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Original research

Characterising heart rhythm abnormalities associated with Xp22.31 deletion

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► Additional supplemental material is published online only. To view, please visit the journal online (<http://dx.doi.org/10.1136/jmg-2022-108862>).

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Received 5 August 2022

Accepted 29 October 2022



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To cite: Wren G, Baker E, Underwood J, *et al.* *J Med Genet* Epub ahead of print: [please include Day Month Year]. doi:10.1136/jmg-2022-108862

ABSTRACT

Background Genetic deletions at Xp22.31 are associated with the skin condition X linked ichthyosis (XLI), and with a substantially increased risk of atrial fibrillation/flutter (AF), in males. AF is associated with elevated thrombosis, heart failure, stroke and dementia risk.

Methods Through: (a) examining deletion carriers with a diagnosis of AF in UK Biobank, (b) undertaking an online survey regarding abnormal heart rhythms (AHRs) in men/boys with XLI and female carriers of XLI-associated deletions and (c) screening for association between common genetic variants within Xp22.31 and idiopathic AF-related conditions in UK Biobank, we have investigated how AHRs manifest in deletion carriers, and have identified associated risk factors/comorbidities and candidate gene(s). Finally, we examined attitudes towards heart screening in deletion carriers.

Results We show that AHRs may affect up to 35% of deletion carriers (compared with <20% of age-matched non-carriers), show no consistent pattern of onset but may be precipitated by stress, and typically resolve quickly and respond well to intervention. Gastrointestinal (GI) conditions and asthma/anaemia were the most strongly associated comorbidities in male and female deletion carriers with AHR, respectively. Genetic analysis indicated significant enrichment of common AF risk variants around *STS* (7 065 298–7 272 682 bp in GRCh37/hg19 genome build) in males, and of common GI disorder and asthma/anaemia risk variants around *PNPLA4* (7 866 804–7 895 780 bp) in males and females, respectively. Deletion carriers were overwhelmingly in favour of cardiac screening implementation.

Conclusion Our data suggest AHRs are frequently associated with Xp22.31 deletion, and highlight subgroups of deletion carriers that may be prioritised for screening. Examining cardiac function further in deletion carriers, and in model systems lacking steroid sulfatase, may clarify AF pathophysiology.

INTRODUCTION

Prenatal screening estimates indicate that genetic deletions within Xp22.31 occur in around 1 in 1500 males and 1 in 750 females.^{1 2} Twenty-five per cent to 60% of males carrying such variants are diagnosed with X linked ichthyosis (XLI (MIM: 308100)), a dermatological condition characterised by skin scaling and resulting from absence of the

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Deletions at Xp22.31 are associated with a substantially increased likelihood of diagnosis of atrial fibrillation/flutter in middle-aged individuals, but how abnormal heart rhythms (AHRs) manifest in this population is unclear.

WHAT THIS STUDY ADDS

⇒ We show that AHR in Xp22.31 deletion carriers are common, comparatively mild, largely responsive to treatment, tend to be precipitated by stress, and are comorbid with gastrointestinal conditions, asthma and anaemia. We identify *STS* as a candidate gene for AHR and *PNPLA4* as a candidate gene for comorbidities. We also show that deletion carriers are in favour of cardiac screening.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The study guides future work in Xp22.31 deletion carriers and suggests that work in steroid sulfatase-deficient model systems may provide insights into the pathophysiology of atrial fibrillation. The feasibility of cardiac screening in individuals carrying Xp22.31 deletions or diagnosed with X linked ichthyosis (particularly those exhibiting gastrointestinal conditions, asthma or anaemia) might be investigated.

steroid sulfatase (*STS*) enzyme.³ In males, typically sized XLI deletions of 1.5–1.7 Mb are associated with extracutaneous symptoms including: corneal opacities,³ testicular maldescent,³ focal epilepsy,⁴ atopic disorders (asthma, eczema and allergic rhinitis),^{5–7} aberrant haemostasis and fibrosis,⁸ and increased neurodevelopmental and mood diagnoses/traits⁹; larger deletions (>2.5 Mb) can be associated with more severe developmental issues. Heterozygous female carriers can also show high neurodevelopmental and mood traits, and around 60% experience delayed or prolonged labour during childbirth, but these individuals do not usually exhibit the physical (skin, eye and neurological) phenotypes seen in males.¹⁰

Using the large UK Biobank sample recruited from the general population of the UK, we have recently shown that middle-aged men carrying

deletions of 0.8–2.5 Mb within Xp22.31 (a region which escapes X-inactivation in females¹¹) are at significantly elevated (approximately fourfold) risk of being diagnosed with atrial fibrillation and/or atrial flutter (AF) (but not other cardiovascular or metabolic conditions) compared with male non-carriers¹²; the excess of AF diagnoses was not apparent in female deletion carriers,¹² nor was it apparent in males with genetic duplication of the same region.¹³ AF is the most common supraventricular arrhythmia, and is characterised by dysregulated and chaotic twitching of the atrium.¹⁴ The severity of the condition can vary from a single, rapidly resolving incident, to recurrent, rapidly resolving episodes ('paroxysmal AF'), episodes resolving more slowly ('persistent AF') or not resolving at all ('permanent AF').¹⁵ Xp22.31 deletions could potentially explain up to 1 in 300 cases of AF in middle-aged men.¹⁵

AF can have significant effects on morbidity and mortality with downstream consequences including: thrombosis due to turbulent cardiac blood flow, embolism and stroke, as well as dementia.¹⁶ Risk factors for idiopathic AF include: age and heart tissue pathology (eg, arising due to hypertension, valvular or congenital heart disease, pericarditis, cardiomyopathy and diabetes), and the condition may be secondary to pulmonary conditions such as asthma.¹⁴ Recent case reports in males with XLI have described paroxysmal supraventricular tachycardia with anaemia,¹⁷ and AF with kidney disease, type 2 diabetes mellitus, hypertension and dyslipidaemia.¹⁸ AF is commonly associated with the sensation of an irregular or very fast heartbeat even while at rest, with palpitations and chest pain, and with breathlessness, fatigue and feelings of dizziness or faintness; however, in some cases, AF is asymptomatic and may be picked up incidentally during medical examinations or check-ups.¹⁵ AF may be screened for, and diagnosed in, 'at-risk' individuals through a combination of ECG and echocardiograms, chest X-ray and blood tests.¹⁵

The purpose of the present study was fourfold. First, we aimed to identify comorbidities in middle-aged male and female carriers of XLI-associated deletions in the UK Biobank who had been diagnosed with AF. Second, we aimed to characterise abnormal heart rhythm (AHR)-related phenotypes and comorbidities in men and boys with XLI, and in female carriers, through an online survey. These initial analyses were intended to enhance our understanding of the nature and course of, and risk factors for, AF in individuals with Xp22.31 deletions with a view to improving AF prediction within this genetic cohort. Convergent findings across our two distinct participant groups (UK Biobank and online samples) would be expected to be robust and generalisable. Third, we aimed to gauge opinion on screening for cardiac abnormalities in patients with confirmed XLI/Xp22.31 deletion. Finally, we aimed to identify candidate genes within the Xp22.31 deletion interval by screening for common risk variants for idiopathic AF and associated conditions in the UK Biobank sample; this would provide clues regarding biological mechanisms for any phenotype–genotype associations and would suggest future studies geared towards clarifying these.

METHODS

Comorbid conditions in carriers of Xp22.31 deletions and AF in UK Biobank sample

The UK Biobank sample comprises almost half a million participants recruited between 2006 and 2010 at ages 40–69 years from the UK general population for which anonymised genotype and extensive phenotype data are available.¹⁹ Descriptive International Classification of Diseases 10th Revision (ICD-10) codes

and self-reported 'blistering/desquamating disorder' (ie, ichthyosis) diagnoses in Xp22.31 deletion carriers (0.8–2.5 Mb around STS) from the UK Biobank sample were compared between individuals diagnosed with AF and those not diagnosed with AF.

Online survey

Participants

Adult (>18 years) men with a confirmed diagnosis of XLI, confirmed adult female carriers and parents of boys with a confirmed diagnosis of XLI were recruited via relevant charities, online patient support groups and social media; diagnosis/carrier status was typically confirmed on the basis of a combination of: family history, assessment of skin condition and biochemical/genetic testing. Participants were directed to an online survey which was open from 2 September to 22 December 2021, and anonymised data were returned to the study team upon completion.

Survey structure

The survey was designed in Qualtrics²⁰ and was available via a specific URL. Participants were initially asked to provide basic demographic information including their (or their son's) age, country of residence and ethnicity, before confirming the basis of their (or their son's) diagnosis/carrier status. Men with XLI, or parents of boys with XLI, were then asked to rate their (or their son's) skin severity across life based on the Congenital Ichthyoses Severity Index (possible scores 2–8),²¹ and check whether their son(s) had been affected by any of the following developmental conditions: testicular maldescent or a neurodevelopmental/neurological condition. Participants were then asked to specify if they (or their sons) had ever experienced, or been diagnosed by a medical professional with, an AHR (and if so, to specify the condition); they were also asked to check whether they (or their sons) had been diagnosed with an array of other cardiovascular or metabolic conditions known to be risk factors/comorbidities for AF (online supplemental text 1), and to confirm whether or not there was a family history of cardiovascular issues. There were then a series of questions about the precipitants, nature, developmental course and severity of any self-reported heart arrhythmias and their treatments. Finally, participants were presented with a short vignette about the potential link between XLI and AF, and the possible complications associated with AF (online supplemental text 2) and were asked to rate the following statements on a 5-point scale ranging from strongly disagree to strongly agree: 'Do you think that risk of AF in XLI males is a significant health concern?', 'Do you think that males should be screened for heart abnormalities routinely following confirmation of XLI?', 'I believe that screening for heart problems in XLI is a good use of healthcare funding', 'I would be happy to attend/bring my son to a doctor's surgery/hospital regularly for heart screening', 'I believe that knowing that I/my son have a heart condition with possible adverse consequences is preferable to not knowing', 'I believe that the benefits associated with screening for, monitoring and treating any heart condition outweigh their risks' and 'I require more information about the relationship between XLI and heart conditions in order to make any judgement about the benefit of screening'. Participants could leave their email address if they wanted further information or were happy to be recontacted.

Survey analysis

For each group, the proportion of individuals reporting AHRs was calculated, and the nature of those abnormalities characterised. Subsequently, each participant group was divided into individuals with and without self-reported AHRs, and the two subgroups were then directly compared across demographic

measures and medical phenotypes to identify factors co-segregating with heart arrhythmias within these populations. Continuous variables were compared between groups with unpaired t-test or Mann-Whitney U test depending on normality of the data, and categorical data were analysed by χ^2 or Fisher's exact test; ORs are presented as a measure of effect size. Where multiple medical phenotypes were assessed, Benjamini-Hochberg False Discovery Rate correction was applied.²²

Molecular genetics in UK Biobank sample

A total of 363 693 white British and Irish individuals remained in our UK Biobank sample after removing related individuals and those who have since chosen to withdraw from the study. Diagnoses were coded according to ICD-10. The UK Biobank contains 3 917 799 imputed SNPs on the X chromosome. These imputed data were quality controlled (QC) by removing rare SNPs with minor allele frequency <0.01, SNPs imputed with poor accuracy (INFO <0.4) and SNPs with missing data proportion >0.05. The data were split into males and females. After these QC steps, 262 278 SNPs in 195 638 females and 267 937 SNPs in 168 055 males remained for analysis.

First, SNPs in the region of interest (ChrX:6 435 064–8 414 482 GRCh37/hg19 genome build, that is, consensus deletion interval for individuals with AF in UK Biobank) were extracted: 3966 and 4060 SNPs remain in females and males, respectively; individual SNPs with $-\log_{10}(p) > 4.90$ ($p < 1.26 \times 10^{-5}$) or $-\log_{10}(p) > 4.91$ ($p < 1.23 \times 10^{-5}$) were regarded as significant following multiple testing correction in females and males, respectively. We then ran multiple association analyses between all SNPs in the region of interest with a number of different phenotypes linked to AF. We investigated AF (ICD-10 code 'I48'), stroke (ICD-10 code 'I64'), acute myocardial infarction (ICD-10 code 'I21'), dementia (combined ICD-10 codes 'F00–03'), asthma (ICD-10 code 'J45'), anaemia (combined ICD-10 codes 'D50–52') and gastrointestinal (GI) disorders (combined ICD-10 codes 'K50–59'). The association analyses were run for each phenotype in males and females separately. Fifteen principal components, array and age were included in the model as covariates. Finally, we used MAGMA²³ to run a gene-based analysis for the different association analyses to determine whether the SNPs within our region of interest had an aggregate effect in a particular protein-coding gene. The SNPs were annotated to genes using the gene location file from the MAGMA website; this contains gene locations from protein-coding genes obtained from the National Center for Biotechnology Information (NCBI) site. SNPs within the transcription start and stop sites were included. The mean χ^2 gene-based analysis was used on the summary statistics from the association analyses, and unadjusted p values are presented.

Availability of data and materials

UK Biobank data are available upon application to that resource.²⁴ Online survey data generated or analysed during this study are included in this published article and the online supplemental file 1, or are available from <http://doi.org/10.17035/d.2022.0230251614>.

RESULTS

Comorbid conditions in carriers of Xp22.31 deletions diagnosed with AF in UK Biobank

Of the 86 male Xp22.31 deletion carriers previously identified in UK Biobank, 9 had been diagnosed with AF. These nine individuals, were on average, significantly older than the remaining 77 (age in 2016: 69.8 ± 1.6 years vs 65.3 ± 0.9 years, $t[11.7] = 2.40$, $p = 0.034$), but the two groups did not differ with respect to

self-reported blistering/desquamating disorder diagnoses (1 of 9 (11%) vs 3 of 77 (4%), Fisher's exact test $p = 0.36$). In the nine subjects with AF, the most common comorbid diagnoses were: GI problems (viral intestinal infection in one individual, and non-infective gastroenteritis and colitis in two individuals) and respiratory conditions (asthma in one individual and pneumonia in two individuals). Comparison of the most common diagnoses in the male deletion carrier AF versus non-AF group revealed that only the prevalence of 'non-infective gastroenteritis and colitis' differed significantly between groups, being more common in the former group (2 of 9 (22%) vs 1 of 77 (1%), $p = 0.028$ Fisher's exact test). Of the 312 female deletion carriers identified in the UK Biobank, just 3 had been diagnosed with AF. All three carriers diagnosed with AF displayed prominent cardiovascular abnormalities and had also been diagnosed with left ventricular failure. The other most common comorbidities in female carriers with AF were dyspnoea (two individuals) and bone/joint conditions (rheumatoid arthritis in one patient and fracture in the second); one individual diagnosed with AF presented with acute renal failure and anaemia.

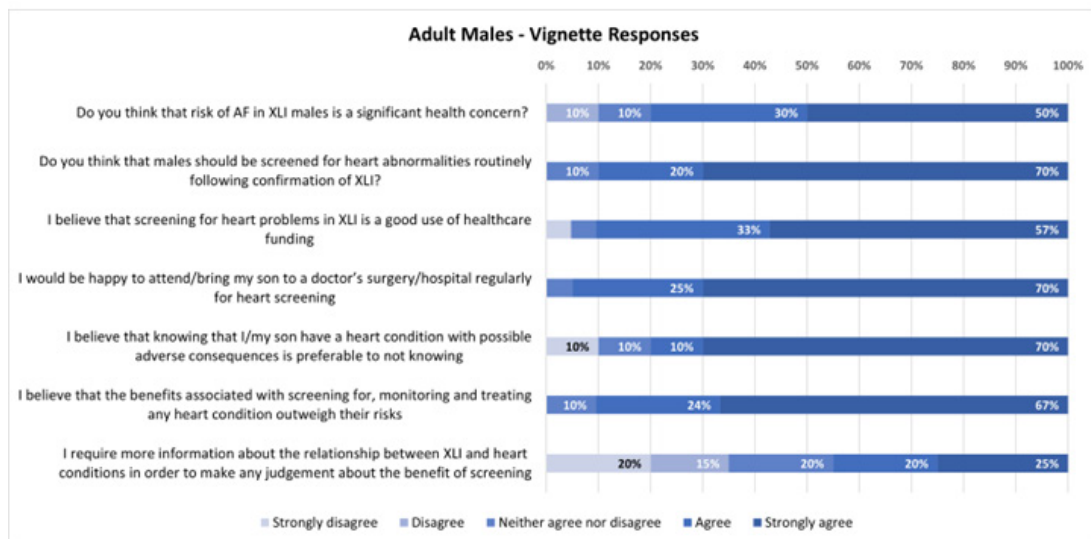
Online survey data

Comparison of demographics and comorbidities in individuals with and without AHR

We recruited a total of 191 participants (43 adult men with XLI, 79 female carriers and 69 boys with XLI), although not all participants completed all aspects of the survey. The prevalence of self (or parent)-reported AHRs was similar across the three groups (35% in adult men with XLI, 28% in adult female carriers and 28% in boys with XLI); across the three groups, individuals affected by AHRs and those unaffected did not differ significantly with respect to age, country of residence and ethnicity, although individuals experiencing AHRs tended to be older on average (online supplemental table 1). Across both male groups combined, severity of the skin condition did not differ between those with AHR and those without (4.1 ± 1.7 and 3.7 ± 1.4 , respectively, $U = 1092.0$, $z = -0.132$, $p = 0.90$). Boys with AHR did not exhibit more developmental conditions than those without (testicular maldescent: 20% vs 10%, respectively, $p = 0.09$; neurodevelopmental disorder: 27% vs 27%, respectively, $p = 1.0$). Consistent with a possible genetic influence on arrhythmia risk, males self-reporting AHRs were more likely to endorse a family history of cardiovascular issues than males not reporting (77% vs 46%, respectively, $p = 0.005$); this pattern of results was maintained when female carriers were also included in the analysis (74% vs 49%, respectively, $\chi^2_{(1)} = 8.88$, $p = 0.003$).

Of all the comorbid medical conditions assessed, only 'gut problem' was significantly more frequently self-reported in males with AHR than males without (OR: 7.0; 95% CI: 1.3 to 38.4, $p = 0.022$). 'Heart murmur' (OR >10, $p = 0.005$), anaemia (OR: 5.0; 95% CI: 1.5 to 17.2, $p = 0.011$) and asthma (OR: 5.0; 95% CI: 1.3 to 18.2, $p = 0.017$) were all significantly more common in female carriers reporting heart rhythm abnormalities than in those without. When adult/young male, and female deletion-carrying groups were all combined, four medical conditions were significantly more common in AHR than in non-AHR groups: 'heart valve disease or malformation' (OR: 10.6 (95% CI: 1.2 to 97.4), $p = 0.025$), 'anaemia' (OR: 3.2 (95% CI: 1.1 to 8.7), $p = 0.027$), 'asthma' (OR: 2.9 (95% CI: 1.3 to 6.4), $p = 0.010$) and 'gut problem' (OR: 4.9 (95% CI: 1.7 to 14.3), $p = 0.004$); of these, only 'gut problem' survived Benjamini-Hochberg False Discovery Rate correction (adjusted $p = 0.1$) (online supplemental table 2A,B).

A



B

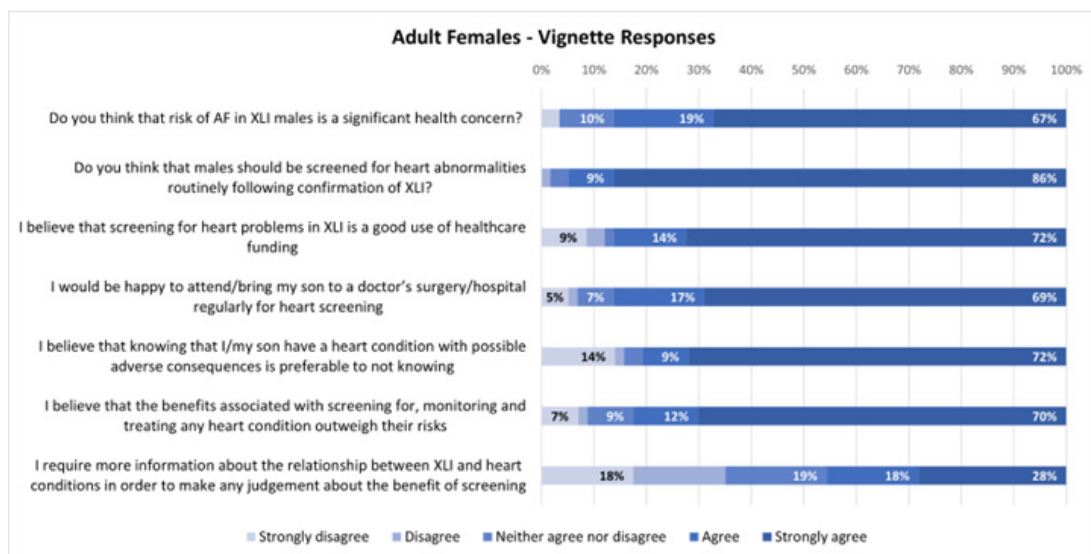


Figure 1 Responses to the vignette in adult men with XLI (n=21) (A) and adult female carriers (n=58) (B). AF, atrial fibrillation/flutter; XLI, X linked ichthyosis.

Nature of rhythm abnormalities in individuals with AHR

In adult men, AF had been diagnosed by a medical professional in 60% of individuals with AHR, and tachycardia in 27%. Thirty per cent of female carriers with AHR had been diagnosed with AF, and 60% with tachycardia. In boys with XLI and AHR, 53% had been diagnosed with tachycardia, 40% with bradycardia and 20% with AF.

Stress was cited in the top three precipitating factors for AHR across all three groups (referred to as a precipitant by 42% of men with XLI, 45% of female carriers and 14% of parents of boys with XLI); other precipitants were less-consistently identified but included 'no obvious cause' (men and boys with XLI), 'exercise' (female carriers) and 'increased body temperature' (boys with XLI) (online supplemental table 3). There was no clear pattern as to when AHRs onset during the day across all three groups (online supplemental table 4). For the majority of participants who provided responses, AHRs resolved spontaneously

within 12 hours (82% of men with XLI, 85% female carriers and 82% boys with XLI), and often within 1 hour (36%, 61% and 55%, respectively). Where interventional strategies (typically breathing exercises) were required, their success was reported as being good (>5 out of 10) in 61% of males with XLI, in 100% of female carriers and in 86% of boys with XLI.

Response to the vignette

A total of ≥80% of men with XLI (figure 1A) and female carriers (figure 1B) agreed or strongly agreed that: (1) risk of AF in XLI was a significant health concern, (2) cardiac screening should be routinely performed following confirmation of XLI, (3) screening for heart problems in XLI is a good use of healthcare funding, (4) they would be happy attending, or bringing their child to attend, heart screening appointments, (5) they would prefer to be made aware of potentially adverse medical

Table 1 A gene-based analysis of SNPs associated with atrial fibrillation/flutter in the consensus Xp22.31 deletion interval in males (4556 cases vs 163 499 controls) and females (2144 cases vs 193 494 controls) from the UK Biobank

Gene	Start (bp)	Stop (bp)	Males			Females		
			NSNPs	NParam	P value	NSNPs	NParam	P value
<i>VCX3A</i>	6 451 659	6 453 159	5	3	0.229	5	3	0.745
<i>HDHD1</i>	6 966 961	7 066 231	225	12	0.061	223	13	0.983
<i>STS</i>	7 065 298	7 272 682	343	20	0.041*	336	20	0.793
<i>VCX</i>	7 810 303	7 812 184	4	2	0.639	3	2	0.259
<i>PNPLA4</i>	7 866 804	7 895 780	39	6	0.633	39	6	0.278
<i>VCX2</i>	8 137 985	8 139 308	11	4	0.444	8	3	0.444

*P<0.05.
NParam, the number of relevant parameters used in the model (essentially the number of independent SNPs in the gene); NSNPs, the number of SNPs in the data annotated to the gene.

conditions to not know, and (6) the potential benefits of heart screening outweighed the risks. Fewer than 50% of individuals from these two groups stated that they required further information regarding the link between XLI and heart arrhythmias.

Molecular genetic analyses

Across all conditions, only two closely linked individual SNPs (rs141750978;chrX:7934924 and rs2051996;chrX:7933380 GRCh37/h19 genome build) exceeded the threshold for significant association ($p=2.24\times 10^{-6}$ and 2.39×10^{-6} , respectively), and this was with GI disorders in males (42 680 cases (25.4%) vs 125 375 controls (74.6%)). These SNPs are located closest to the *PNPLA4* transcriptional start site²⁵ and show evidence for gene expression correlation with *PNPLA4* in small intestine terminal ileum (effect size 0.15, $p=0.009$).²⁶ Gene-based analysis suggested a nominally significant association between *STS* (chrX:7 065 298–7 272 682, GRCh37/h19 genome build) and AF in males (4556 cases (2.7%) vs 163 499 controls (97.3%), $p=0.041$) but not in females (2144 cases (1.1%) vs 193 494 controls (98.9%), $p=0.793$) (table 1 and figure 2). No significant gene-based associations were identified in either males or females for stroke, acute myocardial infarction or dementia. Nominally significant associations between *PNPLA4* and asthma ($p=0.040$) and anaemia ($p=0.013$) were noted in females only. Finally, gene-based analysis suggested significant associations between *VCX3A*, *VCX*, *PNPLA4* and *VCX2* genes and GI conditions in males only (p values between 0.018 and 0.044). Gene-based analysis results for medical conditions related to AF are presented in online supplemental tables 5–10.

DISCUSSION

Using the UK Biobank sample, we previously showed that approximately 10% of middle-aged men carrying Xp22.31 deletions had been diagnosed with AF, a prevalence around four times than that in the non-carrier male population.¹² Theoretically, the increased prevalence of AF in male Xp22.31 deletion carriers might have been due to the administration of retinoid-derived medications sometimes used to treat severe skin scaling which can have extensive side-effect profiles.^{27 28} However, <5% of male Xp22.31 deletion carriers within UK Biobank reported being formally diagnosed with ichthyosis, AHRs are not typically reported as a side effect of acitretin/(iso)retinoin administration,^{27 29} and no male deletion carriers within the UK Biobank sample self-reported receiving acitretin or (iso)retinoin treatment. Therefore, it is likely that the Xp22.31 deletion somehow confers a biological predisposition to AF. In the present study, we aimed to characterise the features and causes of cardiac rhythm

abnormalities associated with this genetic variant more fully. We also aimed to investigate the acceptability of cardiac screening for deletion carriers.

Our online survey suggested self-reported AHRs in 28%–35% of male and female carriers of Xp22.31 deletions, and, consistent with this, many affected carriers had received a cardiac arrhythmia-related diagnosis from a medical professional and reported a family history of cardiovascular issues. This prevalence figure is likely higher than the 10% figure reported previously as it includes multiple causes of AHR in addition to AF and may include subclinical and undiagnosed cases; on the one hand, true prevalence rates may be underestimated given that only perceptible and symptomatic AHRs can be self-reported, but on the other hand, reported rates may be somewhat overinflated due to response bias. While we acknowledge the limitations of self-reported data on AHRs (eg, possible misreporting by participants and challenges associated with parental reporting on their sons' experiences), this assessment method allows interrogation of comparatively large samples and provides information on AHRs occurring at time points across the lifespan as opposed to temporally restricted clinical monitoring via, for example, ECG. While AHR prevalence rates across the three online samples appear broadly similar, we note that the expected pattern of data was obtained, that is, highest rate (and worst response to intervention) in older, hemizygous males and lower rates (and better intervention responses) in heterozygous females and younger males. The prevalence of AHRs in our deletion-carrying population appears elevated compared with that in non-carrier samples, for example, arrhythmia was self-reported in 13.3% of men and 21.9% of women aged 40–49 years in a large European general population sample previously³⁰ and cardiac arrhythmias of any type were detected in 17.2% of >10 000 general dentistry patients.³¹ Conceivably, the novelty of the confirmed XLI–cardiac arrhythmia association, the apparently non-impairing and sporadic nature of AHRs in individuals with XLI/female carriers, and the fact that individuals with XLI are typically managed by clinicians without significant expertise in cardiology may explain why high rates of AHRs within XLI populations have not been recognised previously.

So far as we could determine and bearing in mind lack of power, there was no clear relationship between heart rhythm abnormalities and the severity or presence of other features commonly associated with XLI (skin condition, testicular maldescent, neurodevelopmental disorders) implying partially dissociable causes. Our data suggest that, as with idiopathic cardiac arrhythmias, the prevalence of AHR within the Xp22.31 deletion population increases with age. They also indicate that

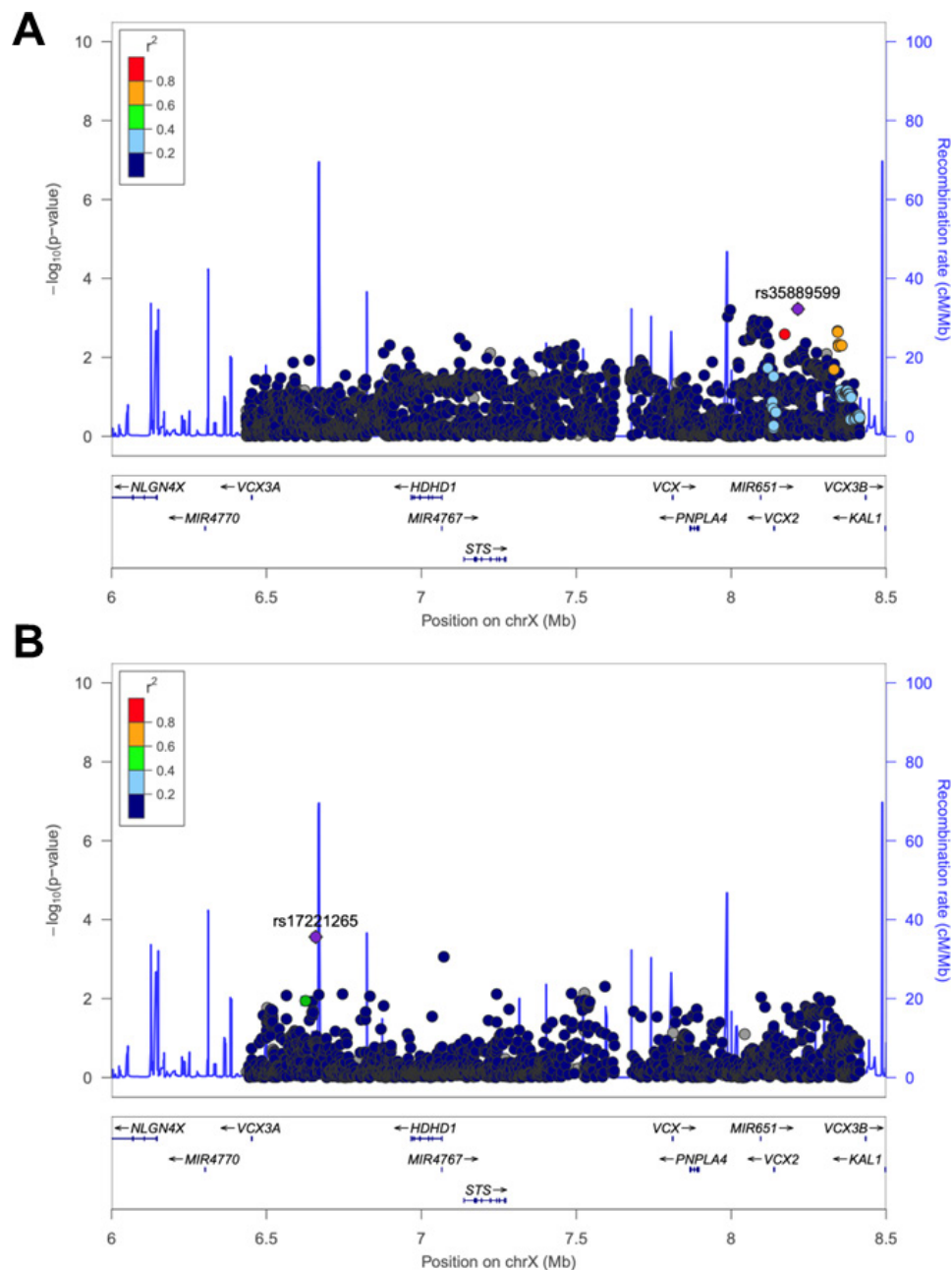


Figure 2 LocusZoom plots showing the SNPs most significantly associated with atrial fibrillation/flutter in the Xp22.31 deletion consensus interval in males (A) and females (B).

AHRs in Xp22.31 deletion carriers typically resolve quickly and respond well to intervention where this is required. The most commonly reported precipitant within this subpopulation was psychological and/or physiological stress.

Gene-based analyses suggested an aggregate effect of SNPs within *STS* on male (but not female) risk of idiopathic AF. These genetic findings, in combination with: (a) our finding of high rates of AHR in female carriers not affected by XLI and boys unlikely to have been on long-term medication, and (b) medication not being reported as a significant contributor to AHR in males with XLI, provide further evidence against the idea that elevated rates of AHR in Xp22.31 deletion carriers are a secondary consequence of pharmacotherapy but instead support the idea of a biological predisposition to risk.

STS deficiency appears a strong functional candidate for AF risk in Xp22.31 deletion carriers given that *STS* is highly

expressed in adult arterial vasculature and at a lower level in the atrial appendage.³² Systemic inhibition of the *STS* enzyme in a cohort of 10 patients with early breast cancer prescreened to exclude a history of cardiac arrhythmia resulted in three grade 2 adverse events (two related to abnormal ECG and one to tachycardia) and one grade 1 adverse event (prolonged QT).³³ The *STS* enzyme affects cardiac valve function³⁴ and fibrotic pathways,^{8 15 35} and regulates androgen and oestrogen production and the balance between sulfated and non-sulfated steroids such as dehydroepiandrosterone sulfate (DHEAS) and DHEA.³⁶ DHEA(S) levels correlate with AF risk in older men in some studies,¹² and levels of these hormones (and the DHEA:DHEAS ratio) increase upon acute psychosocial stress, with the increase in levels correlating with the stress-induced increase in heart rate.³⁷

Despite the UK Biobank and online samples having different ascertainment biases and characteristics, they provided converging evidence that GI problems, asthma and anaemia are the comorbidities most closely associated with AHR in deletion carriers, with GI issues more prominent in male carriers with AHR, and asthma and anaemia more strongly comorbid in female deletion carriers. GI issues are increasingly being recognised as contributors to AF risk via multiple plausible pathways.³⁸ Our genetic analyses provide information pertinent to these sex-specific comorbidity effects. Specifically, they implicate *PNPLA4* in asthma and anaemia risk in females only, and *PNPLA4* in GI disorder risk in males only. *PNPLA4* (previously known as GS2 and iPLA2eta) encodes an enzyme with triacylglycerol lipase and acylglycerol transacylase activities,³⁹ which may play a role in mitochondrial respiratory chain complex function⁴⁰; *PNPLA4* deficiency could feasibly contribute to the lipid metabolism and mitochondrial abnormalities associated with asthma,^{41 42} anaemia^{43 44} and GI disorders.^{45 46} We speculate that loss of *PNPLA4* in female Xp22.31 deletion carriers may predispose to asthma/anaemia while in males loss of *PNPLA4* may predispose to GI issues, and that these medical vulnerabilities may exacerbate any AHR risk incurred as a consequence of STS deficiency. Potentially, effective treatment of GI issues and atopic conditions/anaemia in male and female deletion carriers, respectively, could reduce AHR risk. We did not identify any genetic signatures within Xp22.31 associated with possible downstream consequences of AF including stroke, acute myocardial infarction and dementia; this may be because common variants within Xp22.31 contribute marginally towards AF risk, and AF, in turn, only contributes to the pathogenesis of a relatively small fraction of stroke, acute myocardial infarction and dementia cases.

Work in relevant *in vitro* (eg, cardiomyocyte/neuronal cultures derived from stem cells from patients with XLI) and *in vivo* models (eg, *Sts*-deficient mice), in combination with more focused clinical analyses in Xp22.31 deletion carriers guided by the preliminary results presented here, should clarify the physiological, cellular and molecular mechanism(s) through which genetic variants at Xp22.31 affect risk of AF and other relevant medical conditions. In turn, this should lead to better-informed genetic counselling for deletion carriers.

We have shown that individuals with XLI (or female carriers of associated genetic deletions) are strongly in favour of cardiac screening shortly after diagnosis to mitigate long-term health consequences associated with AHRs for them and their offspring. As such, the utility and viability of screening within these populations, and particularly in individuals with comorbid GI disorders, asthma or anaemia where clinical prognosis appears worse,^{47 48} should be investigated. Our results further indicate that routine targeted cardiac screening of both male and female Xp22.31 deletion carriers may be warranted, irrespective of whether they present with XLI-associated phenotypes or not. Such an approach may identify individuals requiring early clinical intervention to mitigate later-life adverse outcomes.

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Acknowledgements We would like to thank participants in UK Biobank and respondents to our survey, administrators of various social media sites, and the following organisations who aided with advertisement of the study and recruitment of participants: Ichthyosis Support Group UK, Foundation for Ichthyosis and Related Skin Types (FIRST) (USA), DermNet New Zealand Trust and the British Skin Foundation.

Contributors GW developed online survey and analysed data. EB performed association analyses with supervision from VE-P. JU and GK called CNVs in UK Biobank. TH and AT developed the online survey. WD conceived the study, developed the online survey and analysed data, and wrote the first draft. All authors revised the manuscript and approved submission of the final version. WD is guarantor for this study and accepts full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish.

Funding This work was supported by a Cardiff University School of Psychology PhD studentship to GW, UK Dementia Research Institute (supported by the Medical Research Council (UKDRI-3003), Alzheimer's Research UK and Alzheimer's Society) funding to EB, a Wellcome Trust GW4-CAT Clinical Doctoral Fellowship (222849/Z/21/Z) to JU, and the Medical Research Council Centre for Neuropsychiatric Genetics and Genomics (centre grant number MR/L010305/1).

Disclaimer The funding bodies had no role in the design of the study and collection, analysis and interpretation of data and in writing the manuscript.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval This study involves human participants. This research has been conducted using the UK Biobank resource under application numbers 14421 and 15175; ethical approval for UK Biobank was granted by the North West Multicentre Ethics Committee, UK under Research Ethics Committee approval number 11/NW/0382. The online survey was ethically approved by Cardiff University School of Psychology Ethics Committee (EC.21.07.13.6374R), and participants provided online informed consent for participation and publication of anonymised data.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available as follows: UK Biobank data are available upon application to that resource. Online survey data generated or analysed during this study are included in this published article and its online supplemental files, or are available from <https://doi.org/10.17035/d.2022.0230251614>.

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Supplementary Text 1: Cardiovascular and metabolic conditions investigated as potential risk factors, or

comorbidities, for abnormal heart rhythm in our online survey

Coronary artery/heart disease

Pericarditis

Congenital heart disease

Heart failure

Heart attack

Heart murmur

Angina

Stroke

Heart valve disease or malformation

High cholesterol

Anaemia

Hypertension

Cardiomyopathy

Type 1 diabetes

Type 2 diabetes

Rheumatoid arthritis

Inguinal hernia

Pneumonia

Asthma

Gut problem

Lung cancer

Pulmonary embolism

Thyroid gland disorder

Obstructive sleep apnoea

Obesity (BMI>30)

Supplementary Text 2: A vignette regarding our current knowledge of heart rhythm abnormalities in Xp22.31 deletion carriers.

Atrial fibrillation/flutter (AF) is a medical condition characterised by an irregular heart rhythm resulting from disorganised signals to the atria (upper chambers of the heart). The latest evidence suggests that in middle-aged males with XLI, the risk of AF is approximately 4 times greater than in males without XLI (affecting around 10% of males with XLI compared to 2.5% of unaffected males). The scientific literature also describes rare cases of abnormal heart rhythm in young boys with XLI. Individuals with AF are at increased risk of blood clots, and associated disorders including stroke (5x more likely), dementia/cognitive decline (1.5x more likely) and heart failure (3.5x more likely). Stroke, dementia and heart failure can be associated with long-term impairments in mobility and cognitive function, and increased care needs. If identified early, AF can be effectively treated via rate control medication (to lower the heart rate), rhythm control medication (to restore a regular heart rhythm), and/or anti-coagulation (blood clot/stroke prevention) medication, both on a short- and long-term basis. Individuals may be monitored for AF via an electrocardiogram (ECG) (wires attached to the chest, routinely undertaken at local doctors), blood pressure monitoring (using a cuff placed around the arm) and cardiovascular examinations (examining any external physical indicators e.g. skin discolouration, eyes) via hospital appointments from early life, and appropriate interventions administered.

Supplementary Table 1. Demographic variables in individuals with and without self- or parent-reported abnormal heart rhythms

Adult males with XLI (n=43)			
	Age (yrs)	Country of residence	Ethnicity
With abnormal heart rhythm (AHR)	51.5 (95%CI:42.6-60.6)	UK:47% USA:27% Other:26%	White European: 100% Other: 0%
Without abnormal heart rhythm (AHR)	46.2 (95%CI:41.1-51.2)	UK:54% USA:36% Other:10%	White European:86% Other: 14%
	t[40]=1.09, p=0.28	$\chi^2_{[2]}=4.67$, p=0.10	$\chi^2_{[1]}=2.36$, p=0.12
Adult female carriers (n=79)			
	Age (yrs)	Country of residence	Ethnicity
With abnormal heart rhythm (AHR)	43.7 (95%CI: 39.1-48.2)	UK:35% USA:40% Other:25%	White European: 90% Other: 10%
Without abnormal heart rhythm (AHR)	40.9 (95%CI: 38.2=43.6)	UK:37% USA:37% Other:26%	White European: 88% Other: 12%
	t[57]=0.57, p=0.57	$\chi^2_{[2]}=0.51$, p=0.78	$\chi^2_{[1]}=0.77$, p=0.38
Boys with XLI (n=69)			
	Age (yrs)	Country of residence	Ethnicity
With abnormal heart rhythm (AHR)	8.9 (95%CI: 6.3-11.4)	UK:40% USA:40% Other:20%	White European: 80% Other: 20%
Without abnormal heart rhythm (AHR)	7.5 (95%CI: 6.1-9.0)	UK:41% USA:35% Other:24%	White European: 91% Other: 9%
	t[67]=0.40, p=0.69	$\chi^2_{[2]}=0.16$, p=0.92	$\chi^2_{[1]}=1.32$, p=0.25

Supplementary Table 2A. Individuals with or without abnormal heart rhythms (AHR) diagnosed with cardiovascular and metabolic conditions

Condition	Yes/No	XLI males with AHR (n=15)	XLI males without AHR (n=24)	XLI females with AHR (n=20)	XLI females without AHR (n=51)	XLI boys with AHR (n=15)	XLI boys without AHR (n=48)	All males with AHR (n=30)	All males without AHR (n=72)	All participants with AHR (n=50)	All participants without AHR (n=123)
Coronary artery/heart disease	Yes	0	0	1	0	0	0	0	0	1	0
	No	15	24	19	51	15	48	30	72	49	123
Pericarditis	Yes	0	0	0	0	0	0	0	0	0	0
	No	15	24	20	51	15	48	30	72	50	123
Congenital heart disease	Yes	0	0	1	0	1	0	1	0	2	0
	No	15	24	19	51	14	48	29	72	48	123
Heart failure	Yes	0	0	0	0	0	0	0	0	0	0
	No	15	24	20	51	15	48	30	72	50	123
Heart attack	Yes	0	1	0	0	0	0	0	1	0	1
	No	15	23	20	51	15	48	30	71	50	122
Heart murmur	Yes	0	0	4	0	2	7	2	7	6	7
	No	15	24	16	51	13	41	28	65	44	116
Angina	Yes	1	0	0	0	0	0	1	0	1	0
	No	14	24	20	51	15	48	29	72	49	123
Stroke	Yes	0	0	0	0	0	0	0	0	0	0
	No	15	24	20	51	15	48	30	72	50	123
Heart valve disease or malformation	Yes	1	0	2	1	1	0	2	0	4	1
	No	14	24	18	50	14	48	28	72	46	122

High cholesterol	Yes	4	3	3	7	0	0	4	3	7	10
	No	11	21	17	44	15	48	26	69	43	113
Anaemia	Yes	0	0	8	6	1	2	1	2	9	8
	No	15	24	12	45	14	46	29	70	41	115
Hypertension	Yes	5	4	4	10	0	0	5	4	9	14
	No	10	20	16	41	15	48	25	68	41	109
Cardiomyopathy	Yes	0	0	1	0	0	0	0	0	1	0
	No	15	24	19	51	15	48	30	72	49	123
Type I diabetes	Yes	1	0	0	0	0	0	1	0	1	0
	No	14	24	20	51	15	48	29	72	49	123
Type II diabetes	Yes	1	2	2	3	0	0	1	2	3	5
	No	14	22	18	48	15	48	29	70	47	118
Rheumatoid arthritis	Yes	1	0	1	1	0	0	1	0	2	1
	No	14	24	19	50	15	48	29	72	48	122
Inguinal hernia	Yes	1	0	1	3	0	0	1	0	2	3
	No	14	24	19	48	15	48	29	72	48	120
Pneumonia	Yes	2	3	2	3	2	2	4	5	6	8
	No	13	21	18	48	13	46	26	67	44	115
Asthma	Yes	4	5	7	5	4	6	8	11	15	16
	No	11	19	13	46	11	42	22	61	35	107
Gut problem	Yes	4	0	5	4	1	2	5	2	10	6
	No	11	24	15	47	14	46	25	70	40	117

Lung cancer	Yes	0	0	0	0	0	0	0	0	0	0
	No	15	24	20	51	15	48	30	72	50	123
Pulmonary embolism	Yes	0	0	0	2	0	0	0	0	0	2
	No	15	24	20	49	15	48	30	72	50	121
Thyroid gland disorder	Yes	2	1	2	6	1	0	3	1	5	7
	No	13	23	18	45	14	48	27	71	45	116
Obstructive sleep apnoea	Yes	1	3	3	4	0	1	1	4	4	8
	No	14	21	17	47	15	47	29	68	46	115
Obesity (BMI>30)	Yes	2	2	4	9	0	3	2	5	6	14
	No	13	22	16	42	15	45	28	67	44	109

Supplementary Table 2B Associated statistical analysis for **Supp Table 2A** (two-tailed p-values from Fisher Exact Test) *p≤0.05, **p≤0.005

Condition	XLI adult males (AHR vs. no AHR)	Female carriers (AHR vs. no AHR)	XLI boys (AHR vs. no AHR)	All males (AHR vs. no AHR)	All participants (AHR vs. no AHR)
Coronary artery/heart disease	1.000	0.282	1.000	1.000	0.289
Pericarditis	1.000	1.000	1.000	1.000	1.000
Congenital heart disease	1.000	0.282	0.238	0.294	0.082
Heart failure	1.000	1.000	1.000	1.000	1.000
Heart attack	1.000	1.000	1.000	1.000	1.000
Heart murmur	1.000	0.005**	1.000	0.723	0.202
Angina	0.385	1.000	1.000	0.294	0.289
Stroke	1.000	1.000	1.000	1.000	1.000

Heart valve disease or malformation	0.385	0.189	0.238	0.084	0.025*
High cholesterol	0.396	1.000	1.000	0.190	0.265
Anaemia	1.000	0.011*	1.000	1.000	0.027*
Hypertension	0.266	1.000	1.000	0.119	0.322
Cardiomyopathy	1.000	0.282	1.000	1.000	0.289
Type I diabetes	0.385	1.000	1.000	0.294	0.289
Type II diabetes	1.000	0.616	1.000	1.000	0.692
Rheumatoid arthritis	0.385	1.000	1.000	0.294	0.201
Inguinal hernia	0.385	1.000	1.000	0.294	0.627
Pneumonia	1.000	0.616	0.238	0.443	0.356
Asthma	0.711	0.017*	0.231	0.263	0.010*
Gut problem	0.017*	0.105	1.000	0.022*	0.004**
Lung cancer	1.000	1.000	1.000	1.000	1.000
Pulmonary embolism	1.000	1.000	1.000	1.000	1.000
Thyroid gland disorder	0.547	1.000	0.238	0.075	0.332
Obstructive sleep apnoea	0.648	0.394	1.000	1.000	0.746
Obesity (BMI>30)	1.000	1.000	0.574	1.000	1.000

Supplementary Table 3. Percentage of individuals with XLI (or female carriers) reporting involvement of precipitating factor in onset of AHR episodes

Precipitating factor	Adults males with XLI and AHR (n=12)	Female carriers with AHR (n=20)	Boys with XLI and AHR (n=14)
Stress	42	45	14
Medication	8	0	7
Caffeine consumption (tea, coffee, energy drinks)	33	25	7
Smoking	8	0	0
Postural change (e.g. moving from sitting down to standing up)	17	25	7
Sleep disturbance	33	15	7
Infection	8	10	14
Exercise	17	35	14
Increased body temperature e.g. due to inability to sweat	17	15	29
No obvious cause	42	25	50
Other	17	30	14

Supplementary Table 4. Percentage of individuals with XLI (or female carriers) reporting times of onset of AHR episodes

Time of onset	Adults males with XLI and AHR (n=12)	Female carriers with AHR (n=12)	Boys with XLI and AHR (n=7)
Whilst sleeping/in the night	57	42	57
Early morning, shortly after waking	57	8	14
During the day	57	75	86
Late at night, just before sleeping	43	58	29

Supplementary Table 5. A gene-based analysis of SNPs associated with stroke in the consensus Xp22.31 deletion interval in males (3,713 cases vs. 191,861 controls) and females (3,128 cases vs. 164,873 controls) from the UK Biobank. NSNPs= the number of SNPs in the data annotated to the gene; NParam = the number of relevant parameters used in the model (essentially the number of independent SNPs in the gene).

Gene	Start (bp)	Stop (bp)	Males			Females		
			NSNPs	NParam	P-value	NSNPs	NParam	P-value
<i>VCX3A</i>	6451659	6453159	5	3	0.651	5	3	0.859
<i>HDHD1</i>	6966961	7066231	225	12	0.666	223	13	0.208
<i>STS</i>	7065298	7272682	343	20	0.839	336	20	0.115
<i>VCX</i>	7810303	7812184	4	2	0.929	3	2	0.099
<i>PNPLA4</i>	7866804	7895780	39	6	0.084	39	6	0.061
<i>VCX2</i>	8137985	8139308	11	4	0.813	8	3	0.494

Supplementary Table 6. A gene-based analysis of SNPs associated with acute myocardial infarction in the consensus Xp22.31 deletion interval in males (12,186 cases vs. 155,869 controls) and females (3,927 cases vs. 191,711 controls) from the UK Biobank. NSNPs= the number of SNPs in the data annotated to the gene; NParam = the number of relevant parameters used in the model (essentially the number of independent SNPs in the gene).

Gene	Start (bp)	Stop (bp)	Males			Females		
			NSNPs	NParam	P-value	NSNPs	NParam	P-value
<i>VCX3A</i>	6451659	6453159	5	3	0.793	5	3	0.146
<i>HDHD1</i>	6966961	7066231	225	12	0.903	223	13	0.746
<i>STS</i>	7065298	7272682	343	20	0.830	336	20	0.777
<i>VCX</i>	7810303	7812184	4	2	0.483	3	2	0.390
<i>PNPLA4</i>	7866804	7895780	39	6	0.557	39	6	0.795
<i>VCX2</i>	8137985	8139308	11	4	0.318	8	3	0.171

Supplementary Table 7. A gene-based analysis of SNPs associated with dementia in the consensus Xp22.31 deletion interval in males (2,238 cases vs. 165,817 controls) and females (1,892 cases vs. 193,746 controls) from the UK Biobank. NSNPs= the number of SNPs in the data annotated to the gene; NParam = the number of relevant parameters used in the model (essentially the number of independent SNPs in the gene).

Gene	Start (bp)	Stop (bp)	Males			Females		
			NSNPs	NParam	P-value	NSNPs	NParam	P-value
<i>VCX3A</i>	6451659	6453159	5	3	0.228	5	3	0.729
<i>HDHD1</i>	6966961	7066231	225	12	0.843	223	13	0.537
<i>STS</i>	7065298	7272682	343	20	0.865	336	20	0.429
<i>VCX</i>	7810303	7812184	4	2	0.893	3	2	0.433
<i>PNPLA4</i>	7866804	7895780	39	6	0.690	39	6	0.839
<i>VCX2</i>	8137985	8139308	11	4	0.453	8	3	0.390

Supplementary Table 8. A gene-based analysis of SNPs associated with asthma in the consensus Xp22.31 deletion interval in males (22,231 cases vs. 145,824 controls) and females (29,206 cases vs. 166,432 controls) from the UK Biobank. NSNPs= the number of SNPs in the data annotated to the gene; NParam = the number of relevant parameters used in the model (essentially the number of independent SNPs in the gene). *p<0.05

Gene	Start (bp)	Stop (bp)	Males			Females		
			NSNPs	NParam	P-value	NSNPs	NParam	P-value
<i>VCX3A</i>	6451659	6453159	5	3	0.170	5	3	0.484
<i>HDHD1</i>	6966961	7066231	225	12	0.994	223	13	0.909
<i>STS</i>	7065298	7272682	343	20	0.732	336	20	0.880
<i>VCX</i>	7810303	7812184	4	2	0.207	3	2	0.873
<i>PNPLA4</i>	7866804	7895780	39	6	0.196	39	6	0.040*
<i>VCX2</i>	8137985	8139308	11	4	0.150	8	3	0.596

Supplementary Table 9. A gene-based analysis of SNPs associated with anaemia in the consensus Xp22.31 deletion interval in males (7,958 cases vs. 160,097 controls) and females (14,015 cases vs. 181,623 controls) from the UK Biobank. NSNPs= the number of SNPs in the data annotated to the gene; NParam = the number of relevant parameters used in the model (essentially the number of independent SNPs in the gene). *p<0.05

Gene	Start (bp)	Stop (bp)	Males			Females		
			NSNPs	NParam	P-value	NSNPs	NParam	P-value
<i>VCX3A</i>	6451659	6453159	5	3	0.438	5	3	0.551
<i>HDHD1</i>	6966961	7066231	225	12	0.210	223	13	0.981
<i>STS</i>	7065298	7272682	343	20	0.242	336	20	0.971
<i>VCX</i>	7810303	7812184	4	2	0.165	3	2	0.623
<i>PNPLA4</i>	7866804	7895780	39	6	0.584	39	6	0.013*
<i>VCX2</i>	8137985	8139308	11	4	0.120	8	3	0.680

Supplementary Table 10. A gene-based analysis of SNPs associated with gastrointestinal disorders in the consensus Xp22.31 deletion interval in males (42,680 cases vs. 125,375 controls) and females (56,842 cases vs. 138,796 controls) from the UK Biobank. NSNPs= the number of SNPs in the data annotated to the gene; NParam = the number of relevant parameters used in the model (essentially the number of independent SNPs in the gene). *p<0.05

Gene	Start (bp)	Stop (bp)	Males			Females		
			NSNPs	NParam	P-value	NSNPs	NParam	P-value
<i>VCX3A</i>	6451659	6453159	5	3	0.021*	5	3	0.650
<i>HDHD1</i>	6966961	7066231	225	12	0.553	223	13	0.344
<i>STS</i>	7065298	7272682	343	20	0.610	336	20	0.296
<i>VCX</i>	7810303	7812184	4	2	0.044*	3	2	0.283
<i>PNPLA4</i>	7866804	7895780	39	6	0.022*	39	6	0.470
<i>VCX2</i>	8137985	8139308	11	4	0.018*	8	3	0.113