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Dissecting the genetics of cardiomyopathy in India: A tale of ten steps

Reliable data on the prevalence of cardiovascular disease (CVD) from the Indian sub-continent do not exist. However, compared to the global average, the public-health burden attributable to CVD is very high in India (Goyal and Yusuf 2006). Based on cross-sectional surveys conducted in Indian populations (Gupta 2004, 2005), the prevalence of coronary heart disease (CHD) was estimated to be 3–4% in rural areas and 8–10% in urban areas in 2003. These prevalence estimates were two- to six-fold higher than those estimated 40 years ago, and may be underestimates because of incompleteness of our cause-of-death statistics. CVD, as is well known, is caused by an interaction between genetic and environmental factors. Variants in many genes have been found to be associated with CVD in populations, some of which also co-segregate with CVD in families. However, replication of association or linkage findings has been difficult. The difficulties stem from various complexities: heterogeneous nature of the CVD phenotype, possible involvement of multiple genes with variable effects from one family/population to another, limited sample sizes and population stratification. In the dissection of a common disease such as CVD, one way to reduce the complexity arising from heterogeneity of phenotype is to recognize, define and study a sub-phenotype that is clinically more homogeneous. CHD is the most significant component of CVD. Even CHD is clinically heterogeneous. A common cause of heart failure is impaired contraction of one or both ventricles of the heart in the absence of significant coronary artery disease. Impairment of contraction of ventricles may be due to enlargement (dilated cardiomyopathy), thickening (hypertrophic cardiomyopathy) or rigidity (restrictive cardiomyopathy) of the heart muscle. Cardiomyopathy is a leading cause of cardiac transplantation. The high morbidity and mortality associated with cardiomyopathy underscores the need for a better understanding of the underlying molecular events leading to heart failure in cardiomyopathy.

Defective cardiac-myosin binding protein C, a component of filaments in cardiac muscle cells, results in distorted sarcomeres – the smallest functional units of muscle. The gene that produces this protein is called *MYBPC3*. Variants in this gene have been associated with cardiac disease in various world populations. Dhandapany *et al* (2009) has recently identified a 25-bp deletion in an intron of *MYBPC3* that is associated with cardiomyopathy in south Asia. This is an important finding from a clinical and molecular genetic standpoint. A DNA diagnostic test to assess whether an individual has inherited this deletion will have a significant predictive value.

Aside from significance, the study serves as a model for dissection of a complex disease such as CHD. This is to be emphasised. First, the authors reduced the clinical heterogeneity of CHD by focussing on a subphenotype – heart failure due to cardiomyopathy. This strategy enhances the chance of gene identification. True, the identified variant/mutation in *MYBPC3* cannot explain the garden-variety CHD phenotype, but in any case one does not expect a single variant/mutation in a single gene to explain any complex phenotype. At least this strategy serves to identify and helps to predict – and thus possibly prevent – heart failure in a large number of individuals. Second, the authors built their hypothesis of the association of the 25-bp deletion with cardiomyopathy when they observed (possibly a chance observation) an enhancement of the frequency of this deletion in a small number of individuals with cardiomyopathy. Third, the authors then went on to test their hypothesis with much larger sample sizes (354 affected individuals and 238 healthy controls, matched for appropriate covariates). The hypothesis was accepted. The odds-ratio of having cardiomyopathy was 5.3-fold higher if one carried the 25-bp deletion than if one did not. Fourth, the authors went on to cross-validate this inference in an independent

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sample of 446 affecteds and 466 healthy controls. The odds-ratio (6.99) was again statistically highly significant (cross-validation is crucial to guard against false positive inferences). In genetic association studies false positive results can also arise due to heterogeneity of ancestries of selected affecteds and controls (a phenomenon called "population stratification"). The fifth highlight of this study is that the authors generated extensive and relevant genetic data from judiciously selected population groups and carried out appropriate statistical tests to rule out the possibility of having drawn a false positive inference arising from population stratification.

An individual can be either homozygous or heterozygous for the 25-bp deletion. Are the clinical manifestations of possessing two copies of the deletion allele the same as that of possessing one copy of the allele? This was the sixth issue considered in this study. The authors gathered data from 28 unrelated families comprising 120 individuals. Unfortunately, the number of homozygotes was too few to draw any strong conclusions, although the homozygotes seemed to be vulnerable to sudden cardiac death. The heterozygote carriers of the deletion showed an age-dependent phenotype. Young heterozygote carriers were mostly asymptomatic or mildly symptomatic, but ~90% of the older heterozygote carriers in these families were symptomatic. Symptoms in carriers started to develop by about the third decade of life. Some exceptions were noted, indicating phenotypic heterogeneity within the heterozygous genotype.

The seventh highlight of the study was that the authors investigated the risk induced by the deletion. They analysed RNA and protein from the endomyocardial biopsy specimens of two young heterozygous individuals, who were 25 and 32 years old. Their cardiac MyBP-C cDNA revealed two transcript structures: a normal transcript and a mutated allele with exon-skipping. However, the altered protein could not be detected in the biopsied tissue samples. Could the altered protein distort the normal structure of the sarcomere? To investigate this, the authors expressed the myc-tagged altered protein along with wild type (WT) protein in neonatal rat cardiomyocytes, and stained these with antibodies to the myc. The results of this experiment showed that the normal sarcomeric architecture was distorted as a result of aberrant incorporation of the altered protein. This disorganization perhaps leads to pathogenesis. It has been hypothesized that the accumulation of the altered protein might disrupt the cellular homeostasis and initiate late-onset cardiomyopathy; a possible explanation for the vast majority of individuals with the 25-bp deletion remaining healthy until the third decade of their life. Since the altered protein could not be detected in the tissue, this causal explanation could not be confirmed. More evidence is required to prove casuality.

Since carriers of the 25-bp deletion remain asymptomatic for the first three decades of their life, a large number of individuals in a population would not even know that they are carriers. In other words, they may be unknowingly sitting on a volcano, waiting to erupt. What is the frequency of the 25-bp deletion allele in the general Indian population? The authors screened 6,273 individuals belonging to 107 ethnic populations across 35 States of India. They found that the allele frequency across populations ranged from 2% to 8%; southern and western States showed significantly higher frequencies of the deletion allele compared with the north and northeastern States. If indeed possessing even one copy of the deletion allele had deleterious consequences, why was the deletion allele not eliminated? Based on inferred haplotype backgrounds of chromosomes carrying the deletion and the normal alleles, they authors did not find major selective effects. They have argued that since a carrier of the deletion allele remains asymptomatic till about 30–40 years of age, possessing this allele did not impact on family size. Further evidence may be sought from families of carriers who do not adopt family-planning practices: hard to find, but possibly obtainable from rural India.

The deletion allele is common throughout India. Is it also common in other regions of the world? Sampling of populations from 5 continents showed that except for Pakistan, Sri Lanka, Indonesia and Malaysia, the deletion allele is absent from other regions of the world. Did the allele arise in India? Perhaps, yes … about 33,000 years ago, after the initial settlers arrived in India.

The work embodied in this paper represents a model study on dissecting the genetics of a complex disorder, characterized by judicious choice of a nearly-homogeneous sub-phenotype, building on cues obtained from earlier studies, conduct of population and family based studies with large sample sizes to identify an associated genetic variation, cross-validation, excluding a known factor ("population stratification") that leads to false positive inferences, genotype-phenotype correlation, functional validation (which remains somewhat weak in this work), and drawing relevant population-genetic and evolutionary inferences.

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