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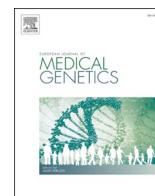
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Sex-linked genetic mechanisms and atrial fibrillation risk

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ABSTRACT

Atrial fibrillation (AF) is a cardiac condition characterised by an irregular heartbeat, atrial pathology and an elevated downstream risk of thrombosis and heart failure, as well as neurological sequelae including stroke and dementia. The prevalence and presentation of, risk factors for, and therapeutic responses to, AF differ by sex, and this sex bias may be partially explained in terms of genetics. Here, we consider four sex-linked genetic mechanisms that may influence sex-biased phenotypes related to AF and provide examples of each: X-linked gene dosage, X-linked genomic imprinting, sex-biased autosomal gene expression, and male-limited Y-linked gene expression. We highlight novel candidate risk genes and pathways that warrant further investigation in clinical and preclinical studies. Understanding the biological basis of sex differences in AF should allow better prediction of disease risk, identification of novel risk/protective factors, and the development of more effective sex-tailored interventions.

1. What is atrial fibrillation?

Atrial fibrillation (AF) is a cardiac condition (the most common type of 'supraventricular arrhythmia') resulting in chaotic twitching, or quivering, of the atrial myocardium; it can be concomitant with dysregulated electrical activity at the sinoatrial node, and is typically diagnosed through a combination of electro- and echo-cardiograms, chest X-ray and blood tests (Zimetbaum, 2017). AF risk increases with age and in the presence of heart tissue pathology, and may also be secondary to pulmonary conditions e.g. asthma; cardiac damage can have multiple precipitants notably hypertension, valvular or congenital heart disease, pericarditis, cardiomyopathy and diabetes (Lip et al., 2016). AF is commonly-associated with the sensation of an irregular or very fast heartbeat even whilst at rest, with palpitations and chest pain, and with breathlessness, fatigue and feelings of dizziness or faintness; in some cases, AF is asymptomatic and is picked up incidentally during medical examinations or check-ups (Lip et al., 2016). In the related, and occasionally co-morbid, condition of atrial flutter, the atrial rhythm is less disorganised and the symptoms may be less severe (Campbell, 1998). AF severity can vary from a single, rapidly-resolving incident, to recurrent episodes resolving quickly (within seven days, 'paroxysmal AF'), resolving more slowly ('persistent AF'), or not resolving at all ('permanent AF'). The turbulent blood flow within the heart associated with both atrial fibrillation and atrial flutter significantly increases the

likelihood of blood clot formation (thrombosis), and the downstream consequences of this including embolism/stroke, heart failure and cognitive decline/dementia; as such, AF is managed as a condition with serious implications for morbidity and mortality (Staerk et al., 2017). Treatments to regulate heart rate and rhythm, and limit the possible consequences of AF, include: breathing exercises to limit the duration of acute episodes, medication such as beta blockers, anti-arrhythmic drugs and anti-coagulants, the application of controlled electrical signals to the heart via electrodes on the chest (cardioversion), and surgical techniques including ablation and pacemaker implantation; lifestyle changes such as improved diet and reduced smoking and alcohol consumption are also beneficial for many patients (Lip et al., 2016). With increasing exposure to cardiovascular risk factors, and an ageing population, AF will represent a severe public health challenge over the coming decades (Lippi et al., 2021). Identifying and characterising environmental and biological risk factors for the condition, and understanding how the two interact, will be key to developing more effective, more targeted, and less invasive interventional strategies for AF to reduce its societal burden.

2. Sex differences in atrial fibrillation

Sex differences have been noted with respect to many facets of AF. First, epidemiological studies around the world have consistently shown

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that the prevalence of the condition differs in middle-aged males and females, with rates in the former sex 1.2–2.7 times higher those in the latter (Berge et al., 2018; Schnabel et al., 2015; Zoni-Berisso et al., 2014; Zhang et al., 2021). In terms of presentation, there is some evidence that women with AF may develop the condition at an older age and be diagnosed later, experience more severe and disabling symptoms, and have a poorer quality of life than affected males (Li et al., 2019; Lip et al., 2015); moreover, whilst women and men with AF exhibit similarly-elevated dementia rates (Chen et al., 2018; Miyasaka et al., 2007), women (and particularly those >75yrs) exhibit greater AF-related mortality which is likely to be largely attributable to an increased risk of thromboembolism (Guo et al., 2016; Piccini et al., 2012; Yoshida et al., 2016; Zhang et al., 2021). Consistent with this, use of direct oral anticoagulant (DOAC) drugs reduced the risk of intracranial hemorrhage and all-cause mortality in women (but not men) with AF (Law et al., 2018). The benefits of particular therapies may also differ by sex. For example, there is some evidence that the benefits of catheter ablation outweigh those associated with medical therapy to a greater extent in males than females with AF (Asad et al., 2019).

The factors underlying sex differences in AF prevalence, presentation and response to therapy are manifold, and the fact that they are likely to interact in complex ways makes assigning causality challenging. Potential underlying mechanisms include socio-cultural determinants which exacerbate sex-biased ascertainment or diagnosis (Regitz-Zagrosek, 2012), and sex biases in predisposing medical conditions or in exposure/response to environmental and lifestyle factors. As examples of the latter, diabetes and advanced age appear to be stronger risk factors for AF in women than in men (Bose et al., 2019), whilst increased physical activity appears to enhance AF risk in men, but mitigate it in women (Zhu et al., 2016). Intrinsic biological differences in sex hormone levels, atrial structure/function, cardiac autonomic modulation, and calcium signalling processes, could also explain sex-biased AF risk; these mechanisms have been comprehensively reviewed elsewhere (Chen et al., 2021; Odening et al., 2019; Winham et al., 2015). AF risk appears to be strongly influenced by the combined action of many genetic and epigenetic factors (Kim et al., 2021; Weng et al., 2017), and biological differences between males and females must ultimately arise from the differential expression of sex-linked genes. Such differentially-expressed genes may be expressed within the heart and act directly upon it, and/or may elicit their effects indirectly e.g. through influencing hormonal production or secretion in a distal tissue. Direct sex-linked genetic effects are likely to account for a comparatively small proportion of sex-biased AF-related phenotypes. To date, convincing evidence for AF-risk variants within two specific X-linked genes (*KCNE5* at Xq23 and *EMD* at Xq28), has been obtained through studies of syndromes in which AF is a variably-associated trait; *KCNE5* encodes a voltage-gated potassium channel ancillary subunit perturbed in Brugada syndrome, whilst *EMD* encodes the nuclear membrane protein emerin perturbed in Emery-Dreifuss muscular dystrophy (Abbott, 2016; Karst et al., 2008). Less robust evidence from a small, candidate gene-based study has suggested a risk polymorphism within the X-linked *ACE2* (angiotensin-converting enzyme 2) gene (Feng et al., 2017).

In the remainder of this review, we discuss general genetic mechanisms arising from the asymmetric inheritance and expression of the X and Y sex chromosomes in mammals, and consider whether, and how, these may play a role in conferring (sex-biased) vulnerability to AF.

3. The sex chromosomes and theoretical genetic risk mechanisms for atrial fibrillation

The X chromosome is an averagely-sized chromosome (156 Mb), harbouring 857 protein-coding genes, 663 non-coding genes, and 888 pseudogenes (Ensembl, 2021a). The Y chromosome is approximately one third the size of the X (57 Mb), and harbours just 64 protein-coding genes, 107 non-coding genes and 395 pseudogenes (Ensembl, 2021b). The X chromosome is enriched for genes mediating development of the

nervous system (Mallard et al., 2021; Zechner et al., 2001), and both sex chromosomes are enriched for genes underlying reproductive function (Heard and Turner, 2011). Given the likely role of abnormal cardiac autonomic function, and gonadal hormones, in AF pathophysiology, the X and Y chromosomes appear good *a priori* candidates for harbouring risk variants. However, to date, there has been little exploration of the role of the sex chromosomes in AF: a PubMed search for “atrial fibrillation” with the terms “X chromosome OR X-linked OR sex-linked OR Y chromosome or Y-linked” yields fewer than 60 papers as of August 2021.

Mammalian males inherit a single X chromosome (invariably from their mother) and a Y chromosome from their father. In contrast, mammalian females inherit two X chromosomes (one from either parent). In each female somatic cell, one X chromosome is largely-silenced through the epigenetically-mediated process of X-inactivation, whilst expression of the non-inactivated chromosome is upregulated; together these processes ensure that X-linked gene dosage is grossly-similar between males and females, and that, within an individual, X-linked and autosomal gene dosage is appropriately matched (Brockdorff and Turner, 2015). Some genes (approximately 20%) on the X chromosome consistently escape X-inactivation, and are therefore expressed from both X chromosomes in female cells (Tukiainen et al., 2017). These so-called ‘X-escapees’ include genes within the pseudoautosomal regions (PARs) at either end of the chromosome, and at discrete clusters across the chromosome (Tukiainen et al., 2017). The extent to which X-escapees are expressed biallelically can vary between individuals, by gene, and in a tissue/cell and developmental stage-specific manner; for genes outside the PARs, the allele on the inactive chromosome tends to be expressed at lower levels than the allele on the active chromosome (Fang et al., 2021; Tukiainen et al., 2017). X-escapees appear to be particularly enriched for roles in nervous system development/function (Zhang et al., 2013).

The idiosyncratic features of the sex chromosomes described above result in several genetic mechanisms that may elicit sex-differentiated effects on AF-related phenotypes (Fig. 1). First, X-linked variants in hemizygous males will exert an effect in every cell, whereas in females the phenotypic effects of such variants may be masked by the presence of a second X-linked allele expressed in some, or all, cells. Moreover, the dosage of X-escapees may differ between males and females, with higher expression in females where the genes are expressed from two, rather than one, X chromosome. Second, X-linked ‘imprinted genes’, which are expressed monoallelically in a parent-of-origin-dependent manner, may be expressed differently in male and female tissues; X-linked imprinted genes expressed solely from the paternally-inherited allele will only be expressed in female tissues, whereas genes expressed solely from the maternally inherited allele will be expressed more highly in male than female tissues if they are subject to X-inactivation (Davies et al., 2006). Third, in female cells, the requirement to maintain one X chromosome in an inactivated, heterochromatic, state, necessitates a large, localised, deployment of epigenetic machinery. In male cells, such a deployment is unnecessary. Thus, female and male cells may differ in terms of the availability of epigenetic factors to regulate autosomal gene transcription (Schaafsma and Pfaff, 2014). Finally, the genes on the non-recombining (i.e. non-pseudoautosomal) region of the Y chromosome (NRY) can only affect male biology. In the following sections, we consider the potential role of each of these mechanisms in conferring AF risk.

4. X-linked gene dosage and atrial fibrillation risk

The phenotypic correlates of X-linked gene dosage can be identified through studying individuals affected by sex chromosome ploidies such as Turner and Klinefelter syndromes.

4.1. Turner syndrome and atrial fibrillation

Turner syndrome (TS) is a developmental condition in females,

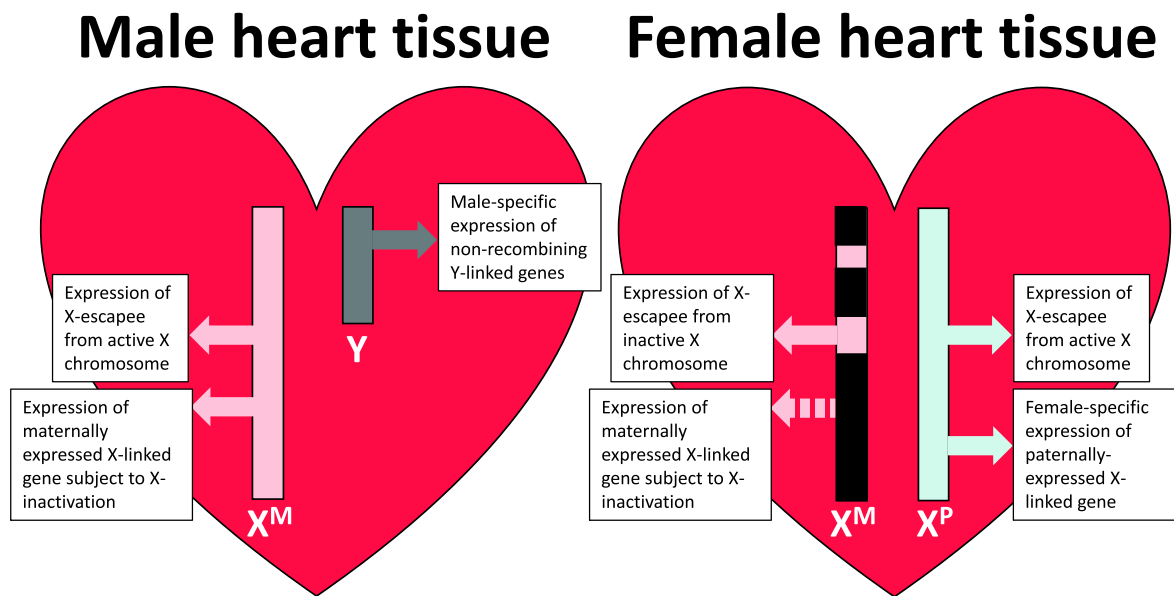


Fig. 1. Male heart cells contain an X chromosome inherited maternally (X^M), and a Y chromosome inherited paternally. Female heart cells contain two X chromosomes, one of which is inherited maternally (X^M) and one paternally (X^P); in approximately 50% of female cells X^M is inactivated (black shading), and in the remainder X^P is inactivated. Genes within the non-recombining region of the Y chromosome are solely expressed in male heart cells (grey arrow), genes which escape X-inactivation are expressed more highly in female than male heart cells, genes which are subject to genomic imprinting (maternally expressed) and X-inactivation are expressed more highly in male than female heart cells, and genes which are subject to genomic imprinting and are paternally expressed are only expressed in female heart cells. The differential requirement for epigenetic machinery localisation to the sex chromosomes in male and female cells (i.e. black shading only present in female cells) can result in sex biases in autosomal gene expression.

caused by partial, or complete (karyotype 45,X), loss of the second X chromosome (Gravholt et al., 2019). 45,X individuals with TS are haploinsufficient for all X-escapees. TS is associated with short stature and ovarian dysfunction (and associated depleted oestrogen levels). In terms of cardiovascular abnormalities, TS is associated with structural heart abnormalities (aortic coarctation, bicuspid aortic valve and hypoplastic left heart syndrome) and metabolic syndrome (abdominal obesity, hypertension, dyslipidemia and elevated fasting glucose) (Allybocus et al., 2018; Davis and Geffner, 2019; Lin et al., 2020; Silberbach et al., 2018). The condition also appears to be associated with a heightened risk of supraventricular arrhythmias including AF (Cho et al., 2020; Choi et al., 2021; Nielsen et al., 2017; Sozen et al., 2008) and stroke (Silberbach et al., 2018). In terms of effect size, a recent study within a young Korean population reported a three-fold elevation in new AF diagnoses within the TS group across a 6.8yr follow-up period compared to a matched control sample (adjusted hazard ratio = 2.75); no strong age-related effects were noted (Cho et al., 2020). The apparent elevated risk in TS could be due to the direct tissue effects arising from haploinsufficiency for X-escapees, lower levels of cardioprotective oestrogens (Odening et al., 2019), or a combination of both factors.

4.2. Klinefelter syndrome and atrial fibrillation

Klinefelter syndrome (KS), in direct contrast to TS, is associated with an excess of X chromosome material, with the most common karyotype being 47,XXY (Kanakis and Nieschlag, 2018). In 47,XXY individuals the additional X chromosome is typically inactivated, although X-escapees will be expressed more highly than in males with the typical 46,XY karyotype. Individuals with KS are typically tall and the prevalence of hypogonadism (with reduced androgen secretion), and metabolic and cardiovascular disease within this population are high (Spaziani and Radicioni, 2020). Major structural heart abnormalities do not seem to be more common in KS than in 46,XY males, although rates of AF appear higher in the former group, and in some cases are associated with hypertrophic cardiomyopathy (Cho et al., 2020; Karagoz et al., 2015; Lv et al., 2019; Sadick et al., 2014; Yoshida et al., 1998). In contrast to TS,

elevated AF risk in KS may arise from higher expression of X-escapees and/or from lower levels of circulating androgens.

4.3. Candidate genes for X-linked gene dosage effects on atrial fibrillation

Although large-scale studies systematically comparing rates of AF in TS, KS and sex-matched controls with typical karyotypes are currently rare, the data above suggest that both under- and over-dosage of one or more X-escapees in relation to typical sex chromosome complement may predispose to the risk factors for, and the occurrence of, AF. Moreover, the fact that many of the genes identified as demonstrating a robust sex-bias in their expression in adult atrial and ventricular tissue in a recent global screen are X-escapees (Oliva et al., 2020) suggests that this class of genes may play an important role in sex-specific aspects of cardiac physiology. Indeed, a recent analysis has suggested that X-escapees, and particularly those with Y-linked homologues, explain the largest proportion of X-linked heritability across a number of human disease phenotypes including self-reported heart arrhythmia (Sauteraud et al., 2021). The three X-linked genes associated with atrial fibrillation previously (*KCNE5*, *EMD* and *ACE2*) appear to be completely, or partially, subject to X-inactivation in adult heart tissue and do not show clear sex biases in this tissue in adulthood (Oliva et al., 2020; Tukiainen et al., 2017).

Recent work within our group has identified a candidate region on the short arm of the X chromosome (Xp22.31) which may be important in conferring AF risk (Brcic et al., 2020). Briefly, we showed, using the UK Biobank resource comprising middle-aged individuals drawn from the general population of the United Kingdom, that males carrying Xp22.31 genetic deletions were approximately four times more likely to have received a clinical diagnosis of atrial fibrillation/flutter (but not of any other cardiovascular or metabolic conditions) than male non-carriers. We further showed that AF diagnoses were not more common in female deletion carriers than in female non-carriers, and that AF diagnoses were not more common in males and females with duplication of the same region compared to non-carrier controls (Gubb et al., 2020). Clearly this initial positive finding requires replication, but

it is supported by evidence from case studies (Lam et al., 2020; Maki et al., 2018). With an estimated prevalence of 1 in 1500 males (Craig et al., 2010; Langlois et al., 2009), Xp22.31 deletions could feasibly explain around 1 in 300 cases of AF in 60 year old men (assuming an AF prevalence of 2% (Mandalenakis et al., 2015)).

Male deletion carriers diagnosed with AF all lacked the protein-coding genes *VCX3A*, *HDHD1/PUDP*, *STS*, *VCX*, *PNPLA4* and *VCX2*, and the microRNAs *MIR4767* and *MIR651*, and deficiency for one or more of these may explain the genotype-phenotype association. On the basis of our current knowledge, deficiency for *STS* appears to be the strongest candidate mechanism. First, gene-based analysis of the critical Xp22.31 deletion interval highlights a significant excess of common risk variants for idiopathic AF within the *STS* gene only in males from the UK Biobank, although the single most significant polymorphism was closest to the *MIR651* gene (unpublished results). In terms of function, *STS* is most highly expressed in adult vasculature (Broad Institute Genotype-Tissue Expression GTEx Portal, 2021a) and encodes an enzyme which cleaves sulfate groups from a variety of steroid hormones (e.g. dehydroepiandrosterone sulfate, DHEAS), allowing the resultant 'free steroids' to act as precursors for a variety of oestrogens and androgens (Mueller et al., 2015). Males with deletions encompassing *STS* show reduced levels of serum testosterone and elevated levels of DHEAS pre-pubertally (Idkowiak et al., 2016). There is some clinical evidence (albeit inconsistent) that oestrogens (notably oestradiol) and testosterone supplementation in individuals with low pre-existing levels may confer a degree of protection against AF (Odening et al., 2019), and that DHEAS levels correlate with AF risk (Krijthe et al., 2014; Magnani et al., 2014; Ravaglia et al., 2002). Work in rodent models has shown that acute inhibition of the *STS* enzyme in adults results in altered expression of the *Ccn2(Ctgf)*, *Ccn3(Nov)* and *miR-133a* genes in the nervous system (Humby and Davies, 2019; Humby et al., 2016); these genes and their associated proteins have been postulated to modulate AF risk through their effects on fibrosis (Diez et al., 2016; Thanigaimani et al., 2017; Thiriet, 2018; Su et al., 2021). Thus, we speculate that *STS* deficiency might confer AF risk through inducing endocrinological changes and/or through inducing the expression of pro-fibrotic factors. Additionally, acute *STS* inhibition in rodents enhances acetylcholine release in the central nervous system (Rhodes et al., 1997), and, given the key role of acetylcholine in the regulation of heart rhythm (Linz et al., 2014), *STS* could exert an effect on AF risk through affecting cholinergic neurotransmission. Furthermore, Aguado et al. (2022) have shown that expression of *STS* (and the X-escapee *BMX*) may influence the development of aortic valve stenosis, an established risk factor for AF (Widgren et al., 2012); thus *STS*-related effects on AF risk could also be secondary to effects on valvular pathology.

In terms of sex differences, *STS* consistently escapes X-inactivation in every tissue including heart (Tukiainen et al., 2017) and although the gene does have a Y homologue, this is a non-expressed pseudogene (Yen et al., 1988). Therefore, in healthy adult humans and non-human primates *STS* expression, and the activity of the associated enzyme, is higher in females than males (Cuevas-Covarrubias et al., 1993; Hirato et al., 1991; Kriz et al., 2005; Lykkesfeldt et al., 1984; Miranda-Duarte et al., 1999; Miyakawa et al., 1994; Muller et al., 1980; Oliva et al., 2020). We propose that lower *STS* activity in males compared to females may increase AF risk in the former sex. Although AF diagnoses in heterozygous deletion-carrying females were not significantly higher than those in female non-carriers in our UK Biobank study, it is possible that this group do exhibit an elevated incidence of related abnormalities which may be ascertained upon further, more intensive, investigation of cardiac function. If so, haploinsufficiency for one or more genes in this X-inactivation-escaping region may also contribute to the higher rates of cardiac arrhythmias reported in TS (Davies, 2021).

5. X-linked genomic imprinting and atrial fibrillation risk

Although genes subject to the epigenetic process of genomic

imprinting are rare (<1% of the genome), they are often highly-conserved across mammalian species, and regulate key developmental and metabolic processes (Tucci et al., 2019). There is some evidence that such genes may contribute towards congenital heart disease susceptibility (Chang et al., 2021a). A role for X-linked imprinted genes in particular aspects of physiology is indicated by distinct phenotypes in X-monosomic individuals with Turner syndrome who inherit their intact X chromosome maternally (45,X^M, around two-thirds of cases) versus those who inherit their single X chromosome paternally (45,X^P). To date, whether cardiac arrhythmias show any such evidence for X-linked parent-of-origin effects has not been systemically investigated, and such studies would be hampered by limited power. Some initial evidence suggested that the parent-of-origin of the X chromosome in Turner syndrome may influence structural/valvular cardiac phenotypes (notably coarctation and bicuspid aortic valves) with 45,X^M individuals being disproportionately affected (Chu et al., 1994) although subsequent, larger studies have disputed this finding (Bondy et al., 2007; Ko et al., 2010; Vrtel et al., 2021). Consistent with the latter studies, no X-linked imprinted genes in humans have yet been unambiguously identified, despite some intriguing phenotypic differences between 45, X^M and 45,X^P individuals (Morison, 2021). In contrast, in mice, where cardiac tissue is easily-accessible throughout life and many of the confounds inherent in the clinical work are not applicable, *Xlr3b* has been identified as an X-linked imprinted gene expressed 10-15-fold more highly in the developing heart of 39,X^MO mice than 39,X^PO mice (Davies et al., 2005). In the 39,XO mouse model, mice inheriting their single X chromosome maternally appear predisposed to aortic valve abnormalities, a finding mirroring (and giving further support to) the initial human parent-of-origin findings (Hinton et al., 2015); *Xlr3b* represents a strong candidate gene for this predisposition. The closest human orthologue to *Xlr3b* is *FAM9B* located at Xp22.31 (Davies et al., 2005). The precise function of the *FAM9B* protein is unknown and its expression does not differ significantly in atrial or ventricular adult heart tissue by sex (Oliva et al., 2020), but it appears to be expressed polymorphically in male and female heart (valve) tissue (Hinton et al., 2015) and lies within the short arm region of the X chromosome implicated in left-sided defects in cardiovascular development (Bondy et al., 2013). Given the established relationship between valvular heart disease and AF risk (Ramchand et al., 2021; Thomas et al., 2017), the role of *FAM9B* in valve development/function and thereafter in AF risk, might be investigated further.

A recent study in mice has identified five X-linked genes (*Msl1*, *Prps2*, *Hccs*, *Tmsb4x*, *Tlr7*) that are apparently genomically-imprinted (maternally expressed), and exhibit sex-biased expression (higher in males) in a strain-specific manner in T-lymphocytes (Golden et al., 2019); whether these genes and their human orthologues are imprinted elsewhere, including in the heart, remains to be determined. In man, *TMSB4X* is expressed highly in all tissues including the adult heart (Broad Institute Genotype-Tissue Expression GTEx Portal, 2021b), plasma levels of the associated thymosin beta-4 protein are specifically elevated in women with heart failure with a preserved ejection fraction (Drum et al., 2017), and the protein may have cardioprotective and regenerative effects (Smart et al., 2011). Although Toll-like receptor (TLR) proteins may play a role in heart failure and atrial fibrillation risk, the specific contribution of TLR7 appears minor (Shahid et al., 2018).

6. Sex-biased gene expression in adult heart tissue

Genes that are differentially expressed in male and female heart, and particularly atrial, tissue represent plausible candidate mediators of sex-biased effects associated with AF. X-linked genes may be expressed differentially in male and female tissues as a direct consequence of the epigenetic features of the X chromosome, whilst autosomal genes may be expressed differentially between the sexes as a downstream consequence of sex-linked processes e.g. hormonal regulation of such loci, sex-specific availability of epigenetic regulators/transcriptional factors, or

sex differences in environmental exposures.

Cross-referencing the 500 most significantly sex-biased genes in adult atrial tissue (Oliva et al., 2020) with a list of genes implicated in AF by the latest transcriptomic studies (Ke et al., 2020; Li et al., 2020; Wang et al., 2021; Wu et al., 2019; Zhang et al., 2022) highlights *NPPA* and *AMPD2* (chromosome 1), *PDGFC* and *CD38* (chromosome 4), *CANX* (chromosome 5), *FZD3* (chromosome 8), *EFNB3* (chromosome 17) and the X-linked *XIST* and *FUNDC1* genes as potential mediators of sex-biased AF-related effects. There is no clear pathway connecting these genes, although *CANX* and *FUNDC1* are both members of the mitochondria-associated endo/sarcoplasmic reticulum (ER/SR) membrane machinery (Silva-Palacios et al., 2020); interestingly, STS is also an ER/SR-localised protein (Stein et al., 1989), and interactions between membrane proteins within these two subcellular organelles, and associated effects on intracellular calcium release, could play a contributory role in sex-biased AF-related phenotypes (Dridi et al., 2020; Schonleitner et al., 2017).

7. The Y chromosome and atrial fibrillation risk

As the Y chromosome is only inherited by males, genetic variants within its non-recombining region can only influence physiology in this sex. Currently, no genetic variants on the Y chromosome have been directly implicated in atrial fibrillation (or indeed any arrhythmia). However, it is likely that Y-linked genes/variants contribute indirectly to the higher risk of AF in males.

Expression of the Y-linked *SRY* gene in the embryonic bipotential gonad stimulates differentiation into testes, organs which subsequently secrete high levels of a variety of androgens including testosterone (Makela et al., 2019); as a consequence of lacking *SRY*, females typically have far lower levels of circulating testosterone. The role of androgens in AF risk, and in arrhythmia risk more generally, has been reviewed comprehensively elsewhere (Chang et al., 2021b; Grouthier et al., 2021; Liu et al., 2010), and so will only be considered briefly here. Epidemiological studies have indicated that testosterone levels correlate to some extent with AF risk in males, with some large studies showing an increased AF risk as testosterone levels decrease with ageing, and others showing the opposite (Chang et al., 2021b). The increased AF risk in Klinefelter syndrome may be partially attributable to chronic low testosterone levels, whilst administration of anabolic steroids also appears to increase AF risk (Chang et al., 2021b). Clearly, the relationship between androgen levels and AF is complex, and may depend upon whether total or bioavailable androgens are being measured, the magnitude and temporality of the androgen excess or insufficiency, and patients' background physiology. At the cellular level, testosterone appears to exert its primary effects through altering the number and function of L-type calcium channels in cardiomyocytes (Chang et al., 2021b).

Perturbed autonomic nervous system function has long been implicated in AF pathophysiology (Rebecchi et al., 2021), and sex-specific perturbations may contribute towards differences in AF risk in males and females (Odening et al., 2019). Studies in preclinical rodent models have indicated that the Y chromosome (and potentially *Sry* itself) can influence blood pressure through adrenal gland-mediated effects on the sympathetic nervous system, whilst clinical studies have suggested that specific Y chromosome haplogroups are associated with numerous cardiovascular conditions, notably hypertension, via effects on adaptive immune function (Khan et al., 2019). However, it should be appreciated that many of these clinical studies are underpowered, and often employ ethnically and geographically-restricted samples limiting the generalisability of their findings.

Finally, the Y chromosome may impact directly, or indirectly (e.g. via hormonal mechanisms), upon aspects of morphology associated with AF risk e.g. tall stature (Wannamethee et al., 2021). For example, individuals with polyploidies such as 47,XXY are typically taller than 46, XY males, probably due to higher expression of the PAR gene *SHOX*

(Ottesen et al., 2010). Moreover, Y chromosome haplogroups have been associated with height (Ellis et al., 2001), although the precise nature of the relationship between Y chromosome genetic variation and height is uncertain (Ye et al., 2018).

8. Conclusions and future work

Many cardiovascular disorders demonstrate a sex bias, and a number of approaches to investigating relevant underlying mechanisms have been suggested previously (Ventura-Clapier et al., 2017). Here, we have proposed that risk of, and recovery from, atrial fibrillation is dependent upon sex, and that sex-linked genes are likely to play some fundamental role - in Table 1, we summarise X-linked candidate genes of particular interest. A key question is: to what extent, and exactly how, do sex chromosome effects contribute to AF-related phenotypes? This is challenging to answer in clinical and human studies, but may be addressed to some extent in mammalian models. A recent proteomic screen in cardiomyocytes of XO and XYY mice highlighted a suite of expression differences from sex-matched XX and XY controls, many of which arose from X-escapee genes and occurred prior to the onset of gonadal differentiation (Shi et al., 2021); clearly the orthologues of these represent candidates for sex-biased cardiovascular phenotypes in man. In the 'Four-Core Genotypes (FCG)' mouse model the relative contributions of sex chromosome complement and gonadal type (i.e. hormonal factors) to sex-biased aspects of anatomy and physiology may be distinguished (Arnold, 2020). Whilst the FCG model has been predominantly used in brain and behavioural research so far, some studies have investigated clinically-relevant cardiovascular measures including aortic aneurysm, atherosclerosis, stroke, hypertension and cardiac ischaemia. Caeiro et al. (2011) showed an effect of sex chromosome complement on heart rate (bradycardic baroreflex response), whilst Shi et al. (2021) followed up their XO/XYY studies by using the FCG model to demonstrate that 159 protein expression changes co-segregated with sex chromosome complement. Besides utility in investigating sex-linked molecular dependencies, the FCG model could be exploited to examine the extent to which sex chromosome complement and gonadal type contribute to atrial development, and to anatomy and (electro)physiology in healthy adult animals; how sex chromosome complement and gonadal type modulate pathophysiological processes could be determined through crossing the FCG model to established genetic mouse models of relevance to AF e.g. *Pitx2* knockout (Syeda et al., 2017). Should promising sex chromosome complement effects be identified, there are a number of chromosomal mutant, and genetically-altered, mouse models available to narrow down the list of candidate genes (Arnold, 2020).

In man, there is a need to carefully document the prevalence and presentation of AF and associated risk factors/medical conditions in individuals with sex chromosome ploidies, or with sex chromosome copy number or polymorphic variants (e.g. Xp22.31 deletions) in large samples from diverse backgrounds; through examining patients with a range of chromosomal abnormalities, it may be possible to better define genomic regions housing causal risk loci. Understanding the environmental and biological risk mechanisms underlying any genotype-phenotype associations, and clarifying their relative contributions, is also important; environmental risk factors might include exposures associated with the condition of interest e.g. medications used to treat Turner syndrome. Biological mechanisms might be investigated using *in vitro* and *in vivo* approaches e.g. atrial cardiomyocytes derived from induced pluripotent stem cells (iPSCs) originating from patients' tissues (Feki et al., 2020; Zuo et al., 2017), or mammalian animal models e.g. mice deficient for the *Sts* gene, or in which the STS enzyme is acutely inhibited (Davies et al., 2014).

Human molecular genetic studies, in combination with transcriptomic/proteomic datasets, will be useful for specifying risk variants within candidate genomic regions/genes of interest and their putative mechanisms of action, whilst increasingly highly-powered large-scale genome-wide association/copy number variant studies stratified by sex

Table 1
X-linked genes with an established or putative role in atrial fibrillation risk.

Gene	Chromosomal location	Protein name and function	Escape from X-inactivation in man	Evidence linking gene/protein to atrial fibrillation
<i>KCNE5</i>	Xq23	Potassium Voltage-Gated Channel Subfamily E Regulatory Subunit 5	No	Altered in Brugada syndrome, a condition in which AF is a variably-associated trait (Abbott, 2016)
<i>EMD/STA</i>	Xq28	Emerin nuclear membrane protein	No	Altered in Emery-Dreifuss muscular dystrophy, a condition in which AF is a variably-associated trait (Karst et al., 2008)
<i>ACE2</i>	Xp22.2	Angiotensin Converting Enzyme 2	Partial	Association in a candidate gene study (Feng et al., 2017)
<i>STS</i>	Xp22.31	Steroid sulfatase	Yes	Males with deletions encompassing <i>STS</i> show elevated rate of AF (Brcic et al., 2020); highly expressed in vasculature and key role in estrogen and androgen production
<i>FAM9B</i>	Xp22.31	Family with sequence similarity 9 Member B	Variable/partial	Closest human orthologue of mouse X-linked imprinted gene <i>Xlr3b</i> ; gene lies within the short arm region of the X chromosome implicated in left-sided defects in cardiovascular development (Bondy et al., 2013) and is variably-expressed in heart valve tissue (Hinton et al., 2015)
<i>FUNDC1</i>	Xp11.3	FUN14 Domain Containing 1	Yes	Expressed more highly in female than male adult atrial tissue; member of the mitochondria-associated endo/sarcoplasmic reticulum (ER/SR) membrane machinery (Silva-Palacios et al., 2020)

(Gao et al., 2015; Ventura-Clapier et al., 2017), and Y chromosome haplotype associations with AF risk (and correlates such as testosterone levels and height), will point to novel sex-linked risk factors for AF which will require characterisation. Potentially, future genetic studies may signpost the role of previously-unanticipated biological pathways in AF pathophysiology, and may allow more accurate predictive risk models incorporating polygenic scores to be developed. Finally, it will be interesting to test, using genetic epidemiology techniques such as Mendelian Randomisation (Bennett and Holmes, 2017), the extent to

which genetic risk factors for AF (including those on the sex chromosomes) overlap with those associated with stroke, dementia and heart failure, and the extent to which AF risk loci are causal for these debilitating conditions.

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