Framingham's Contribution to Gene Identification for CV Risk Factors and Coronary Disease

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SUMMARY

Genome-wide association studies have been published since 2005 and remain exemplary in translating knowledge fostered by the human genome project into genomic lessons on health and disease. Although our understanding of the basis of complex disease remains by far incomplete, the knowledge of the genetic basis of cardiovascular risk factors and their end organ damage has been significantly improved. The Framingham Heart Study was one of the earliest population-based studies to apply genomic methods and is an important contributor to large disease-based consortia as the International Consortium for Blood Pressure Genome-Wide Association Studies, the Global Lipids Genetics Consortium, the Diabetes Genetics Replication and Meta-Analysis Consortium, and the Coronary Artery Disease Genome-Wide Replication and Meta-Analysis Consortium. The variability of these cardiovascular risk factors is partly genetic and knowledge on the genetic basis originated largely from analysis of monogenic disease in rare syndromes before the use of genome-wide, common single nucleotide polymorphism analysis. Genome-wide association studies have identified ~45 common variants associated with systolic and diastolic blood pressure, ~65 common variants for type 2 diabetes, and ~95 common variants for lipid traits. One major type of end organ damage is coronary heart disease, and ~25 loci could be shown to be associated. Risk scores using multiple cardiovascular risk factor single nucleotide polymorphisms are clearly correlated with cardiovascular outcome. This review summarizes recent findings by genome-wide association studies and the contributions by the Framingham Heart Study on the basis of seminal papers and gives an outlook on some of the future experiments.

After theoretical considerations on the usefulness of genome-wide association studies (GWAS) [1], the first clearly significant genome-scans in 2005 suggested the possibility that GWAS using common variants might explain a large proportion of trait variabilities [2–4]. The very next year, the second set of GWAS published [5] indicated that only little of the trait variability, QT interval length in this case, is explained by common variants, although the sample sizes of these early experiments were small. Based on The National Human Genome Research Institute GWAS catalog [6], 1,443 GWAS have been published in the meantime [7]. Of 237 unique associated single nucleotide polymorphisms (SNPs) currently in the database for which effect sizes are provided in units of standard deviation, the average effect size is 0.09 SD units and only 4% (9 of 236) have an effect size of greater than 0.2 SD units. This observation emphasizes a key finding that effect sizes of individual genetic variants are small in the great majority of cases.

There is no doubt that GWAS have contributed to our understanding of complex genetic disease, even though little of phenotypic variance is explained so far for most traits. It is very clear now that for the 3 principal modifiable cardiovascular risk factors (smoking is not considered here), there exist genetic variants with large effect sizes that are rare (for lipids and diabetes) to extremely rare (for blood pressure [BP]) and these are typically encountered in a syndromic, familial context. Given the population frequencies of variants with large effect sizes identified in family studies, the phenotype variance explained is very small, as only few individuals carry these variants. Therefore, the great majority of genetic variability is due to other types of genetic variation. GWAS explore this type of variation.

This review highlights some of the principal GWAS findings for BP, blood lipids, diabetes, and coronary artery disease (CAD) by the International Consortium for Blood Pressure Genome-Wide Association Studies (ICBP), the Global Lipids Genetics Consortium (GLGC), the Diabetes Genetics Replication and Meta-Analysis Consortium (DIAGRAM), and the Coronary Artery Disease Genome-Wide Replication and Meta-Analysis Consortium (CARDIoGRAM). Common variants associated with these phenotypes discovered by GWAS have small effect sizes, but collectively they explain a sizable fraction of the phenotypic variability (Fig. 1). The review concentrates on 4 seminal reports that represent to date the largest GWAS efforts on these phenotypes. The Framingham Heart Study (FHS) is an important contributor in all 4 studies. The review summarizes the loci identified across studies, but it does not systematically include further information from additional studies.

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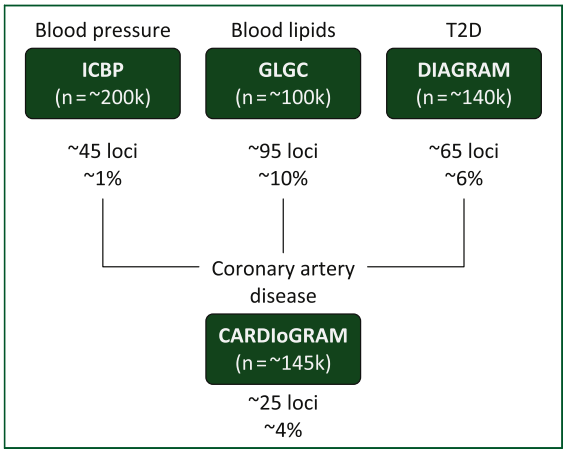

FIGURE 1. Key features of cardiovascular risk and coronary artery disease (CAD) genome-wide association studies consortia. The consortium name is given in bold type. For each consortium, the total maximal sample size, the number of loci discovered, and the percent of total trait variance explained is indicated. CARDIoGRAM, Coronary Artery Disease Genome-Wide Replication and Meta-Analysis Consortium; DIAGRAM, Diabetes Genetics Replication and Meta-Analysis Consortium; GLGC, Global Lipids Genetics Consortium; ICBP, The International Consortium for Blood Pressure Genome-Wide Association Studies; T2D, type 2 diabetes.

OPPORTUNITIES BY GWAS FOR OUR UNDERSTANDING OF CARDIOVASCULAR RISK FACTORS

Individuals and patients are made unequally: Some become centenarians with little medical intervention; others die young of a potentially preventable disease such as myocardial infarction. Many efforts are made in medicine to change the "nurture" side of this equation, but it is interesting to remind the reader that "nature" [8] explains close to half of the phenotypic variation of cardiovascular risk factors [9–12] (Table 1). The precise estimates have been subject to critique [13], but it appears unlikely that heritability estimates are very far from the true underlying heritability. Surprisingly, despite the huge impact of major cardiovascular risk factors on population health, most of the risk factors' pathogenesis remains poorly understood, despite considerable progress documented in other papers of this series. Further improvement of the understanding of the mechanisms of cardiovascular risk factors and their relationship with outcomes will likely lead to improved chances for intervention.

TABLE 1. Heritability of principal cardiovascular risk factors and coronary artery disease

	Heritability [reference]
SBP	42% [9]
DBP	39% [9]
T2D	26% [10]
TC	64% [11]
LDL-C	66% [11]
HDL-C	58% [11]
TG	42% [11]
CAD	56% [12]

For SBP and DBP, single-visit heritability is indicated. The estimates for long-term average phenotypes appear considerably higher (0.57 for SBP and 0.56 for DBP) [9]. CAD, coronary artery disease; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; T2D, type 2 diabetes; TC, total cholesterol; TG, triglycerides.

For a number of years, there has been a great opportunity to use genomics in cardiovascular medicine to help better understand the pathogenesis as well as the relationship of cardiovascular risk factors with outcomes. One beautiful thing about GWAS is their unbiased approach to discovery [14]. GWAS approaches have been criticized, and much of the critique is due to characteristics necessary to obtain very large sample sizes. The sample assembly and data file review stage of a typical experiment might sometimes resemble "factory-like" science, but these are necessary means to reach the objective of sufficient statistical power by large sample sizes.

GWAS have explained only a small fraction of total heritability so far for most traits, and currently a major proportion of heritability is unexplained by common variants ("missing heritability") [15]. Although the explanations formulated to explain this observation are multiple, experimental proof is outstanding. A corollary is that more of the heritability will be explained by future experiments, and GWAS-based and non-GWAS methods still have great potential.

STATISTICAL POWER IN GWAS AND RANGES OF TARGETED ALLELE FREQUENCIES

One important point for GWAS is the necessity to overcome the burden of multiple testing. Therefore, considerations of statistical power are central to GWAS. Typically, many more than 1 million genetic variants are tested, but genetic variants are correlated (linkage disequilibrium). It is generally accepted that for common variants in participants of European ancestry, the correlation between SNPs leads to ~1 million effective tests, even if nominally more variants are tested, leading to a typical p value significance threshold of 5×10^{-8} (Bonferroni correction of $p = 0.05$ divided by the effective number of tests [0.05/1000,000]). Given that such significance thresholds can only be reached with a large number of participants, most recent GWAS are meta-analyses of multiple smaller studies and include at least hundreds, often many thousands of participants.

The 3 determinants of statistical power are: 1) sample size; 2) allele frequency; and 3) the effect size of the variants. Table 2 shows the necessary sample sizes to reach 80% statistical power with effect sizes typically observed in

BP GWAS (0.05 SD) at 2 scenarios of allele frequencies. The reader can observe that the required sample size is large, but in the reach of current studies using common variants for the phenotypes described in this review. There is the hope that the effect sizes will be larger for more rare variants that will be evaluated in future experiments by genotyping or sequencing. The effect size increases while the minor allele frequency decreases [16], and it is unclear to date how far the increase in effect size can compensate the decrease in allele frequency as both are determinants of statistical power.

LOCI ASSOCIATED WITH SYSTOLIC AND DIASTOLIC BP

GWAS on BP and hypertension have been difficult, and the FHS has contributed to the first efforts under the guidance of FHS leadership [17]. The reason for the difficulties to find BP-associated variants in these first studies can be attributed today to low statistical power given the small effect sizes of an individual variant. A sample size of >30,000 is necessary to reach 80% power with an effect size of 0.05 SD, which is typical for BP GWAS (see Table 2), even at maximal minor allele frequencies (0.5). The first studies by the Wellcome Trust Case Control Consortium and the early FHS studies were clearly underpowered given their large, but limited sample size. Therefore, it became clear quickly that even the large population-based studies do not reach sufficient sample sizes individually. Consequently, multiple visionary studies confederated in 2007 to form consortia with a sample size reaching the critical threshold. The FHS was among the founding members of the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium (CHARGE) consortium [18,19], a consortium that has been extremely successful not least because of synergies across multiple phenotype groups. The other 4 founding members of CHARGE are: the Age, Gene, Environment, Susceptibility Study - Reykjavik (AGES), the ARIC (Atherosclerosis Risk in Communities Study), the CHS (Cardiovascular Health Study), and the Rotterdam Study (RS). Currently, the total number of participating studies is larger than 10, which increases the total sample size to more than 70,000 participants.

TABLE 2. Statistical power in GWAS

	Sample Size Needed for 80% Statistical Power
eff. size: 0.05 SD, MAF 0.5	31,681
eff. size: 0.05 SD, MAF 0.1	88,003
eff. size: 0.5 SD, MAF 0.01	8,001
eff. size: 0.5 SD, MAF 0.001	79,282

The sample size needed to reach 80% statistical power is indicated for different scenarios of effect sizes (eff. size) of a variant (expressed in standard deviations [SD] of the phenotype) and the minor allele frequency (MAF) of the variant. Alpha is 5×10^{-8}. GWAS, genome-wise association studies.

The initial GWAS meta-analysis of the CHARGE-BP working group under the leadership of Dr. Dan Levy could identify 8 loci associated with systolic blood pressure (SBP) or diastolic blood pressure (DBP) at a genome-wide significant level in 29,136 participants [20]. The study was published in 2009 conjointly with a study by the Global Blood Pressure Genetics Consortium (Global BPgen) that had identified 8 partially overlapping loci using a similar sample size [21]. Two of the 8 top SNPs in CHARGE-BP were in strong linkage disequilibrium with a non-synonymous coding SNP. Other key findings of the 2 studies were that effect sizes of individual variants are small, ~1 mm Hg for SBP and ~0.5 mm Hg for DBP, although the joined effect of multiple risk alleles observed in the population was shown to be several mm Hg, which is substantial given that observational data indicate a prolonged increase of 5 mm Hg in DBP to be associated with a 34% increase in the risk for stroke and 21% increase in the risk of coronary events [22]. The total phenotypic variance explained by the 8 variants identified by the CHARGE-BP working group was ~1%. Most of the genes near the identified variants are in pathways completely unsuspected to be associated with BP before these studies.

The most comprehensive experiment to date is the joint meta-analysis of data by the CHARGE-BP and Global BPgen consortia, including additional studies, that came together and formed the ICBP with a total sample size of up to 200,000 samples, again including the FHS [23]. Figure 2 indicates the 29 SNPs identified to be associated with SBP and DBP in this study by physical position, including all the variants published by CHARGE-BP previously. The key additional findings of ICBP were that a risk score based on the 29 variants is associated with hypertension, left ventricular wall thickness, stroke, and CAD, but not with any of the 5 parameters of kidney disease or kidney function tested. This might be explained by a weaker association between the risk score and the kidney phenotypes versus with stroke and CAD, or by a reverse causal relationship in that BP is a consequence rather than a cause of kidney disease. Most of the associated SNPs could be shown to be associated with BP in multiple ethnicities (East Asian, South Asian, and participants of African origin were tested), suggesting a surprising transethnic effect. Eight of the 29 SNPs were in strong LD ($r^2 > 0.8$) with a nonsynonymous coding SNP, suggesting a functional role. There was some evidence for enrichment of expression SNPs near the 29 SNPs identified. Body mass index- and sex-interaction effects, metabolomic signatures, or enrichments of pathways could not be demonstrated to be significant.

ASSOCIATIONS WITH BLOOD LIPIDS

There are 4 major clinically used blood lipid levels: total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides. The FHS has contributed importantly to the current

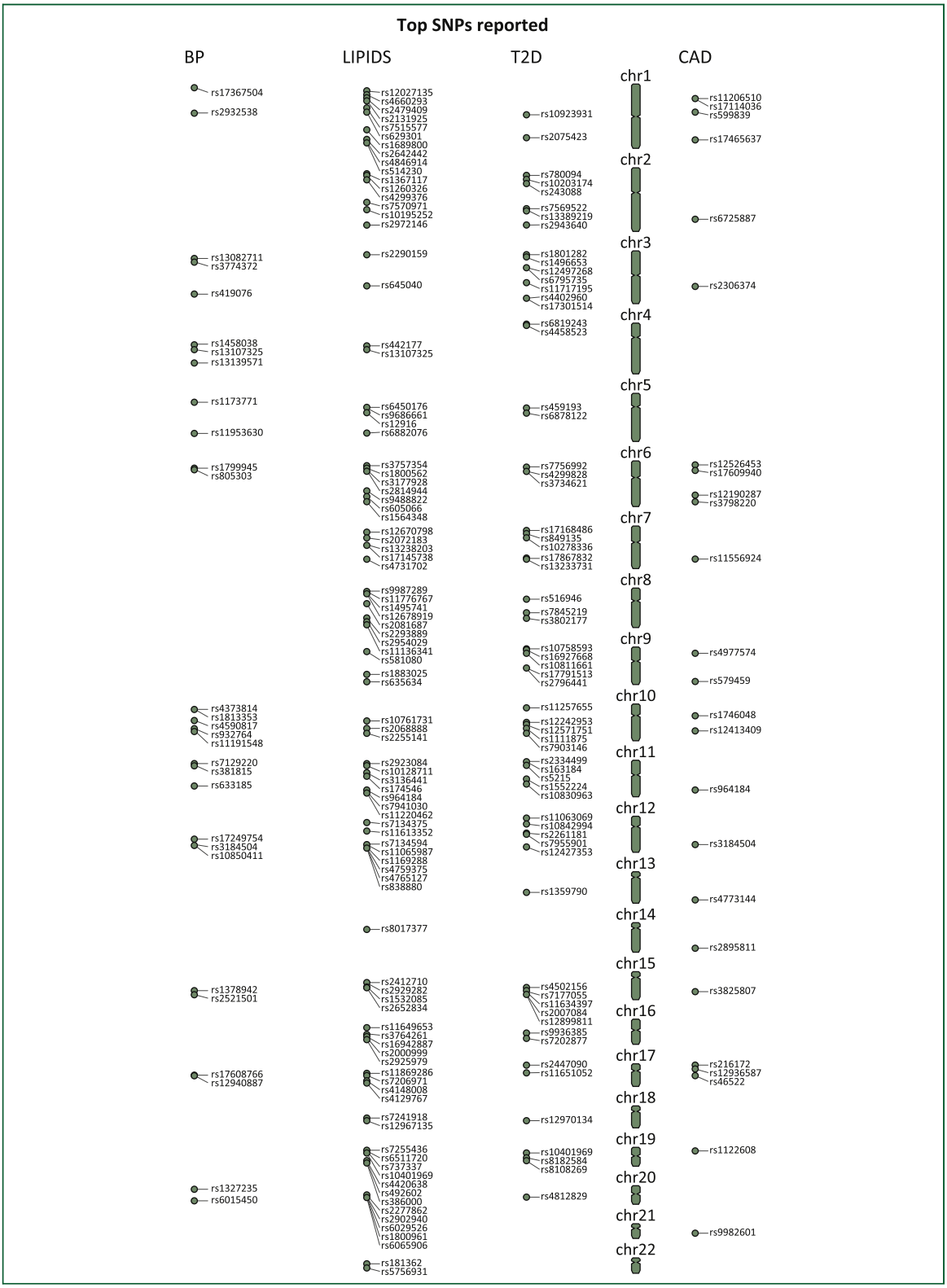

FIGURE 2. Top genetic variants identified for risk factors (blood pressure [BP], blood lipids [LIPIDS], type 2 diabetes [T2D]) and coronary artery disease by physical position. SNPs, single nucleotide polymorphisms.

knowledge on the blood lipid epidemiology and the clinical impact of cholesterol levels, for example, total cholesterol is part of the Framingham risk score [24]. Only the contribution of the FHS to the genomics of blood lipid levels is discussed in more detail here.

After publications based on smaller sample sizes, among them the FHS [25], to date the largest publication of blood lipid level loci is by the GLGC [26]. In this experiment on ~100,000 individuals of European origin in discovery, 95 lipid loci were discovered and 59 were new (the variants identified are shown in Fig. 2). The general principles that emerge from these data on lipid traits follow. 1) Collectively, the 95 loci explain 10% to 12% of the total trait variance in the FHS, a much larger fraction of total variance when compared with the analyses on BP (see Fig. 1). 2) Comparing the effect of the directions of the association signals in different ancestries, the investigators were able to provide evidence that lipid variants show association in individuals of East Asian, South Asian, and African American ancestry based on an analysis of individuals with extreme blood lipid levels, suggesting, again, that the variants identified act across multiple ethnicities. 3) Of the genes near the 95 loci identified, a number are known lipid regulators (*CYP7A1, NPC1L1, SCARB1*), but the great majority was unsuspected to be linked to blood cholesterol levels previously. 4) Three genes (*GALNT2, PPP1R3B,* and *TTC39B*) near discovered variants were demonstrated to increase lipid levels in mouse models. Among the additional findings were that 4 of the 95 loci identified in the primary analyses showed a significant sex-interaction effect. Expression quantitative trait loci close to the associated SNP in the liver, the omental fat, and the subcutaneous fat were identified. Using an allelic dosage risk score, the GLGC investigators could show that top and bottom quartile contrasts show a 13× increased risk for elevated LDL-C blood levels.

It is clearly established that elevation of blood LDL-C levels plays a causal role in CAD. There is clear evidence that triglycerides and HDL-C levels are associated with CAD, but a causal role has been questioned, particularly for HDL-C for which blood level lowering treatment has failed to show cardioprotective effects [27], and there is more recent genetic evidence in the form of Mendelian randomization studies [28]. Fourteen SNPs identified to be associated with blood lipids in the GLGC study were also associated with CAD, and most of these were SNPs identified using LDL-C levels, in line with the existing evidence that LDL-C is causally linked to CAD. Four of the SNPs associated with CAD showed exclusive significant association with HDL-C or triglycerides, but not with LDL-C, opening potential future avenues for investigations of the phenomenon.

TYPE 2 DIABETES LOCI

The DIAGRAM Consortium analyzing type 2 diabetes (T2D) has completed GWAS analyses based on the HapMap backbone [29] and is the first of the large phenotype consortia discussed here to have published a meta-analysis of data based on the Human Cardio-Metabo BeadChip (Illumina, San Diego, CA) [30]. Among the advantages of the CardioMetabochip platform is a relatively favorable cost that enables genotyping in very large sample sizes.

The GWAS experiment had a total sample size of 47,117 in discovery (8,130 cases and 38,987 control cases) with follow-up in an additional 94,337 individuals (34,412 cases and 59,925 control cases), among them the FHS. In the GWAS study, 12 additional T2D loci were identified in addition to the ~25 T2D loci previously known and another 8 loci were identified in the recent CardioMetabochip study, bringing the total number to 64 (see Figs. 1 and 2). The general principles that emerge from these studies are as follows. 1) Overall, ~6% of the total phenotypic variance is explained by the loci discovered. 2) The effect sizes are small (odds ratio of 1.06 to 1.14 for autosomes). 3) Many of the loci discovered were previously unsuspected to be associated with T2D, but some had previous evidence from monogenic T2D. 4) One T2D locus is located on the X-chromosome. 5) Two loci (TCF7L2 and BCL11A) showed effect size heterogeneity when analyzed stratified by obese and nonobese individuals, whereas no strong evidence for age-of-diagnosis effects was found. In the CardioMetabochip analysis, 2 loci showed sex-differentiated association. Additional findings were that 7 of the 12 loci of the GWAS analysis are also associated with phenotypes other than T2D. Not unexpectedly, a positive correlation between the SNP-association results with T2D and the SNP-association results with anthropometric traits (GIANT Consortium) [31] and continuous measures of glucose blood levels (MAGIC Consortium) [32,33] were observed. In pathway and protein-protein interaction analyses, a signal on cell-cycle regulation emerged across different approaches (additionally *CREBBP*-related transcription, adipocytokine signaling, and other pathways). Analyses in the CardioMetabochip effort suggest a large number of currently nonsignificant variants with small effect sizes.

CAD GWAS

One major disease consequence of the cardiovascular risk factors mentioned previously that is discussed briefly here is CAD. Currently, the largest GWAS on CAD included 22,233 cases and 64,762 control subjects of European ancestry [34] in the CARDIoGRAM Consortium to which the FHS contributed. The findings were replicated by follow-up in 56,682 additional individuals. This study identifies 13 novel loci for CAD, bringing their total number to ~25 (Figs. 1 and 2). The principal findings were as follows. 1) Most of the loci are in regions previously unsuspected to be associated with CAD. 2) The effect sizes are small and increase risk by 6% to 17% per allele, and ~4% of the phenotypic variance is explained. 3) Three of the loci are also associated with traditional cardiovascular risk factors; 5 show pleiotropic effects; and 3 top SNPs were

expression SNPs. 4) A decile contrast of a weighted allelic risk score shows an increase of CAD risk of ~3×.

OUTLOOK

A significant increase in the number of loci discovered to be associated with each of the phenotypes discussed in this review is to be expected in the near future starting with the publication of the results based on CardioMetabochip genotyping by the GLGC, the ICBP, and the CARDIoGRAM Consortium. Subsequent experiments will address different allele-frequency ranges (exome chip, sequencing) [35]. The field is waiting for creative experiments to identify still more of the missing heritability of the major cardiovascular risk factors. The current clinical utility of the variants identified for cardiovascular risk factors and stroke is largely undefined.

CONCLUSIONS

Starting from little, an impressive body of data on the genetic basis of common cardiovascular disease and cardiovascular risk factors has accumulated over the last 5 years, as shown by the synopsis of variants identified for BP, blood lipids, T2D, and CAD in Figure 2. The hope is that the improved understanding of the mechanisms of cardiovascular disease and cardiovascular risk factors will permit improved patient treatment and the discovery of new, potentially more efficient, therapeutic agents. This is also particularly important also for low- and middle-income countries where 80% of all cardiovascular deaths occur [36].

After the tremendous impact that the FHS has had on the understanding of cardiovascular disease on the epidemiological level, the FHS has used and continues to use the unique resource available for important contributions to the discovery of the genetic origin of cardiovascular disease to which it should be firmly congratulated.

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