THERAPEUTIC IMPLICATIONS OF GENETIC RISK VARIANTS FOR CORONARY ARTERY DISEASE

Rajiv Kumar Srivastava¹, Naushaba Dulare Sayyed², Kshitija Chandrashekhar Rane³, Dattatraya Shankar Joshi⁴

¹Associate Professor, Department of Cardiovascular and Thoracic Surgery, MGM Medical College, Kamothe, Navi Mumbai. ²Scientific Assistant, Department of Genetics, MGM Medical College, Kamothe, Navi Mumbai. ³Ph.D. Scholar, Department of Genetics, MGM Medical College, Kamothe, Navi Mumbai. ⁴Professor, Department of Genetics and Biotechnology, MGM Medical College, Kamothe, Navi Mumbai.

ABSTRACT

BACKGROUND

This review covers therapeutic implication of genetic risk variant responsible for coronary artery disease by utilising the highdensity single-nucleotide microarrays to screen the entire human genome. The sequence of the human genome provides the blueprint for life. Approximately, 99.5% of the human genome Deoxyribonucleic Acid (DNA) sequence is identical among humans with 0.5% of the genome sequence (15 million bps) accounting for all individual differences.

MATERIALS AND METHODS

The new technology of the computerised chip array of millions of Single-Nucleotide Polymorphisms (SNPs) as Deoxyribonucleic Acid (DNA) markers makes it possible to study and detect genetic predisposition to common polygenic disorders such as Coronary Artery Disease (CAD). The sample sizes required for these studies are massive and large; worldwide consortiums such as Coronary Artery Disease Genome-wide Replication and Meta-Analysis (CARDIoGRAM) study have been formed to accommodate this requirement. After the identification of 9p21 progress to detect genetic predisposition has been remarkable.

RESULTS

There are currently a total of 50 genetic risk variants predisposing to CAD of genome-wide significance with confirmation in independent populations. Rare variants (Minor Allele Frequency, MAF <5%) will require direct sequencing to detect genetic predisposition.

CONCLUSION

We can develop new biomarkers for detecting early CAD as well as unique targets for novel therapy. The challenge for the future will be to identify the molecular mechanisms mediating the risk of those genetic risk variants that act through nonconventional risk factors. The ultimate objective for the future is the sequencing and functional analysis of the causative polymorphisms for its therapeutic implications.

KEYWORDS

Human Genome, Single-Nucleotide Polymorphisms, Coronary Artery Disease, Deoxyribonucleic Acid.

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BACKGROUND

Coronary Artery Disease (CAD) and its most severe complication Myocardial Infarction (MI) are leading causes of death and disability worldwide.^{1,2} Approximately, 99.5% of the human genome Deoxyribonucleic Acid (DNA) sequence is identical among humans with 0.5% of the genome sequence (15 million bps) accounting for all individual susceptibility for disease. The advance technology of the computerised chip array containing up to millions of Single-Nucleotide Polymorphisms (SNPs) as

Financial or Other, Competing Interest: None. Submission 26-12-2016, Peer Review 12-01-2017, Acceptance 26-01-2017, Published 09-02-2017. Corresponding Author: Dr. Rajiv Kumar Srivastava, No. 303/A, AUM Shree Palace, M. B. Road, MIRA Road- E, Thane-401107, Maharashtra. E-mail: rajiv0207@gmail.com DOI: 10.18410/jebmh/2017/133 COOSO Deoxyribonucleic Acid (DNA) markers makes possible genome-wide association studies to detect genetic predisposition to common polygenic disorders such as Coronary Artery Disease (CAD). The sample sizes required for these studies are massive and large; worldwide consortiums such as Coronary Artery Disease Genome-wide Replication and Meta-Analysis (CARDIoGRAM) study have been formed to accommodate all requirement. After the identification of 9p21 progress to detect genetic predisposition has been remarkable. There are currently a total of 50 genetic risk variants predisposing to CAD of genome-wide significance in independent populations. It is expected that most of the common variants (minor allele frequency, MAF >5%) will be identified for CAD within the next two to three years. Rare variants (MAF <5%) will require direct sequencing to detect genetic predisposition. For its therapeutic implications, further objective is the sequencing and functional analysis of the causative polymorphisms. Recent Genome-Wide Association Studies (GWAS) of CAD have discovered multiple chromosomal

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regions.³ The first genetic risk variant for CAD was discovered in 2007 located on the short arm (p) of chromosome 9, now commonly referred to as 9p21.4,5,6,7 Simultaneously and independently, the deCODE group also discovered 9p21.8,9 9p21 was confirmed by multiple investigators around the world.¹⁰⁻¹³ Further technological advances markedly facilitated the pursuit of genetic risk for CAD and mapping of more than 16 million SNPs to their chromosomal location. Several GWAS were performed for CAD as well as other diseases and by 2009.¹⁴ Multiple genetic risk variants contribute to CAD each associated with only mild-to-moderate genetic influence. Two association signals reached genome-wide significance. These included one locus (4 SNPs on 9p21.3) previously identified in populations of European descent and one newly-discovered locus (rs9268402 at C6orf10-BTNL2) in Chinese population. After meta-analysis of the discovery, eight additional variants were associated with CAD at a significance level. When combining the data, ten SNPs at seven regions associated with CAD at a prespecified threshold were found. Among these SNPs, the four (rs9632884, rs10757274, rs1333042, rs1333049) on 9p21.3, rs9349379 on 6p24.1 in PHACTR1 and rs11066280 on 12q24.13 near C12orf51 confirmed associations with CAD previously reported in Europeans.⁵⁻¹¹ In addition, rs12524865 on 6q23.2 near TCF21 also showed association previously reported in Europeans,12 which nearly reached the genome-wide significance threshold in the combined analyses. Moreover, four new CAD loci in Chinese were identified.

AIMS AND OBJECTIVES

Our main aim is to identify the molecular pathways for early detection of CAD. We can develop new biomarkers for detecting early CAD as well as targets for novel therapy. Identification of these molecular pathways will provide early detection and effective prevention of CAD. Our objective is to identify the molecular mechanisms mediating the risk of those genetic risk variants.

MATERIALS AND METHODS Inclusion Criteria

Based on our conceptual model and practical considerations, we developed literature review methods that included criteria to identify potentially relevant articles. Our focus was on articles that provided definitive primary data from randomised-controlled trials for information not covered by the primary randomised-controlled trial reports. The objective of our search strategy was to identify all published randomised trials and all ongoing research. For the literature review, we used standard search strategies involving the querying of online databases (MEDLINE®, Embase and Cochrane) using keywords followed by evaluation of the bibliographies of relevant articles.

Exclusion Criteria

We have excluded all those articles, which are not related to provide definitive primary data from randomised-controlled trials.

RESULTS

An international consortium was formed dedicated to the pursuit of discovering genes associated with CAD¹⁵ that is the largest collaboration in the history of cardiology. The initial international consortium was referred to as the Coronary Artery Disease Genome-Wide Replication and Meta-Analysis (CARDIoGRAM) study. This provided a sample size of 86,995 individuals of European ancestry for the discovery genotyping in an population size of 56,682. The study led to the discovery of 13 new genetic risk variants for CAD.¹⁶ This was followed by the results from the Coronary Artery Disease C4D Genetics Consortium, which identified four additional genetic risk variants for CAD.¹⁷ The IBC 50K CAD Consortium identified three additional risk variants for CAD.¹⁸ Subsequently, CARDIoGRAM joined with the C4D group to become CARDIoGRAM plus C4D with a sample size of 1,90,000 individuals. Analysis of this sample size led to the discovery and confirmation of 46 genetic risk variants associated with CAD.¹⁹ There are currently a total of 50 genetic risk variants predisposing to CAD of genome-wide significance with confirmation in independent populations.

DISCUSSION

We performed a meta-analysis of 2 genome-wide association studies of coronary artery disease comprising 1,515 cases with coronary artery disease and 5,019 controls, followed by de novo replication studies in 15,460 cases and 11,472 controls, all of Chinese Han descent. We successfully identified four new loci for coronary artery disease reaching genome-wide significance (P <5×10-8), which mapped in or near TTC32-WDR35, GUCY1A3, C6orf10-BTNL2 and ATP2B1. We also replicated four loci previously identified in European populations (PHACTR1, TCF21, CDKN2A/B and C12orf51). These findings provide new insights into biological pathways for the susceptibility of coronary artery disease in Chinese Han population.

The value of genomic inflation factors indicated population stratification effects were negligible in study samples. We further examined whether hypertension could mediate the effect on CAD. After adjustment for hypertension, the associations with CAD remained genome-wide significant rs1842896 is located to 76.4 kb upstream of the GUCY1A3 locus. The GUCY1A3 gene encodes the a subunit of Soluble Guanylyl Cyclase (sGC), an important enzyme of nitric oxide signaling pathway, which is the pathogenesis of CAD and atherosclerosis. Preclinical studies have explored the therapeutic potential of sGC stimulators. ¹⁵ rs7136259 is near ATP2B1, which encodes PMCA1, a plasma membrane calcium ATPase, which pumps calcium (Ca2+) ions out of the cytosol into the extracellular milieu.¹⁶

The C6orf10-BTNL2 on 6p21.32 is a hotspot associated with immune-related diseases.¹⁷⁻¹⁹ BTNL2 is a member of the immunoglobulin superfamily that probably functions as a T cell costimulatory molecule. It is noteworthy that rs2076530, a truncating splice site mutation in BTNL2 gene¹⁷ is in strong LD with rs9268402. BTNL2 gene polymorphisms have been found associated with susceptibility to Kawasaki Disease (KD)²⁰ with increased risk of developing ischaemic

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heart disease in the future.²¹ rs2123536 on 2p24.1 is located to ~150kb downstream of TTC32 and WDR35. TTC32 encodes the protein containing the tetratricopeptide repeat motif to bind other peptides.²² WDR35 encodes a member of the WD,²³ which involves cell cycle progression and gene regulation.

The chromosome 12q24 region is of particular interest. All variants on 12q24 associated with CAD^{10,11} in Europeans are not polymorphic in Chinese, whereas all CAD-associated variants on 12q24 in Chinese are monomorphic in Europeans. rs11066280 also showed significant evidence of association with high-density lipoprotein, triglycerides, total cholesterol and blood pressure. The 4 SNPs showing significant CAD association in 9p21.3 region were in almost perfect LD in European descent.

To examine the effect of 9 SNPs in aggregate on the risk of CAD, a CAD risk score was calculated by using weighted sum across the SNPs combining effect. The mean CAD risk score of cases was significantly higher than that of controls. Logistic regression model was applied to test the association of risk score categories with CAD. Compared with bottom quintile, individuals in the top quintile of CAD risk score had greater than twofold increased risk for CAD.

We assessed both the known and new CAD susceptibility loci for overlap of associations with a number of relevant traits-lipid levels (GLGC),¹⁶ blood pressure (ICBPG),¹⁷ diabetes (DIAGRAM),¹⁸ glucometabolic traits (fasting insulin and fasting glucose concentrations, HOMA-B (homeostatic model assessment-β score) and HOMA-IR (insulin resistance) MAGIC¹⁹ and anthropometric traits (GIANT).^{20,24} After applying a Bonferroni correction for the 51 independent CAD-associated alleles tested, 12 loci showed evidence of association between the lead CAD risk SNP and one or more plasma lipid trait in the expected direction (the CAD risk allele was associated with higher total cholesterol). These lead SNPs were most strongly associated with LDL cholesterol concentration at 8 loci (APOB, ABCG5-ABCG8, PCSK9, SORT1, ABO, LDLR, APOE and LPA) with triglyceride concentration at two loci (TRIB1 and the APOA5 cluster) and with HDL cholesterol concentration at one locus (ANKS1A). All loci except LPA and ANKS1A showed genome-wide significance for association with a lipid trait. At the SH2B3 locus, the CAD risk allele for rs3184504 was associated with both lower LDL cholesterol and HDL cholesterol concentration; one likely explanation is the presence of independent variants for CAD and LDL cholesterol. Two known CAD risk loci (CYP17A1-NT5C2 and SH2B3) and two of the new CAD susceptibility loci (GUCY1A3and FES) have previously been associated with systolic (SBP) and diastolic (DBP) blood pressure.¹⁷ In contrast to the results for lipid concentration and blood pressure, there was no significant association of any of the loci tested with type 2 diabetes (T2D). Suggestive associations with Body Mass Index (BMI) and waist-hip ratio were observed in the CYP17A1-CNNM2-NT5C2 and RAI1-PEMT-RASD1 loci, respectively.

The genome-wide significance threshold, we used is the accepted criteria for reporting individual association signals, as for each experiment it controls the error rate among common variants to less than 5%. SNPs showing association with a phenotype, but not this genome-wide threshold are likely to include additional true positive signals in wellpowered studies. Such SNPs may also be informative in predicting CAD risk and in constructing CAD-associated biological networks. To identify such variants, we undertook an FDR analysis to assess the proportion of false-positive signals in a set of (nominally) significant SNPs.²⁵ The Metabochip array contains both SNPs with priors in terms of association to CAD (CARDIoGRAM study) and blocks of SNPs of fine-mapping regions. To normalise the distribution of SNPs considered for FDR analysis, we (i) removed all SNPs in the CAD fine-mapping regions and LD-pruned SNPs in the non-CAD fine-mapping regions and (ii) adjusted the combined SNPs with priors in stage 1 using fixed-effect inverse variance-weighted meta-analysis P values for all other SNPs. We obtained 104 SNPs at an FDR threshold of 5% and LD threshold of r2 <0.2. The median OR for CAD for these SNPs was 1.054 per risk allele.

On the basis of a heritability estimate of 40% for CAD, the combination of the known and newly-associated SNPs within the 45 susceptibility loci explains approximately 6% of the genetic variance of CAD. By addition of the 104 SNPs from FDR analysis increased the fraction explained to 10.6%.

CONCLUSION

The genetic risk variants for CAD are very common occurring on an average in 50% of the population with a frequency varying from 2% to 91%. The relative increased risk of each genetic variant is small averaging 18% with an odds ratio varying from 2% to 90%. For CAD as well as other common polygenic disorders, multiple genetic risk variants are inherited by everyone. Those at high genetic risk for CAD have a greater genetic risk burden due to inheritance of a greater number of common risk variants. In a CARDIoGRAM analysis of 23 genetic risk variants for CAD, the average number inherited per individual was 17. Most of the genetic risk variants for CAD are located in DNA sequences that do not code for protein. The risk variant mediates its risk for CAD directly or indirectly through regulation of DNA sequences that do code for protein. All DNA genetic risk variants need only be assessed once. Genetic risk variants do not vary with time, meals, drugs or gender. It is important to search new genetic risk factors with the hope of identifying new pathways that lead to CAD. We can develop new biomarkers for detecting early CAD as well as unique targets for therapy. Identification of these molecular pathways will provide for early detection and more effective prevention of this disease.

Further challenge will be to identify the molecular mechanisms mediating the risk of those genetic risk variants that do not act through known conventional risk factors.

ABBREVIATIONS

CAD- Coronary artery disease; GWAS- Genome wide association studies; MI- Myocardial infarction; PRISMA-Preferred reporting items for systematic reviews and meta-

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analyses statement; DPI- Duke CAD prognostic index; Δ MLD- Change in minimum lumen diameter; ECG-Electrocardiography; WMD- Weighted mean difference; RR-Relative risk; Single-nucleotide polymorphisms (SNPs) as DNA markers CARDIOGRAM (coronary artery disease genome-wide replication and meta-analysis) study.

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