease (CAD) in worldwide populations, using novel approaches to quantify relationships between polygenic selection signals and CAD genetic risk. We identified new candidate adaptive loci that appear to have been directly modified by disease pressures given their significant associations with CAD genetic risk. These candidates were all uniquely and consistently associated with many different male and female reproductive traits suggesting selection may have also targeted these because of their direct effects on fitness. This suggests the presence of widespread antagonistic-pleiotropic tradeoffs on CAD loci, which provides a novel explanation for the maintenance and high prevalence of CAD in modern humans. Lastly, we found that positive selection more often targeted CAD gene regulatory variants using HapMap3 lymphoblastoid cell lines, which further highlights the unique biological significance of candidate adaptive loci underlying CAD. Our study provides a novel approach for detecting selection on polygenic traits and evidence that modern human genomes have evolved in response to CAD-induced selection pressures and other early-life traits sharing pleiotropic links with CAD.

Author Summary

How genetic variation contributes to disease is complex, especially for those such as coronary artery disease (CAD) that develop over the lifetime of individuals. One of the fundamental questions about CAD — whose progression begins in young adults with arterial plaque accumulation leading to life-threatening outcomes later in life — is why natural selection has not removed or reduced this costly disease. It is the leading cause of death worldwide and has been present in human populations for thousands of years, implying considerable pressures that natural selection should have operated on. Our study provides new evidence that genes underlying CAD have recently been modified by natural selection and that these same genes uniquely and extensively contribute to human reproduction, which suggests that natural selection may have maintained genetic variation contributing to CAD because of its beneficial effects on fitness. This study provides novel evidence that CAD has been maintained in modern humans as a by-product of the fitness advantages those genes provide early in human lifecycles.

Introduction

It is well established that modern human traits are a product of past evolutionary forces that have shaped heritable phenotypic and molecular variation, but we are far from understanding what diseases have driven natural selection and how this process has left its imprint across the genome. Although many recent genome-wide multi-population scans have searched for signatures of positive selection [1-9], these studies have detected few signals of selection on candidate loci associated with traits or diseases [10-12]. This suggests that classic 'selective sweeps' have been relatively rare in recent human history [13, 14] and that the tools currently used miss most of the smaller selection signals caused by diseases associated with polygenic traits [12]. This limits our understanding of how natural selection has acted on variation underlying complex diseases. In this study, we aimed to comprehensively identify positive selection signals underlying coronary artery disease (CAD) loci with methods designed to detect signals of recent positive selection. We also compared quantitative selection signals in 12 worldwide populations (HapMap3) with patterns of disease risk to identify signals of selection linked to CAD pressure.

Classic population genetics theory describes positive selection with the selective-sweep (or hard-sweep) model, in which a strongly advantageous mutation increases rapidly in frequency (often to fixation) resulting in reduced heterozygosity of nearby neutral polymorphisms due to genetic hitch-hiking [15, 16] and a longer haplotype with higher frequency. Many methods have been developed to detect these signatures [17, 18], including traditional tests that detect differentiation in allele frequencies among population (i.e. Wright's fixation index, Fst [19]) and more recently developed within population tests for extended haplotype homozygosity (i.e. integrated haplotype score, iHS [9]). Some of the most convincing examples of human adaptive evolution have been uncovered for traits influenced by single loci with large effects. For example, the lactase persistence (*LCT*) and Duffy-null (*DARC*) mutations affecting expression of key proteins in milk digestion [10] and malarial resistance [20] both display hallmarks of selective sweeps. Other loci that are not clearly monogenic but also show selective sweeps are associated with high-altitude tolerance (*EPAS1* [21]) and skin pigmentation (*SLC24A5* and *KITLG* [22]). These previous studies showed that rapid selective sweeps occurred around loci where alleles that were previously rare or absent in populations had large effects on phenotypes.

Motivated by these initial successes and the increasing availability of global population data genotyped on higher resolution arrays (i.e. HapMap Project, 1000 Genomes Project), many genome-wide scans for candidate adaptive loci have recently been performed [11]. These studies suggest that selection may have operated on a variety of biological processes [10] in ways that differ among populations (i.e. local adaptation) [23], has been prevalent in genetic variation linked to metabolic processes [24], and may have often targeted intergenic regions and gene regulatory variants rather than protein-coding regions [12]. However, only the larger signals underlying monogenic traits are typically captured due to the lack of statistical power imposed by the need to correct for genome-wide multiple testing [18]. Most of these candidates also are not yet convincing due to inconsistencies between studies that utilized the same data [14], cannot be validated due to the absence of biological or functional information [25, 26], and perhaps because selective sweeps have actually been rare in human populations [27, 28].

In contrast to population genetics, in quantitative genetics rapid adaptation typically involves selection acting on quantitative traits that are highly polygenic [29, 30]. Under the 'infinitesimal (polygenic) model', such traits are likely to respond quickly to changing selective pressures through smaller frequency shifts in many polymorphisms already present in the population [13, 31]. Such alleles would not necessarily sweep to fixation, would produce smaller changes in

surrounding heterozygosity, and would thus be hard to detect with most current population genetic methods [14, 26, 32]. Note that polygenic and classic sweep models are not mutually exclusive [13, 33], for alleles with small- and large-effects may both underlie a polygenic trait. Thus the degree to which candidate alleles will be detectable after a selective event will vary. Given that most common diseases are highly polygenic [34], this suggests a need to improve how we detect and understand adaptive signatures in the loci associated with polygenic traits.

Recent selection studies investigating polygenic traits have taken two approaches. The first scans for significant selection signals within genome-wide significant disease effect SNPs. For example, Ding and Kullo [35] found significant population differentiation (Fst) for 8 of 158 index SNPs underlying 36 cardiovascular disease phenotypes, and Raj et al. [36] observed elevated positive selection scores (Fst, iHS) for 37 of 416 index susceptibility SNPs underlying 10 inflammatory-diseases. The second approach tests if aggregated shifts in genome-wide significant allele frequencies are associated with phenotypic differences by population, latitudinal, or environmental gradients, which might indicate local adaptation. For example, Castro and Feldman [37] used 1300 index SNPs underlying many polygenic traits and found elevated adaptive signals (Fst and iHS) above background variation, and Turchin et al. [38] demonstrated moderately higher frequency of 139 height-increasing alleles in a Northern (taller) compared to Southern (shorter) European populations. These approaches all assume that the variants with the most significant p values are the most probable selection targets, but many if not most such variants are tagging tested or untested causal variants, which may themselves be of lower frequencies. This suggests an approach sensitive to more subtle signals of selection and disease risk is needed for polygenic selection.

We chose CAD as a model for examining polygenic selection signals underlying complex disease because it has (and continues to) impose considerable disease burden (selection pressure) in humans [39], its underlying genetic architecture has been extensively studied [40, 41] and many of its risk factors (cholesterol, blood pressure) have been under recent natural selection [42] related to potential pleiotropic effects or tradeoffs with CAD. Antagonistic pleiotropy describes gene effect on multiple linked traits where selection on one may cause fitness tradeoffs (i.e. disease, survival) in the other due to their negative genetic association [43]. Two common misconceptions are that CAD is exclusively late age of onset and only occurs at appreciable frequency in contemporary humans. If that were true, selection might not have had either the opportunity or sufficient time to affect genetic variation associated with CAD. However, CAD manifests early in life [44, 45] and can be detected even in adolescence through degree of atherosclerosis [45, 46] and myocardial infarction events [47]. CAD is also a product of many heritable risk factors (cholesterol, weight, blood pressure) whose variation is expressed during the reproductive period, when CAD could drive selection directly or indirectly. Furthermore, CAD has impacted human populations since at least the ancient Middle Kingdom period, with studies finding the presence of atherosclerosis in Egyptian mummies [48]. This suggests that there has been enough time for genomic signatures of selection related to CAD to develop and be detectable in modern humans.

By combining several 1000 Genomes-imputed datasets including HapMap3 and Finnish SNP data, a large genetic meta-analysis of CAD, and HapMap3 gene expression data, we sought to address the reason(s) why CAD exists in humans by answering the following questions: 1) Has selection recently operated on CAD loci 2) How do selection signals underlying CAD loci vary among populations and are they enriched for gene regulatory effects? 3) Do candidate adaptive signatures overlap directly with CAD genetic risk and is this useful for highlighting disease-linked selection signals? 4) Do CAD-linked selection signals display functional effects and

evidence of antagonistic pleiotropy, in that they are also linked to biological processes or traits influencing reproduction?

Results

To test for selection signals for variants directly linked with CAD, we utilized SNP summary statistics from 56 genome-wide significant CAD loci in Nikpay et al. [41], the most recent and largest CAD case-control GWAS meta-analysis to date, to identify 76 candidate genes for CAD (**Supplementary Materials and Methods**). Nikpay et al. used 60,801 CAD cases and 123,504 controls from a mix of individuals of mainly European (77%), south (13% India and Pakistan) and east (6% China and Korea) Asian, Hispanic and African American (~4%) descent with genetic variation imputed to a high-density using the 1000 Genomes reference panel. By investigating all SNPs in candidate CAD genes, we aimed to improve detection of smaller polygenic selection signals for the range of functional genic variants and short-range intergenic regulatory variants that would be missed with approaches that only consider genome-wide significant SNPs.

Signals of positive selection within coronary artery disease loci

We utilised the integrated Haplotype Score (iHS) to estimate positive selection for each SNP underlying candidate CAD genes within each population separately. Because iHS is typically used to detect candidate adaptive SNPs where the selected alleles may not have reached fixation [9], this estimate is well suited for detecting recent signals of selection as opposed to other measures [18]. iHS is also better suited for detecting selection acting on standing variation in polygenic traits [18, 49].

Candidate selection signals were found for many of the 76 CAD genes within each of the 12 worldwide populations (11 HapMap3 populations and Finns; Fig. 1A for top 40 based on their association with CAD log odds genetic risk, Fig. S1 for all 76). These were defined as 'peaks' of significantly elevated iHS scores across SNPs within each gene-population combination, with the apex approximating the likely positional target of positive selection.

In the sample of all populations (Fig. 1A, largest iHS scores), most candidate selection signals were relatively small, but a few larger signals were detected. For example, out of the 912 gene-by-population combinations (Fig. S1), 354 (38%) contained weak-moderate candidate selection signals (significant iHS between 2-3), 84 (9%) contained moderate-strong signals (significant iHS between 3-4), and 6 (0.6%) had very strong signals (significant iHS > 4). The 6 largest selection signals were found in the following gene-population combinations: *BCAS3* in GIH (iHS=4.45), MEX (iHS=4.23) and CEU (iHS=4.86), *PEMT* in MKK (iHS=4.24), *ANKS1A* in LWK (iHS=4.03), and *CXCL12* in JPT (iHS=4.10), with all iHS p values <0.0001. Six genes (*BCAS3*, *SMG6*, *PDGFD*, *KSR2*, *SMAD3*, *HDAC9*) exhibited candidate selection signals consistently within all populations (Fig. 1A), and many genes also contained consistent selection signals for all populations within similar ancestral groups (e.g. African, European etc, Fig. 1A).

Within CAD genes, multiple candidate selection signals were sometimes present (particularly within larger genes, within separate linkage disequilibrium (LD)-blocks); these varied between and sometimes within a population. For example, in *PHACTR1* (~0.57mb in size, 14 introns)

there are three main candidate selection signals in introns 4, 7 and 11 (see Fig. S2, comparing cross-population selection signals in *PHACTR1*) that were in separate LD-blocks (see Fig. 3C, LD plots). Within most populations, there was a broad and relatively weak set of candidate selection signals in intron 4 (the largest *PHACTR1* intron, ~300kb in length). Intron 4 is also the location of the published CAD index SNP (rs9369640) for *PHACTR1*. Three of the African populations had the highest iHS score for the same SNP in intron 4 (rs8180558) including ASW (iHS=2.4, P<0.05), LWK (iHS=2.8, P<0.01) and YRI (iHS=2.2, P<0.05), which is ~18kb upstream from the index CAD SNP (r^2 between rs8180558 and rs9369640 in *PHACTR1*: ASW=0.12; LWK=0.03; YRI=0.04). Peaks of *PHACTR1* selection signals within the three Asian populations were at rs4715043 in CHB (iHS=2.3, P<0.05) and rs6924689 in both CHD (iHS=2.9, P<0.01) and JPT (iHS=3.0,P<0.01). The GIH population contained the largest selection signal, also in intron 4, with an apex at rs4142300 (iHS=3.7, P<0.001, 75kb downstream of/r^2=0.07 with index CAD SNP rs9369640). This corresponded with the same apex SNP in intron 4 for TSI, though the TSI signal was weaker and non-significant (rs4142300, iHS=1.84); rs4142300 was also close to the apex SNP in CEU (rs9349350, iHS=2.0, P<0.05, r^2=0.92) and MEX (rs2015764, iHS=2.1, P<0.05, r^2=0.30). Other significant candidate selection signals were also present in intron 7 for three of the African populations (ASW, LWK, MKK), the CHD and GIH populations, with the largest intron 7 signal within MKK (SNP rs13191209, iHS=3.0, P<0.001). The last significant candidate selection signal within *PHACTR1* was found within intron 11 with the largest signal at rs9349549 (MKK iHS=2.9, P<0.01; CEU iHS=2.7, P<0.01; TSI iHS=3.0, P<0.01). Other interesting candidate selection signals present in other CAD genes (Fig. S1) are not discussed here. Such patterns suggest that candidate selection signals are complex and often do not correspond to the alleles with largest effect on CAD.

Relationship between CAD genetic risk and selection across populations

For each CAD gene within each population, we used a mixed effects linear model to regress SNP-based estimates of CAD log odds genetic risk (ln(OR), obtained from cardiogramplusc4d.org) against iHS selection scores (**Supplementary Materials and Methods**). We accounted for LD structure by including the first eigenvector from an LD matrix of correlations (r^2) between SNPs within each gene as a random effect.

For a subset of CAD loci, we found significant quantitative associations between disease risk and selection signals and for each of these the direction of this association was often consistent between populations (Fig. 1B). Furthermore, when compared to a null distribution of genes selected randomly from the genome, the strength of the CAD log odds versus selection signal at most loci was statistically significant (Fig. 1C). Fig. 1B shows 40 genes ranked based on those that showed the most consistent number of significant associations across the 12 populations, with those that showed fewer than four significant associations excluded. Positive and negative associations indicate elevated selection signals present in regions with higher or lower CAD log odds genetic risk, respectively.

In the comparison across populations, directionality of significant selection-risk associations tended to be most consistent for populations within the same ancestral group (Fig. 1B). For example, in *PHACTR1*, negative associations were present within all European populations (CEU, TSI, FIN), and in *NT5C2* strong positive associations were present in all East Asian populations (CHB, CHD, JPT). Other negative associations that were consistent across all populations within an ancestry group included five genes in Europeans (*COG5*, *ABO*, *ANKS1A*, *KSR2*, *FLT1*) and four genes (*LDLR*, *PEMT*, *KIAA1462*, *PDGFD*) in East Asians.

Additional consistent positive associations included four genes (*CNNM2*, *TEX41*, *NT5C2*, *MIA3*) in East Asians, three (*BCAS3*, *RAI1*, *KCNK5*) in Europeans, and one (*PPAP2B*) in Africans. In comparison to other ancestral groups, African populations showed fewer significant selection-risk associations (27.9% of all 76-gene x 12-population combinations) than Asians (31.5%) or Europeans (32.8%). Some associations were consistent in all but one population (e.g. *CNNM2*, *ABCG8* in Europeans; *BCAS3*, *KCNK5* in Asians; *CNNM2*, *TEX41* in Africans) or unique to one population within an ancestral group (e.g. *TEX41* in FIN, *COG5* in ASW).

Below we focus on *BCAS3* (Fig. 2) and *PHACTR1* (Fig. 3), two of the strongest selection-risk associations which, when adjusting for LD (**Supplementary Materials and Methods**), displayed varying directionality between at least two populations.

Genetic risk of CAD vs positive selection in BCAS3

The genetic risk of CAD for variants in *BCAS3* were positively correlated with an extremely large candidate adaptive signal in all European and two of three East Asian populations (Fig. 1B). For example in CEU, the largest iHS score was 4.85 and highly significant, and was elevated across most of *BCAS3* (Fig. 2B CEU, spanning introns 1-18 and various LD-blocks, Fig. 2C), which matched the approximate trends in CAD log odds giving rise to a highly significant positive correlation (Fig. 2A CEU). In contrast, in YRI there was no detectable selection signal close to the index SNP (Fig. 2B YRI), but weak-moderate signals were present towards the end of *BCAS3* (Fig. 2B YRI, introns 18-19, smaller LD-blocks Fig. 2C), which also corresponded with lower CAD log odds (Fig. 2B, YRI) thus giving rise to a significant negative correlation in Fig. 2A.

Genetic risk of CAD vs positive selection in PHACTR1

For all European populations, *PHACTR1* (see CEU example, Fig. 3A) selection peaks were typically located within regions of consistently lower CAD log odds (Fig. 3B). This contrasted with most other non-European populations where the highest candidate selection peaks were located within regions with elevated CAD log odds (including the index CAD SNP rs9369640, intron 4). The largest selection peak in GIH (Fig. 3B) overlapped the CAD log odds peak in *PHACTR1* giving rise to the strong positive association seen in Fig. 3A. The two distinctive selection peaks in both CEU and GIH were separated by different LD-blocks (Fig 3C), suggesting that these may have developed independently within *PHACTR1*. Interestingly, the negative association found for the MKK population was due to the location of the selection peaks more closely matching those of the European populations in intron 11 (Fig. S2).

Enrichment of gene regulatory variants under selection at CAD loci

To establish whether variants with evidence of selection in CAD genes also showed evidence of function, we performed an eQTL scan in 8 HapMap3 populations with matched LCL gene expression. We compared all SNPs in each CAD locus against expression for each focal gene within each population. We found that SNPs with significant integrated Haplotype Scores (iHS) were often also involved in gene regulation, compared to SNPs with non-significant selection scores (Fig. 4, Kolmogorov-Smirnov test p value <0.001). To assess which biological pathways were enriched for the highest-ranked genes according to Fig. 1B, i.e. those where selection scores were most closely associated with CAD log odds genetic risk, we included the top 10 genes into the Enrichr analysis tool [50] and found that these genes are especially enriched in pathways related to metabolism, focal adhesion and transport of glucose and other sugars. More interestingly, we found connections to reproductive phenotypes in the associations of these genes with pathways, ontologies, cell types and transcription factors. For example, we found links to

ovarian steroidogenesis and genes expressed in specific cell types and tissues including the ovary, endometrium and uterus (see Table S4 for Enrichr outputs).

Discussion

This study has identified many candidate adaptive signals which suggests that selection on CAD loci is much more widespread than previously appreciated (also see Supplementary Discussion). It has previously been suggested [12] and demonstrated [51] that selection on gene expression levels has been an important element of human adaptation in general. We confirm this result for CAD associated loci. Positive selection signals within CAD loci were more likely than random SNPs to be associated with gene expression levels in *cis* (Fig. 4).

We found evidence that some of these signals may be a result of selection pressures induced directly by CAD itself. This finding is important for highlighting genes that may have been modified directly by selection on disease phenotypes and also for our general understanding of how quickly human genomes can respond to selection induced by changing environments. Subsequent biological process analyses and a thorough literature assessment (below) demonstrated that the loci most consistently associated with CAD genetic risk are also often linked to human reproduction, which suggests both their potential to respond to natural section and their possible role via antagonistic pleiotropy in the reproductive tradeoffs that would help to explain why CAD exists in human populations.

Coronary artery disease-induced changes to human genomes

One of our most interesting findings was the significant association between selection signals and CAD log odds genetic risk. This approach of integrating genome scans of positive selection with genome-wide genotype-phenotype data has been promoted previously as a tool to uncover biologically meaningful selection signals of recent human adaptation [12, 51] but has rarely been applied. Among the exceptions, Jarvis et al. [52] found a cluster of selection and association signals coinciding on chromosome 3 that included genes *DOCK3* and *CISH*, which are known to affect height in Europeans.

For highly-ranked genes (according to the number of significant associations present within the 12 populations) in Fig. 1B such as *BCAS3*, *CNNM2*, *TEX41*, *SMG6* and *PHACTR1*, the consistent overlap between selection and genetic risk of CAD suggests that many of these may have been modified by CAD-linked selective pressures. If so, then two conditions must have been met. Firstly, CAD was present for long enough to be involved in these genetic alterations, an evolutionary process which generally takes thousands of years. Indeed, precursors of CAD (i.e. atherosclerosis) are detectable in very early civilizations [48]. Secondly, the effects of CAD were directly or indirectly expressed during the reproductive period and trait variation was under natural selection due to its effects on reproductive success.

It is only possible for natural selection to directly act on CAD if those outcomes modify individual fitness relative to others in the same population. As outlined in the introduction, this is possible as CAD outcomes (i.e. myocardial infarction) do occur in young adults. However, early-life CAD outcomes are relatively rare, suggesting selection is more likely to operate indirectly on CAD via its risk factors (or other pleiotropically linked traits, discussed below), which provides a

more likely explanation for the close associations we found between positive selection and genetic risk. Supporting this, phenotypic selection has been found operating on CAD risk factors [42], suggesting that these selection pressures are still present in modern humans.

Some genes had large signals of selection but showed weak or no consistent overlap with CAD genetic risk. For example *HDAC9* (Histone Deacetylase 9) shows extensive evidence for having undergone recent selection within most populations, especially those of European or Mexican decent, but little or no overlap with CAD risk was evident in most populations. This suggests positive selection has operated on this gene due to its effects on a trait unrelated to CAD, which may not be surprising given *HDAC9*'s broad biological roles (as a transcriptional regulator, cell-cycle progression) and association with other very different phenotypes including ulcerative colitis [53] and psychiatric disorders [54]. This further demonstrates that this approach is useful for separating candidate selection signals important for the disease or phenotype of interest from those that aren't.

Pleiotropic effects that establish the genetic foundations of tradeoffs

To further investigate whether top candidate adaptive loci for CAD modify fitness or share pleiotropic links with other traits that may modify fitness, we performed an extensive systematic literature search on the 40 top-ranked genes in Fig. 1 and a random set of 20 genes. If they have been under selection recently, they might still be associated with reproductive variation (i.e. fitness) in modern environments. We found that all 40 CAD genes shared at least one (often more) connection with fitness (Table S1-S2). Some appear to directly influence fitness (offspring number, age at menarche, menopause, survival), while many were associated with early-life reproductive traits that are likely to indirectly correlate with fitness including variation in ability to fertilize/conceive or fetal growth, development and survival. To test the novelty of this, we randomly chose 20 genes that were approximately the same size as the top 20 genes in Fig. 1. We only found three (out of 20) random genes with at least one potential link with fitness (Table S3). This suggests there are unique pleiotropic links between CAD and traits that have likely been under selection earlier in life.

Evidence for direct links between CAD genes and fitness (Table S1-S2) included genes associated with reproductive (*PPAP2B*, [55]) or twinning (*SMAD3*, [56]) capacity and number of offspring produced (e.g. *KIAA1462*, [57], *SLC22A5*, [58]). *PHACTR1*, *LPL*, *SMAD3*, *ABO* and *SLC22A5* may contribute to reproductive timing (menarche, menopause) in women [59-61] and animals [62]. Expression of *PHACTR1* [63], *KCNK5* [64], *MRAS* and *ADAMST7* [65] appear to regulate lactation capacity. Some gene deficiencies also cause pregnancy loss (e.g. *LDLR*, [66], *COL4A2*, [67]). Evidence for antagonistic links were much more common and included these: 25 genes shared links with traits expressed during pregnancy (Table S1-S2), i.e. variation that can negatively influence the health and survival outcomes of both the fetus and mother [68]. For example, a variant of *CDKN2B-AS1* significantly contributes to risk of fetal growth restriction [69], both *FLT1* [70] and *LPL* [71] are significantly differentially expressed in placental tissues from pregnancies with intrauterine growth restriction (IUGR), and preeclampsia and *LDLR*-deficient mice had litters with significant IUGR [72]. A further 29 and 19 genes were linked to traits that can directly influence female and male fertility, respectively (13 influence both) (Table S1-S2). For example, *BCAS3* and *PHACTR1* are highly expressed during human embryogenesis [73, 74], *SWAP70* is intensely expressed at the site of implantation [75], and *PHACTR1* may play a role in receptivity to implantation [76]. For *ABCG8* and *KSR2,* animal models provide further support as gene expression deficiency can cause infertility in females (*ABCG8*, [77]) and males (*KSR2*, [78]).

Pleiotropic connections were also apparent in the classification of specific disorders or from studies investigating single-gene effects. For example, women with polycystic ovarian syndrome (PCOS) have higher rates of infertility due to ovulation failure and modified cardiovascular disease risk factors (i.e. diabetes, obesity, hypertension [79]). A number of CAD genes in this study (e.g. *PHACTR1*, *LPL*, *PDGFD*, *IL6R*, *CNNM2*) are found differentially expressed in PCOS women [80-84], suggesting possible links between perturbed embryogenesis and angiogenesis. In males, this can be demonstrated with a mutation in *SLC22A5* that causes both cardiomyopathy and male infertility due to altered ability to break down lipids [85, 86]. More generally, many recent studies link altered cholesterol homeostasis with fertility, which is most apparent in patients suffering from hyperlipidemia or metabolic syndrome [87, 88].

To facilitate interpretation of selection occurring on early-life traits or CAD phenotypic risk factors that share pleiotropic connections and possible evolutionary tradeoffs with coronary artery disease, we present a conceptual figure (Fig. 5). These pleiotropic effects are important because many of them affect traits expressed early in life, some extremely early in life. Any allele that increases reproductive performance enough early in life to more than compensate for a loss of associated fitness late in life will be selected [43]. Such a mechanism has been recently suggested to help explain the maintenance of polymorphic disease alleles in modern human populations [89]. Some previous studies have tested for such tradeoffs in humans using direct fitness-related phenotypes (e.g. [90]) although evidence for such a mechanism influencing human disease is currently lacking. Our approach examining antagonistic fitness effects for disease genes that displayed consistent selection-genetic risk associations in diverse worldwide populations provides support for such a mechanism influencing CAD. Here we have presented multiple cases in which such antagonistic pleiotropy appears to be present for genes associated with CAD, which may help to explain our vulnerability to the disease.

Study limitations

There are also some limitations to our approach. We utilized CAD genetic risk estimated from a meta-analysis based on predominantly European (77%) with smaller contributions from south/east Asian (19%), Hispanic and African American (~4%) ancestry [41]. Genetic risk variation for CAD might be different in the un-represented (i.e. Mexican) or less-represented (i.e. African) populations in this meta-analysis. If that were the case, it would reduce the usefulness of comparing selection and risk estimates in those populations. We also saw fewer significant selection-risk associations in the African populations (Fig. 1B), however this may be due to selection signals in the African populations being less obvious than those in East Asian and European populations, perhaps due to lesser linkage disequilibrium, as is consistent with results from previous studies [91]. Calculating disease risk and selection variation from populations within the same ancestral group might help resolve this, however it only represents a potential shortcoming for our cross-population analyses and not observations of antagonistic pleiotropy.

Summary

In this study, we found evidence that natural selection has recently operated on CAD associated variation. By comparing positive selection variation with genetic risk variation at known loci underlying CAD, we were able to identify and prioritize genes that have been the most likely targets of selection related to this disease across diverse human populations. That selection signals and the direction of selection-risk relationships varied among some populations suggests

that CAD-driven selection has operated differently in these populations and thus that these populations might respond differently to similar heart disease prevention strategies. The pleiotropic effects that genes associated with CAD have on traits associated with reproduction that are expressed early in life strongly suggests some of the evolutionary reasons for the existence of human vulnerability to CAD.

Methods

Defining loci linked to coronary artery disease

We started with the 56 lead index SNPs from Supplementary Table 5 in Nikpay et al. [41] corresponding to 56 CAD loci. When the index SNP was genic, all SNPs within that gene were extracted (using NCBI's dbSNP) including directly adjacent intergenic SNPs ±5000bp from untranslated regions (UTR) in LD>0.7 (with any respective genic SNP). When the index SNP was intergenic, that SNP and other directly adjacent SNPs ±5000bp and in LD>0.7 (with the index SNP) were extracted and combined with SNPs from the respective linked gene listed in Nikpay et al. [41] including SNPs ±5000bp from UTR regions in LD>0.7 with that gene. This resulted in SNP lists for 56 genes. To further explore other genes not directly connected with lead index SNPs, but that were found within the CAD loci identified by Nikpay et al. [41], we extracted SNPs within each of those genes (plus SNPs ±5000bp from UTR regions in LD>0.7 with that gene). This resulted in SNP lists for a further 20 genes, bringing the total number of candidate genes for CAD to 76.

The per-SNP log odds (ln(OR)) values for the 76 genes were obtained from Nikpay et al. [41] available at http://www.cardiogramplusc4d.org/downloads and used in the analysis described below.

Preparation of HapMap3 samples

Genotype data (1,457,897 SNPs, 1,478 individuals) were downloaded for 11 HapMap Phase 3 (release 3) populations (http://www.hapmap.org [92]) including: Yoruba from Ibadan, Nigeria (YRI), Maasai in Kinyawa, Kenya (MKK), Luhya in Webuye, Kenya (LWK), African ancestry in Southwest USA (ASW), Utah residents with ancestry from northern and western Europe from the CEPH collection (CEU), Tuscans in Italy (TSI), Japanese from Tokyo (JPT), Han Chinese from Beijing (CHB), Chinese in Metropolitan Denver, Colorado (CHD), Gujarati Indians in Houston, TX, USA (GIH), and Mexican ancestry in Los Angeles, CA, USA (MEX). We also included another HapMap3 population, the Finnish in Finland (FIN) sample (ftp://ftp.fimm.fi/pub/FIN_HAPMAP3 [93]). These data had already been pre-filtered, i.e. SNPs were excluded that were monomorphic, call rate < 95%, MAF<0.01, Hardy-Weinberg equilibrium P $<1x10^{-6}$ etc.

Before phasing and imputation, we performed a divergent ancestry check with flashpca [94] to check accuracy of population assignments, converted SNP data from build 36 to 37 with UCSC LiftOver (https://genome.ucsc.edu/cgi-bin/hgLiftOver), checked strand alignment in Plink v1.9 [95] to ensure all genotypes were reported on the forward strand, and kept only autosomal SNPs. To speed up imputation, data were first pre-phased with Shapeit v2 [96] using the duoHMM option that combines pedigree information to improve phasing and default values for window size

(2Mb), per-SNP conditioning sates (100), effective population size (n=15000) and genetic maps from the 1000 Genomes Phase 3 b37 reference panel (ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/).

Phased data were imputed in 5 Mb chunks across each chromosome with Impute v2 [97]. We then removed any multiallelic SNPs (insertions, deletions etc) from the imputed data and excluded SNPs with call rate < 95%, HWE P $<1x10^{-6}$ and MAF<1%. The final dataset was then phased with Shapeit v2, and alleles were converted to ancestral and derived states using python script. Ancestral allele states came from 1000 Genomes Project FASTA files and derived 6-primate (human, gorilla, orangutan, chimp, macaque, marmoset) Enredo-Pecan-Ortheus alignment [98] from the Ensembl Compara 59 database [99].

Estimating signatures of recent selection

Integrated Haplotype Score (iHS): Using the package rehh [100] in R version 3.1.3, per SNP iHS scores were calculated within each population (after excluding non-founders) using methods described previously [9]. iHS could not be calculated for SNPs without an ancestral state, or whose population minor allele frequency is <5%, or for some SNPs that are close to chromosome ends or large regions without SNPs [9]. Rehh was also used to standardize (mean 0, variance 1) iHS values empirically to the distribution of available genome-wide SNPs with similar derived allele frequencies. For analyses in the main text, we considered a SNP to have a candidate selection signal if it had an absolute iHS score > 2, a permuted p value <0.05, and was within a 'cluster' of SNPs that also had elevated iHS scores. Although permuting p values is computationally more intensive, it provides more flexibility to detect smaller selection signals that may be incorrectly classified with the more stringent Bonferroni correction that is often applied to these estimates. For the analyses described below, even though we only used iHS estimates for the SNPs defined in the CAD genes (and additional SNPs for permutation purposes), we calculated per-SNP iHS scores genome-wide (rather than locally, i.e. within 1MB regions around focal SNPs), for this provides more accurate estimates because final adjustments are made relative to other genome-wide SNPs of similar sized derived allele frequency classes. P values for iHS scores were permuted based on comparison of nominal p values against 10000 randomly selected estimates from within the same derived allele frequency classes.

Comparing CAD genetic risk and quantitative selection signals

We first tested the null hypothesis that there is no association between CAD genetic risk and signals of positive selection for CAD genes. For each gene within each population, we used a mixed effects linear model to regress SNP-based estimates of CAD log odds (ln(OR)) genetic risk against selection scores (iHS) resulting in 912 separate regressions. To account for LD structure (and potential confounding of highly correlated SNPs) within each gene, we also included the first eigenvector derived from an LD matrix of correlations (r^2) between SNPs within each gene as a random effect. We chose to model LD structure with mixed-effects models rather than LD-prune because for many genes, the sample would have been too small for regression analyses. Also, it would be very difficult to properly capture both selection and the CAD log odds peaks needed to compare these variables. We accounted for multiple testing by permuting p values for each regression based on comparing each nominal p value against 10000 permuted p values derived from shuffling iHS scores.

Genes were then ranked based on the number of significant associations summed across the 12

populations. The 40 genes with at least four or more significant associations are shown in Fig. 1B. To illustrate the positional architecture of these selection-risk associations, plots for selected highly-ranked genes are shown in Fig. 2-3. By demonstrating how CAD genetic risk peaks and valleys correspond to variation in the magnitude of selection scores (iHS), this allowed visual assessment of potential modifications made to the phenotype-genotype map by selective pressures imposed directly or indirectly by CAD. It also helped us localize selection peaks within genes and compare them between populations. Similar peaks suggested similar selection and different peaks suggested local adaptation. This way of presenting the results also allowed us to detect the smaller adaptive shifts in allele frequencies typically expected to underlie selection on polygenic traits.

We then tested a second null hypothesis: that the selection-risk associations using the CAD genes are not unique compared to non-CAD associated loci. For each of the 76 CAD genes, we randomly (without replacement) chose 100 genes of similar length across the genome and performed the same mixed effects regression procedure described above for each gene by population combination using both CAD log odds values from Nikpay et al. [41], iHS scores estimated from the SNP data, and the first LD eigenvector from SNPs within a gene. Permuted p values were derived by comparing the nominal p value for each CAD gene against the 100 null distribution p values from the non-CAD associated genes. Results are shown in Fig. 1C.

Identifying functional targets of selection

To examine whether candidate adaptive signals within each gene corresponded to a gene's regulatory variation, we regressed SNPs within focal genes and gender against that gene's probe expression levels, which had previously been quantified in lymphoblastoid cell lines using Illumina's Human-6 v2 Expression BeadChip for eight of the 12 populations [101]. While selection related to CAD may have targeted regulatory variants important for other tissues/cell-types, gene expression data was only available for this cell-type. Given the central importance of circulating lymphoblastoid cells in CAD and its risk factors, we might expect this cell type a good candidate to search for association between selection signals and regulatory variants important for these genes. The raw gene microarray expression data had previously been normalized on a log2 scale using quantile normalization for replicates of a single individual then median normalization for each population [101]. P values for each SNP-probe association were permuted using 10000 permutations by randomly shuffling gene probes expression. P values were then extracted for the most significant iHS score for each gene-population combination and compared to the same number of p values randomly drawn from different LD blocks underlying SNPs with non-significant iHS scores across each gene-population combination. A Kolmogorov-Smirnov test was used to compare the distribution of p values from each. To examine what biological processes were associated with the top ranked genes from Fig. 1, we uploaded the top 10 genes into Enrichr (http://amp.pharm.mssm.edu/Enrichr/) to define associated pathways (i.e. KEGG 2016, kegg.jp/kegg), ontologies (MGI Mammalian phenotypes, informatics.jax.org), cell types (Cancer cell line Encyclopedia, broadinstitute.org/ccle) and transcription factors (ChEA 2015, amp.pharm.mssm.edu/lib/chea.jsp).

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Author Contributions

Conceptualization, S.G.B. and M.I.; Methodology, S.G.B. and M.I.; Formal analysis, S.G.B. and Q.H.; Literature review, S.G.B.; Writing – original draft, S.G.B. and M.I.; Writing – review & editing, S.G.B., Q.H., L.G., S.R., G.A., S.C.S and M.I.; Visualization, S.G.B.; Funding acquisition, M.I.; Supervision, M.I.

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Figure legends

Figure 1. Association of coronary artery disease (CAD) genetic risk and positive signatures of selection in 12 worldwide populations. The 40 of 76 CAD genes investigated are shown that have at least four significant selection-risk associations in Panel B across all 12 populations. **Panel A.** Magnitude and significance of largest positive selection signal (integrated haplotype score, iHS) within each gene-population combination. P values (circles within squares) were obtained from 10000 permutations. Bonferroni corrected p-value limit also shown (α=0.05/76=0.000657) with closed circles. **Panel B.** Null hypothesis: no association between CAD genetic risk and positive selection, tested using mixed effects model with SNP estimates of CAD log odds genetic risk and iHS while accounting for gene LD structure as a random effect (first eigenvector from LD matrix per gene). Scaled regression coefficients were obtained directly from regressions, each p value from 10000 permutations. **Panel C.** Null hypothesis: association between genetic risk and positive selection for SNPs within CAD genes no different than non-CAD associated genes. Permuted p values were estimated by comparing each p value in Panel B against 100 nominal p values obtained by randomly choosing (without replacement) 100 non-CAD associated genes of similar size across the genome and using the same mixed effects model setup as described above. **Populations**. Grouped by ancestry, African (ASW, African ancestry in Southwest USA; MKK, Maasai in Kinyawa, Kenya; YRI, Yoruba from Ibadan, Nigeria; LWK, Luhya in Webuye, Kenya), East-Asian (CHB, Han Chinese subjects from Beijing; CHD, Chinese in Metropolitan Denver, Colorado; JPT, Japanese subjects from Tokyo), European (CEU, Utah residents with ancestry from northern and western Europe from the CEPH collection; TSI, Tuscans in Italy; FIN, Finnish in Finland), GIH (Gujarati Indians in Houston, TX, USA), MEX (Mexican ancestry in Los Angeles, CA, USA).

Figure 2. Quantitative links between coronary artery disease risk and selection signals in *BCAS3*. A. Correlation between selection signals (iHS) and coronary artery disease (CAD) log odds genetic risk (log odds, ln(OR)), both represented as absolute values. Red line/upper right value, β from mixed effects regression. **B.** Base pair positional comparison of selection signals and CAD genetic risk across *BCAS3*. Blue points, CAD log odds values; grey-orange or non-significant-significant points, iHS scores. Horizontal bar shows *BCAS3* gene (and intron) span and location of lead index SNP. Blue/orange lines are smoothed lines estimated with loess function in R. **C.** LD plots, r^2. Populations: CEU, Utah residents with ancestry from northern and western Europe from the CEPH collection; YRI, Yoruba from Ibadan, Nigeria.

Figure 3. Quantitative links between coronary artery disease risk and selection signals in *PHACTR1*. A. Correlation between selection signals (iHS) and coronary artery disease (CAD) log odds genetic risk (ln(OR)), both represented as absolute values. Red line/upper right value, β from mixed effects regression. **B.** Base pair positional comparison of selection signals and CAD genetic risk across *PHACTR1*. Blue points, CAD log odds values; grey-orange or non-significant-significant points, iHS scores. Horizontal bar shows *PHACTR1* gene (and intron) spans and location of index SNP if present. **C.** LD plots, r^2. Populations: CEU, Utah residents with ancestry from northern and western Europe from the CEPH collection; GIH, Gujarati Indians in Houston, TX, USA.

Figure 4: Comparing positive selection with gene regulation. Summary distribution of permuted eQTL p values for SNPs with (left) or without (right) a significant selection signal. SNPs with a significant selection signal (iHS) were chosen by taking the largest significant positive selection signal (if one was present) within each gene-population combination. The same number of SNPs without a significant selection signal were also randomly drawn across all gene-population combinations for comparison. These SNPs were used in an eQTL analysis where they were regressed (including gender as a covariate) against their associated gene probe's expression.

Figure 5. Conceptual figure of potential evolutionary tradeoffs between coronary artery disease (CAD) burden and other phenotypes as a consequence of antagonistic pleiotropy (AP) [43]. As a simple example, AP describes gene effect on two traits (pleiotropy) that oppositely (antagonistic) affect individual fitness at different ages. Selection on that gene conferring a fitness advantage and disadvantage at different ages depends on the size and timing of the effects. An advantage during the ages with the highest probability of reproduction (between~20-45 years of age in humans) would increase fitness (lifetime reproductive success) more than a similarly sized disadvantage at later ages would decrease it. This concept is part of the well-known evolutionary theory of ageing, which describes tradeoffs in energy invested into growth, reproduction and survival [102]. In the figure above, intense natural selection occurring on CAD loci as a result of fitness advantages (+ signs, red text callout box 1.) conferred by genetically correlated risk factors ('CAD risk factors' box) or early-life traits ('early-life traits' box) trades off with the deleterious effects of these genes on fitness (i.e. CAD burden) later in life (- sign, red text callout box 2.) where the intensity of selection is weak. This occurs because of the negative relationship between genetic effects on early vs late-life traits (- sign, red text callout box 3.), which could help explain the high prevalence and maintenance of CAD in modern human populations. Over shorter timescales, lifetime probability of CAD is modified by a combination of genetic and environmental risk factors (e.g. [103]). There is a good evidence that such antagonistic effects have operated on CAD loci given: significant associations between CAD genetic risk and selection we found (Fig 1-2); CAD genes also underlie many early-life traits known to modify fitness (Table S2); phenotypic selection has been found operating on CAD phenotypic risk factors [42].

Figure 1

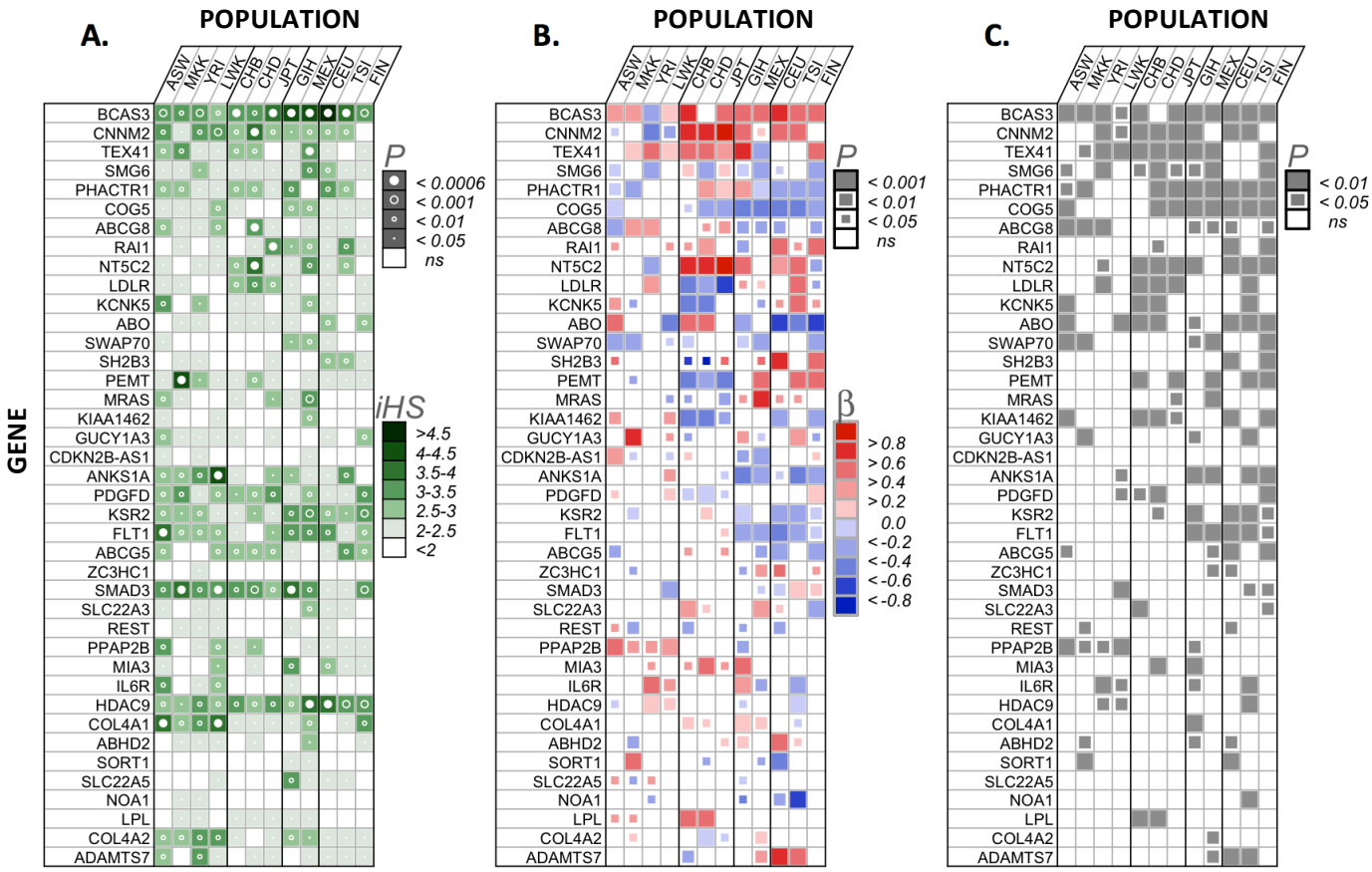


Figure 2

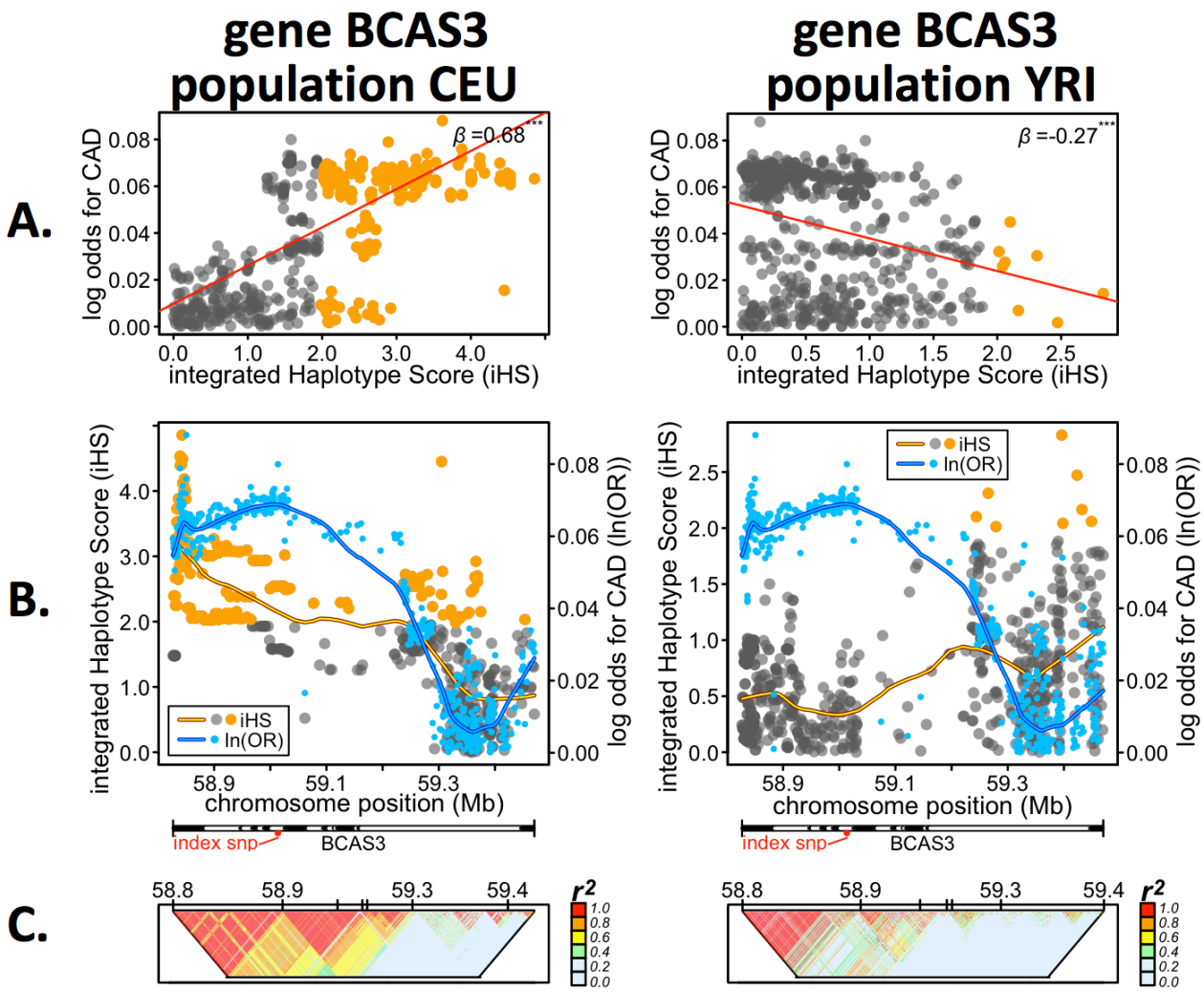


Figure 3

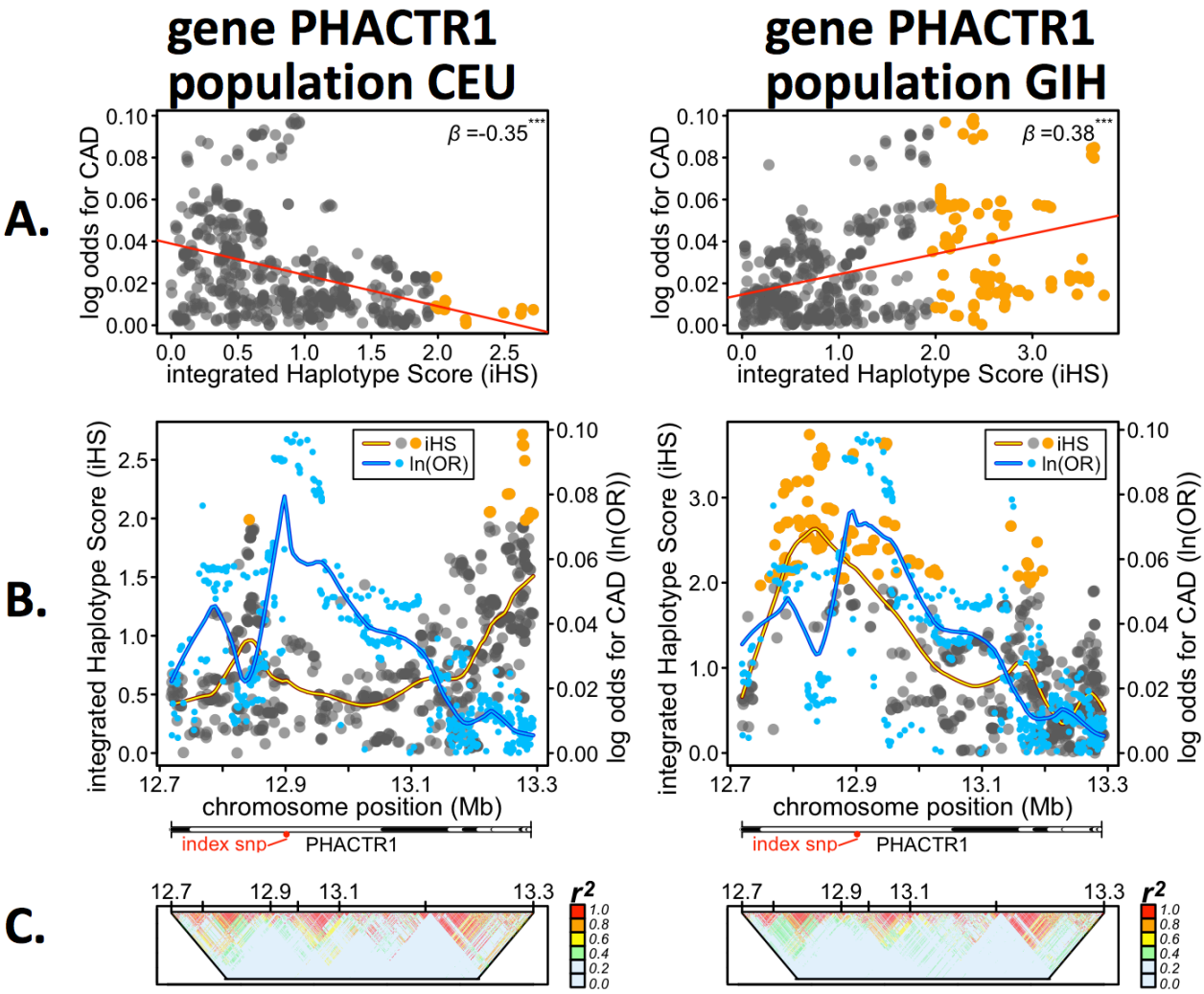


Figure 4

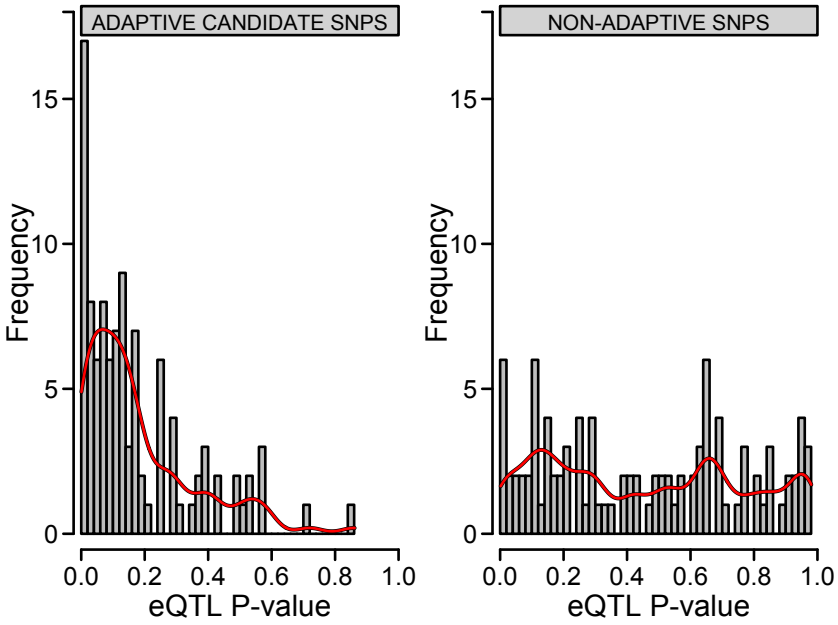
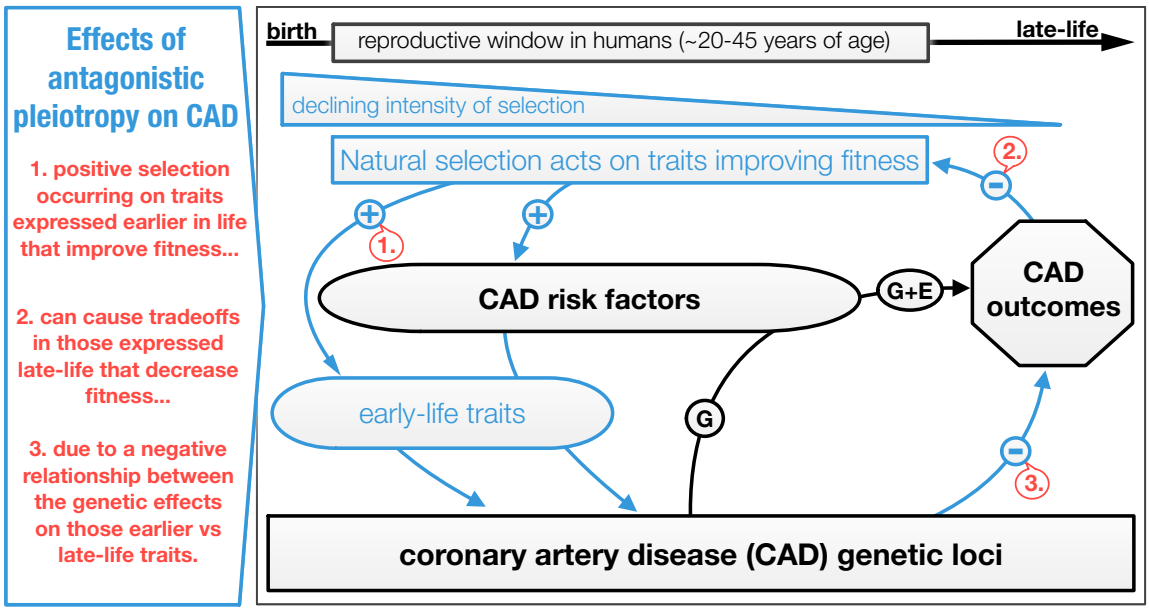


Figure 5



Supplementary Materials - Contents

Figure S1

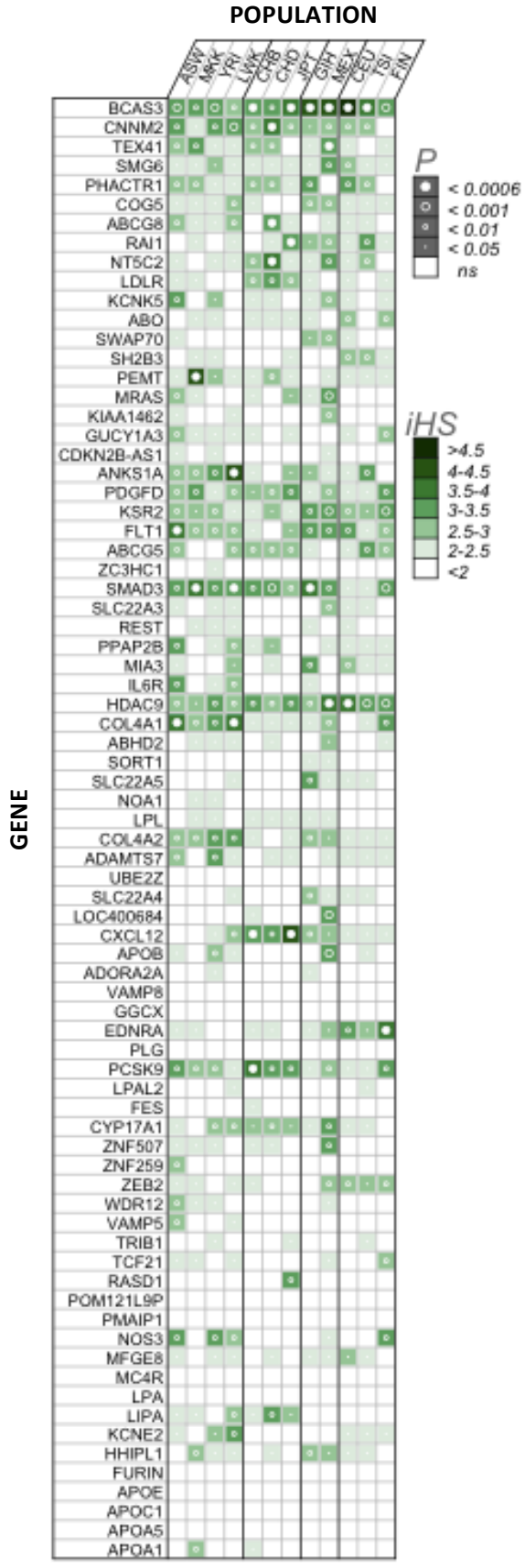


Figure S1: Association of coronary artery disease (CAD) risk and genomic signatures of selection in 12 worldwide populations. All 76 genes are shown ranked according to Fig. 1B. Boxes show magnitude and significance of largest positive selection signal (integrated haplotype score, iHS) within each gene-population combination. P values (circles within squares) were obtained from 10000 permutations. Bonferroni corrected p value limit also shown (α=0.05/76=0.000657) with closed circles. **Populations**. Grouped by common ancestry, African (ASW, African ancestry in Southwest USA; MKK, Maasai in Kinyawa, Kenya; YRI, Yoruba from Ibadan, Nigeria; LWK, Luhya in Webuye, Kenya), East-Asian (CHB, Han Chinese subjects from Beijing; CHD, Chinese in Metropolitan Denver, Colorado; JPT, Japanese subjects from Tokyo), European (CEU, Utah residents with ancestry from northern and western Europe from the CEPH collection; TSI, Tuscans in Italy; FIN, Finnish in Finland), GIH (Gujarati Indians in Houston, TX, USA), MEX (Mexican ancestry in Los Angeles, CA, USA).

Figure S2

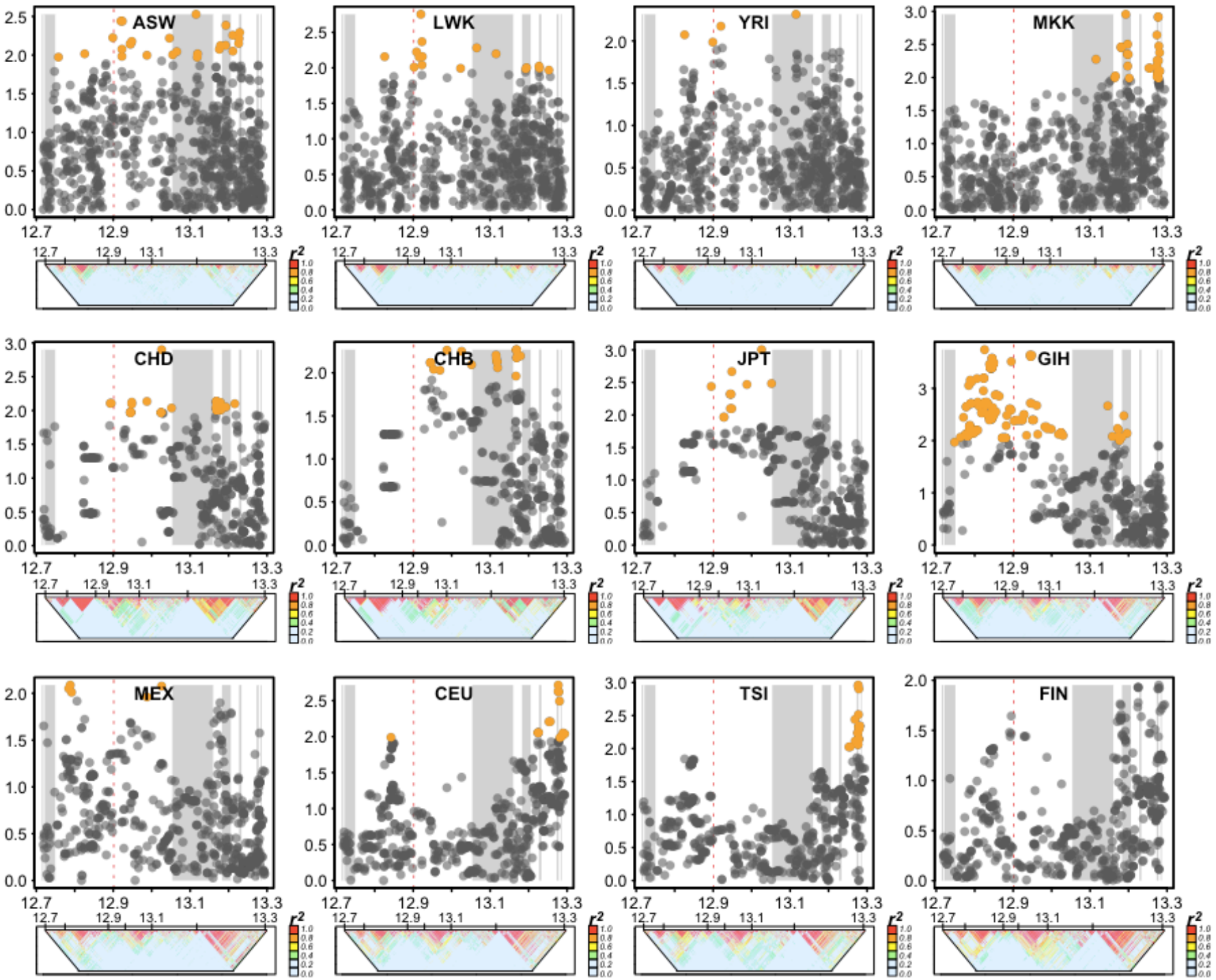

ASW
LWK
YRI
MKK
CHD
CHB
JPT
GIH
MEX
CEU
TSI
FIN
12.7
12.9
13.1
13.3
r2

Figure S2: Comparing cross-population candidate selection signals in *PHACTR1*. Per-SNP integrated Haplotype Scores (iHS) plotted by chromosome position within *PHACTR1* (including LD plots below each) for 12 worldwide populations. Permuted p value significance for each score coded by color (grey, non-significant; orange, $p < 0.05$). Red dashed line indicates position of index SNP for *PHACTR1*. Grey columns in background represent intron spans. Populations are clustered by common ancestry, African (ASW, African ancestry in Southwest USA; MKK, Maasai in Kinyawa, Kenya; YRI, Yoruba from Ibadan, Nigeria; LWK, Luhya in Webuye, Kenya), East-Asian (CHB, Han Chinese subjects from Beijing; CHD, Chinese in Metropolitan Denver, Colorado; JPT, Japanese subjects from Tokyo), European (CEU, Utah residents with ancestry from northern and western Europe from the CEPH collection; TSI, Tuscans in Italy; FIN, Finnish in Finland), GIH (Gujarati Indians in Houston, TX, USA), MEX (Mexican ancestry in Los Angeles, CA, USA).

Table S1

Table S1. Pleiotropic links between coronary artery disease (CAD) and early-life fitness-related traits due to shared genetic loci. The table below provides extensive support (143 studies) that antagonistic pleiotropy is likely to be present for CAD genes due to their consistent connections with fitness-related traits expressed early in life. See Fig. 5 for discussion and conceptual overview of these potential effects. Fitness-related traits include fertility potential, reproductive outcomes, pregnancy outcomes, fetal growth and survival, i.e. affecting the ability of an organism to reproduce and transfer genes to the next generation. The first 3 columns give CAD gene rank (no.; based on rank of 40 genes from Fig. 1B), name and full name. Columns 4-8 provide key details of each study where CAD genes also contribute to traits that influence fitness, including what species that was demonstrated in, what biological process or fitness effects that gene is impacting, what fitness class that effect is likely to impact (e.g. dysfunctional spermatogenesis or embryogenesis will affect male and female fertility, ability to conceive), what the observed genetic effect or mechanism that gene was associated with.

no.	CAD gene	full name	species	fitness effects	fitness class*	observed genetic effect or mechanism	ref
1	***BCAS3***	Breast Carcinoma Amplified Sequence 3	human/ mouse	embryogenesis	female potential fertility	*BCAS3* highly expressed in developing oocytes	[1]
	BCAS3		mouse	embryogenesis	female potential fertility	*BCAS3* significantly up-regulated in developmentally incompetent mouse oocytes	[2]
2	***CNNM2***	Cyclin And CBS Domain Divalent Metal Cation Transport Mediator 2	human	pregnancy-related blood pressure	pregnancy outcomes	*CNNM2* significantly differentially expressed	[3]
	CNNM2		mouse	pregnancy complications, hypoxia	pregnancy outcomes	*CNNM2* significantly down-regulated (-2.5 fold change) during pregnancy	[4]
3	***TEX41***	Testis Expressed 41 (Non- Protein Coding)	human	fetal IUGR, developmental delays	pregnancy outcomes	triplication involving *TEX41* causes IUGR	[5]
4	***SMG6***	Nonsense Mediated MRNA Decay Factor	mouse	altered embryogenesis	female potential fertility	*SMG6* essential for normal embryogenesis based on gene knock-down study	[6]
5	***PHACTR1***	Phosphatase And Actin Regulator 1	human	reproductive timing	reproductive outcomes	*PHACTR1* genetic variation	[7]
	PHACTR1		human	oocyte function	female potential fertility	*PHACTR1* highly significantly expressed	[8]
	PHACTR1		human	placental inflammatory responses	pregnancy outcomes	*PHACTR1* significantly down-regulated	[9]
	PHACTR1		human	endometrium implantation receptivity	female potential fertility	*PHACTR1* 8-fold significantly up-regulated	[10]
	PHACTR1		mouse	uterus functioning	female potential fertility	*PHACTR1* significant 1.4-1.9 fold change	[11]
	PHACTR1		rat	lactation	reproductive outcomes	*PHACTR1* significantly expressed (4.7 fold change) in mammary tissues	[12]

Table S1

6	***COG5***	Component of Oligomeric Golgi Complex 5	*Drosophila*	spermatogenesis	male potential fertility	*COG5* expression required for normal spermatogenesis	[13]
	COG5		human	intrauterine growth	pregnancy outcomes	*COG5* expression required for normal fetal growth	[14]
7	***ABCG8***	ATP-Binding Cassette, Sub-Family G, Member 8	mouse	infertility	female potential fertility	Knockout mice deficient *Abcg8* are infertile	[15]
	ABCG8		human	fetal distress, asphyxial events, intrauterine death	pregnancy outcomes	*ABCG8* involved in intrahepatic cholestasis of pregnancy (ICP), enterohepatic circulation, specifically for exportation of cholesterol	[16]
8	***RAI1***	Retinoic Acid Induced 1	human/ mouse	growth retardation, embryonic-postnatal development	pregnancy outcomes	knock-out mouse model for Smith-Magenis syndrome shows involvement of *RAI1*	[17]
	RAI1		mouse	growth retardation, impaired motor and sensory coordination, smaller litter size (direct reproductive fitness)	pregnancy outcomes	Transgenic mice over-expressing *RAI1* have developmentally impaired offspring	[18]
9	***NT5C2***	Nucleotidase, Cytosolic II	human	female reproduction	female potential fertility	*NT5C2* is over-expressed in fallopian tube, uterine endometrium, endocervix, ectocervix	[19]
	NT5C2		human	fetal growth, birthweight, postnatal growth & metabolism	pregnancy outcomes	*NT5C2* genetic variation affects birthweight	[20]
10	***LDLR***	Low Density Lipoprotein Receptor	human/ mouse	IUGR in offspring of *LDLR*-/- mice. Childhood obesity.	pregnancy outcomes	*LDLR* involved in fetal/offspring growth	[21]
	LDLR		human	Placental regulation of cholesterol	pregnancy outcomes	Maternal lipid profile affecting placental protein expression of *LDLR*	[22]
	LDLR		mouse	Placental regulation of cholesterol	pregnancy outcomes	LDLR involved in maternal-fetal transfer of lipids	[23]
	LDLR		rat	Pregnancy loss	fetal/offspring mortality	*LDLR* rat model for diabetes	[24]
11	***KCNK5***	Potassium Channel, Two Pore Domain Subfamily K, Member 5	human	Fertility - sperm volume	male potential fertility	*KCNK5* involved in protein and mRNA levels in sperm	[25]
	KCNK5		human	Male infertility	male potential fertility	*KCNK5* involvement in sperm inability to fertilize egg	[26]
	KCNK5		mouse	Male infertility	male potential fertility	*KCNK5* involved in sperm volume	[27]
	KCNK5		primate	Male fertility	male potential fertility	*KCNK5* involved in sperm function	[28]
	KCNK5		mouse	Female fertility	female potential fertility	*KCNK5* involved in oocyte survival/viability	[29]
	KCNK5		cattle	Lactation	reproductive	*KCNK5* expression	[30]

Table S1

					outcomes		
12	***ABO***	ABO Blood Group (Transferase A, Alpha 1-3-N-Acetylgalactosaminyltransferase; Transferase B, Alpha 1-3-Galactosyltransferase)	human	Birth weight, maternal age at child-bearing	pregnancy outcomes	*ABO* variation effects	[31]
	ABO		human	Fetal growth restriction	pregnancy outcomes	*ABO* variation effects	[32]
	ABO		human	fetal hypoxia, pregnancy complications, hemolytic disease of fetus/newborn, fetal death	pregnancy outcomes	*ABO* incompatibility effects	[33]
	ABO		human	protection against malaria	pregnancy outcomes	*ABO* variation effects	[34]
	ABO		human	Age at menarche	reproductive outcomes	*ABO* blood group phenotypes	[35]
	ABO		human	male infertility	male potential fertility	*ABO* involved in sperm concentration/function	[36]
	ABO		human	pregnancy complications	pregnancy outcomes	*ABO* involved in preeclampsia	[37]
	ABO		human	female fertility, embryo implantation	female potential fertility	*ABO* variation effects	[38]
13	***SWAP70***	SWAP Switching B-Cell Complex 70kDa Subunit	monkey	female fertility, implantation, placentation	female potential fertility	*SWAP-70* expression effects	[39]
	SWAP70		human	fetal growth restriction	pregnancy outcomes	*SWAP70* involved in preeclampsia	[40]
14	***SH2B3***	SH2B Adaptor Protein 3	human	intrauterine/postnatal growth	pregnancy outcomes	*SH2B3* variation effects	[41]
	SH2B3		human	male testicular function	male potential fertility	*SH2B3* variation effects	[42]
15	***PEMT***	Phosphatidylethanolamine N-Methyltransferase	human	fetal growth, placental function	pregnancy outcomes	Choline metabolism/*PEMT* expression effects	[43]
	PEMT		human	premature birth	pregnancy outcomes	*PEMT* variation [744CC genotype] effects	[44]
	PEMT		human	sperm quality	male potential fertility	*PEMT* variation [27774G.C] effects	[45]
	PEMT		human	fetal growth, placental function	pregnancy outcomes	mRNA levels of *PEMT* involved in fetal/placental function	[46]
	PEMT		mouse	embryo survival/viability during pre-implantation	female potential fertility	*PEMT* expression effects	[47]
	PEMT		human	fetal development	pregnancy outcomes	*PEMT* expression effects	[48]
16	***MRAS***	Muscle RAS Oncogene Homolog	mouse	male testicular function	male potential fertility	*MRAS* expression effects	[49]
	MRAS			embryo implantation	female potential	*MRAS* regulation by androgen	[50]

Table S1

					fertility	and progesterone receptors	
	MRAS		mouse	embryo pluripotency	female potential fertility	*MRAS* expression effects	[51]
	MRAS	Muscle RAS Oncogene Homolog	human	breastfeeding capacity	reproductive outcomes	*MRAS* expression effects	[52]
17	***KIAA1462***	*KIAA1462*	bird	offspring number	reproductive outcomes	*KIAA1462* expression effects	[53]
	KIAA1462		human	birth-related myometrial gene expression	pregnancy outcomes	*KIAA1462* expression effects	[54]
	KIAA1462		mouse	female reproduction	female potential fertility	*KIAA1462* highly expressed in oocytes & ovaries	[55]
	KIAA1462		pig	fetal growth	pregnancy outcomes	*KIAA1462* expression effects	[56]
	KIAA1462		human	embryo implantation	female potential fertility	*KIAA1462* significantly differentially expression	[57]
18	***GUCY1A3***	Guanylate Cyclase 1, Soluble, Alpha 3	cattle	embryo implantation	female potential fertility	*GUCY1A3* expression effects	[58]
	GUCY1A3			embryo implantation	female potential fertility	*GUCY1A3* expression effects	[59]
	GUCY1A3		human	placental functioning	pregnancy outcomes	*GUCY1A3* expression effects	[60]
	GUCY1A3		human	birth weight	pregnancy outcomes	*GUCY1A3* expression effects	[61]
	GUCY1A3		human	fetal growth, birthweight, postnatal growth & metabolism	pregnancy outcomes	*GUCY1A3* fetal genotype involved in fetal development	[62]
19	***CDKN2B-AS1***	CDKN2B Antisense RNA 1	human	fertility	female potential fertility	*CDKN2B-AS1* linked endometriosis	[63]
	CDKN2B-AS1		human	fetal growth restriction	pregnancy outcomes	*CDKN2B-AS1* variation	[64]
20	***ANKS1A***	Ankyrin Repeat And Sterile Alpha Motif Domain Containing 1A	cattle	fertility	female potential fertility	*ANKS1A* significant expression in endometrium and corpus luteum	[65]
	ANKS1A		cattle	fertility	female potential fertility	*ANKS1A* 6.7-fold significantly up-regulated in blastocysts	[66]
	ANKS1A		human/ mouse	male fertility	male potential fertility	*ANKS1A* expression	[67]
21	***PDGFD***	Platelet Derived Growth Factor D	human	female fertility	female potential fertility	*PDGFD* involved in ovarian hyperstimulation	[68]
	PDGFD		human	female reproduction	female potential fertility	*PDGFD* significantly expressed in oocytes	[69]
	PDGFD		mouse	male/female reproduction	female potential fertility	*PDGFD* significantly expression	[70]
	PDGFD			female reproductive function	female potential fertility	*PDGFD* significantly down-regulated in endometrium	[71]
	PDGFD		rat	female reproductive function	female potential fertility	*PDGFD* significantly expressed	[72]
	PDGFD		human	pregnancy complication, preeclampsia	pregnancy outcomes	*PDGFD* significantly down-regulated in placenta	[73]
22	***KSR2***	Kinase Suppressor Of Ras 2	mouse	male fertility	male potential fertility	*KSR2* -/- knockout mouse model for spermatogenesis	[74]
	KSR2		cattle	female reproductive	female potential	*KSR2* significantly up-	[75]

Table S1

				function	fertility	regulated in epithelial cells	
	KSR2		mouse	offspring growth	fetal/offspring mortality	*KSR2* -/- knockout mouse model	[76]
23	***FLT1***	Fms-Related Tyrosine Kinase 1	human	fetal development	pregnancy outcomes	*FLT1* expression effects	[77]
	FLT1		mouse	offspring viability, fetal growth	pregnancy outcomes	*FLT1* knockdown effects	[78]
	FLT1		human	pregnancy loss	fetal/offspring mortality	FLT1 involved in immune responses to placental malaria	[79]
	FLT1			female reproduction	female potential fertility	*FLT1* significantly expression in oocytes	[80]
	FLT1		human	intrauterine growth restriction	pregnancy outcomes	*FLT1* significantly up-regulated	[81]
	FLT1		human	fetal growth	pregnancy outcomes	*FLT1* significantly expressed in placenta	[82]
	FLT1		human	female reproduction	female potential fertility	*FLT1* significantly expressed in oocytes	[83]
	FLT1		human	female reproduction, implantation	female potential fertility	*FLT1* significantly expressed in uterus	[84]
	FLT1		human	female reproduction	pregnancy outcomes	*FLT1* significantly expressed in placenta, fetal tissues	[85]
	FLT1		human	intrauterine growth restriction	pregnancy outcomes	*FLT1* significantly expressed during pregnancy	[86]
24	***ABCG5***	ATP-Binding Cassette, Sub-Family G, Member 5	rat	intrauterine growth restriction	pregnancy outcomes	rat model of IUGR	[87]
	ABCG5			trophoblast, blastocyst development	female potential fertility	*ABCG5* gene expression effects	[88]
25	***ZC3HC1***	Zinc Finger, C3HC-Type Containing 1		male fertility	male potential fertility	meiosis disruptors	[89]
	ZC3HC1		mouse	pregnancy establishment, maintenance, conceptus survival	female potential fertility	*ZC3HC1* expression, 1.57-fold significantly changed	[90]
26	***SMAD3***	SMAD Family Member 3		folliculogenesis	female potential fertility	*SMAD3* expression effects	[91]
	SMAD3		mouse/rat	oocyte function	female potential fertility	*SMAD3* expression effects	[92]
	SMAD3			estrogen receptor interactions	female potential fertility	*SMAD3* expression effects	[93]
	SMAD3		rat	testis function	male potential fertility	*SMAD3* expression effects	[94]
	SMAD3		human	age at natural menopause	reproductive outcomes	*SMAD3* interaction effects	[95]
	SMAD3		human	twinning capacity	reproductive outcomes	*SMAD3* genotype (rs17293443-C) effects	[96]
	SMAD3		human	female fertility and fecundity	female potential fertility	*SMAD3* promotes proliferation and steroidogenesis of human ovarian lutenized granulosa cells	[97]
	SMAD3		mouse	embryo viability	female potential fertility	*SMAD3* signalling effects	[98]

Table S1

	SMAD3		human	spermatogenesis, male reproduction	male potential fertility	*SMAD3* expression effects	[99]
27	***SLC22A3***	Solute carrier family 22, extra neuronal monoamine transporter	human	placental functioning	pregnancy outcomes	*SLC22A3* expression effects	[100]
	SLC22A3		human	fetal development, fetal-placental resource provisioning	pregnancy outcomes	*SLC22A3* expression effects	[101]
	SLC22A3		human	fetal-placental functioning	pregnancy outcomes	*SLC22A3* expression changes during pregnancy	[102]
	SLC22A3		human	fetal-placental functioning	pregnancy outcomes	*SLC22A3* significantly expressed by trimester	[102]
28	***REST***	RE1-Silencing Transcription Factor	mouse	embryo functioning	female potential fertility	*REST* regulatory network effects	[103]
29	***PPAP2B***	Phospholipid Phosphatase 3	human	endometriosis, female fertility	female potential fertility	*PPAP2B* -1.69-fold significantly changed	[104]
	PPAP2B		human/rodent	gametogenesis	male potential fertility	*PPAP2B* expression effects	[105]
	PPAP2B		sheep	breeding capacity	reproductive outcomes	*PPAP2B* association effects	[106]
	PPAP2B		human	pregnancy complications	pregnancy outcomes	*PPAP2B* 1.36 -fold significantly up-regulated in placental tissues of preeclamptic mothers	[107]
	PPAP2B		human	embryo viability	female potential fertility	*PPAP2B* involved in spontaneous abortion due to parthenogenesis	[108]
	PPAP2B		human	embryo implantation	female potential fertility	*PPAP2B* differential expression effects	[57]
	PPAP2B			female reproductive function	female potential fertility	*PPAP2B* up-regulated in endometrium	[71]
30	***MIA3***	Melanoma Inhibitory Activity Family, Member 3	cattle	ovarian functioning	female potential fertility	*MIA3* 4.6-fold significantly up-regulated	[109]
	MIA3		mouse	placental (dys)function	female potential fertility	*MIA3* expresed in early trophoblast differentiation	[110]
31	***IL6R***	Interleukin 6 Receptor	pig	endometrium functioning	pregnancy outcomes	*IL6R* significantly differentially expressed in endometrium	[111]
	IL6R		cattle	endometrium functioning	pregnancy outcomes	*IL6R* 3.38-fold significantly up-regulated during pregnancy	[112]
	IL6R		human	endometrium functioning in PCOS women	female potential fertility	*IL6R* significantly up-regulated	[113]
	IL6R		human	pre-term birth SNP variation	pregnancy outcomes	*IL6R* significantly associated with pre-term birth	[114]
32	***HDAC9***	Histone Deacetylase 9	human	oocyte function	female potential fertility	*HDAC9* expression effects	[115]
	HDAC9		cattle	male fertility	male potential fertility	*HDAC9* involved in germ cell production	[116]
	HDAC9		pig	birth weight	pregnancy outcomes	*HDAC9* expression effects	[117]

Table S1

	HDAC9		human/ mouse	oocyte function	female potential fertility	*HDAC9* expression effects	[118]
	HDAC9		human	birth-related myometrial gene expression	pregnancy outcomes	*HDAC9* expression effects	[119]
33	***COL4A1***	Collagen, Type IV, Alpha 1	pig	neonate survival	pregnancy outcomes	*COL4A1* expression effects	[120]
	COL4A1		human	testis function	male potential fertility	*COL4A1* expression effects	[121]
	COL4A1		mouse	folliculogenesis	female potential fertility	*COL4A1* expression effects	[122]
	COL4A1		human	fetal survival	fetal/offspring mortality	*COL4A1* mutation effects	[123]
	COL4A1			fetal/placenta growth and development	pregnancy outcomes	*COL4A1* expression effects	[124]
34	***ABHD2***	Abhydrolase Domain Containing 2		male fertility	male potential fertility	*ABHD2* expression effects	[125]
35	***SORT1***	Sortilin 1	human	endometrium functioning	pregnancy outcomes	*SORT1* significantly expressed during labour	[54]
	SORT1			ovarian functioning	female potential fertility	*SORT1* significantly up-regulated	[126]
	SORT1		rat	ovarian functioning	female potential fertility	*SORT1* expression effects	[127]
	SORT1		human	embryo implantation	female potential fertility	*SORT1* differential expression effects	[128]
36	***SLC22A5***	Solute Carrier Family 22 (Organic Cation/Carnitine Transporter), Member 5	mouse	male infertility	male potential fertility	*SLC22A5* mutation related to male infertility	[129]
	SLC22A5		pig	reproductive variation, offspring born alive and total born	reproductive outcomes	*SLC22A5* genotype effects on reproductive capacity	[130]
	SLC22A5		pig	age at puberty	reproductive outcomes	*SLC22A5* genotype effects	[131]
37	***NOA1***	Nitric Oxide Associated 1	human	male fertility, testicular functioning	male potential fertility	*NOA1* expression effects	[132]
	NOA1		mouse	embryo/trophoblast viability	female potential fertility	*NOA1*-deficient mouse model	[133]
38	***LPL***	Lipoprotein Lipase	human	pregnancy complications	pregnancy outcomes	*LPL* expression effects	[134]
	LPL		human	male infertility	male potential fertility	sperm DNA fragmentation related to *LPL* expression	[135]
	LPL		human	reproductive timing	reproductive outcomes	*LPL* expression effects	[7]
	LPL		human	intrauterine growth restriction	pregnancy outcomes	*LPL*-mediated fetal-placental nutrient transfer	[136]
	LPL		human/ mouse	placental functioning	pregnancy outcomes	*LPL* expression effects	[137]
	LPL			fetal/placental resource transfer, pregnancy	pregnancy outcomes	*LPL* expression effects	[138]

Table S1

				complications			
	LPL		human	testis/spermatogenesis	male potential fertility	*LPL* expression effects	[139]
	LPL		mouse	placental regulation of cholesterol	pregnancy outcomes	*LPL* involved in maternal-fetal transfer of lipids	[23]
39	***COL4A2***	Collagen, Type IV, Alpha 2	mouse	fetal viability	fetal/offspring mortality	mouse knockout model for *COL4A2*	[140]
	COL4A2		human	testis function	male potential fertility	*COL4A2* expression effects	[121]
	COL4A2		human	offspring viability	fetal/offspring mortality	*COL4A2* expression effects	[141]
40	***ADAMST7***	ADAM Metallopeptidase With Thrombospondin Type 1 Motif, 7	mouse	embryogenesis	female potential fertility	*COL4A2* expression effects	[142]
	ADAMST7		dog	mammary tissue functioning	reproductive outcomes	*ADAMST7* significantly up-regulated in mammary tissues	[143]
	ADAMST7		human	breastfeeding capacity	reproductive outcomes	*ADAMST7* expression effects	[52]

Table footnotes:

***'fitness class' column defined further:**

male potential fertility - includes processes affecting spermatogenesis, sperm motility, volume or function that ultimately affect probability of successful egg fertilization.

female potential fertility - includes processes affecting embryogenesis (i.e. oocyte viability, survival), functioning of uterus (i.e. implantation receptivity, endometrium functioning), placentation (trophoblast cell motility) that ultimately affects initial successful establishment of pregnancy.

pregnancy outcomes - includes processes affecting regulation of blood pressure, nutrient and oxygen transfer between fetal and placental tissues during pregnancy that ultimately influences fetal growth, development and survival.

fetal/offspring mortality - includes processes linked to pregnancy defects, resistance to pathogens, affecting survival of fetus during pregnancy or perinatal mortality.

reproductive outcomes - includes effects on age at maturity, reproductive timing, potential number of offspring, breastfeeding capacity.

Search criteria:

- For each CAD gene, Google scholar was used to search for studies using the 'Search terms' (below) and the gene name (*BCAS3* is used as an example)
- For each search, only the first page of results was considered. Search results most consistent with all search terms are ranked by page, thus the most relevant results were always on the first page. This approach was also employed to keep this literature search tractable in terms of time (i.e. a search for each of the terms below for one gene usually took ~1 hour).
- We also used the GWAS Catalog (https://www.ebi.ac.uk/gwas/) using the gene name to search for further potential links to fitness related traits

Search terms (example using gene *BCAS3*):

- "*BCAS3*" and "reproduction" and gene and -"noncommercial use, distribution, and reproduction in any"
- "*BCAS3*" and "fitness" and gene
- "*BCAS3*" and "fertility" and gene
- "*BCAS3*" and "menarche" and gene
- "*BCAS3*" and "menopause" and gene
- "*BCAS3*" and "birth" or "birth weight"
- "*BCAS3*" and "pregnancy" and gene
- "*BCAS3*" and "placenta" and gene

Table S1

- "*BCAS3*" and "implantation" and gene
- "*BCAS3*" and "oocyte" and gene
- "*BCAS3*" and "sperm" and gene
- "*BCAS3*" and "testis"

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Table S2. Summary of types of pleiotropic connections between coronary artery disease (CAD) and fitness-related traits. Counts are based on Table S1, 'fitness class' column. Most fitness-related traits were related to female potential fertility (29 of 40 genes had these effects) and pregnancy outcomes (25 of 40 genes had these effects). Some genes had broad or specific effects on fitness-related traits. For example, number of fitness classes affected ranged from 6 for *ABO* (had fitness effects across all classes) to 1, for example *CNNM2* (evidence for fitness effects in pregnancy outcomes class).

no.	CAD Gene	male potential fertility	female potential fertility	sum(both male and female fertility)	pregnancy outcomes	reproductive outcomes	fetal/offspring mortality	sum (all columns)
1	***BCAS3***		1		1			**2**
2	***CNNM2***				1			**1**
3	***TEX41***				1			**1**
4	***SMG6***		1					**1**
5	***PHACTR1***		1		1	1		**3**
6	***COG5***	1			1			**2**
7	***ABCG8***	1	1	1	1		1	**5**
8	***RAI1***				1			**1**
9	***NT5C2***		1		1			**2**
10	***LDLR***				1		1	**2**
11	***KCNK5***	1	1	1		1		**4**
12	***ABO***	1	1	1	1	1	1	**6**
13	***SWAP70***		1		1			**2**
14	***SH2B3***	1			1		1	**3**
15	***PEMT***	1	1	1	1			**4**
16	***MRAS***	1	1	1		1		**4**
17	***KIAA1462***		1		1	1		**3**
18	***GUCY1A3***		1		1		1	**3**
19	***CDKN2B-AS1***		1		1			**2**
20	***ANKS1A***	1	1	1				**3**
21	***PDGFD***		1		1			**2**
22	***KSR2***	1	1	1			1	**4**
23	***FLT1***		1		1		1	**3**
24	***ABCG5***		1		1			**2**
25	***ZC3HC1***	1	1	1				**3**
26	***SMAD3***	1	1	1		1		**4**
27	***SLC22A3***				1			**1**

Table S2

28	***REST***		1					**1**
29	***PPAP2B***	1	1	1	1	1		**5**
30	***MIA3***		1					**1**
31	***IL6R***		1		1			**2**
32	***HDAC9***	1	1	1	1			**4**
33	***COL4A1***	1	1	1	1		1	**5**
34	***ABHD2***	1						**1**
35	***SORT1***		1		1			**2**
36	***SLC22A5***	1				1		**2**
37	***NOA1***	1	1	1				**3**
38	***LPL***	1			1	1		**3**
39	***COL4A2***	1					1	**2**
40	***ADAMST7***		1			1		**2**
	sum	**19**	**29**	**13**	**25**	**10**	**9**	**106**
							average	**2.7**

Table S3

Table S3. Pleiotropic links between randomly chosen genes and early-life fitness-related traits. Fitness-related traits include fertility potential, reproductive outcomes, pregnancy outcomes, fetal growth and survival, i.e. affecting the ability of an organism to reproduce and transfer genes to the next generation. The first column gives coronary artery disease (CAD) gene (first 20 of 40 CAD genes from Fig. 1B/Table S1). Columns 2-3 give name (abbreviated, full) of randomly chosen genes matched for approximate length for each CAD gene. Columns 4-8 provide key details of each study where random genes also contribute to traits that influence fitness, including what species that was demonstrated in, what biological process or fitness effects that gene is impacting, what fitness class that effect is likely to impact (e.g. dysfunctional spermatogenesis or embryogenesis will affect male and female fertility, ability to conceive), what the observed genetic effect or mechanism that gene was associated with.

CAD gene	Random Gene	full name	species	fitness effects	fitness class*	observed genetic effect or mechanism	ref
BCAS3	***STPG2***	Sperm Tail PG-Rich Repeat Containing 2	-	-	-	-	
CNNM2	***CFAP44***	Cilia And Flagella Associated Protein 44	-	-	-	-	
TEX41	***SHISA9***	Shisa Family Member 9	-	-	-	-	
SMG6	***TANGO6***	Transport And Golgi Organization 6 Homolog	-	-	-	-	
PHACTR1	***SUMF1***	Sulfatase Modifying Factor 1	mouse	embryogenesis	female potential fertility	*SUMF1* significantly up-regulated in developmentally incompetent mouse oocytes	[1]
COG5	***FRMD5***	FERM Domain Containing 5	-	-	-	-	
ABCG8	***ASIC5***	Acid Sensing Ion Channel Subunit Family Member 5	-	-	-	-	
RAI1	***ZNF516***	Zinc Finger Protein 516	human	endometriosis	female potential fertility	*ZNF516* appears to be involved in endometriosis	[2]
NT5C2	***LANCL1-AS1***	LANCL1 Antisense RNA 1	-	-	-	-	
LDLR	***SYT13***	Synaptotagmin 13	-	-	-	-	
KCNK5	***FAM53A***	Family With Sequence Similarity 53 Member A	-	-	-	-	
ABO	***TTC22***	Tetratricopeptide Repeat Domain 22	-	-	-	-	
SWAP70	***RNF157***	Ring Finger Protein 157	cattle	oocyte/follicle maturation (oocyte quality)	female potential fertility	In cattle model, *RNF157* 2.24 significantly differentially up-regulated between BCB+ and BCB- oocytes	[3]
	RNF157		human	early peripheral blood gene	pregnancy complications/ou	*RNF157* is -1.65 fold significantly (P=0.01)	[4]

Table S3

				expression during pregnancy related to preeclampsia	tcomes	down-regulated in peripheral blood
SH2B3	***PLBD1-AS1***	PLBD1 Antisense RNA 1	-	-	-	-
PEMT	***WAC***	WW Domain Containing Adaptor With Coiled-Coil	-	-	-	-
MRAS	***TMEM178A***	Transmembrane Protein 178A	-	-	-	-
KIAA1462	***PLEKHD1***	Pleckstrin Homology And Coiled-Coil Domain Containing D1	-	-	-	-
GUCY1A3	***MACC1***	Metastasis Associated In Colon Cancer 1	-	-	-	-
CDKN2B-AS1	***CACNA2D4***	Calcium Voltage-Gated Channel Auxiliary Subunit Alpha2delta 4	-	-	-	-
ANKS1A	***NWD2***	NACHT And WD Repeat Domain Containing 2	-	-	-	-

Table footnotes:

***'fitness class' column defined further:**

male potential fertility - includes processes affecting spermatogenesis, sperm motility, volume or function that ultimately affect probability of successful egg fertilization.

female potential fertility - includes processes affecting embryogenesis (i.e. oocyte viability, survival), functioning of uterus (i.e. implantation receptivity, endometrium functioning), placentation (trophoblast cell motility) that ultimately affects initial successful establishment of pregnancy.

pregnancy outcomes - includes processes affecting regulation of blood pressure, nutrient and oxygen transfer between fetal and placental tissues during pregnancy that ultimately influences fetal growth, development and survival.

fetal/offspring mortality - includes processes linked to pregnancy defects, resistance to pathogens, affecting survival of fetus during pregnancy or perinatal mortality.

reproductive outcomes - includes effects on age at maturity, reproductive timing, potential number of offspring, breastfeeding capacity.

Search criteria:

- For each random gene, Google scholar was used to search for studies using the 'Search terms' (below) and the gene name (*STPG2* is used as an example)
- For each search, only the first page of results was considered. Search results most consistent with all search terms are ranked by page, thus the most relevant results were always on the first page. This approach was also employed to keep this literature search tractable in terms of time (i.e. a search for each of the terms below for one gene usually took ~1 hour).
- We also used the GWAS Catalog (https://www.ebi.ac.uk/gwas/) using the gene name to search for further potential links to fitness related traits

Search terms (example using gene *STPG2*):

- "*STPG2*" and "reproduction" and gene and -"noncommercial use, distribution, and reproduction in any"
- "*STPG2*" and "fitness" and gene
- "*STPG2*" and "fertility" and gene
- "*STPG2*" and "menarche" and gene

Table S3

- "*STPG2*" and "menopause" and gene
- "*STPG2*" and "birth" or "birth weight"
- "*STPG2*" and "pregnancy" and gene
- "*STPG2*" and "placenta" and gene
- "*STPG2*" and "implantation" and gene
- "*STPG2*" and "oocyte" and gene
- "*STPG2*" and "sperm" and gene
- "*STPG2*" and "testis"

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Table S4. Selected Enrichr analysis outputs for top 10-ranked CAD genes with highest genetic risk-selection associations from Fig. 1B. Enrichr outputs includes KEGG 2016 Pathways (http://www.kegg.jp/kegg/download/), MGI Mammalian Phenotype Level 3 (http://www.informatics.jax.org/), Cancer Cell Line Encyclopaedia (http://portals.broadinstitute.org/ccle/data/browseData), and ChEA 2015 (http://amp.pharm.mssm.edu/lib/cheadownload.jsp).

PATHWAYS - KEGG 2016 Pathways

Index	Name	P-value	Adjusted p-value	Z-score	Combined score
1	Bile secretion_Homo sapiens_hsa04976	0.0006325	0.008222	-1.77	8.48
2	Ovarian steroidogenesis_Homo sapiens_hsa04913	0.02878	0.07482	-1.85	4.79
3	Fat digestion and absorption_Homo sapiens_hsa04975	0.02374	0.07482	-1.76	4.57
4	Nicotinate and nicotinamide metabolism_Homo sapiens_hsa00760	0.01700	0.07482	-1.75	4.53
5	Aldosterone synthesis and secretion_Homo sapiens_hsa04925	0.04596	0.09556	-1.87	4.39
6	ABC transporters_Homo sapiens_hsa02010	0.02542	0.07482	-1.65	4.28
7	Toxoplasmosis_Homo sapiens_hsa05145	0.06617	0.09558	-1.71	4.01
8	Hepatitis C_Homo sapiens_hsa05160	0.07427	0.09655	-1.61	3.76
9	Pyrimidine metabolism_Homo sapiens_hsa00240	0.05911	0.09558	-1.60	3.75
10	mRNA surveillance pathway_Homo sapiens_hsa03015	0.05145	0.09556	-1.56	3.66

ONTOLOGIES - MGI Mammalian Phenotype Level 3

Index	Name	P-value	Adjusted p-value	Z-score	Combined score
1	IGR1_SKIN	0.002319	0.1055	-1.95	4.38
2	HEYA8_OVARY	0.03735	0.1638	-2.31	4.18
3	OVK18_OVARY	0.003838	0.1055	-1.79	4.03
4	HTK_HAEMATOPOIETIC_AND_LYMPHOID_TISSUE	0.03195	0.1638	-2.13	3.85
5	HS944T_SKIN	0.04174	0.1638	-2.08	3.76
6	MFE296_ENDOMETRIUM	0.04564	0.1638	-2.05	3.71
7	HS746T_STOMACH	0.05146	0.1638	-2.03	3.67
8	WM983B_SKIN	0.05724	0.1638	-1.96	3.54
9	NCIH650_LUNG	0.08805	0.1638	-1.95	3.53
10	TE10_OESOPHAGUS	0.05387	0.1638	-1.94	3.51

Table S4

CELL TYPES - Cancer Cell Line Encyclopedia

Index	Name	P-value	Adjusted p-value	Z-score	Combined score
1	MP0000003_abnormal_adipose_tissue_	0.01005	0.1061	-2.31	5.19
2	MP0003718_maternal_effect_	0.01434	0.1061	-2.10	4.71
3	MP0005395_other_phenotype_	0.01434	0.1061	-1.88	4.21
4	MP0002139_abnormal_hepatobiliary_system_	0.005946	0.1061	-1.86	4.16
5	MP0009389_abnormal_extracutaneous_pigme_	0.03729	0.1769	-1.87	3.23
6	MP0002168_other_aberrant_phenotype_	0.03869	0.1769	-1.67	2.90
7	MP0001764_abnormal_homeostasis_	0.01658	0.1061	-1.22	2.74
8	MP0005501_abnormal_skin_physiology_	0.05987	0.2395	-1.60	2.29
9	MP0000358_abnormal_cell_content/_	0.07746	0.2680	-1.41	1.86
10	MP0005253_abnormal_eye_physiology_	0.09214	0.2680	-1.37	1.81

TRANSCRIPTION - ChEA 2015

Index	Name	P-value	Adjusted p-value	Z-score	Combined score
1	CTNNB1_20460455_ChIP-Seq_HCT116_Human	4.305e-7	0.00005554	-2.06	20.15
2	TRIM28_17542650_ChIP-ChIP_NTERA2_Human	0.4994	0.5017	-24.98	17.23
3	ESR1_20079471_ChIP-ChIP_T-47D_Human	0.0007167	0.009462	-2.99	13.94
4	FOXP1_22492998_ChIP-Seq_STRATIUM_Mouse	0.005027	0.03393	-4.06	13.72
5	ESR1_22446102_ChIP-Seq_UTERI_Mouse	0.0002684	0.005797	-2.46	12.65
6	FOXA2_19822575_ChIP-Seq_HepG2_Human	0.000003903	0.0002810	-1.20	9.82
7	TFEB_21752829_ChIP-Seq_HELA_Human	0.0003687	0.007099	-1.92	9.50
8	BCL11B_21912641_ChIP-Seq_STHDH STRIUM _Mouse	0.008180	0.04530	-2.86	8.85
9	ARNT_22903824_ChIP-Seq_MCF7_Human	0.0007447	0.009462	-1.83	8.55
10	CIITA_18437201_ChIP-ChIP_Raji B and iDC_Human	0.01114	0.05347	-2.91	8.53

Supplementary Discussion

Widespread signals of positive selection on CAD loci

Evidence of candidate positive selection signals for CAD loci were widespread, with many genes having significant iHS scores of small-medium size (i.e. iHS score range: 2-3) with four genes (*BCAS3*, *ANKS1A*, *CXCL12*, *PEMT*) harboring large selection signals (iHS >4), two of which had previously been identified as having strong selection signals including *BCAS3* (Breast Carcinoma Amplified Sequence 3) in the HapMap3 CEU population [1] and *PHACTR1* (phosphatase and actin regulator 1) across the ASW, CEU and CHB/CHD HapMap3 populations [2]. Twelve genes contained SNPs with selection scores that remained significant after correction for multiple testing (Fig. 1A). The consistency of smaller, less significant selection signals for several genes within most populations (i.e. *CNNM2*, *PHACTR1*, *PDGFD*) strongly suggest that these may be smaller and possibly valid incomplete selective sweeps that are typically missed due to stringency of multiple-correction thresholds and lack of validation across multiple populations.

These patterns match expectations from the polygenic model of selection that predicts that selection on complex traits mostly involves smaller shifts in many underlying loci; it is the likely reason why so few large selection signals have been found underlying complex traits in general [3, 4] and those underlying cardiovascular disease phenotypes in particular [5, 6]. For example, Kullo & Ding 2007 [6] found that 110 out of 364 genes in pathways associated with cardiovascular disease (i.e. inflammation, insulin, p53, Ras, cholesterol biosynthesis etc) had significantly higher Fst (empirical $P<0.05$) in at least one SNP between 4 populations, but none remained significant after correction for multiple testing. In a later study, Ding & Kullo 2011 [5] found that 8 out of 158 genome-wide significant SNPs in genes for 36 cardiovascular disease phenotypes and related traits (CHD, hypertension, stroke, BMI, lipids etc) had significantly elevated Fst between 52 populations in the Human Genome Diversity Project.

It is difficult to compare selection candidates we found in the 76 CAD associated genes with results from these two previous studies as full sets of gene lists and Fst estimates were not available for either, and they used loci underlying much broader cardiovascular disease phenotypes than our more current list of specific CAD loci [7]. Nevertheless, due to fine-scale imputation with the 1000 Genomes Panel, our study suggests that many more loci related to cardiovascular disease have been recently modified by natural selection than previously identified. The larger sample of SNPs also likely improved reliability of iHS p values, with many more estimates available per MAF bin used to standardize iHS measures [8].

The Fst measures used in the Ding and Kullo studies also differ qualitatively from the iHS scores we used. Fst captures allele frequency differences between populations and is less sensitive to detecting alleles that have undergone recent selection [9], while the iHS statistic detects whether common alleles are carried on unusually long haplotypes within populations and should be better at capturing more recent smaller selection signals [8]. Lastly, by considering not just genome-wide significant index SNPs, we were able to detect smaller selection signals within CAD loci that were consistent across populations and would have otherwise been missed. *PHACTR1* is a good example of this – several smaller candidate selection signals were found (iHS ranging from 2-3.8) where peak selection signals did not span the index SNP location - sometimes signals were in different introns within the same locus (Fig. S2).

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