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Genetics of cardiovascular disease: Importance of sex and ethnicity

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ABSTRACT

Sex differences in incidence and prevalence of and morbidity and mortality from cardiovascular disease are well documented. However, many studies examining the genetic basis for cardiovascular disease fail to consider sex as a variable in the study design, in part, because there is an inherent difficulty in studying the contribution of the sex chromosomes in women due to X chromosome inactivation. This paper will provide general background on the X and Y chromosomes (including gene content, the pseudoautosomal regions, and X chromosome inactivation), discuss how sex chromosomes have been ignored in Genome-wide Association Studies (GWAS) of cardiovascular diseases, and discuss genetics influencing development of cardiovascular risk factors and atherosclerosis with particular attention to carotid intima-medial thickness, and coronary arterial calcification based on sex-specific studies. In addition, a brief discussion of how ethnicity and hormonal status act as confounding variables in sex-based analysis will be considered along with methods for statistical analysis to account for sex in cardiovascular disease.

1. Introduction

Sex differences in incidence, prevalence, morbidity and mortality from cardiovascular diseases are well documented and represent important health disparities [1–3]. These sex differences can be classified as those that are sex-specific such as erectile dysfunction in men and hypertensive pregnancy disorders in women, or conditions common to both sexes but which show sex differences in presentation and outcomes such as hypertension, atherosclerosis, angina, and stroke.

Sex differences in cardiovascular diseases result from a complex interaction among genetic, hormonal and environmental factors that provide a profile of individual risk and phenotypic presentation of disease. Therefore, there is an increased interest in identifying the genetic components of disease to optimize treatments and outcomes. However, investigations into the genetics of cardiovascular diseases in men and women are hampered by the failure to include the sex chromosomes in genome-wide association studies (GWAS), to account for sex as a variable in targeted genetic analyses, and to examine hormone-gene interactions. In this paper, we will provide general background on the X and Y chromosomes, discuss how they have been ignored in GWAS of cardiovascular disease, and discuss genetic factors contributing to sex differences in conventional cardiovascular risk factors and atherosclerosis with examples of sex-specific studies of carotid intima-medial thickness, and coronary arterial calcification. Confounding factors of ethnicity and hormonal status also will be considered along with some proposed methods for statistical analysis for future studies.

2. Background: the genetics of sex

In humans, sex is determined via the X and Y chromosomes. Each somatic cell of the human body contains 23 pairs of chromosomes, 22 of which are the same in both sexes (autosomes); however females have two copies of the X chromosome, whereas males have one copy of the X and one copy of the Y [4]. Therefore, differences between the sexes result from the particular combination of sex chromosomes an individual possesses, as well as, differing levels of the sex hormones. Often early studies of sex differences compared persons with XX and XY genotypes to those with sex chromosome monosomy (e.g. XO karyotype in Turner syndrome) or trisomy (e.g. XXY karyotype in Klinefelter syndrome) to distinguish whether observed sex differences were due to sex

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chromosome dosage effects independent of hormonal influences [5].

As genotyping technologies have rapidly improved, studies of the genetic basis of sex differences are no longer limited to investigating global differences in sex chromosome dosage effects, but can directly examine the individual genes and genetic variants on the X and Y chromosomes. In humans, the larger X chromosome contains 813 protein coding genes, whereas the much smaller Y chromosome contains 143 protein-coding genes, although many of the Y genes are multi-copy genes or present in both males and females (Ensemble Genome Browser, http://www.ensembl.org); only 46 genes are on the male-specific region of the Y (MSY). The sex chromosomes evolved from ancestral autosomes [6,7], and over the course of evolution, the X has retained many of the ancestral genes (98% retained), but the Y lost many (3% retained) [8]. Only 14 ancestral X–Y gene pairs are still present in humans, and many of these have important regulatory functions in regards to transcription, translation, and protein stability [8,9]. Some of the genes in the ancestral gene pairs retain the same function across the X and Y, but others have diverged. Additionally, both the X and Y have acquired new genes through evolution, with an emphasis on reproductive function [10,11]. The Y chromosome contains the SRY gene, or sex-determining region Y, which encodes the testes determining factor [12]. Many other genes on the Y chromosome are related to male sexual development and reproduction, including multicopy gene families expressed exclusively in the testes [8,11]. The X chromosome also includes reproductive-related genes, such as the androgen receptor [13], and genes important for brain development, blood clotting, and visual pigmentation [14].

The X and Y chromosomes have developed unique properties as compared to the autosomes, as illustrated schematically in Fig. 1 (this schematic diagram does not reflect the large difference in genetic content between the X and Y chromosomes, such as the large block of non-coding heterochromatin on the Y located in the MSY region designated in the figure at * [11]). Unlike autosomal pairs, the X and Y do not generally undergo recombination due to evolutionary divergence. However, there are two regions on each end of the X and Y chromosomes that are homologous, called the pseudoautosomal regions (PAR), which behave like the autosomes and do recombine [6] (Fig. 1A). For most genes in the PAR, both copies are expressed across both sex chromosomes (XX for females or XY for males). However, for other regions, that is, the genes on the non-pseudo-autosomal regions, the aneuploidy causes unequal patterns of expression across the sexes, which requires some form of dosage compensation across males and females [15]. One method is upregulation of X-linked genes [16]. In humans, one of the female copies of the X chromosome is silenced during embryogenesis to achieve dosage compensation during a process called X chromosome inactivation [6] (Fig. 1B). The X-linked gene *XIST* is expressed from the inactive chromosome, triggering DNA methylation that is responsible for the chromosome-wide silencing [17]. The inactive chromosome remains silent in somatic cells, but is reactivated during meiosis. This inactivation is tissue and cell specific, such that the maternally inherited allele may be expressed in some cells, while the paternally inherited allele may be expressed in others [18]. This process occurs randomly so that usually each chromosome is inactivated in 50% of cells, although preferentially (skewed) inactivation of one chromosome can occur either globally or in specific tissues, as demonstrated in cardiac muscle of mice [18]. Skewed X-inactivation has been observed in samples from human arteries, suggesting that the cells in the plaque arose from a single or similar group of cells [19]. To further complicate matters, approximately 15% of X-linked genes escape inactivation in humans [20,21]. For these 'escapee genes,' both copies are active in females (whereas males may have a single active copy if there is no homologous gene on the Y). Mechanisms of dosage compensation, whether concerning X or Y specific genes undergoing upregulation or X inactivation, or shared homologous X–Y gene pairs or pseudoautosomal genes, may be related to differential gene expression across the sexes and are important considerations for sex specific disease risk.

In contrast to the PAR on the X and Y chromosomes that is present in both sexes, the non-PAR region of the Y chromosome (MSY) encompasses the vast majority of the Y chromosome [11]. This region does not undergo recombination, and is inherited from father to son as a single haplotype, reflecting the paternal lineage only. Many of these genes are widely-expressed (at the RNA level), exhibiting global regulatory functions in transcription, translation, and other biological processes (non-reproductive), and hence may be important to men's health beyond sex determination and sexual reproduction [8].

Both X and Y chromosomes contribute to disease. Many rare Mendelian genetic disorders are linked to the sex chromosomes. According to the Online Mendelian Inheritance in Man (OMIM) catalog of genetic disorders in humans, approximately 7% of cataloged phenotypes are X-linked [22]. XY males are at increased risk compared to XX women for X-linked disorders, such as learning disabilities and mental retardation caused by X-linked genes important in brain development and function [14]. Hearing impairment (DFNY1, MIM 400043) reported in a Chinese family was the only documented Mendelian disorder showing Y-linkage in humans [23]. However, subsequent analysis found that the condition was related to an insertion of chromosome 1 into the Y gene rather than a mutation of a Y-chromosomal gene [24].

Much work to link the sex chromosomes to disease has focused on such rare disorders caused by a single gene, and less is known about the influence on complex traits with multiple genetic and etiological components. In particular, variants on the X chromosome are understudied for complex traits, with significantly fewer genome-wide associations than the autosomes [22] (Fig. 2). Of the over 2800 genome-wide significant associations reported for over 300 traits in the NHGRI GWAS Catalog [25], only 15 published associations reside on the X despite representing approximately 5% of the human genome, including 153 million nucleotide base pairs and 1669 genes [26]. This representation is in stark contrast to the similarly sized chromosome 7, which holds 120 published associations [22]. There are a number of reasons for this discrepancy, including reduced power due to the use of sex-specific analyses or low data quality, reflecting poor genotyping accuracy on current genome-wide arrays and quality control issues. However, an important reason is also that although the X chromosome is routinely included in standard genotyping arrays, it is simply excluded from analysis. Indeed, of the first 53 studies that have released publically available GWAS data, only 31 include the X chromosome [22]. Since the number of copies of X chromosome variants is confounded with sex, special analysis methods and computational tools are required (discussed below); therefore the sex chromosomes have historically been excluded to simplify analyses, setting a critical precedent that needs revision.

Important issues regarding the genetics of sex differences are not limited to large scale GWAS studies in humans but also are critical concerning preclinical work involving model organisms. In particular, basic science research utilizes experiments in model organisms as critical tools to investigate human disease. However, the genetics and biology of the sex chromosomes differs from species to species regarding genes that are conserved and mechanisms of dosage compensation [8,15]. Therefore the choice of the model organism may be critical to the study of sex differences. The mouse has become a favorite model organism for preclinical disease research because many genes are shared with humans,