Phenotypes associated with female X chromosome aneuploidy in UK Biobank: an unselected, adult, population-based cohort

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Abstract

Women with X chromosome aneuploidy such as 45,X (Turner syndrome) or 47,XXX (Triple X syndrome) present with a range of characteristics including differences in stature, an increased risk of cardiovascular disease and premature ovarian insufficiency. Many women with X chromosome aneuploidy undergo lifetime clinical monitoring for possible complications. However, biased ascertainment of cases may mean that the penetrance of phenotypes is overestimated. We aimed to characterise the prevalence and phenotypic consequences of X chromosome aneuploidy in a large population of older adults. We detected 30 women with 45,X, 186 with mosaic 45,X/46,XX and 110 with 47,XXX in 245,203 women from UK Biobank, using SNP array data. The phenotypic features of women with full aneuploidy (whether 45,X or 47,XXX) were similar to those previously reported. Consistent with the recognised Turner syndrome phenotype, those with 45,X were 17.2cm shorter than controls and 53% did not go through menarche. Similarly, the phenotype of women with 47,XXX included increased height (on average 5.3cm taller than controls, P = 1

x 10^{-18}), earlier menopause age (on average 5.12 years earlier than controls, $P = 1.2 \times 10^{-14}$) and a lower fluid intelligence (on average 24% lower than controls, $P = 3.7 \times 10^{-8}$). In contrast, women with 45,X/46,XX mosaicism had a very mild phenotype; were not as short, had a normal reproductive lifespan and birth rate, with no reported cardiovascular complications. This study characterises X chromosome aneuploidy phenotypes in an adult population-based sample of older individuals and suggests that clinical management of women with a 45,X/46,XX mosaic karyotype should be minimal, particularly those identified incidentally.

Introduction

X chromosome aneuploidy is a common chromosome abnormality that may be detected as an incidental finding[1]. It can be difficult to determine the clinical importance of the abnormality, as population based studies of X chromosome aneuploidy are rare and are often from prenatal screening with little or no follow-up[2]. Cohorts recruited through clinical features of X chromosome aneuploidy syndromes may suffer from ascertainment bias, because features reported in the literature are often present and only individuals with those features are being tested. It is increasingly important to determine the phenotypic consequences of genetic abnormalities in unbiased populations, as more genomic testing is carried out on individuals in the general population, including direct to consumer testing, plus more extensive whole genome analyses in individuals with recognised conditions[3].

Women with a 47,XXX chromosome complement are reported to be taller than average and to have earlier menopause, but the evidence to support these associations is limited to potentially biased collections of cases reaching medical attention[4]. Girls with trisomy X are also at increased risk of developmental delay, particularly problems with speech and language[4]. In contrast women with a 45,X chromosome complement or Turner syndrome are generally short (20cm below population mean), may have physical features such as a webbed neck and about 80% have primary amenorrhea[5]. Women with Turner syndrome are at increased risk of hearing difficulties and cardiac disorders, particularly dissection of the aorta[6; 7 8; 9]. Hypergonadotrophic hypogonadism in Turner syndrome means that pregnancy is often difficult to achieve spontaneously, but women may opt for *in vitro* fertilisation with donor eggs. However, pregnancy in women with Turner syndrome is considered high risk, predominantly due to the increased risk of cardiac complications[10].

Trisomy X occurs in about 1/1000 newborn females and Turner syndrome in about 1/2500[11]. Both karyotypes can exist in mosaic form with a mixture of aneuploid and 46,XX cell types in varying proportions. The presence of a normal 46,XX cell line is generally associated with a less severe phenotype, but may depend on tissue specific variability in degree of mosaicism. X chromosome loss can also occur with normal ageing, with 7.3% of lymphocytes having a 45,X karyotype in women over 65 years old[12]. Traditionally X chromosome aneuploidy was detected by cytogenetic testing, which is expensive and labour intensive, making large population based studies unfeasible. In this study, we tested over 245,000 women from the UK Biobank – a population based study of adults aged 40-70, for copy number variation using SNP array data and identified 326 individuals with whole X chromosome imbalances. We used the detailed phenotype data available in UK Biobank to characterise the features of females with X chromosome aneuploidy from this population based cohort.

Methods

UK Biobank cohort

UK Biobank recruited over 500,000 individuals aged 37-73 years (99.5% were between 40 and 69 years) between 2006-2010 from across the UK. Participants provided a range of information via questionnaires and interviews (e.g. demographics, health status, lifestyle) and anthropometric measurements, blood pressure readings, blood, urine and saliva samples were taken for future analysis: this has been described in more detail elsewhere[13]. SNP genotypes were generated from the Affymetrix Axiom UK Biobank array (~450,000 individuals) and the UKBiLEVE array (~50,000 individuals). This dataset underwent extensive central quality control (http://biobank.ctsu.ox.ac.uk). We based our study on 451,099 individuals of white European descent as defined by Principal Components analysis (PCA). Briefly, principal components were generated in the 1000 Genomes Cohort using high-confidence SNPs to obtain their individual loadings. These loadings were then used to project all of the UK Biobank samples into the same principal component space and individuals were then clustered using principal components 1 to 4. We removed 7 participants who withdrew from the study, and 348 individuals whose self-reported sex did not match their genetic sex based on relative intensities of X and Y chromosome SNP probe intensity. These steps resulted in

245,203 females who were carried forward for subsequent analyses. Basic characteristics for these females are given in **supplemental table S1**.

Identification of women with chromosome X aneuploidy

Log R Ratio (LRR) and B Allele Frequency (BAF) values for each non-pseudoautosomal SNP probe were provided by UK Biobank. For each female, we calculated the mean LRR across all 18,725 SNP probesets on chromosome X and the number of probesets falling inside the expected BAF heterozygous range (values between 0.49 and 0.51). We classified females as having X chromosome aneuploidy if they were outliers in both the cohort mean LRR distribution and the expected heterozygous BAF count distribution (**Supplemental Figs S1-S4**, **Fig. 1**). Women who were outliers for both mean-LRR and BAF heterozygote count were identified using the standard definition of a value more than 1.5 times the interquartile range away from the mean value in the study[14]. Finally, we checked for relatedness amongst the X chromosome aneuploidy cases and we found no UK Biobank defined 1st-3rd degree related individuals.

Approximate levels of mosaicism in 45,X/46,XX individuals was estimated from the LRR. The mosaicism was derived by dividing mean LRR by the lowest detected mean LRR value for those with an LRR less than zero, and the maximum positive mean LRR value for those greater than zero. We defined 45,X females as mosaic if the estimate was ≤ 0.80.

Validation of methodology

To validate the use of SNP genotype dosage for determining 45,X mosaicism, we tested six lymphocyte DNA samples from an independent source. The DNA was from individuals tested at the Wessex Regional Genetics Laboratory with a range of 45,X mosaicism, as determined by traditional karyotyping. The samples included a full 45,X sample, a 46,XX sample and four 45,X/46,XX mosaics. The six samples were genotyped using both the Illumina Infinium HTS assay on Global Screening array, and the Affymetrix Axiom UK Biobank array. Processing and derivation of LRR and BAF were carried out using Illumina Genome Studio for the Illumina array data. PennCNV-Affy was used to normalise and derive LRR/BAF values from the raw probe intensity data from the Affymetrix array together with 1,000 randomly selected UK Biobank samples. The percentage dosage was derived for Illumina and Affymetrix data separately by dividing mean LRR by the smallest detected

mean LRR value amongst the 6 samples. We checked for concordance blindly between independent karyotyping results and mean LRR across chromosome X and then visually inspected the LRR/BAF plots (**Supplemental Fig. S5**).

Association testing for a range of phenotypes

A range of phenotypes were available in UK Biobank, derived from self-reported questionnaire data, ICD10 diagnoses recorded in Hospital Episodes Statistics data and measurements taken at baseline clinic visits as part of the study. We tested the association of X chromosome aneuploidy with a range of phenotypes and traits including: anthropometric, reproductive, cardiovascular, learning/memory and incidence of various diseases (**supplemental table S1**). X chromosome aneuploidy was stratified into 4 groups: all 45,X and 45,X/46,XX samples, 45,X samples only, 45,X/46,XX samples only and 47,XXX samples. Controls were all other women in UK Biobank (max N=244,738). Association testing was carried out with inverse normalised phenotypes to account for any skewed distributions, using linear regression models in STATA 13, adjusting for SNP chip type (UKB Axiom or UK BiLEVE), ancestry-principal components 1 to 5 supplied by UK Biobank, test centre and age (or year of birth for age at menarche) with the exception of three traits: hypertension, hypothyroidism and household income. Hypertension and hypothyroidism were tested using logistic regression and household income was tested using ordinal logistic regression. The logistic and ordinal logistic models were adjusted using the same covariates as the linear regression model.

Results

We detected 326 women with whole X chromosome imbalances, 110 with a higher dosage indicating an additional X chromosome and 216 with a lower dosage, suggesting only one X chromosome in some or all cells (**Fig. 1 and Supplemental Figs S2-S4**). Of the 216 suspected 45,X women there was a range of estimated dosages with mean LRRs ranging from -0.5 to -0.07 (mean = -0.23). Of these, 30 individuals had an estimated X chromosome dosage in the lowest quintile based on LRR, suggesting the majority of cells had only one X and therefore had Turner syndrome with a 45,X karyotype. There were fourteen individuals in UK Biobank with a prior medical diagnosis of 'Turner syndrome' within the genotyped

individuals as determined by ICD10 code. Eight of those with a prior diagnosis of Turner syndrome were among the 216 women we detected with X chromosome loss, all were in the 'non-mosaic' 45,X category, with an estimated X chromosome loss in more than 80% of cells. Of the remaining 6 cases with a prior diagnosis of Turner syndrome; 3 were excluded from the analysis (1 was of non-European ancestry but would have been scored as 45,X and 2 were classified as sex mismatches by UKBiobank and indeed the X chromosome profiles were similar to those of male samples as they were heterozygous for a region on Xq, and hemizygous elsewhere, we excluded samples with similar profiles), 1 had a normal 46,XX SNP array profile and 2 were not outliers for whole X chromosome dosage, but had deletion of the p arm and duplication of the q arm, suggesting an isochromosome (**Supplemental Fig. S6**).

Validation of 45,X mosaicism

For 186 individuals the log R ratio dosage and B allele frequency plot indicated a mosaic 45,X/46,XX karyotype. The estimated percentage 45,X cells per sample ranged from 15-79%. We validated the accuracy of the SNP array method for determining 45,X mosaicism by testing 6 independent samples which had been tested by conventional cytogenetic techniques, using two different SNP arrays (**Table 1, Supplemental Fig. S5**). There was high level of concordance between the arrays and cytogenetic estimates, with all samples differing by <10%. A perfect correlation between the two methods may be unrealistic as cytogenetic analysis is carried out on cultured lymphocytes, rather than whole blood and thus could be subject to clonal expansion of one cell population over the other. We predicted that age-related loss of the X chromosome would not be detected by the SNP array methodology. As age-related loss is a random process, with a different X being lost in each cell the BAF pattern would not differ from controls and the change in dosage as measured by LRR would be relatively small and beyond the limit of detection by our method. However, the SNP array method can detect 45,X mosaics representing a clonal 45,X cell line as the same X chromosome will be absent in each 45,X cell. In summary we were able to verify that the SNP array methodology was able to accurately call 45,X genetic abnormalities down to about 15% 45,X cells, but not age-related loss of the X.

45,X and 45,X/46,XX mosaicism and height

The group of 216 women with evidence of X chromosome loss were on average 5.6cm shorter than 46,XX females in UK Biobank ($P = 3.1 \times 10^{-37}$), but this was largely driven by the 30 non-mosaic 45,X cases, who had a mean height 17.2cm shorter than the 46,XX controls (**Tables 2 and 3, Fig. 2**). The 186 mosaic 45,X/46,XX cases were shorter than 46,XX females but only by 3.8cm on average ($P = 4.0 \times 10^{-14}$). The difference compared to 45,X individuals was much greater, who were on average 13.4cm shorter than the 186 mosaic 45,X/46,XX individuals ($P = 8.6 \times 10^{-17}$). Height ranged from 139-182cm in the mosaic group, with 7% of women being taller than 1SD from the mean height in controls. There was a weak correlation between percentage mosaicism and height in the women with mosaic 45,X/46,XX (r=0.23).

45,X and 45,X/46,XX mosaicism and ovarian function

Primary amenorrhea was not specifically coded in UK Biobank, but 5 of the 30 non-mosaic 45,X women answered 'don't know' and 11 responded 'Prefer not to answer' to the question "How old were you when your periods started?", which were the only options apart from an age in years, a higher proportion than in controls (OR = 39.52, $P = 1.5 \times 10^{-17}$). We therefore assume that a large proportion of these women did not go through menarche. For the other 14 non-mosaic cases, menarche ages of between 12 and 19 years were recorded. It is possible that menarche was induced with exogenous hormones in these 14 individuals, but there was no data to indicate this. In contrast, the 186 mosaic 45,X/46,XX cases reported a menarche age in all but 9 cases, and the mean menarche timing was 13.2 years in those 177 individuals who recorded an age, not different from 46,XX controls ($P = 0.12$).

Ninety-five of the 45,X/46,XX mosaic women had also gone through natural menopause (**Table 2, Fig. 2**), at an average age of 50.6 years (range 25-59 years), again not different from the mean age in control 46,XX females (mean = 50 years (range 18-65); $P = 0.23$). The recruitment ages of the 30 non-mosaic 45,X cases ranged from 42-69 years, but only 5 reported a natural menopause age (compared to controls, OR = 3.98, $P = 0.0026$).

Most of the mosaic 45,X/46,XX cases reported a pregnancy, with only 37 of 186 reporting they had never been pregnant, the remainder having between 1 and 10 pregnancies. The mean number of pregnancies in the 45,X/46,XX group was 2.2, not different from the control

46,XX individuals ($P = 0.09$). There was also no increased incidence of pregnancy loss in this group of women, either from miscarriage, termination or stillbirth. Only four of the thirty non-mosaic 45,X cases had ever been pregnant, much fewer than in controls (OR = 32.35, $P = 8.98 \times 10^{-17}$), having 1 or two pregnancies each.

45,X and 45,X/46,XX mosaicism and cardiac function

Heart defects are a common reported feature of Turner syndrome, but we found no substantial increased incidence of heart defects in this cohort of 45,X women, based on self-reported incidence of disease and ICD10 codes. Blood pressure and hypertension were slightly elevated in the non-mosaic 45,X group (hypertension OR = 3.14, $P = 5 \times 10^{-3}$) but not in the women with 45,X/46,XX (hypertension OR = 1.02, $P = 0.88$) (**Table 4**), while arterial stiffness was lower in the 45,X group ($P = 0.001$). Of the 30 women in the 45,X group there were 4 individuals who had a medically diagnosed heart condition, including coarctation/dissection of the aorta and myocardial infarction and 13 were on blood pressure medication. The 45,X/46,XX mosaic cases had not had more cardiac operations and were not more likely to be on blood pressure medication than controls.

X chromosome loss and additional abnormalities

There was an increased incidence of diagnosed hypothyroidism in the full 45,X women (OR = 6.61, $P = 1.4 \times 10^{-6}$), but not in the mosaic 45,X/46,XX women (OR = 1.31, $P = 0.26$). Bone mineral density was slightly reduced in the full 45,X group compared to 46,XX ($P = 2.3 \times 10^{-4}$), but not in the mosaic women (**Table 4**). There was an increased incidence of hearing abnormalities in the full 45,X group, with more women wearing a hearing aid (OR = 35.43, $P = 1.2 \times 10^{-15}$), reporting 'hearing problems' (OR = 6.54, $P = 1.7 \times 10^{-06}$) and having poorer measured hearing (mean speech recognition threshold 1.84 units higher, $P = 0.0034$) (**Table 4**). Similar problems were also found in the 45,X/46,XX mosaic group, but with lower incidence or effect size. There were more 45,X/46,XX women wearing a hearing aid (OR = 2.54, $P = 1.1 \times 10^{-3}$) or reporting 'hearing problems' (OR = 1.74, $P = 5.1 \times 10^{-4}$), but no difference in measured hearing ($P > 0.05$) (**Table 4**).

Trisomy X phenotype

Trisomy X was detected in 110 women in the UK Biobank cohort and a further two appeared to be mosaic 46,XX/47,XXX. The two mosaic cases were excluded from further analyses

because there were too few in this category to analyse separately. We found an association between trisomy X and adult height ($P = 5.8 \times 10^{-20}$), with 47,XXX women being 5.3cm taller on average than 46,XX controls (**Fig. 2, Tables 2 and 3**). Trisomy X women had normal age at menarche, but an average decrease of 5.12 years in natural menopause age ($P = 1.2 \times 10^{-14}$) (**Tables 2 and 3**, **Fig. 2**). Women with 47,XXX had an average number of pregnancies (mean = 1.9, range 0-10) and no higher frequency of pregnancy loss. We also found that women with trisomy X had a lower fluid intelligence ($P = 3.7 \times 10^{-8}$) and a decreased household income ($P = 8.7 \times 10^{-17}$) (**Tables 2 and 3**). Sixty three percent of women in the 47,XXX group had a household income less than £18,000 per year, compared to 24% of control women. Women with 47,XXX were more likely to live alone ($P = 7 \times 10^{-5}$) with 37/110 (35.2%) living alone compared to 19.8% of 46,XX controls living alone. The women with 47,XXX also had a higher BMI than controls, by on average over 2 BMI units ($P = 6.3 \times 10^{-6}$).

Discussion

We identified 326 women in UK Biobank with whole X chromosome dosage anomalies: 216 consistent with having a 45,X or 45,X/46,XX mosaic karyotype and 110 with 47,XXX. We detected only two cases of 46,XX/47,XXX mosaicism, but these are less common than 45,X/46,XX mosaics and would be more difficult to detect with the array method[15]. Previous studies of X chromosome aneuploidy have likely been biased towards the characterisation of women with more severe phenotypes, more likely to reach clinical presentation. The availability of >245,000 older women with SNP array data in a single population based study has provided us with an important opportunity to assess the phenotype of these relatively common genetic abnormalities in later life.

Women with 45,X/46,XX mosaicism had a phenotype similar to controls.

Our data would suggest that many women with a 45,X cell line are not at increased risk of pregnancy loss or of cardiac complications and do not necessarily need the healthcare intervention that is currently recommended[16]. The international clinical practice guidelines do not readily differentiate mosaic from full 45,X yet our data suggest that level of mosaicism is an important clinical indicator of Turner syndrome features and related complications. There is less information in the literature about women with a mosaic 45,X/46,XX karyotype, as

these generally constitute a smaller proportion of any reported series of cases, commonly combined with 45,X cases. In the UK Biobank there were 186 women who appeared to be mosaic for X chromosome loss, i.e. retaining two X chromosomes in a proportion of cells. Surprisingly, the phenotype in these women was unremarkable: while they were shorter on average, many of the women in this category were of average height, with the tallest individual being 182cm, despite 20% of blood cells being 45,X (**Supplemental Fig. S3ah**). This group of women, went through menarche and menopause at an average age, had an average number of children, were not at increased risk of pregnancy loss and there was no evidence of increased risk of cardiac complications.

We detected a 45,X or 45,X/46,XX mosaic karyotype in 1/1,133 women in UK Biobank, a population-based cohort of individuals not selected for any phenotypic features or disease. The majority of these cases were mosaic. This is approximately double the prevalence of Turner syndrome estimated by cytogenetic screening and may be explained by the high proportion of mosaic cases in our series, who have few phenotypic features of Turner syndrome and would likely go undetected in clinical practice. As more individuals are obtaining genomic information either as part of healthcare assessments or through direct-to-consumer genetic testing, the likelihood of detecting X chromosome imbalance is increasing and it is important to be able to counsel individuals appropriately regarding the implications of such findings. Currently any women identified as having a 45,X cell line, not thought to be due to age-related loss, would be diagnosed as having Turner syndrome and would be offered extensive monitoring, particularly during pregnancy[16]. While it is recognised that mosaic cases may be less likely to have abnormalities than full 45,X Turner syndrome cases they are still considered high risk with respect to cardiac anomalies and hypertension during pregnancy for instance. In young women one of the main concerns will be the likelihood of infertility associated with a diagnosis of Turner syndrome. Our data suggest that in fact the presence of a 45,X cell line in blood does not affect reproductive lifespan or fertility adversely in most cases, as long as more than 20% of cells have two X chromosomes.

Of the 326 women in UK Biobank with X chromosome aneuploidy, 30 had a complete loss of one X chromosome, consistent with a diagnosis of Turner syndrome.

Eight of these 30 women had a pre-existing medical diagnosis of Turner syndrome. None of the women with a previous diagnosis of Turner syndrome were in the mosaic 45,X/46,XX

group confirming that a high proportion of X chromosome loss is required to generate a substantial effect on phenotype, such that would be identified by clinicians. Women with 45,X had a phenotype that was characteristic of that reported for this chromosomal abnormality, i.e. short stature and primary amenorrhea being the main features. Cardiac abnormalities were not very common in this group, but were found in some individuals, confirming the need for continued screening programmes. There were no data available on physical characteristics, such as neck webbing.

Women with 47,XXX are taller, have earlier menopause and are of below average cognitive ability.

These findings are important because they suggest that ascertainment bias has not dramatically influenced the current consensus of the 47,XXX phenotype. Our findings agree with published literature on the phenotype associated with 47,XXX syndrome in a cohort without the ascertainment bias of having a clinical referral. Traditionally 47,XXX syndrome has been identified by cytogenetic testing, which is a costly and labour intensive procedure and requires fresh tissue to enable culture of dividing cells. Thus the majority of studies on 47,XXX have been on women tested for a clinical presentation such as learning difficulties, or identified through prenatal testing as an incidental finding. Cohorts of older women with 47,XXX are uncommon as prenatal chromosome testing has been relatively recently adopted and thus most longitudinal studies of 47,XXX women ascertained at birth have not yet reached post-menopausal ages. The first reported case of 47,XXX was an infertile woman and infertility or premature ovarian failure is often a reason for referral for 47,XXX testing[17]. However premature ovarian insufficiency (POI) affects 1% of women and 47,XXX is a relatively common finding too (1/1000) and thus the association could be due to ascertainment bias. Our data suggests that there is a genuine effect on reproductive lifespan in women with 47,XXX. This effect appears to be limited to the end of reproductive life however, as these women had average menarche age, but went through menopause on average more than 5 years earlier than controls, with 10 women meeting the criteria for POI, with menopause before 40 years. The frequency of POI in 47,XXX women is therefore 9%, approximately 10 times that seen in controls. We found no evidence for an impact of 47,XXX on reproductive function throughout life, as these women had an average number of pregnancies and no significant increase in pregnancy loss.

Dosage of the sex chromosomes is known to influence human height[18]. Individuals with a single X chromosome and Turner syndrome are typically on the 0.1 centile for height and an additional X in men with Klinefelter syndrome increases adult height by about one standard deviation. The UK Biobank women with 47,XXX were on average 5.3cm taller than 46,XX controls. Adult height is associated with puberty timing, with earlier puberty resulting in shorter adult height. The 47,XXX effect on adult height is however unlikely to be driven by later puberty timing, as menarche age was not significantly different from controls in 47,XXX women. It is more likely that the increase in height is caused by dosage sensitive genes responsible for post-pubertal growth.

The IQ of girls with 47,XXX is reported to be within the normal range, but approximately 10-15 points below their sibling average. Lower IQ is also reported in adult cases of 47,XXX, along with psychosocial features, such as low self-esteem, language difficulties and increased prevalence of psychiatric disorders[19]. Few series of older adults with 47,XXX have been reported previously, but a study from Denmark found reduced socio-economic status in a series of 108 women with 47,XXX[20]. In the UK Biobank study we found a substantial reduction in fluid intelligence compared to controls. 'Fluid intelligence' describes the capacity to solve problems that require logic and reasoning ability, independent of acquired knowledge. We observed an increase in BMI in women with 47,XXX, that is not reported as a typical feature of this chromosome abnormality, and may be related to the observed lower IQ. There was also a reduction in household income in women with 47,XXX. The association with lower household income was not a consequence of general lower socio economic status as Townsend deprivation index was not significantly associated with 47,XXX status. Ability to work and type of employment taken by younger women with 47,XXX has been reported in other studies, but with much smaller sample sizes. Thus the reduced IQ observed in adolescents with 47,XXX continues into older age and appear to have a lifelong effect on income.

Limitations. The samples tested in this study were from peripheral blood and levels of mosaicism are likely to differ between tissue types. Thus the mosaicism we detected may not reflect the level present in brain, ovary or heart for example. Our study is also biased in favour of healthier individuals with a more benign phenotype[21]. We know for instance that there are only sixteen cases of Down syndrome in UK Biobank, while we would expect

nearer 280 individuals in a population of 500,000 people between 40-70 years old[22]. UK Biobank participants are all over 40 years old and thus cases of Down syndrome may be underrepresented due to reduced life expectancy, nevertheless it is likely that individuals with Down syndrome were less likely to volunteer for the study due to the learning difficulties associated with the condition. Biobank volunteers are more likely to be female, have higher IQ and be from higher socioeconomic group than the general population[21]. Cases of X chromosome aneuploidy with a more severe phenotype may therefore be underrepresented in the Biobank cohort. Thus our study does not represent a comprehensive assessment of X chromosome aneuploidy in the population, but it goes a long way to redressing the bias seen in many published series of cases ascertained through clinical referrals and therefore more likely to have a clinical phenotype.

Validation. While we were not able to karyotype any of the samples from UK Biobank to validate our methodology, we did use two platforms of array-based technology to test an independent series of patients with various levels of 45,X mosaicism and found very close correlation between the array method and cytogenetic assessment. Further validation came from finding eight previously known Turner syndrome cases in UK Biobank among the 30 individuals who we classified as full 45,X with the array dosage data. The array method and analysis pipeline we used will not detect random age-related X chromosome loss and thus the cases we have identified are true clonal mosaics. Karyotype analysis cannot distinguish between age-related loss and presence of a 45,X cell line in patients over 30 years old. One previously diagnosed case of Turner syndrome appeared to have a normal 46,XX SNP array profile, this may be a sample mix-up, or a suspected Turner syndrome case that was not confirmed by genetic testing, or perhaps has a more complex genetic abnormality that was not detected by the array method. Indeed two additional previously known Turner syndrome cases were not detected by the SNP array method because they had loss of Xp and gain of Xq making the overall X chromosome dosage not significantly different from normal 46,XX. We suspect that these are isochromosomes, which contribute to about 15% of Turner syndrome cases in other published series[23; 24]. We did not seek to include additional cases with this anomaly or other X chromosome imbalances known to cause Turner syndrome such as ring X or Xp deletions, as we were not able to validate these findings with an independent technology. We are confident that the cases we have identified do not include false positives,

but there may be X chromosome imbalances with Turner or 47,XXX phenotypes that are not included in our cases.

Conclusions. Our population-based cohort data confirms a dosage effect on adult height associated with X chromosome aneuploidy. An additional X chromosome was associated with early menopause and reduced fluid intelligence. However X chromosome aneuploidy was not always associated with an adverse phenotype: 45,X/46,XX mosaic females had a normal reproductive lifespan and birth rate, with no reported cardiovascular complications compared to controls. This study characterises X chromosome aneuploidy phenotypes in a population-based sample of older individuals and presents implications for future management of women with a 45,X/46,XX mosaic karyotype, particularly those identified incidentally.

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Ethics UK Biobank

This study was conducted using the UK Biobank resource. Details of patient and public involvement in the UK Biobank are available online (www.ukbiobank.ac.uk/about-biobank-uk/ and https://www.ukbiobank.ac.uk/wp-content/uploads/2011/07/Summary-EGF-consultation.pdf?phpMyAdmin=trmKQlYdjjnQIgJ%2CfAzikMhEnx6). No patients were specifically involved in setting the research question or the outcome measures, nor were they involved in developing plans for recruitment, design, or implementation of this study. No patients were asked to advise on interpretation or writing up of results. There are no specific plans to disseminate the results of the research to study participants, but the UK Biobank disseminates key findings from projects on its website.

Tables

Table 1. Degree of X chromosome loss in samples tested by conventional cytogenetics, compared to SNP arrays.

Sample	Cytogenetic estimate	Illumina array estimate	Affymetrix array estimate
1	0% 45,X	0% 45,X	0% 45,X
2	11% 45,X*	2% 45,X	13% 45,X
3	24% 45,X	29% 45,X	30% 45,X
4	26% 45,X	25% 45,X	23% 45,X
5	50% 45,X	48% 45,X	67% 45,X+
6	100% 45,X	100% 45,X	100% 45,X

* Cytogenetics report stated that this could be due to age-related loss of the X, this was the only control sample from a patient possibly old enough to demonstrate age-related loss (34 years old) and was referred in her mid-thirties for infertility. Both SNP arrays estimated a low level of mosaicism consistent with the cytogenetic estimate, but the LRRs and BAFs were not sufficiently outside the normal range for a sample such as this to be included in our case series. Sample 2 therefore either represents a sample outside the level of detection for this method, or that this individual has age-related loss of the X [12].

+ While the LRR was consistent with the cytogenetic estimate of % mosaicism, the BAF plot was inconsistent across the X chromosome in the Affymetrix data and would have been excluded from our case series, as we selected only individuals with evidence of whole X chromosome imbalance (**Supplemental Fig. 5k**).

Table 2. Characteristics of UK Biobank females stratified by X chromosome aneuploidy status. '45,X (all)' are individuals with any detectable X chromosome loss, so includes mosaic 45,X/46,XX and full 45,X. '45,X (> 80%)' are individuals assumed to be full 45,X. '45,X (<80%)' are individuals assumed to be mosaic 45,X/46,XX. '46,XX' are controls, and '47,XXX' are trisomy X individuals. 16 and 25 full 45,X cases did not report menarche or menopause ages respectively. (N=number of individuals included in analysis, SD = standard deviation)

Trait	Status	N	Mean	SD	Min. Value	Max. Value
Age at Recruitment (Years)	45,X (all)	216	59.02	8.13	42	70
	45,X (> 80%)	30	54.84	7.77	42	69
	45,X (<80%)	186	59.69	8.00	42	70
	46,XX	244,522	57.09	7.94	40	71
	47,XXX	110	56.83	7.06	41	69
Height (cm)	45,X (all)	216	157.03	8.65	134.5	182
	45,X (> 80%)	30	145.47	5.72	134.5	159
	45,X (<80%)	186	158.89	7.52	139	182
	46,XX	244,037	162.66	6.23	121	199
	47,XXX	110	167.98	5.52	156	181
Menarche Age (Years)	45,X (all)	191	13.31	1.79	9	19
	45,X (> 80%)	14	15.36	2.59	12	19
	45,X (<80%)	177	13.15	1.61	9	18
	46,XX	237,744	12.96	1.61	5	25
	47,XXX	104	12.73	1.96	7	17
Natural Menopause Age (Years)	45,X (all)	100	50.32	5.49	25	59
	45,X (> 80%)	5	45.40	6.43	37	51
	45,X (<80%)	95	50.58	5.35	25	59
	46,XX	108,479	50.02	4.53	18	65
	47,XXX	60	44.90	5.10	32	56
Number of Pregnancies	45,X (all)	215	1.95	1.78	0	10
	45,X (> 80%)	29	0.24	0.64	0	2
	45,X (<80%)	186	2.22	1.75	0	10
	46,XX	240,046	2.30	1.58	0	31
	47,XXX	107	1.93	1.77	0	10
Fluid Intelligence Score (0-13)	45,X (all)	90	5.97	2.27	2	12
	45,X (> 80%)	11	5.82	1.94	3	9
	45,X (<80%)	79	5.99	2.32	2	12
	46,XX	118,776	5.80	2.04	0	13
	47,XXX	51	4.39	1.72	1	8

Household Income Category (1-5)	45,X (all)	177	2.28	1.17	1	5
	45,X (> 80%)	21	2.24	1.18	1	5
	45,X (<80%)	156	2.28	1.17	1	5
	46,XX	203,115	2.53	1.18	1	5
	47,XXX	78	1.50	0.75	1	4
Townsend Deprivation Index	45,X (all)	216	-1.26	2.84	-6.11	6.71
	45,X (> 80%)	30	-1.32	2.54	-6.11	5.70
	45,X (<80%)	186	-1.26	2.89	-5.39	6.71
	46,XX	244,238	-1.51	2.93	-6.26	11.00
	47,XXX	110	-0.54	3.38	-5.95	6.81
Birthweight (kg)	45,X (all)	103	3.32	0.40	2.55	4.28
	45,X (> 80%)	14	3.07	0.28	2.72	3.40
	45,X (<80%)	89	3.36	0.40	2.55	4.28
	46,XX	130,961	3.35	0.42	2.5	4.5
	47,XXX	56	3.27	0.45	2.55	4.37
Body Mass Index(kg/m^2)	45,X (all)	215	26.76	4.88	18.36	46.25
	45,X (> 80%)	30	27.19	4.30	18.36	35.94
	45,X (<80%)	185	26.70	4.97	18.64	46.25
	46,XX	243,502	27.02	5.14	12.12	74.68
	47,XXX	110	29.18	5.48	17.86	46.48

Table 3. Association between X chromosome ploidy and nine phenotypes. The effect of the aneuploidy on each trait is given in SD units compared to 46,XX controls and an association p value (N=number of cases included in analysis, SD = standard deviation, se = standard error). *P* values that pass the threshold for statistical confidence, corrected for multiple testing, are highlighted (i.e. Equivalent to *P*<0.05).

Trait	Status	N	Effect (SD)	se	Low 95% CI (SD)	High 95% CI (SD)	*P*
Height (cm)	45,X (all)	216	-0.84	0.07	-0.97	-0.71	**3.1×10^{-37}**
	45,X (> 80%)	30	-2.73	0.18	-3.07	-2.38	**1.5×10^{-53}**
	45,X (<80%)	186	-0.54	0.07	-0.68	-0.40	**4.0×10^{-14}**
	47,XXX	110	0.84	0.09	0.66	1.03	**5.8×10^{-20}**
Menarche Age (Years)	45,X (all)	191	0.21	0.07	0.06	0.35	4.5×10^{-3}
	45,X (> 80%)	14	1.33	0.27	0.81	1.86	**5.9×10^{-7}**
	45,X (<80%)	177	0.12	0.08	-0.03	0.26	0.12
	47,XXX	104	-0.15	0.10	-0.34	0.05	0.14
Natural Menopause Age (Years)	45,X (all)	100	0.07	0.10	-0.13	0.26	0.51
	45,X (> 80%)	5	-0.99	0.44	-1.86	-0.13	0.024
	45,X (<80%)	95	0.12	0.10	-0.08	0.32	0.23
	47,XXX	60	-0.98	0.13	-1.23	-0.73	**1.2×10^{-14}**
Number of Pregnancies	45,X (all)	215	-0.31	0.07	-0.44	-0.18	**4.8×10^{-06}**
	45,X (> 80%)	29	-1.50	0.18	-1.86	-1.14	**3.3×10^{-16}**
	45,X (<80%)	186	-0.12	0.07	-0.27	0.02	0.09
	47,XXX	107	-0.26	0.10	-0.44	-0.07	0.0076
Fluid Intelligence Score (0-13)	45,X (all)	90	0.06	0.10	-0.14	0.26	0.56
	45,X (> 80%)	11	-0.17	0.29	-0.74	0.39	0.55
	45,X (<80%)	79	0.09	0.11	-0.12	0.30	0.39
	47,XXX	51	-0.74	0.13	-1.00	-0.48	**3.7×10^{-08}**
Household Income Category (1-5)	45,X (all)	177	-0.30	0.14	-0.57	-0.03	0.032
	45,X (> 80%)	21	-0.89	0.39	-1.66	-0.12	0.024
	45,X (<80%)	156	-0.21	0.15	-0.51	0.08	0.15
	47,XXX	78	-1.98	0.24	-2.45	-1.51	**8.7×10^{-17}**
Townsend Deprivation Index	45,X (all)	216	0.13	0.06	0.01	0.26	0.036
	45,X (> 80%)	30	0.13	0.17	-0.20	0.47	0.43
	45,X (<80%)	186	0.13	0.07	0.00	0.27	0.052
	47,XXX	110	0.24	0.09	0.07	0.42	0.0067
Birthweight (kg)	45,X (all)	103	-0.07	0.10	-0.27	0.12	0.45
	45,X (> 80%)	13	-0.67	0.27	-1.19	-0.14	0.013
	45,X (<80%)	89	0.02	0.11	-0.19	0.23	0.86

	47,XXX	56	-0.21	0.13	-0.47	0.05	0.11
Body Mass Index (kgm^2)	45,X (all)	215	-0.06	0.07	-0.21	0.08	0.39
	45,X (> 80%)	30	0.13	0.20	-0.26	0.51	0.51
	45,X (<80%)	185	-0.09	0.08	-0.25	0.06	0.23
	47,XXX	110	0.46	0.10	0.26	0.66	**6.3×10^{-06}**

Table 4. Arterial stiffness, blood pressure, deafness, bone mineral density, hypothyroidism and hypertension in women with X chromosome aneuploidy compared to controls with 46,XX karyotype. The effect of the aneuploidy on each trait is given in SD units compared to 46,XX controls and an association p value (N = number of individuals included in analysis, SD = standard deviation, se = standard error).

Phenotype	Status	N*	Mean	SD	Min. Value	Max. Value	Effect in SD (se)+	*P*
Pulse wave	45,X (all)	67	8.72	3.57	3.1	18.8	-0.14 (0.12)	0.26
Arterial	45,X (>80%)	10	6.31	2.55	3.1	10.7	-1.02 (0.32)	0.001
Stiffness index	45,X (<80%)	57	9.14	3.57	4.8	18.8	0.02 (0.13)	0.9
	46,XX	78,897	8.81	3.93	1	530		
Diastolic blood	45,X (all)	216	84.67	14.14	50.5	126	0.04 (0.06)	0.56
pressure	45,X (>80%)	30	89.10	14.83	69	117	0.52 (0.17)	0.002
(mm/Hg)	45,X (<80%)	186	83.95	13.93	50.5	126	-0.04 (0.07)	0.55
	46,XX	243,408	84.08	13.23	44.5	147		
Systolic Blood	45,X (all)	216	144.56	26.31	86	232	0.03 (0.06)	0.6
Pressure	45,X (>80%)	30	150.45	25.65	109	203	0.50 (0.16)	0.003
(mm/Hg)	45,X (<80%)	186	143.61	26.36	86	232	-0.04 (0.07)	0.52
	46,XX	243,858	140.59	24.28	72	252.5		
Hypertension	45,X (all)	96/119					OR = 1.20	0.22
	45,X (>80%)	10/20					OR = 3.15	0.005
	45,X (<80%)	86/99					OR = 1.02	0.88
	46,XX	128,474/ 114,539						
Left ear Speech	45,X (all)	63	-6.20	2.07	-9	-0.5	0.16 (0.12)	0.19
Reception	45,X (>80%)	9	-4.78	2.67	-9	-0.5	0.84 (0.31)	0.0075
Threshold (SNR)	45,X (<80%)	54	-6.44	1.88	-9	-1	0.04 (0.13)	0.75
	46,XX	76,887	-6.71	1.90	-11.25	8		
Right ear Speech	45,X (all)	64	-5.77	2.55	-9.5	5.5	0.32 (0.12)	0.0068
Reception	45,X (>80%)	9	-4.83	1.80	-7	-2	0.92 (0.31)	0.0034
Threshold (SNR)	45,X (<80%)	55	-5.92	2.63	-9.5	5.5	0.22 (0.13)	0.083
	46,XX	76,816	-6.67	1.92	-11.5	8		
Bone Mineral	45,X (all)	213	0.49	0.12	0.23	0.97	-0.18 (0.06)	0.0062
Density (g/cm2)	45,X (>80%)	30	0.45	0.12	0.23	0.70	-0.63 (0.17)	2.3×10^{-4}
	45,X (<80%)	183	0.50	0.12	0.27	0.97	-0.10 (0.07)	0.14
	46,XX	240,063	0.52	0.12	0.00	1.82		
Hypothyroidism	45,X (all)	186/30					OR = 1.7	0.003
	45,X (>80%)	20/10					OR = 6.61	1.4×10^{-6}

	45,X (<80%)	166/20	OR = 1.31	0.26
	46,XX	225,349/ 19,173		

*N shows the number of individuals within the group except for Hypothyroidism and Hypertension which give N-controls/N-cases.

+Effects given in in SD (se) unless otherwise stated as odds ratio (OR) for logistic regression

Figures

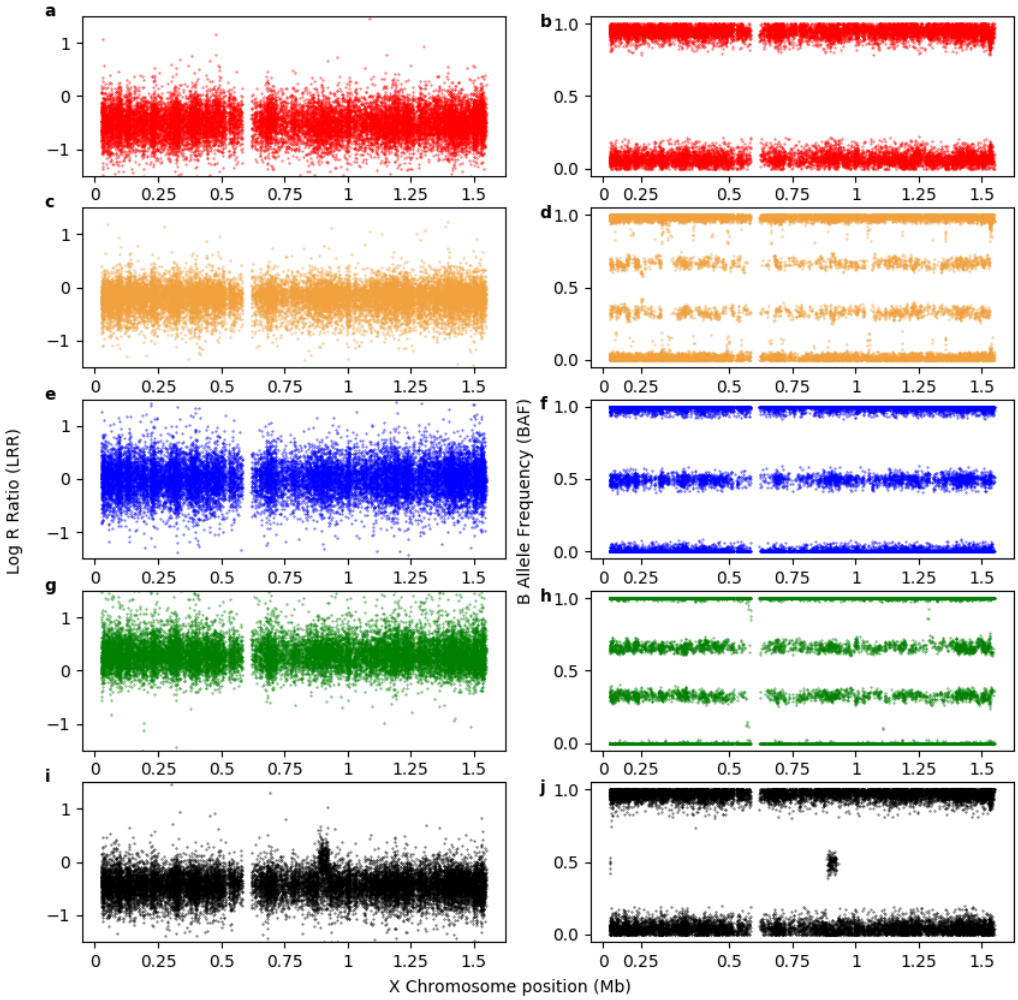

Figure 1. Exemplar Log R Ratio (LRR) and B Allele Frequency (BAF) plots for each detected X chromosome state. (a) and (b) show the respective LRR and BAF for a > 80% dosage 45,X individual, (c) and (d) represent the LRR and BAF for a mosaic (< 80%) dosage 45,X/46,XX individual respectively, (e) and (f) represent the respective LRR and BAF of a 46,XX individual and (g) and (h) represent the respective LRR and BAF of a 47,XXX individual. Finally for comparison, plots (i) and (j) represent the respective LRR and BAF of a 46,XY male illustrating a similar LRR dosage to 45,X - note the 2-copy 4.5Mb dosage region at 88.5Mb on chromosome X which is homologous to the Y chromosome present in all males (PAR3).

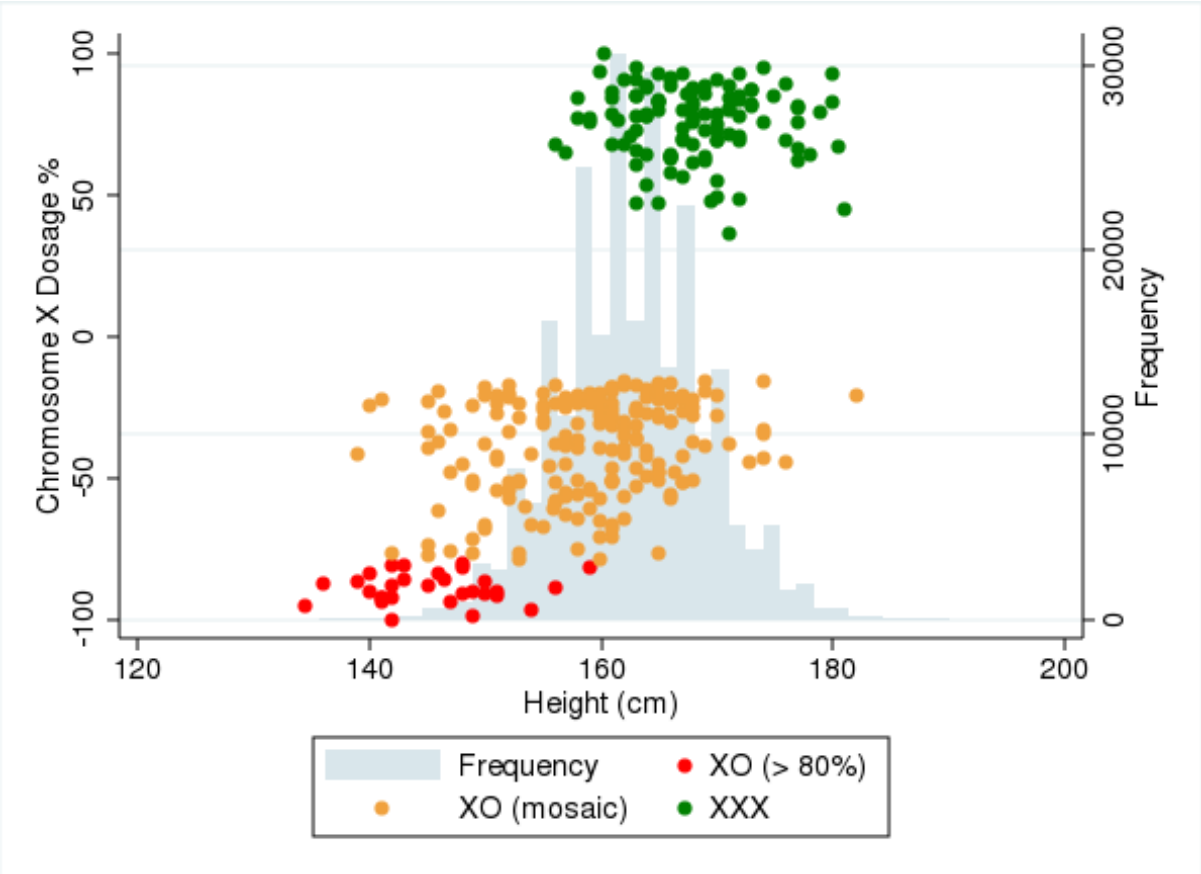

Figure 2a. Distribution of adult height in UK Biobank women indicating the mean Log R Ratio in 45,X and 47,XXX females. X chromosome dosage is colour coded (see key) for each category tested in the analysis. Distribution of height in controls is shown in grey histogram.

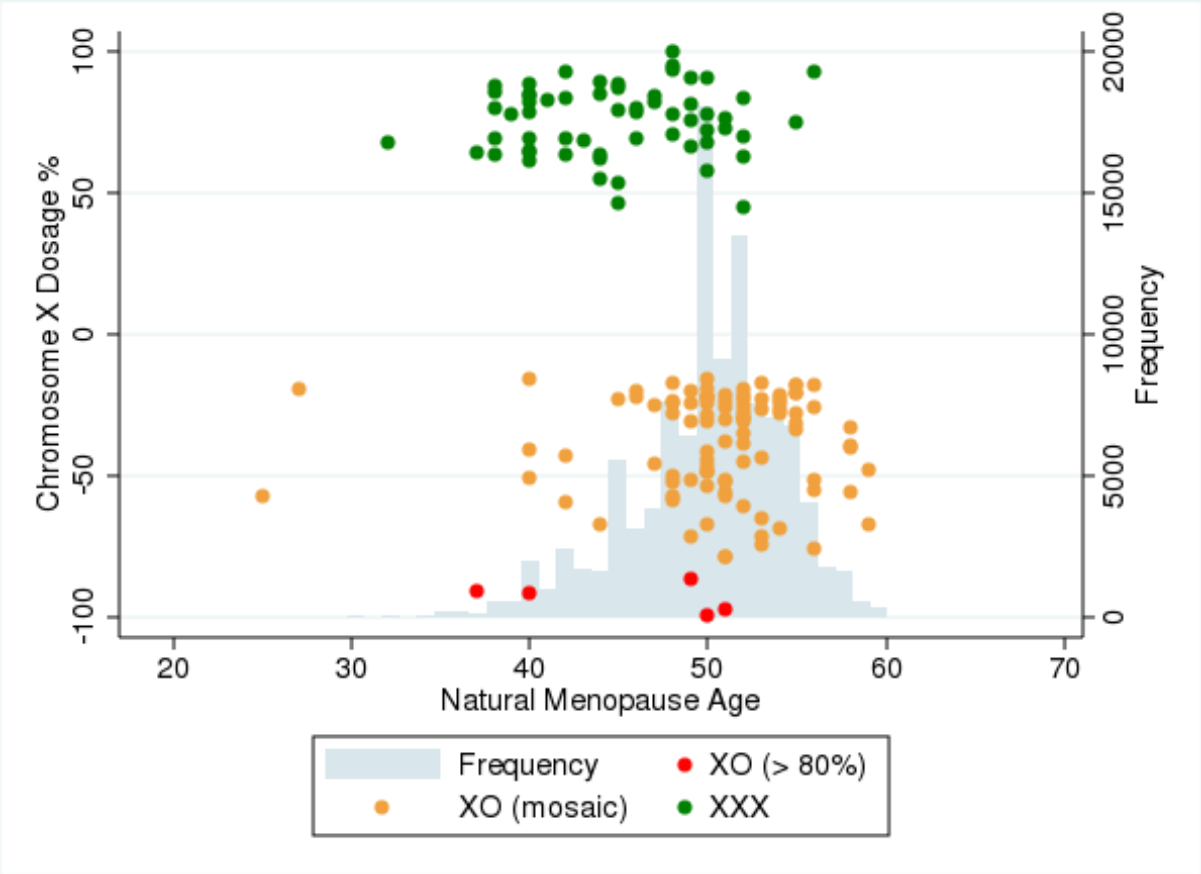

Figure 2b. Distribution of natural menopause age in UK Biobank indicating the mean Log R Ratio in 45,X and 47,XXX females. X chromosome dosage is colour coded (see key) for each category tested in the analysis. Distribution of menopause age in controls is shown in grey histogram.

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