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Identification of intellectual disability genes in female patients with a skewed X inactivation pattern

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Abstract

Intellectual disability (ID) is a heterogeneous disorder with an unknown molecular etiology in many cases. Previously, X-linked ID (XLID) studies focused on males due to the hemizygous state of their X chromosome. Carrier females are generally unaffected due to the presence of a second normal allele, or inactivation of the mutant X chromosome in most of their cells (skewing). However, in female ID patients, we hypothesized that the presence of skewing of X-inactivation would be an indicator for an X chromosomal ID cause. We analysed the X-inactivation patterns of 288 females with ID, and found that 22 (7.6%) had extreme skewing (>90%), which is significantly higher than observed in the general population (3.6%; p=0.029). Whole exome sequencing of 19 females with extreme skewing revealed causal variants in 6 females in the XLID genes *DDX3X, NHS, WDR45, MECP2* and *SMC1A*. Interestingly, variants in genes escaping X-inactivation presumably cause both XLID and skewing of X-inactivation in 3 of these patients. Moreover, variants likely accounting for skewing only, were detected in *MED12, HDAC8* and

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Conflict of interest

The authors declare no conflict of interest.

TAF9B. All tested candidate causative variants were *de novo* events. Hence, extreme skewing is a good indicator for the presence of X-linked variants in female patients.

Keywords

Escape genes; intellectual disability; skewing of X-inactivation; exome sequencing

INTRODUCTION

Intellectual disability (ID) is a very clinically and genetically heterogeneous disorder with a prevalence of 2–3% in the general population of developed countries [Leonard and Wen 2002; Ropers and Hamel 2005]. ID can be the sole major clinical feature observed (non-syndromic ID) but can also be associated with other clinical features (syndromic ID). In about 40% of these cases the etiology remains unknown. Since 30% more males than females present with ID [Herbst and Miller 1980] and because there is an overrepresentation of the expression of X chromosomal genes in the central nervous system in comparison to autosomal genes [Nguyen and Disteche 2006], the search for ID associated genes initially focused on the X chromosome (X-linked ID, XLID) [de Brouwer et al., 2007]. To date, over 100 XLID genes have already been identified [Piton et al., 2013]. Traditionally, XLID studies focused on males since X-linked mutations in males are always expressed because of the hemizygous state of the X chromosome. In females two X chromosomes are present and X-inactivation of one of them takes place in every cell. Females can be carrier of an X-linked mutation but are generally not or mildly affected due to compensation by their second normal allele or because of inactivation of the mutant X chromosome in most of their cells (skewing). Therefore, in contrast to this study, mutations in X-linked genes are generally not searched for in female ID patients unless structural defects (e.g. X-autosome translocations) on the X chromosome are detected.

The process of X-inactivation entails the random transcriptional silencing of one of the two X chromosomes present in female somatic cells and takes place during the blastocyst stage of early development [Barakat et al., 2011; Belmont 1996]. Once X-inactivation is initiated, the chosen X chromosome remains inactive for the rest of that cell's life and the X-inactivation pattern is passed on to its daughter cells. Various epigenetic mechanisms are involved in the inactivation process, including DNA methylation, *XIST* RNA-mediated gene silencing and chromatin modification [Barakat et al., 2010]. However, not all genes on the inactivated X chromosome are completely silenced. About 15% of human genes 'escape' from X-inactivation and the proportion of genes that escape can vary between different regions of the X chromosome. An additional 10% of X-linked genes show variable patterns of inactivation and are expressed to different extents from inactive X chromosomes [Carrel and Willard 2005]. Some of the genes that escape have a Y-linked paralog and therefore these genes behave as autosomal genes [Disteche 1997]. In about 14% of females, skewing of X-inactivation occurs [Amos-Landgraf et al., 2006]. This refers to the situation where one X chromosome becomes preferentially inactivated in >80% of all somatic cells [Nesterova et al., 2003]. The current cut off for biologically significant and potentially clinically relevant skewing is often set at >90%, but to avoid confusion, this threshold is referred to as

'extreme' skewing of X-inactivation in this study. In the general population, the X-inactivation ratio is subjected to a Gaussian distribution and extreme skewing was reported to be present in 3.6% of females [Amos-Landgraf et al., 2006]. However, X-inactivation is measured in blood cells and might thus not always reflect the situation in other somatic cells and tissues.

Skewing of X-inactivation can occur by chance, but can also be caused by primary or secondary stochastic or genetic processes [Morey and Avner 2011]. In primary skewing, the inactive X chromosome is chosen before silencing is initiated [Morey and Avner 2011]. An example of primary skewing is a potential harmful mutation in *XIST* (MIM# 314670) [Nesterova et al., 2003], which precludes the cell from silencing that X chromosome carrying the mutation. However, mutations in *XIST* are very rare. The most common cause of secondary skewing is post-inactivation cell selection, due to an X chromosome mutation that affects cell proliferation [Morey and Avner 2011]. Therefore, skewed X-inactivation may point to a carrier status of an X-linked mutation.

In this study, we investigated skewing of X-inactivation as a screening method to detect (novel) XLID mutations in female patients. In females with skewing the same parentally-inherited X chromosome will be active in almost all of their (blood) cells. Therefore, if a detrimental mutation is present on the active X chromosome it will be fully expressed. This means that the masking effect established by random X-inactivation would no longer be present and we expect the female patient to manifest the full blown phenotype. This is similar to the situation in males who have no choice but to express their single X chromosome. In this report, we investigated the exomes of 19 female patients with ID and >90% skewing using whole exome sequencing (WES). We detected variants in the ID genes *DDX3X* (MIM# 300160), *NHS* (MIM# 300457), *SMC1A* (MIM# 300040), *WDR45* (MIM# 300526), *MECP2* (MIM# 300005), *MED12* (MIM# 300188), *HDAC8* (MIM# 300269), *TAF9B* (MIM# 300754), *EP300* (MIM# 602700) and *SYNGAP1* (MIM# 603384) that could be responsible for the patients ID phenotypes, for skewing or for both.

MATERIALS AND METHODS

Samples

Genomic DNA samples from 223 female patients with sporadic and syndromic ID were obtained from the Center for Human Genetics (Leuven), and 93 samples were received from genetic centers outside Belgium. Only female patients with extreme skewed X-inactivation ratios (>90%) were selected for further analysis (N=22). We focused on sporadic individuals because families with multiple affected females more likely have an autosomal cause of their ID, and we selected patients with more severe syndromic phenotypes due to the higher chance of finding genetic defects when compared to more subtly affected patients with isolated ID [Ropers 2010]. Finally, due to an increase in the degree of skewing with age [Busque et al., 1996], only females of 35 years or younger were included. The screening protocols were approved by the appropriate Institutional Review Board of the respective University Hospitals and informed consent was obtained from the parents of the affected patients.

Genomic DNA from patients, their parents, if available, and control females was isolated from peripheral blood according to standard procedures and stored at 4°C. Primer sequences are provided in Supp. Table S1.

X-inactivation

Lymphocyte-derived genomic DNA was subjected to the standard androgen receptor (MIM# 313700) assay to determine X-inactivation ratios [Allen et al., 1992]. In non-informative cases with homozygous *AR* alleles, the more recently developed *PGK1* (MIM# 311800) [van Kamp et al., 1991] and *PCSK1N* (MIM# 300399) [Bertelsen et al., 2011] X-inactivation assays were used.

XIST sequencing and X array-CGH

PCR followed by Sanger sequencing was performed on the highest conserved region of the 14 kb *XIST* gene, which contains the minimal promoter region and the beginning of exon 1, covering a total region of 3.2 kb in size (ChrX: 73,069,528-73,072,728; UCSC Hg19). Five overlapping PCR products were generated and Sanger sequenced. Only patients with >95% X chromosome inactivation (XCI) were analysed because mutations in *XIST* are expected to lead to complete skewing of X-inactivation. Oligo-based X chromosome array-CGH analysis was performed as described in [Fieremans et al., 2015].

Whole exome sequencing (WES)

For 7 samples exome sequencing was done at the Genomics Core Facility, KU Leuven, Leuven, Belgium. Exome capture was performed with the SeqCap EZ Human Exome Library v3.0 (NimbleGen). These samples were subsequently sequenced in a paired end 101 bp run on a HiSeq 2000] instrument (Illumina, San Diego, CA). Another 12 patients were whole exome sequenced at Baylor College of Medicine as published [Lupski et al., 2013; Yang et al., 2014]. Data analysis was done using an in-house pipeline of the Genomics Core Facility, KU Leuven. For patients 1, 2 and 8, reads were mapped with BWA version 0.6.2 and BWA aln options –q15 [Li and Durbin 2010], then merged, de-duplicated, realigned and recalibrated with GATK [DePristo et al., 2011]. Variants were called with Unified Genotyper (GATK version 2.4.9). For patients 3 and 7, BWA version 0.7.5a and BWA mem options –q15 were used together with GATK version 3.1.1. Haplotype Caller was used for variant calling. For the remaining patients 4, 5, 6, 9 and 10, BWA version 0.7.8 and BWA mem options –q15 were used together with GATK version 3.2.2. Again, Haplotype Caller was used for variant calling. All variants were annotated using Annovar version 11-02 2013 (http://www.openbioinformatics.org/annovar/ [Wang et al., 2010]). All non-exonic, non-splice site and synonymous variants, as well as segmental duplications, were excluded. Additionally, variants with a frequency ≤0.05 and ≥0.95 in the 1000 genomes project (http://www.1000genomes.org) and in less than 1% of samples in the NGS-Logistics database (https://ngsl.esat.kuleuven.be/) [Ardeshirdavani et al., 2014] were kept. The NGS-Logistics Standard Min Confidence Threshold For Calling was set at 50, Standard Min Confidence Threshold For Emitting at 0 and dcov at 200. For autosomal variants only those in known ID genes [Gilissen et al., 2014] and with a frequency ≤0.01 or ≥0.99 in the 1000 genomes project were included. Variants that were predicted to be benign by the Clinvar database (http://www.ncbi.nlm.nih.gov/clinvar/) were excluded. Nonsynonymous variants were also

evaluated based on predicted pathogenicity using *in silico* analysis including SIFT [Kumar et al., 2009], Poly Phen-2 [Adzhubei et al., 2010], PhyloP [Pollard et al., 2010], GERP++ [Cooper et al., 2005], LRT [Chun and Fay 2009] and Mutation Taster [Schwarz et al., 2014]. Gene function and available genotype-phenotype correlations were used to further prioritize the variants (NCBI, OMIM, Pubmed). Variant confirmation was done by PCR and Sanger sequencing in the patients and their parents if DNA was available. X-linked variants that were paternally inherited were excluded from further analysis. Autosomal variants that were inherited from either parent were also excluded if no other potentially damaging variants were detected in that gene. Escape of X-inactivation was evaluated based on the study of Carrel and Willard [Carrel and Willard 2005]. Variants reported in this study have been submitted to dbVar (http://www.ncbi.nlm.nih.gov/dbvar) with entry SUB1311146.

Expression analysis

Total RNA was extracted from white blood cells or low-passage Epstein Barr virus-transformed peripheral blood lymphocytes (EBV-PBLs). Expression analysis was performed on cDNA by PCR and Sanger sequencing as described previously [Froyen et al., 2012]. For X-linked variants, the expression level, or lack of expression could serve as an indication of whether the variant was located on the predominantly active or inactive X chromosome, respectively.

RESULTS

X-inactivation analysis

In total 316 female patients with sporadic and syndromic ID were subjected to the *AR* X-inactivation assay. In 9 out of 37 females who were homozygous at the *AR* locus, the *PGK1* and/or *PCSK1N* assays were informative. From the 288 informative female patients, 22 (7.6%) had extreme skewing. The X-inactivation results are summarized in Table 1, Supp. Figure S1. Compared to a set of 415 control females reported by Amos-Landgraf and colleagues [Amos-Landgraf et al., 2006], there is a significantly higher prevalence of extreme skewing in the female ID patient cohort (p-value = 0.029). The control group used by Amos-Landgraf and colleagues was subjected to the *AR* assay and consisted mostly of women of child-bearing age and a few younger adults older than 13 years [Amos-Landgraf et al., 2006]. This is similar in constitution and age distribution to our cohort of female patients under 35 years of age.

Mutation detection by exome sequencing

From the 22 female patients with extreme skewing, 19, with a sufficient amount of DNA available were subjected to WES. In 10/19 patients a total of 11 likely pathogenic variants were detected, 9 on the X chromosome and 2 on autosomes. All variants were confirmed by Sanger sequencing (Supp. Table S2), were absent in the NGS-Logistics databank, and were predicted to be detrimental by online prediction programs (Table 2). Moreover, all of these variants, besides the indel in *TAF9B*, were located in known ID genes. If the same variant had been reported previously in dbSNP (http://www.ncbi.nlm.nih.gov/SNP/) or in the literature, this observation is depicted in the ‘Remarks’ column of Table 2. The XCI pattern of each patient and the major clinical features are also included. In case parental DNA was

available, the mode of inheritance, XCI pattern of the mother and whether the predominantly active or inactive X of the mother (based on the *AR/PGK1* XCI assays) was inherited by the daughter, was also determined (Table 2; Supp. Table S2). Information on whether these variants are located in genes that escape X-inactivation is provided and, where available, patient cDNA was used to determine if the variant was presumably located on the predominantly active or inactive X chromosome (Table 2; Supp. Table S2). The most important clinical features are reported in Table 2. Additional relevant clinical information as well as the detected variants is presented below. Family or individual history is only mentioned if relevant. Oligo-based X array results were normal for all 10 patients.

Patient 1 had severe ID, with ataxic gait. Biometry with weight and head circumference is between p3 and p10 and her height is p25–50. She had brachycephaly, prominent eyes, protruding ears, a wide mouth and a cleft uvula. She previously had surgery for vesicoureteral reflux. WES detected a nonsynonymous (NM_001193417:p.G286S) variant in the escape gene *DDX3X* that was absent in her mother. DNA from the father was not available but it is rather unlikely that he would be a carrier as males with *DDX3X* variants are reported to present with ID Snijders Blok et al., 2015.

Patient 2 had mild to moderate ID and hypotonia. During gestation, intrauterine growth restriction was noted. At birth she weighed 2385 g, her head circumference was 31 cm and her length was 46 cm. Autonomous walking was acquired at 18 months and speech at 4 years. Besides dysmorphic facial features (Supp. Figure S2) other anomalies include short stature, failure to thrive (height centile 10, weight < 3rd centile), slender fingers, hirsutism in face and trunk, low posterior hairline, pes cavus with hallux valgus and sub-clinical hypothyroidism. MRI, EEG and mutation screening of *NIPBL* (MIM# 608667) were negative. WES revealed a *de novo* stop variant (NM_001193417:p.G177X) in *DDX3X*. Only the reference G allele was present at cDNA level (Supp. Table S2).

Patient 3 had a mild Cornelia de Lange syndrome (MIM# 122470) phenotype (Supp. Figure S3). Her clinical features are listed in Supp. Table S3. In patient 3, a *de novo* recurrent *SMC1A* variant (NM_006306:p.I784T) was detected. *SMC1A* partially escapes X-inactivation and at cDNA level both alleles were present although the variant allele was present at a lower level than the wild-type allele (Supp. Table S2).

Patient 4 was clinically examined at the age of 29 years. She had severe ID. Her weight was 34 kg and she had short stature with a height of 145 cm. She also presented with pronounced hyperkyphosis with the lower segment at 78 cm. Her head circumference was 52.4 cm. Additional features include low implanted ears and small hands and feet with proximally implanted thumbs. She steps with assistance and showed progressive spasticity of the lower limbs with clonus and hypotrofy. A CT scan of her brain at age 31 revealed increased density of the pedunculi. WES revealed a *de novo* frameshift variant (NM_001029896:p.T260Lfs*27) in *WDR45*, a gene that does not escape X-inactivation. At cDNA level the mutant allele was almost exclusively used (Supp. Table S2).

Patient 5 had mild ID, autism spectrum disorder, and microphthalmia. She had dental surgery for correction of supernumerary enclosed elements (19/29/49) at the age of 10

weeks, which was complicated by a brief cardiac arrest. When seen at age 36 years, she showed a phenotype consistent with a diagnosis of Nance-Horan syndrome, which was not diagnosed clinically. Her teeth were long and partly tulip shaped. Additional features included prominent low-set ears, a flat midface, a long philtrum, and a broad lower part of her thorax. WES detected a *de novo* stop variant (NM_001136024:p.Q55X) in *NHS* that partially escapes X-inactivation.

Patient 6 was first seen at 53 years. She could walk at a younger age but there was no relevant further information on her individual history. Patient 6 harbors a recurrent stop variant (NM_004992:p.R294X) in *MECP2*, reported 216 times in RettBASE and known as a pathogenic variant in dbSNP (rs61751362).

Patient 7 was diagnosed with autism spectrum disorder and severe ID. She had infantile scoliosis. Developmental delay was first noticed around the age of 6 to 9 months. Her first steps were at 22 months and her first words at three years. She presented with clinodactyly of the fifth fingers, short fifth toes, small dorsally rotated ears, sparse lateral eyebrows, deep set eyes and short upper limbs. WES revealed a nonsynonymous *de novo* variant (NM_005120:p.R521H) in *MED12*. At cDNA level only the reference G-allele was present.

Patient 8 (Supp. Figure S4) had synophrys, micrognathia, growth delay, postnatal low weight and height (<p3), gastroesophageal reflux, hypotonia, seizures (hypertonic crisis), moderate ID with language delay and autistic traits. The patient does not have features consistent with a phenotype reported for patients with *HDAC8* mutations that result in Cornelia De Lange syndrome (MIM# 300269). MRI and EEG results were normal. Patient 8 carries a nonsynonymous *de novo* variant (NM_018486:p.G320R) in *HDAC8*, located at Xq13.1. At cDNA level only the reference G-allele was present (Supp. Table S2). Interestingly, this exact variant was reported previously in a severely affected male patient [Deardorff et al., 2012].

Patient 9 presented with mild developmental delay. At the age of 16 months she weighed 8.6 kg (P3), had a body length of 72 cm (<P3) and her head circumference was 44 cm (<P3). She had trigonocephaly and an asymmetrical face. She had a large naevus flammeus in the neck, the roof of her nose and upper lip, which disappeared gradually. She has a broad nasal bridge with hypertelorism, an antimongoloid eye slant, narrow eye slits, and a long hypoplastic philtrum. Her ears were dysplastic and there was a crease in her left earlobe. She had syndactyly of her feet (IIe and IIIe radius). At the age of 4 years and 5 months she weighed 16 kg (P25) and had a head circumference of 47.5 (<P3). She is a calm and cheerful child and can only speak a few words and sentences. Her skin was dry and prone to eczema. Discrete lines of Blashko were visible on her upper left arm. Her hands were small and she has interdigital webbing. Karyotyping and mutation analysis of *CREBBP* were normal. NMR of the brain and 1 Mb molecular karyotyping were normal as well. This patient harbors an in-frame deletion of 12 nucleotides in *EP300* (NM_001429:p.2189_2193del) located on chromosome 22. Parental DNA was unavailable.

Patient 10 was diagnosed with nonsyndromic mild to moderate ID and psychiatric problems (psychosis). WES revealed a nonsynonymous variant (NM_006772:p.S1165L) in

SYNGAP1 on chromosome 6 as well as an indel (NG_012570:g.7766_7770delinsAA) in *TAF9B*, located at Xq21.1. Parental DNA was unavailable.

XIST sequencing

For 6 patients with ≥95% skewing and for whom sufficient DNA was available, we also performed *XIST* sequence analysis. No variants that likely could lead to severe skewing were detected (data not shown).

DISCUSSION

We hypothesized that extreme skewing of X-inactivation (>90%) is an indicator for the underlying genetic defect to be located on the X chromosome. The observation of a two-fold increased prevalence of extreme skewing in our female ID patient population compared to the control female cohort suggests that in approximately half of our female patients who demonstrated extreme skewing, we can expect an X-linked cause for ID that is related to skewing. Our hypothesis is supported by the identification of 11 X-linked variants in 19 female patients of which at least 6 were causal variants present in known XLID genes. These variants were divided into different categories based on whether they are likely to cause ID, skewing of X-inactivation, or both (Table 2). A detailed discussion of the results and the relevant literature can be found in the Supp. Discussion.

Variants that likely cause XLID and skewing

DDX3X escapes X-inactivation and therefore, the variants observed (p.G286S and p.G177X in patients 1 and 2, respectively) can cause ID irrespective of being located on the active or inactive X chromosome. Mutations in *DDX3X* are predicted to be responsible for 1–3% of unexplained ID in female patients, probably through haploinsufficiency Snijders Blok et al., 2015. Interestingly, Snijders Blok and colleagues also reported that 7/15 females with *DDX3X* mutations and ID had almost complete skewing of X-inactivation (>95%), which is much higher than would be expected simply by chance. In patient 3, a *de novo SMC1A* variant p.I784T) was detected. *SMC1A* is reported to partially escape X-inactivation and two other females with ID were reported with the same variant [Gervasini et al., 2013; Limongelli et al., 2010]. In both, the variant allele expression was roughly 50% lower than that of the wild-type allele. Importantly, our cDNA expression data in patient 3 suggested a similar trend (Supp. Table S2). Besides their similar clinical characteristics, all three females had extreme skewing against the mutant X chromosome in blood cells.

In patient 4, a *de novo* frameshift variant (p.T260Lfs*27) was detected in *WDR45*. Mutations in this gene result in beta-propeller protein-associated neurodegeneration (MIM# 300894) [Haack et al., 2012]. At the cDNA level the mutant allele was almost exclusively expressed in our female patient. Interestingly, in lymphoblastoid cell lines derived from *WDR45* mutation carrying female patients described in the literature, 4/5 subjects also exclusively expressed the mutant transcript [Saitsu et al., 2013]. Furthermore, 7/17 female patients had XCI patterns above 90:10 in peripheral blood. This *WDR45* variant could therefore be an example of 1 “hit” in a non-escape gene causing secondary skewing, although the possible involvement of *WDR45* mutations in skewing remains to be further

elucidated. An overview of the likely pathogenic variants detected in WDR45 is listed in Supp. Table S4.

In these four patients (1 to 4) the cause for ID appears to be directly related to skewing and therefore only one "hit" was sufficient to cause both ID and skewing. Interestingly, 3/4 suggested "one-hit" variants were located in escape genes (*DDX3X* and *SMC1A*) thus suggesting that detrimental variants in escape genes are not only an important cause of X-linked ID in female patients, but can result in skewing as well.

Variants that likely cause XLID

In patients 5 and 6 we detected variants that likely caused XLID but did not affect the X-inactivation pattern. These two patients harbor variants in *NHS* and *MECP2*, respectively. In both cases we speculate that these variants are located on their active X chromosomes because their clinical features are similar to those observed in male patients with variants in these genes, but more severe than in other reported carrier female patients [Li et al., 2015; Lundvall et al., 2006].

Variants that likely cause skewing

Patients 7 and 8 harbor variants in *MED12* and *HDAC8*, respectively. In both patients the variants are located on their preferentially inactivated X-chromosomes, which is in line with reported data where female patients tend to skew against mutations in these genes [Kaiser et al., 2014; Risheg et al., 2007]. Escape of X-inactivation was not observed for either variant. We therefore speculate that in both patients, these variants caused preferential X-inactivation in favor of the normal X chromosome. It is of note that the pattern of skewing observed in peripheral blood cells may not be representative for other tissues. Therefore, the XCI pattern in the brain could be random and lead to a phenotype and although unlikely we cannot unequivocally exclude a role for these variants in their ID phenotypes. An overview of the variants detected in MED12 and HDAC8 together with information on XCI are provided in Supp. Table S5 and Supp. Table S6, respectively.

Autosomal causes of ID

Additionally, we also found variants in autosomal ID genes in two females with skewing, demonstrating that skewing can occur independently from ID as well. However, an influence of autosomal variants on the X-inactivation process cannot be excluded. Patient 9 presents with a Rubinstein–Taybi syndrome (RSTS)-like phenotype and harbors an in-frame deletion of 12 nucleotides in *EP300*. Since mutations in *EP300* are responsible for roughly 8% of patients with RSTS (MIM# 613684) with a milder phenotype [Rusconi et al., 2015], we believe this novel *EP300* variant on chromosome 22 is responsible for her ID as well as other clinical features. Patient 10 had a nonsynonymous variant (p.S1165L) in *SYNGAP1* on chromosome 6. Mutations in *SYNGAP1* are known to cause autosomal dominant nonsyndromic ID (MIM# 612621), which is similar to the phenotype of patient 10. Other causal variants identified in SYNGAP1 are summarized in Supp. Table S7. In the same patient, we also detected an indel over the exonintron boundary in *TAF9B*, located on Xq21.1. *TAF9B* is a core promoter factor and regulates neuronal gene expression [Herrera et al., 2014]. To our knowledge, this is the first report of a *TAF9B* variant in a patient and we

speculate that the *TAF9B* variant may be responsible for skewing, although a modifier effect on the phenotype cannot be excluded.

Potential mechanisms of the genetic causes of ID in female patients with skewing

There are several mechanisms that could lead to XLID in female patients with skewing. These potential mechanisms are summarized in Table 3.The first possibility is that only one X-linked gene is affected. In this one-hit model, there are three possibilities: First, the mutation causing the phenotype also influences the inactivation process of that X-chromosome. For example, escape of X-inactivation is often partial and incomplete with a lower expression from the allele located on the inactive X chromosome. Cells with the mutation on their inactive X chromosomes could therefore have a growth advantage in comparison to cells with the mutation on their active X chromosomes [Miyake et al., 2013]. In our cohort, potential variants that fall into this category were detected in the escape genes *DDX3X* and *SMC1A* and in the non-escape gene *WDR45*. Another mechanism where one hit can be responsible for ID as well as skewing is illustrated by X:autosome translocations with the breakpoint in an XLID gene. Here, the selection is against the normal X chromosome in 95% of cases because if the derivative X chromosome were to be inactivated, then genes in the translocated autosomal region would also be inactivated. Hence, the female can present with the full blown phenotype that is also seen in males carrying a mutation in that gene [Frints et al., 2008; Hagens et al., 2006]. Second, cells with the mutation are only viable in case of extreme (but not complete) secondary skewing against this mutation with the prime example of incontinentia pigmenti (MIM# 308300) [Woffendin et al., 1999]. Third, a mutation causing ID can be accompanied by skewing that had occurred by chance.

The second possibility is that mutations are present in two genes (two-hit model). The effects of these two mutations can be independent of each other, for example a mutation in an autosomal gene causes ID and a mutation in an X-linked gene causes skewing. However, one mutation might have an influence on the expression of the other such that one mutation causes skewing and forces a second X-linked mutation, responsible for ID, to be expressed. Most likely the two mutations are located on different X chromosomes although it is also possible that they both reside on the same X chromosome as inactivating mutations in *XIST* can render that X chromosome incapable of being inactivated [Penny et al., 1996]. Another aspect to take into account is that if the second ID-causing mutation is in an escape gene, then it can also cause XLID irrespective of whether it is located on the active X or not [Miyake et al., 2013]. Recently, female patients with mutations in X-linked genes and presenting with predominant X-inactivation of the apparent normal X chromosome have been reported for multiple disorders. These include *MECP2* duplication [Fieremans et al., 2014], Wiskott-Aldrich syndrome (MIM# 614493) [Boonyawat et al., 2013; Daza-Cajigal et al., 2013], Duchenne muscular dystrophy (MIM# 310200) [Juan-Mateu et al., 2012], Fabry disease (MIM# 301500) [Bouwman et al., 2011] and mucopolysaccharidosis type II (MIM# 309900) [Kloska et al., 2011; Pina-Aguilar et al., 2013]. These findings strongly suggest that this ‘female X-linked two-hit model’ can result in several X-linked conditions. In patients 5 to 10, we expect the variant responsible for ID to be different from the variant causing skewing. We therefore expect two different mutations to be required to cause both ID and

skewing in these patients. However, we did not identify two different potential harmful X-linked variants in any of these patients. This inability could be due to the limitations of exome sequencing if e.g. the variant is located in distant regulatory elements, or these apparently missed variants may not have been predicted to be detrimental based on current detection pipelines. Finally, unknown mechanisms might obscure our current way of thinking.

In this study we identified an X-linked cause for ID in 6/19 female patients with extreme X-inactivation. A yield of roughly 30% is very high compared to previous large scale exome sequencing studies on thousands of samples in which the contribution of disease-causing variants on the X chromosome ranged from 2.5–17% [DDD 2015; de Ligt et al., 2012; Gilissen et al., 2014; Grozeva et al., 2015; Hamdan et al., 2014; Rauch et al., 2012; Redin et al., 2014; Tzschach et al., 2015; Vissers et al., 2010; Yang et al., 2014]. If autosomal causes of ID are included we detected a disease-causing variant in 8/19 patients. An overall diagnostic yield of approximately 40% is well in line with previous data where diagnostic yields of up to 42% were also obtained through whole exome and whole genome sequencing analyses [DDD 2015; de Ligt et al., 2012; Gilissen et al., 2014; Grozeva et al., 2015; Hamdan et al., 2014; Redin et al., 2014]. On the other hand, Hu and colleagues [Hu et al., 2015] predicted that the X-exome sequencing of XLID families may resolve up to 58% of Fragile X-negative males, which is almost double the amount of resolved cases in our female cohort. However, considering the small number of samples in our cohort, and due to the notable differences in study design, inclusion criteria and the fact that studies were performed on patient cohorts consisting mainly of males, these numbers cannot be directly compared. Also, considering that X-linked forms of ID are thought to account for 5–10% of ID in all male patients [Lubs et al., 2012], a yield of 30% causal X-linked variants in female patients with skewing shows that skewing is in fact a good indicator of an underlying X-linked defect. Although we did not investigate the frequency of mutations in XLID genes in an unselected cohort of female patients with syndromic ID, we believe that the rate of establishing an etiological molecular diagnosis will be substantially lower than in our female cohort.

De novo events appear to be an important cause of ID in our cohort considering that all of the variants where parental DNA was available have occurred *de novo*. These results are similar to WES studies on mixed gender samples where 39–55% of severe ID cases, not including CNV's, were shown to be caused by *de novo* events [Gilissen et al., 2014; Rauch et al., 2012]. This high incidence of *de novo* variants is not surprising as our cohort consisted solely of sporadic patients with ID. *De novo* events are an important cause for skewing as well and we did not detect any inherited variants responsible for either primary or secondary skewing. Interestingly, in the two mothers with XCI patterns >80%, the cause for skewing in their daughters was *de novo*.

Our data strongly suggests that severe skewing of X-inactivation in female ID patients is a good indicator for a causal variant in an X-linked gene. Moreover, unfavorable X-inactivation patterns can contribute to the severity of the disease phenotype and therefore, XCI analysis in female patients can help to unravel the modifying effect of XCI in females with X-linked disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. A method and server for predicting damaging missense mutations. Nat Methods. 2010; 7:248–249. [PubMed: 20354512]

Allen RC, Zoghbi HY, Moseley AB, Rosenblatt HM, Belmont JW. Methylation of HpaII and HhaI sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. Am J Hum Genet. 1992; 51:1229–1239. [PubMed: 1281384]

Amos-Landgraf JM, Cottle A, Plenge RM, Friez M, Schwartz CE, Longshore J, Willard HF. X chromosome-inactivation patterns of 1,005 phenotypically unaffected females. Am J Hum Genet. 2006; 79:493–499. [PubMed: 16909387]

Ardeshirdavani A, Souche E, Dehaspe L, Van Houdt J, Vermeesch JR, Moreau Y. NGS-Logistics: federated analysis of NGS sequence variants across multiple locations. Genome Med. 2014; 6:71. [PubMed: 25328540]

Barakat TS, Gunhanlar N, Pardo CG, Achame EM, Ghazvini M, Boers R, Kenter A, Rentmeester E, Grootegoed JA, Gribnau J. RNF12 activates Xist and is essential for X chromosome inactivation. PLoS Genet. 2011; 7:e1002001. [PubMed: 21298085]

Barakat TS, Jonkers I, Monkhorst K, Gribnau J. X-changing information on X inactivation. Exp Cell Res. 2010; 316:679–687. [PubMed: 20083102]

Belmont JW. Genetic control of X inactivation and processes leading to X-inactivation skewing. Am J Hum Genet. 1996; 58:1101–1108. [PubMed: 8651285]

Bertelsen B, Tumer Z, Ravn K. Three new loci for determining x chromosome inactivation patterns. J Mol Diagn. 2011; 13:537–540. [PubMed: 21726665]

Boonyawat B, Dhanraj S, Al AF, Zlateska B, Grunenbaum E, Roifman CM, Steele L, Meyn S, Blanchette V, Scherer SW, Swierczek S, Prchal J, et al. Combined de-novo mutation and non-random X-chromosome inactivation causing Wiskott-Aldrich syndrome in a female with thrombocytopenia. J Clin Immunol. 2013; 33:1150–1155. [PubMed: 23943155]

Bouwman MG, Rombach SM, Linthorst GE, Poorthuis BJ, Deprez RH, Aerts JM, Wijburg FA. Early cerebral manifestations in a young female with Fabry disease with skewed X-inactivation. Clin Genet. 2011; 80:500–502. [PubMed: 22243051]

Busque L, Mio R, Mattioli J, Brais E, Blais N, Lalonde Y, Maragh M, Gilliland DG. Nonrandom X-inactivation patterns in normal females: lyonization ratios vary with age. Blood. 1996; 88:59–65. [PubMed: 8704202]

Carrel L, Willard HF. X-inactivation profile reveals extensive variability in X-linked gene expression in females. Nature. 2005; 434:400–404. [PubMed: 15772666]

Chun S, Fay JC. Identification of deleterious mutations within three human genomes. Genome Res. 2009; 19:1553–1561. [PubMed: 19602639]

Cooper GM, Stone EA, Asimenos G, Green ED, Batzoglou S, Sidow A. Distribution and intensity of constraint in mammalian genomic sequence. Genome Res. 2005; 15:901–913. [PubMed: 15965027]

Daza-Cajigal V, Martinez-Pomar N, Garcia-Alonso A, Heine-Suner D, Torres S, Vega AK, Molina IJ, Matamoros N. X-linked thrombocytopenia in a female with a complex familial pattern of X-chromosome inactivation. Blood Cells Mol Dis. 2013; 51:125–129. [PubMed: 23689198]

DDD. Large-scale discovery of novel genetic causes of developmental disorders. Nature. 2015; 519:223–228. [PubMed: 25533962]

Deardorff MA, Bando M, Nakato R, Watrin E, Itoh T, Minamino M, Saitoh K, Komata M, Katou Y, Clark D, Cole KE, De Baere E, et al. HDAC8 mutations in Cornelia de Lange syndrome affect the cohesin acetylation cycle. Nature. 2012; 489:313–317. [PubMed: 22885700]

de Brouwer AP, Yntema HG, Kleefstra T, Lugtenberg D, Oudakker AR, de Vries BB, van Bokhoven H, Van Esch H, Frints SG, Froyen G, Fryns JP, Raynaud M, et al. Mutation frequencies of X-linked mental retardation genes in families from the EuroMRX consortium. Hum Mutat. 2007; 28:207–208. [PubMed: 17221867]

de Ligt J, Willemsen MH, van Bon BW, Kleefstra T, Yntema HG, Kroes T, Vulto-van Silfhout AT, Koolen DA, de Vries P, Gilissen C, del Rosario M, Hoischen A, et al. Diagnostic exome sequencing in persons with severe intellectual disability. N Engl J Med. 2012; 367:1921–1929. [PubMed: 23033978]

DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, Philippakis AA, del Angel G, Rivas MA, Hanna M, McKenna A, Fennell TJ, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet. 2011; 43:491–498. [PubMed: 21478889]

Disteche CM. The great escape. Am J Hum Genet. 1997; 60:1312–1315. [PubMed: 9199551]

Fieremans N, Bauters M, Belet S, Verbeeck J, Jansen AC, Seneca S, Roelens F, De Baere E, Marynen P, Froyen G. De novo MECP2 duplications in two females with intellectual disability and unfavorable complete skewed X-inactivation. Hum Genet. 2014; 133:1359–1367. [PubMed: 25037250]

Fieremans N, Van Esch H, de Ravel T, Van Driessche J, Belet S, Bauters M, Froyen G. Microdeletion of the escape genes KDM5C and IQSEC2 in a girl with severe intellectual disability and autistic features. Eur J Med Genet. 2015; 58:324–327. [PubMed: 25858702]

Frints SG, Lenzner S, Bauters M, Jensen LR, Van Esch H, des Portes V, Moog U, Macville MV, van Roozendaal K, Schrander-Stumpel CT, Tzschach A, Marynen P, et al. MCT8 mutation analysis and identification of the first female with Allan-Herndon-Dudley syndrome due to loss of MCT8 expression. Eur J Hum Genet. 2008; 16:1029–1037. [PubMed: 18398436]

Froyen G, Belet S, Martinez F, Santos-Reboucas CB, Declercq M, Verbeeck J, Donckers L, Berland S, Mayo S, Rosello M, Pimentel MM, Fintelman-Rodrigues N, et al. Copy-number gains of HUWE1 due to replication- and recombination-based rearrangements. Am J Hum Genet. 2012; 91:252–264. [PubMed: 22840365]

Gervasini C, Russo S, Cereda A, Parenti I, Masciadri M, Azzollini J, Melis D, Aravena T, Doray B, Ferrarini A, Garavelli L, Selicorni A, et al. Cornelia de Lange individuals with new and recurrent SMC1A mutations enhance delineation of mutation repertoire and phenotypic spectrum. Am J Med Genet A. 2013; 161A:2909–2919. [PubMed: 24124034]

Gilissen C, Hehir-Kwa JY, Thung DT, van de Vorst M, van Bon BW, Willemsen MH, Kwint M, Janssen IM, Hoischen A, Schenck A, Leach R, Klein R, et al. Genome sequencing identifies major causes of severe intellectual disability. Nature. 2014; 511:344–347. [PubMed: 24896178]

Grozeva D, Carss K, Spasic-Boskovic O, Tejada MI, Gecz J, Shaw M, Corbett M, Haan E, Thompson E, Friend K, Hussain Z, Hackett A, et al. Targeted Next-Generation Sequencing Analysis of 1,000 Individuals with Intellectual Disability. Hum Mutat. 2015; 36:1197–1204. [PubMed: 26350204]

Haack TB, Hogarth P, Kruer MC, Gregory A, Wieland T, Schwarzmayr T, Graf E, Sanford L, Meyer E, Kara E, Cuno SM, Harik SI, et al. Exome sequencing reveals de novo WDR45 mutations causing a phenotypically distinct, X-linked dominant form of NBIA. Am J Hum Genet. 2012; 91:1144–1149. [PubMed: 23176820]

Hagens O, Dubos A, Abidi F, Barbi G, Van Zutven L, Hoeltzenbein M, Tommerup N, Moraine C, Fryns JP, Chelly J, van Bokhoven H, Gecz J, et al. Disruptions of the novel KIAA1202 gene are associated with X-linked mental retardation. Hum Genet. 2006; 118:578–590. [PubMed: 16249884]

Hamdan FF, Srour M, Capo-Chichi JM, Daoud H, Nassif C, Patry L, Massicotte C, Ambalavanan A, Spiegelman D, Diallo O, Henrion E, Dionne-Laporte A, et al. De novo mutations in moderate or severe intellectual disability. PLoS Genet. 2014; 10:e1004772. [PubMed: 25356899]

Herbst DS, Miller JR. Nonspecific X-linked mental retardation II: the frequency in British Columbia. Am J Med Genet. 1980; 7:461–469. [PubMed: 7211956]

Herrera FJ, Yamaguchi T, Roelink H, Tjian R. Core promoter factor TAF9B regulates neuronal gene expression. Elife. 2014; 3:e02559. [PubMed: 25006164]

Hu H, Haas SA, Chelly J, Van Esch H, Raynaud M, de Brouwer AP, Weinert S, Froyen G, Frints SG, Laumonnier F, Zemojtel T, Love MI, et al. X-exome sequencing of 405 unresolved families identifies seven novel intellectual disability genes. Mol Psychiatry. 2015; 21:133–148. [PubMed: 25644381]

Juan-Mateu J, Rodriguez MJ, Nascimento A, Jimenez-Mallebrera C, Gonzalez-Quereda L, Rivas E, Paradas C, Madruga M, Sanchez-Ayaso P, Jou C, Gonzalez-Mera L, Munell F, et al. Prognostic value of X-chromosome inactivation in symptomatic female carriers of dystrophinopathy. Orphanet J Rare Dis. 2012; 7:82. [PubMed: 23092449]

Kaiser FJ, Ansari M, Braunholz D, Concepcion Gil-Rodriguez M, Decroos C, Wilde JJ, Fincher CT, Kaur M, Bando M, Amor DJ, Atwal PS, Bahlo M, et al. Loss-of-function HDAC8 mutations cause a phenotypic spectrum of Cornelia de Lange syndrome-like features, ocular hypertelorism, large fontanelle and X-linked inheritance. Hum Mol Genet. 2014; 23:2888–2900. [PubMed: 24403048]

Kloska A, Jakobkiewicz-Banecka J, Tylki-Szymanska A, Czartoryska B, Wegrzyn G. Female Hunter syndrome caused by a single mutation and familial XCI skewing: implications for other X-linked disorders. Clin Genet. 2011; 80:459–465. [PubMed: 21062272]

Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc. 2009; 4:1073–1081. [PubMed: 19561590]

Leonard H, Wen X. The epidemiology of mental retardation: challenges and opportunities in the new millennium. Ment Retard Dev Disabil Res Rev. 2002; 8:117–134. [PubMed: 12216056]

Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics. 2010; 26:589–595. [PubMed: 20080505]

Li A, Li B, Wu L, Yang L, Chen N, Ma Z. Identification of a novel NHS mutation in a Chinese family with Nance-Horan syndrome. Curr Eye Res. 2015; 40:434–438. [PubMed: 25266737]

Limongelli G, Russo S, Digilio MC, Masciadri M, Pacileo G, Fratta F, Martone F, Maddaloni V, D'Alessandro R, Calabro P, Russo MG, Calabro R, et al. Hypertrophic cardiomyopathy in a girl with Cornelia de Lange syndrome due to mutation in SMC1A. Am J Med Genet A. 2010; 152A: 2127–2129. [PubMed: 20635401]

Lubs HA, Stevenson RE, Schwartz CE. Fragile X and X-linked intellectual disability: four decades of discovery. Am J Hum Genet. 2012; 90:579–590. [PubMed: 22482801]

Lundvall M1, Samuelsson L, Kyllerman M. Male Rett phenotypes in T158M and R294X MeCP2-mutations. Neuropediatrics. 2006; 37:296–301. [PubMed: 17236109]

Lupski JR, Gonzaga-Jauregui C, Yang Y, Bainbridge MN, Jhangiani S, Buhay CJ, Kovar CL, Wang M, Hawes AC, Reid JG, Eng C, Muzny DM, et al. Exome sequencing resolves apparent incidental findings and reveals further complexity of SH3TC2 variant alleles causing Charcot-Marie-Tooth neuropathy. Genome Med. 2013; 5:57. [PubMed: 23806086]

Miyake N, Koshimizu E, Okamoto N, Mizuno S, Ogata T, Nagai T, Kosho T, Ohashi H, Kato M, Sasaki G, Mabe H, Watanabe Y, et al. MLL2 and KDM6A mutations in patients with Kabuki syndrome. Am J Med Genet A. 2013; 161A:2234–2243. [PubMed: 23913813]

Morey C, Avner P. The demoiselle of X-inactivation: 50 years old and as trendy and mesmerising as ever. PLoS Genet. 2011; 7:e1002212. [PubMed: 21811421]

Nesterova TB, Johnston CM, Appanah R, Newall AE, Godwin J, Alexiou M, Brockdorff N. Skewing X chromosome choice by modulating sense transcription across the Xist locus. Genes Dev. 2003; 17:2177–2190. [PubMed: 12952890]

Nguyen DK, Disteche CM. Dosage compensation of the active X chromosome in mammals. Nat Genet. 2006; 38:47–53. [PubMed: 16341221]

Penny GD, Kay GF, Sheardown SA, Rastan S, Brockdorff N. Requirement for Xist in X chromosome inactivation. Nature. 1996; 379:131–137. [PubMed: 8538762]

Pina-Aguilar RE, Zaragoza-Arevalo GR, Rau I, Gal A, Alcantara-Ortigoza MA, Lopez-Martinez MS, Santillan-Hernandez Y. Mucopolysaccharidosis type II in a female carrying a heterozygous stop mutation of the iduronate-2-sulfatase gene and showing a skewed X chromosome inactivation. Eur J Med Genet. 2013; 56:159–162. [PubMed: 23232253]

Piton A, Redin C, Mandel JL. XLID-causing mutations and associated genes challenged in light of data from large-scale human exome sequencing. Am J Hum Genet. 2013; 93:368–383. [PubMed: 23871722]

Pollard KS, Hubisz MJ, Rosenbloom KR, Siepel A. Detection of nonneutral substitution rates on mammalian phylogenies. Genome Res. 2010; 20:110–121. [PubMed: 19858363]

Rauch A, Wieczorek D, Graf E, Wieland T, Endele S, Schwarzmayr T, Albrecht B, Bartholdi D, Beygo J, Di Donato N, Dufke A, Cremer K, et al. Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study. Lancet. 2012; 380:1674–1682. [PubMed: 23020937]

Redin C, Gerard B, Lauer J, Herenger Y, Muller J, Quartier A, Masurel-Paulet A, Willems M, Lesca G, El-Chehadeh S, Le Gras S, Vicaire S, et al. Efficient strategy for the molecular diagnosis of intellectual disability using targeted high-throughput sequencing. J Med Genet. 2014; 51:724–736. [PubMed: 25167861]

Risheg H, Graham JM Jr, Clark RD, Rogers RC, Opitz JM, Moeschler JB, Peiffer AP, May M, Joseph SM, Jones JR, Stevenson RE, Schwartz CE, et al. A recurrent mutation in MED12 leading to R961W causes Opitz-Kaveggia syndrome. Nat Genet. 2007; 39:451–453. [PubMed: 17334363]

Ropers HH. Genetics of early onset cognitive impairment. Annu Rev Genomics Hum Genet. 2010; 11:161–187. [PubMed: 20822471]

Ropers HH, Hamel BC. X-linked mental retardation. Nat Rev Genet. 2005; 6:46–57. [PubMed: 15630421]

Rusconi D, Negri G, Colapietro P, Picinelli C, Milani D, Spena S, Magnani C, Silengo MC, Sorasio L, Curtisova V, Cavaliere ML, Prontera P, et al. Characterization of 14 novel deletions underlying Rubinstein-Taybi syndrome: an update of the CREBBP deletion repertoire. Hum Genet. 2015; 134:613–626. [PubMed: 25805166]

Saitsu H, Nishimura T, Muramatsu K, Kodera H, Kumada S, Sugai K, Kasai-Yoshida E, Sawaura N, Nishida H, Hoshino A, Ryujin F, Yoshioka S, et al. De novo mutations in the autophagy gene WDR45 cause static encephalopathy of childhood with neurodegeneration in adulthood. Nat Genet. 2013; 45:445–449. 449e1. [PubMed: 23435086]

Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods. 2014; 11:361–362. [PubMed: 24681721]

Snijders Blok L, Madsen E, Juusola J, Gilissen C, Baralle D, Reijnders MR, Venselaar H, Helsmoortel C, Cho MT, Hoischen A, Vissers LE, Koemans TS, et al. Mutations in DDX3X Are a Common Cause of Unexplained Intellectual Disability with Gender-Specific Effects on Wnt Signaling. Am J Hum Genet. 2015; 97:343–352. [PubMed: 26235985]

Tzschach A, Grasshoff U, Beck-Woedl S, Dufke C, Bauer C, Kehrer M, Evers C, Moog U, Oehl-Jaschkowitz B, Di Donato N, Maiwald R, Jung C, et al. Next-generation sequencing in X-linked intellectual disability. Eur J Hum Genet. 2015; 23:1513–1518. [PubMed: 25649377]

van Kamp H, Landegent JE, Jansen RP, Willemze R, Fibbe WE. Clonal hematopoiesis in patients with acquired aplastic anemia. Blood. 1991; 78:3209–3214. [PubMed: 1683796]

Vissers LE, de Ligt J, Gilissen C, Janssen I, Steehouwer M, de Vries P, van Lier B, Arts P, Wieskamp N, del Rosario M, van Bon BW, Hoischen A, et al. A de novo paradigm for mental retardation. Nat Genet. 2010; 42:1109–1112. [PubMed: 21076407]

Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 2010; 38:e164. [PubMed: 20601685]

Woffendin H, Jakins T, Jouet M, Stewart H, Landy S, Haan E, Harris A, Donnai D, Read A, Kenwrick S. X-inactivation and marker studies in three families with incontinentia pigmenti: implications for counselling and gene localisation. Clin Genet. 1999; 55:55–60. [PubMed: 10066033]

Yang Y, Muzny DM, Xia F, Niu Z, Person R, Ding Y, Ward P, Braxton A, Wang M, Buhay C, Veeraraghavan N, Hawes A, et al. Molecular findings among patients referred for clinical whole-exome sequencing. JAMA. 2014; 312:1870–1879. [PubMed: 25326635]

Table 1

Number of females with preferential X-inactivation ≥80% and ≥90% skewing in the general population (Amos-Landgraf et al., 2006) compared to our patient population

	Controls (n=415)		ID females (n=288)	
	#	%	#	%
Preferential X-inactivation ≥80%	59	14.2	52	18.06
Preferential X-inactivation ≥90% (skewing)	15	3.60	22	7.6

n= total number of females; # = number of females in group

Table 2

Summary of results for the 11 candidate variants detected by WES in 19 female ID patients with extreme skewing

		Patient number	XCI pattern	Gene	Location	Type of variant	Variant	Inheritance	NGS-Logistics	XCI pattern mother	PhyloP SIFT PolyPhen2 LRT MutationTaster	On Xa or Xi?	Escape? Carrel et al 2005)	Major clinical features	Remarks
Candidate causes XLID	and skewing	1	99 mat	***DDX3X***	X:41203531	ns	NM_001193417.2: c.856G>A:p.G286S	likely *de novo*	0/1115	78	C / D D /	?	9/9	Severe ID, ataxic gait, cleft uvula, facial dysmorphism, vesicoureteric reflux	
		2	92 pat	***DDX3X***	X:41202502	stop	NM_001193417.2: c.529G>T:p.G177X	*de novo*	0/734	52	C / / D /	?	9/9	Mild to moderate ID, pre- and postnatal growth restriction, hirsutism, hypertelorism, facial features, hallux valgus with foot arches	
		3	93 pat	***SMC1A***	X:53430567	ns	NM_006306.3: c.2351T>C:p.I784T	*de novo*	0/870	76	C D D D D	Xi	7/9	Severe psychomotor delay, intrauterine and postnatal growth restriction, synophrys, hirsutism, dysmorphism, microcephaly, hypertonia, congenital heart anomalies, gastroesophageal reflux, hearing loss, hallux valgus, talus valgus, dysplastic nails	2 female ID patients with skewing and same variant (Gervasini et al. 2013; Limongelli et al. 2010)
		4	93 mat	***WDR45***	X:48933072	fr del	NM_001029896.1: c.777delT:p.T260Lfs*27	*de novo*	0/?	81	/ / / / /	Xa	0/9	Severe ID, progressive spasticity, short stature (145 cm at 29 years)	
		5	95 mat	***NHS***	X:17705990	stop	NM_001136024.3: c.163C>T:p.Q55X	*de novo*	0/836	72	C / / D A	?	3/9	Mild ID, ASD, congenital cataract microphthalmia, abnormal teeth	
		6	94 ?	***MECP2***	X:153296399	stop	NM_004992.3: c.880C>T:p.R294X		0/801		C / / D /	?	0/9	Severe ID, spastic quadriplegia, microcephaly (OFC – 3.3)	rs61751362
Candidate causes skewing		7	98 mat	***MED12***	X:70343021	ns	NM_005120.2: c.1562G>A:p.R521H	*de novo*	0/811	79	C D D D D	Xi	0/9	Severe ID, ASD	
		8	93 mat	***HDAC8***	X:71681901	ns	NM_018486.2: c.958G>A:p.G320R	*de novo*	0/711	87	C D P D D	Xi	NR	Moderate ID, postnatal growth restriction, hypotonia, seizures, autistic traits	male patient with same variant (Deardorff et al. 2012)
Candidate causes autosomal ID		9	96 ?	*EP300*	22:41574281	in-frame del	NM_001429.3: c.6567_6578del:p.2189_2193del		0/?		/ / / / /	?	/	Mild developmental delay, hearing disability, relative microcephaly, dysmorphism	
Candidate causes skewing		10	93 ?	***SYNGAP1*** ***TAF9B***	6: 33412306 X:77392414	ns indel affecting splicing	NM_006772.2: c.3494C>T:p.S1165L NG_012570.1:g.7766_7770delinsAA		0/802 0/?	?	C T D D D / / / / /	?	/ 0/9	Nonsyndromic mild to moderate ID, schizophrenia	

X-linked genes are indicated in bold; ID: intellectual disability; XCI: X chromosome inactivation; mat/pat: maternally/paternally inherited predominantly inactivated X chromosome; XLID: X-linked ID; NR: not reported; ns: nonsynonymous; fr: frameshift; del: deletion; ins: insertion; stop: stop variant; C: conserved; /: not applicable; D: damaging/deleterious/disease causing; T: tolerated; P: possibly damaging; A: disease automatic; Xi: inactive X; Xa: active X; ASD: autism spectrum disorder; OFC: occipitofrontal head circumference

Table 3

Basic mechanisms of the genetic causes of XLID in female patients with skewing

	Skewing	XLID
One-hit model	1. Mutation in XLID gene that escapes XCI and causes skewing (on Xa/Xi)	
	2. Mutation in XLID gene with insufficient skewing to rescue complete phenotype	
	3. Chance	Mutation in XLID gene that does not affect XCI (on Xa)
		Mutation in XLID gene that escapes XCI and does not affect XCI (on Xa/Xi)
Two-hit model	Mutation causing skewing (Xi)	Mutation in XLID gene (on Xa)
	Mutation causing skewing (Xi)	Mutation in XLID gene that causes escape (on Xa/Xi)
	Mutation in XCI machinery (on Xa/Xi)	Mutation in XLID gene (on Xa)
		Mutation in XLID gene that escapes XCI (on Xa/Xi)

Mechanisms relying on secondary skewing are shaded in grey and mechanisms caused by primary skewing are not highlighted. Xa = active X chromosome; Xi = inactive X chromosome.