Review

The Impact of X-Chromosome Inactivation on Phenotypic Expression of X-Linked Neurodevelopmental Disorders

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Abstract: Nearly 20% of genes located on the X chromosome are associated with neurodevelopmental disorders (NDD) due to their expression and role in brain functioning. Given their location, several of these genes are either subject to or can escape X-chromosome inactivation (XCI). The degree to which genes are subject to XCI can influence the NDD phenotype between males and females. We provide a general review of X-linked NDD genes in the context of XCI and detailed discussion of the sex-based differences related to MECP2 and FMR1, two common X-linked causes of NDD that are subject to XCI. Understanding the effects of XCI on phenotypic expression of NDD genes may guide the development of stratification biomarkers in X-linked disorders.

Keywords: X-chromosome inactivation; MECP2; FMR1; Rett syndrome; fragile X syndrome; FXTAS; POI; neurodevelopmental disorders

1. Introduction

Several genes on the X chromosome are specifically expressed in the brain and are essential for neuronal plasticity and cognitive processes [1]. Of these, nearly 20% have been linked to neurodevelopmental disorders (NDD) and the dissimilar phenotype in males and females is due in part to differences in the pattern of gene expression [2]. The hemizygosity of most X-linked genes reveals recessive phenotypes in males, thus accounting for the disproportionately large number of affected males [3], while females with the same pathogenic variant are often unaffected or mildly affected. The sex differences found in X-linked NDDs are influenced by X-chromosome inactivation (XCI), a method of X-chromosome dosage compensation that ensures that X-linked genes are expressed at the same level in females as in males. Very early in female development, random inactivation of either the paternal or the maternal X chromosomes occurs in each cell, and the pattern of inactivation is transmitted to all daughter cells via mitosis. This results in the mosaic expression of X-linked genes in females, which can confer protection against disease. Generally, the ratio of the expression of maternal and paternal alleles is about 50:50 in females; however, deviation from the 50:50 ratio, known as skewed XCI, is also seen. Skewed XCI occurs when the inactivation of one X chromosome is favored over the other, and the ratio is commonly considered skewed if it is ≥65:35.

In this review we provide a general overview of X-linked NDD genes, their phenotype and association with XCI, and a focused discussion of the phenotypes associated with methyl-CpG binding protein 2 (MECP2) and fragile X mental retardation 1 (FMR1), two genes that are subject to XCI.
2. X-Linked NDD Genes

The majority of X-linked NDD genes are subject to XCI, resulting in phenotypic variability between males and females. In Table 1, we show a representative sample of genes that have a well-established association with an NDD phenotype as noted in OMIM [4] and discussed in the review by Migeon [5]. Several factors influence clinical presentation in females; these include whether the gene escapes XCI or is subject to skewing, the variant type, as well as the inheritance pattern. About 15% of the genes on the X chromosome escape inactivation and are expressed from both the active and inactive chromosomes [4]. The degree of the escape from XCI is reported to vary between genes, tissues, and individuals and likely contributes to phenotypic heterogeneity [6,7]. For genes that escape XCI, the NDD phenotype may be lethal in males and is generally more severe when compared with symptomatic females. This pattern is seen in X-linked disorders associated with the following genes: SMC1A, USP9X, LAMP2, IQSEC2, DCX, DDX3X, and OFD1 (Table 1).

Of the genes that are subject to XCI, skewing towards expression of the allele with the pathogenic variant or the normal allele may occur. When skewing is towards the variant allele, females and males show a similar affected phenotype, as seen in WDR45-related disorders. Conversely, when there is skewing towards expression of the normal allele, females are typically asymptomatic. An exception to this pattern is seen with the ABCD1 gene, where there can be a less severe phenotype in females, despite skewing towards expression of the variant allele [8].

| Gene | X-Linked Disorder | Inheritance | Male | Female | Gene Subject to X-Inactivation | References |
|------|------------------|-------------|------|--------|-------------------------------|------------|
| ABCD1 | Adrenoleukodystrophy/Adrenomyeloneuropathy | Recessive | Death first decade/progressive stiffness and weakness in the legs, development of cognitive and behavioral disturbance beginning in the 2nd decade | unaffected/late onset adrenomyeloneuropathy | Yes | PMID: 23469258 [9]; PMID: 22280810 [10] |
| AFF2 | Intellectual developmental disorder, X-linked | Recessive | Global developmental delay/ID/behavioral dx | mild or unaffected | N.D. | n/a |
| ALG13 | Developmental and epileptic encephalopathy 36 | Recessive | early-onset epileptic encephalopathy, severe intellectual disability | unaffected carrier females | Yes | PMID: 32337346 [11] |
| ARHGEF9 | Intellectual and epileptic encephalopathy 8 | Recessive | profound ID, epilepsy | intellectual disability, unaffected carriers | ND | n/a |
| ABHD1 | Alpha-thalassemia/ID | Dominant | Severe ID and dysmorphic features | mild ID | Yes | PMID: 16100724 [15] |
| ATX | Intellectual Disability, X-linked 93 | Recessive | Intellectual Disability, X-linked 93 | unaffected | Yes | PMID: 16955409 [16] |
| BMD1 | Intellectual Disability and microcephaly with pontine and cerebellar hypoplasia | Dominant | ID, microcephaly, pontine, cerebellar hypoplasia | unaffected carrier females, ASD | Yes | PMID: 28944139 [17] |

Table 1. Impact of XCI on NDD.
| Gene          | X-Linked Disorder                                                      | Inheritance | Male                      | Female                           | Gene Subject to X-Inactivation | References                  |
|--------------|-----------------------------------------------------------------------|-------------|---------------------------|----------------------------------|---------------------------------|------------------------------|
| CDKL5        | Early infantile epileptic encephalopathy early death                  | Dominant    | milder phenotype, epilepsy and profound ID | severe ID, early onset epilepsy, microcephaly, less severe mild learning disabilities | Yes                             | PMID: 24564546 [18]         |
| CLCN4        | Raynaud-Claes syndrome                                                | Dominant    | Severe ID and epilepsy    | epilepsy, microcephaly, developmental delay syndromic ID | learning disability generally unaffected or mild phenotype | Yes                          | PMID: 27550844 [19]         |
| CNKSR2       | Hough type                                                           | Recessive   | no affected males         | mild ID, seizure                  | N.D.                            | n/a                         |
| CUL4B        | Cullin Ring Cabezas type                                              | Recessive   | moderate ID               | unaffected                         | Yes                             | PMID: 3141730 [20]          |
| CXorf56      | CXorf56-Associated ID                                                  | Recessive   | ID                        | unaffected                         | Yes                             | PMID: 17273978 [21]         |
| DCX          | Lissencephaly                                                         | ND          | ID                        | epilepsy, brain malformation       | Yes                             | PMID: 31822863 [22]         |
| DOX3X        | Snijders Blok                                                         | Recessive   | mild ID                   | unaffected                         | Yes                             | PMID: 1283518 [23]          |
| DLM4         | Intellectual Disability, X-linked                                      | Recessive   | late onset tremor, ataxia, cognitive decline | FXATAS in 10% of premutation carriers POI in 25% of premutation carriers | Yes                             | PMID: 26609701 [28]         |
| DMD          | Duchenne, Muscular dystrophy                                           | Recessive   | mild ID                   | unaffected                         | Yes                             | PMID: 27098336 [26]         |
| DMD          | Intellectual developmental disorder, X-linked, syndromic, Armfield type | Recessive   | ID                        | unaffected                         | N.D.                            | n/a                         |
| DFG1         | Aarskog-Scott syndrome                                                | Not reported | ID                        | short stature                      | N.D.                            | n/a                         |
| DFG13        | Developmental and epileptic encephalopathy 90                         | Dominant/Recessive | epilepsy, developmental delay | epilepsy, developmental delay     | N.D.                            | n/a                         |
| FMR1         | Fragile X syndrome                                                    | Dominant    | ID                        | mild                              | Yes                             | PMID: 8825946 [27]          |
| FMR1         | Fragile X Tremor Ataxia                                               | Dominant    | late onset tremor, ataxia, cognitive decline | FXATAS in 10% of premutation carriers POI in 25% of premutation carriers | Yes                             | PMID: 26609701 [28]         |
| FMR1         | Premature Ovarian Failure                                             | n/a         | ID                        | unaffected                         | Yes                             | PMID: 30098699 [29]         |
| FRMDP4       | Intellectual Disability, X-linked 104                                 | Reccessive  | ID                        | unaffected                         | N.D.                            | n/a                         |
| GRIA3        | Intellectual Disability, X-linked 9/44                                 | Recressive  | ID                        | unaffected                         | N.D.                            | n/a                         |
| GPC3/GPC4    | Simpson-Golabi-Behmel                                                | Recessive   | ID                        | generally unaffected               | Yes                             | PMID: 30028822 [30]         |
| HCF1         | Methylmalonic acidemia                                                | Recessive   | ID                        | unaffected                         | Yes                             | PMID: 22889586 [31]         |
| HCPA9        | Cornelia de Lange, 5                                                  | Dominant    | ID                        | unaffected                         | N.D.                            | n/a                         |
| HPRT         | Lesch-Nyhan syndrome                                                  | Recessive   | ID, spastic cerebral palsy and SIB | not affected                      | Yes                             | PMID: 6585829 [32]          |
| HUWE1        | Intellectual Disability, X-linked                                      | Not reported | moderate - profound syndromic ID | Chiar malformation, ID, dysmorphism | N.D.                            | n/a                         |
| IGBP1        | Corpus callosum, agenesis of, with Intellectual Disability, ocular coloboma and microgathia | Recessive  | ID                        | unaffected                         | N.D.                            | n/a                         |
| IL1RAPL1     | Intellectual Disability, X-linked 2/34                                 | Recessive   | ID                        | unaffected                         | N.D.                            | n/a                         |
| IQSEC2       | Intellectual Disability, X-linked 1/78                                 | Dominant    | ID                        | unaffected                         | Yes                             | PMID: 19449417 [33]         |
| KDM5C/ JARIDC/ SMCX | Claes-Jensen                                 | Recessive   | ID, microcephaly          | non-syndromic ID                  | Yes                             | PMID: 32564198 [34]         |
| KDM6A/ (UTX) | Kabuki syndrome 2                                                      | Dominant    | symmetric ID              | similar to males                  | escapes X inactivation          | PMID: 9202598 [7]           |
| KLM1L15      | Intellectual Disability, X-linked                                      | Recessive   | ID, epilepsy, brain malformation | mild or unaffected               | Yes                             | PMID: 24817361 [35]         |
| L1CAM        | Hydrocephalus, X-linked aqueductal stenosis                           | Recessive   | ID, spastic paraplegia    | late onset                         | N.D.                            | n/a                         |
| LAMP2        | Danon disease                                                         | Dominant    | ID and myopathy           | not affected                       | escapes X inactivation          | PMID: 30871455 [24]         |
| MAOA         | Monoamine oxidase A def                                               | Recessive   | mild ID, behavioral difficulties | ID, epilepsy, microcephaly, gait and language disorder | Yes                             | PMID: 18361425 [38]         |
| MECP2        | Rett syndrome                                                         | Dominant    | early infantile epileptic encephalopathy/death first 2-4 yrs of life | early infantile epileptic encephalopathy/death first 2-4 yrs of life | Yes                             | PMID: 3142717 [39]          |
| Gene       | X-Linked Disorder                      | Inheritance | Male                                                                 | Female                                                                 | Gene Subject to X-Inactivation | References          |
|------------|----------------------------------------|-------------|----------------------------------------------------------------------|------------------------------------------------------------------------|------------------------------|---------------------|
| MECP2      | MECP2 Dup Syndrome                     | Recessive   | profound Intellectual Disability, infantile hypotonia, autistic features, seizures, progressive spasticity, and recurrent infections | mild neuropsychiatric features, such as anxiety.                       | Yes                           | PMID: 29141583 [40] |
| MECP2      | PPMX                                   | Recessive   | ID, spasticity, tremor, hyperkinetic behavior moderate ID, marfanoid habitus, ID, ptosis, cryptorchidism                                  | unaffected carrier females                                             | N.D.                         | n/a                 |
| MED12      | MED12-Related Disorders                | Recessive   | ID, spasticity, tremor, hyperkinetic behavior moderate ID, marfanoid habitus, ID, ptosis, cryptorchidism                                  | unaffected carrier females                                             | Yes                           | PMID: 33244166 [41] |
| NEXM1F     | neurite extension & migration          | Dominant    | severe ID, epilepsy                                                  | unaffected carrier females                                             | Yes                           | PMID: 27358180 [42]; PMID: 29717186 [43] |
| NHS        | Nance–Horan Syndrome                   | Dominant    | Congenital cataract, microphthalmia, and mild or moderate            | mild vision impairment                                                  | N.D.                         | n/a                 |
| NKAP       | Intellectual developmental disorder, X-linked, syndromic, Hackman-Di Donato type | Recessive   | ID                                                                    | unaffected carrier females                                             | N.D.                         | n/a                 |
| NLGN3      | Autism risk                            | Recessive   | ASD                                                                   | unaffected carrier females                                             | Yes                           | PMID: 18361425 [38] |
| NLGN4X     | Intellectual Disability, X-linked      | Recessive   | no affected males                                                     | ID, epilepsy and language disorder                                     | Yes                           | PMID: 32562284 [44] |
| NONO       | X-linked, syndromic 34                 | Recessive   | ID, congenital cardiac malformation                                   | unaffected carrier females                                             | N.D.                         | n/a                 |
| NR2C1      | Lowe syndrome                          | Recessive   | ID, catacauts                                                        | not affected                                                           | N.D.                         | n/a                 |
| OFD1       | Simpson-Golabi- Behmel                 | Recessive   | early lethality, severe ID                                            | not affected                                                           | Yes                           | PMID: 31243241 [46] |
| OFD1       | Joubert 10                             | Recessive   | ID, congenital malformation                                          | unaffected carrier females                                             | N.D. random X-inactivation    | PMID: 25136351 [47] |
| OGT        | Intellectual Disability, X-linked      | Recessive   | syndromic ID                                                         | not affected                                                           | Yes                           | PMID: 2105372 [48] |
| OPHN1      | ID, X-linked Congenital Cerebellar hypoplasia | Recessive | ID, hypotonia, ataxia, seizures, macrocephaly, strabismus, dismorphic features | not reported; mild ID, dysmorphic features, strabismus                  | Yes                           | PMID: 22091964 [49] |
| PAK3       | Intellectual Disability, X-linked      | Recessive   | ID                                                                    | unaffected carrier females                                             | N.D.                         | n/a                 |
| PCDH19     | Early infantile epileptic encephalopathy |
|            |                                        | Not reported | Mosaic males early lethality, brain malformation, infantile/childhood onset Leith mild ataxia | ID, autism, infantile seizures                                        | Yes                           | PMID: 31673819 [50] |
| PDHA1      | PDC deficiency                         | Dominant    | ID, hypotonia, ataxia, seizures, macrocephaly, strabismus, dismorphic features | not reported; mild ID, dysmorphic features, strabismus                  | Yes                           | PMID: 2105372 [48] |
| PFA6       | Borjeson–Forssman–Lehmann syndrome     | Recessive   | ID, epilepsy                                                         | mild ID                                                                | Yes                           | PMID: 15994662 [51]; PMID: 12415272 [52]; PMID: 22190899 [53] |
| PFA6       | Sydromic X-linked intellectual disability Siderus type | Recessive | SYNDROMIC ID                                                         | not affected                                                           | Yes                           | PMID: 1849374 [54] |
| PEC9       | Focal dermal hypoplasia                | Recessive   | ID, hypotonia, ataxia, seizures, macrocephaly, strabismus, dismorphic features | not reported; mild ID, dysmorphic features, strabismus                  | Yes                           | PMID: 2105372 [48] |
| PQBP1      | Rempenning syndrome                    | Recessive   | ID, microcephaly                                                     | unaffected                                                             | Yes                           | PMID: 1581616 [59]; PMID: 31840929 [60]; PMID: 2452855 [61] |
| PRPS1      | Arts syndrome                          | Recessive   | ID, ataxia                                                           | milder phenotype generally unaffected                                   | Yes                           | PMID: 29728703 [62] |
| RCLIM2     | Rett Syndrome                          | Recessive   | ID, GDD, Autism, congenital malformation                             | generally unaffected                                                  | Yes                           | PMID: 29728703 [62] |
| RAB39B     | Waisman Syndrome                      | Recessive   | ID, epilepsy, Parkinson disease                                      | Parkinson disease and unaffected                                       | N.D.                         | n/a                 |
| RPS6KA3    | Coffin-Lowery syndrome                  | Recessive   | ID, hypotonia, ataxia, seizures, macrocephaly, strabismus, dismorphic features | not reported; mild ID, dysmorphic features, strabismus                  | Yes                           | PMID: 13876666 [55]; PMID: 12297985 [56]; PMID: 17546030 [57]; PMID: 17546031 [58] |
| RPS6KA3    | X-Linked MR19                          | Dominant    | Mosaic males                                                         | syndromic ID                                                           | Yes                           | PMID: 1581616 [59]; PMID: 31840929 [60]; PMID: 2452855 [61] |
| SCLC5A2    | Congenital disorder of glycosylation, type II | Recessive | ID, hypotonia, ataxia, seizures, macrocephaly, strabismus, dismorphic features | not reported; mild ID, dysmorphic features, strabismus                  | Yes                           | PMID: 2105372 [48] |
| SLC6A8     | Creatine transporter deficiency        | Recessive   | ID, epilepsy                                                         | mild                                                                   | Yes                           | PMID: 18342287 [66] |
| Gene   | X-Linked Disorder                          | Inheritance | Male                      | Female                        | Gene Subject to X-Inactivation | References          |
|--------|-------------------------------------------|-------------|---------------------------|-------------------------------|-------------------------------|---------------------|
| SLC9A7 | Intellectual developmental disorder, X-linked | Recessive   | ID                         | unaffected carrier females    | N.D.                          | n/a                 |
| SMCT2A | DEE85; Cornelia de Lange, 2               | Dominant    | Lethal in males; ID, limb malformations, dysmorphic | ID, midline brain defects, seizures | escape X inactivation | PMID: 30871455 [24] |
| SOX3   | X-linked, with isolated growth hormone deficiency | X-linked | ID, pancytopenia           | unaffected carrier females    | N.D.                          | n/a                 |
| STAG2  | Holoprosencephaly 13, X-linked Epilepsy, X-linked, with variable learning disabilities and behavior disorders | Dominant, Recessive | early lethality, brain malformation, ID | ID, brain malformation/unaffected | N.D.                          | n/a                 |
| SYN1   | Dominant, Recessive                       | ID, epilepsy, ASD | epilepsy and ASD          | N.D.                          | n/a                           |
| SYP    | X-linked, X-linked syndromic, Nascimento-type | Recessive   | ID, epilepsy               | unaffected carrier females    | N.D.                          | n/a                 |
| TAF1   | XLID 33                                  | Recessive   | ID, syndromic ID          | unaffected carrier females    | Yes                           | PMID: 26637982 [67] |
| THOC2  | XLID 12/35                               | Recessive   | mild - moderate ID        | not affected                  | Yes                           | PMID: 26166480 [68] |
| TSPAN7 | X-linked, X-linked, X-linked 58           | Recessive   | ID                        | unaffected carrier females    | N.D.                          | n/a                 |
| UBE2A  | X-linked syndromic, Nascimento-type       | Recessive   | ID, epilepsy               | unaffected carrier females    | Yes                           | PMID: 16090393 [69] |
| UPF38  | XLID 14                                  | Recessive   | Severe non-syndromic ID, Autism | not affected                  | Yes                           | PMID: 19238151 [70] |
| USP9X  | Syndromic XLID 99                         | Dominant    | ID, autism, maybe Lethal in males | mild or unaffected or ID, multiple congenital anomalies | escapes X inactivation | PMID: 29022598 [7] |
| USP27X | Intellectual Disability, X-linked 105     | Recessive   | ID                        | unaffected carrier females, static encephalopathy, adult onset | N.D.                          | n/a                 |
| WDR45  | NBIA5                                    | Dominant    | lethal, mosaics - affected | neurodegeneration, infantile spasms, developmental delay, ID | Yes                           | PMID: 23176820 [71] |
| ZDHHC9 | Raymond type XLMR                         | X-linked    | non-syndromic ID          | unaffected                    | N.D.                          | n/a                 |

### 3. MECP2

The MECP2 gene is predominantly expressed in the brain where MeCP2 binds to methylated DNA via methyl-CpG pairs and acts as both a transcriptional repressor and an activator of gene expression [10]. MECP2 is important for prenatal neurogenesis, postnatal development of synaptic connections and function, synaptic plasticity, and adult neural function [72]. Variants involving the MECP2 gene may result in MECP2 duplication syndrome, Rett syndrome (RTT), X-linked intellectual disability or autism spectrum disorder (ASD).

Loss-of-function or missense variants in MECP2 may result in syndromic or non-syndromic intellectual disability, Rett syndrome, or ASD without RTT (Figure 1). RTT is seen almost exclusively in females and is lethal in most males by age 2. Individuals with classic RTT generally present with normal early growth and development followed by developmental stagnation between 6 and 18 months and a period of developmental regression affecting social skills, speech, gait, and purposeful hand use between 1 and 4 years old. During the period of regression, distinct hand movements, seizures, and irregular respirations emerge. In addition to RTT, loss-of-function MECP2 variants can also cause a non-specific X-linked intellectual disability in males and females [73]. Females often have mild intellectual disability, while males may develop mild to severe intellectual disability, including PPM-X syndrome marked by psychosis and bipolar disorder, parkinsonism, increased muscle tone, exaggerated reflexes, and abnormal enlargement of the testes [74].
Loss-of-function or missense variants in MECP2 are associated with a more severe phenotype than missense variants, and individuals with truncations show earlier development of hand stereotypies, increased muscle tone, exaggerated reflexes, and abnormal enlargement of the testes [12]. Truncating variants in MECP2 are associated with a more severe phenotype than missense variants, and individuals with truncations show earlier development of hand stereotypies, increased muscle tone, exaggerated reflexes, and abnormal enlargement of the testes [12].

The disease severity and variability of the phenotype is influenced both by the location and type of the variant as well as by genetic background and cellular environment [39]. Truncating variants in MECP2 are associated with a more severe phenotype than missense variants, and individuals with truncations show earlier development of hand stereotypies, decreased height z-scores, paucity of speech [75], a higher incidence of awake respiratory dysfunction [76], and overall higher clinical severity [77].

Variant type and location do not adequately explain phenotypic variability, as individuals with the same pathogenic variant have clinical presentations varying from ASD, ID, and RTT (Figure 1), and XCI has been proposed to be an important factor in the onset and severity of RTT [78,79]. Phenotypic variation ranging from classical RTT to normal individuals with protective skewing of the X chromosome have been reported [78]. Zhang et al. described a Chinese family with Rett syndrome and X-linked intellectual disability [79]. They reported eight individuals with MECP2 variants in six families.

A family made up of a mother, daughter, and son had the identical MECP2 variant c.397C > T. The daughter was diagnosed with a preserved speech variant of RTT, the son was diagnosed with X-linked mental retardation (XLMR), while the mother was healthy. XCI studies showed that the mother had skewing towards the normal allele, while the daughter had random XCI. Another mother and daughter pair were found to have the same c.397C > T MECP2 variant. However, although they both had random XCI, the daughter was diagnosed with RTT, and the mother had learning difficulties and autistic behaviors [79]. While the variability in phenotypes between the mothers and their daughters with the same MECP2 variant may be due to the difference in the pattern of XCI, not all clinical presentations can be explained by the pattern of XCI, given that the clinical symptoms of the mother with random XCI were milder than those of her daughter with the same variant and degree of XCI. Consistent with this report, Xiol et al. found no substantial correlation between the XCI patterns in the blood and the clinical presentation of RTT. In their study of 221 RTT patients with nine recurrent MECP2 variants or a large deletion in MECP2, 17 out of 174 patients had a skewed XCI pattern, and there were no consistent increases or decreases in the clinical severity score of RTT patients with a preferential inactivation of the wild-type or mutated alleles [39]. In addition, the XCI pattern in blood and cortex was different for two patients included in their study. A similar finding was reported by Bao et al., who showed no statistically significant relationship between clinical severity and pattern of XCI [80].

### Table: MECP2 Variants

| N-Term | MBD | ID | TRD | C-Term |
|--------|-----|----|-----|--------|
| p.Arg133Cys** | p.Glu137Gly | p.Arg168X | p.Pro225Arg | p.Pro376Ser |
| p.Ala140Val | p.Ala201Val | p.Gly232Ala* | p.Arg255X | p.Glu397Lys* |
| p.Pro152Arg | p.Arg270X | p.Arg294X | p.Arg306Cys | p.Pro399Leu |
| p.Thr158Met | p.Lys284Glu | p.Asc453QGln | p.Glu406X | p.Asc453QGln |
| p.Thr160Ser | p.Arg306Cys | | |

** = mutation seen in patients with either Rett or ASD

** = mutation seen in patients with either ASD, Rett, or ID

**Autism Spectrum Disorder Disorder mutations**

**Rett Syndrome mutations**

**Intellectual disability**

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MECP2 duplication results in a gain-of-function phenotype that is inherited in a recessive manner, predominantly affects males, and is characterized by severe to profound intellectual disability and limited or absent speech. Individuals with this syndrome have early-onset hypotonia and have progressive spasticity affecting the lower limbs. Additionally, 50% of affected males have epileptic seizures, and many have a predisposition to recurrent infections [81–83]. The X chromosome carrying the duplication is often preferentially silenced in most asymptomatic carriers [84]; however, some females have a mild phenotype, despite inactivation of the variant chromosome [85]. Symptomatic females exhibiting random XCI or skewing with preferential expression of the duplicated chromosome may present with varying severity and can exhibit learning disabilities, intellectual disability, autistic features, or psychiatric symptoms [86,87].

4. FMR1

The FMR1 gene encodes the fragile X mental retardation protein (FMRP), an RNA-binding protein that is highly expressed in the brain and reproductive organs. FMRP regulates the translation, transport, and stability of mRNAs and plays important roles in neuronal development and synaptic plasticity [88]. FMR1-related disorders include fragile X syndrome (FXS), fragile X tremor/ataxia syndrome (FXTAS), and premature ovarian insufficiency (POI), and result from expansion of the trinucleotide CGG repeat in the 5′ untranslated region. The repeat is categorized into four groups based on the size of the repeat: normal alleles (5–44 repeats), intermediate alleles (45–54 repeats), premutation alleles (55–200 repeats), and full-mutation alleles (>200 repeats). The FMR1 premutation is associated with FXTAS and POI, while the full mutation is associated with FXS. Normal alleles are typically transmitted from parent to offspring in a stable manner without any increase or decrease in repeat number. Intermediate alleles may expand into the premutation range when transmitted by the mother [89], while premutation alleles are unstable and tend to expand into a full mutation when transmitted from mother to offspring. It is estimated that about 1 in 850 males and 1 in 300 females have the premutation and 1 in 7000 males and 1 in 11,000 females have the FMR1 full mutation [90].

4.1. FMR1 Full Mutation

The phenotype of the full mutation results from hypermethylation of the CGG expanded region, thus causing the loss of FMR1 transcription and the absence of FMRP. Most males with FXS have intellectual disability, macrocephaly, facial dysmorphism, high arched palate, joint hyperlaxity, hypotonia, otitis media, pes planus, connective tissue problems, and pectus excavatum. The behavioral features typically include attention-deficit/hyperactivity disorder (ADHD), anxiety, depression, emotional lability, gaze avoidance, stereotypic movements, echolalia, and sensory processing differences. ASD is present in 50–70% of individuals with FXS [91], and epilepsy is present in 10–20% of individuals and begins between ages 4 and 10 years [92]. The physical and behavioral features seen in males with FXS are present in females with the full mutation but are typically less severe. The female phenotype is more commonly associated with learning disabilities, behavioral problems, anxiety, depression, shyness, and difficulties in establishing social interactions [93], and about half of females with FXS are diagnosed with intellectual disability [91].

The differences in phenotype among females with full FMR1 mutations can be attributed to differences in X-chromosomal inactivation as shown in a case study of three females with the full mutation from the same family [94]. Patient III-1 had complete inactivation of the normal allele and physical traits of FXS and presented with hand-flapping, short attention span, tactile defensiveness, shyness, and poor eye contact. Less than 10% of the normal allele was inactive in patient II-1 who presented with normal intelligence, while 50% of the normal allele was inactive in patient II-2, and she presented with mild physical traits and intellectual disability [94]. Another case study described two sisters with FMR1 full mutations with different fragile X phenotypes. One sister had severe
intellectual disability and phenotypic traits like those observed in males with FXS. She had complete inactivation of the normal X chromosome, while her sister with learning disabilities had the normal X chromosome active in 70% of her cells [95]. Martorell et al. described a consanguineous Moroccan family in which the four sisters were compound heterozygote for full and pre-mutation in \textit{FMR1}. The proband had complete inactivation of normal X chromosome and presented with autistic-like features and had severe intellectual disability, while the sisters had a random XCI pattern and had learning disabilities and emotional problems with mildly affected IQ [96]. These findings support the hypothesis that the different phenotypes in female carriers with full mutations are primarily caused by unequal X-chromosomal inactivation.

4.2. \textit{FMR1} Premutation

4.2.1. Premature Ovarian Insufficiency (POI)

Female carriers of the fragile X premutation have an increased risk for development of premature ovarian insufficiency (POI), a condition in which women experience infertility, irregular menstruation, and menopause prior to 40 years old. Although it has been hypothesized that the development of POI in fragile X premutation carriers is due to skewed XCI, this has not been supported by published literature. Using the polymorphic androgen receptor (AR) gene assay, Spath et al. compared the inactivation patterns in female premutation carriers with POI (\(n = 37\)) to those of female premutation carriers without POI (\(n = 64\)) and women with idiopathic POI (\(n = 25\)). They found that the degree of skewed XCI did not differ significantly between female premutation carriers with POI, female premutation carriers without POI, and females with idiopathic POI [97]. Similarly, Rodriguez-Revenga et al., using the same methodologic approach, compared the XCI patterns from 220 control female samples, 40 female premutation carriers with POI, and 220 female premutation carriers without POI. Their results showed no significant difference in the prevalence of skewed XCI among non-POI and POI \textit{FMR1} premutation carriers [98]. These findings were further substantiated by a study of monozygotic twins with similar sized \textit{FMR1} premutations who had discordant phenotypes for POI and similar X-inactivation ratios [99]. The idea that the development of POI is related to CGG repeat size was proposed by Sullivan et al. In their study of 507 women, they showed that repeat sizes in the medium premutation range (80–99 repeats) were associated with the highest risk for POI, and the risk of developing POI appears to plateau, or perhaps decrease, among women with very high repeats (>or =100 repeats) [100].

4.2.2. Fragile X-Associated Tremor/Ataxia Syndrome (FXTAS)

\textit{FMR1} premutation research has primarily focused on FXTAS and POI in adults; however, children with \textit{FMR1} premutation are also at increased risk for several health concerns. Bailey et al. reported on 256 children with a \textit{FMR1} premutation and their co-
occurring conditions. Premutation males, when compared with the control group, were more likely to have developmental delay, attention problems, aggression, seizures, ASD, and anxiety, while premutation females were more likely to have attention problems, anxiety, depression, and developmental delay [103]. These findings are corroborated by Renda et al. [104] and Farzin et al. [105] but were in contrast to work by Myers et al. [106]. In their study of 28 children, they found no significant difference between children with and without the premutation. Although several medical conditions seem to be related to the FMR1 premutation in children, none of the studies published to date have examined the effect of XCI, thus further study is needed.

5. Conclusions

Symptoms of X-linked disorders are variable among females, with some presenting the full disease phenotype, while others present with a milder phenotype or as asymptomatic carriers. Skewing of XCI provides a mechanism for the diversity of phenotypes observed in X-linked disorders, as shown by our discussion of MECP2- and FMR1-related disorders; however, it does not account for all phenotypic variability as seen in the cases of POI. The lessons learned from these disorders can be extended to other X-linked NDDs, as shown in Table 1, where the phenotypic expression of many X-linked genes is regulated by XCI. Skewed XCI may be required for survival, as it is observed in a majority of heterozygous females [107]; however, the impact of skewed XCI on phenotype is not well understood.

The studies included in this review have primarily relied on blood for XCI studies. While blood is the most assessable tissue, the pattern of XCI may not correlate well with XCI in the brain. This, in addition to the small sample size are limitations of the XCI studies and indicates that XCI pattern in blood is not a useful predictor of phenotype.

Despite these challenges, targeted reactivation of genes on the inactive X chromosome could represent a therapeutic approach in heterozygous females affected by X-linked diseases, and several groups are exploring this possibility in rodent models and in vitro cell lines [108,109]. Sex chromosomal dosage compensation is an important developmental process, and disturbing XCI could have severe consequences for females since overexpression of genes, such as MECP2, results in MECP2 duplication syndrome. While more work remains to be done, these preliminary studies show promise and may lead to meaningful interventions.

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