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The Relationship between Skewed X-chromosome Inactivation and Neurological Disorders Development

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ABSTRACT

X-chromosome inactivation (XCI) is a process by which one of the copies of the X chromosome in mammalian female cells is inactivated. The XCI causes a balanced X-linked gene quantity between male and females; moreover, it results mosaic females which have paternal active X in some cells and maternal active X in others. Cellular mosaicism is a noteworthy phenomenon and lowers the risk of X-linked diseases in women because the presentation of a mutation on both X chromosomes is unlikely. Therefore, in heterozygous females, the XCI will be present only on the half of the X genome. In contrast, a similar mutation will present in all of the cells of men. Female carriers of some neurological disorders such as autism, Rett syndrome**,** adreno-leukodystrophy and X-linked mental retardation are reported to present XCI. These observations underscore the important role of X chromosome in the brain which may be related to the existence of a chromosomal signature of gene expression associated with the X-chromosome for neurological conditions not normally associated with that chromosome. In this review, we focused on latest investigations on the role of XCI in neurodevelopmental disorders and how these investigations can be effective in the treatment of neurodevelopmental disorders.

Keywords: Adreno-leukodystrophy; Rett Syndrome; X Chromosome Inactivation, X-linked mental retardation

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INTRODUCTION

According to the Lyon hypothesis, after the random inactivation of paternal or maternal X chromosome in a cell, all of the daughters of that cell will have a similar inactivated X. This process results a mosaic females which have paternal active X in some cells and maternal active X in others [1]. Somatic cells have a stable X chromosome inactivation (XCI); however, germline cells have a cyclic XCI. If one of the X was heterochromatinized and compressed during meiosis, pairing and recombination would not completely achieved. Hence, while both X are active during Oogenesis, one of them will be inactivated during mitotic phase of gametogenesis (Figure 1).

Skewing of the normal pattern of XCI

Intermediate phenotypes in women who carry X-linked diseases are considered as evidence of skewed XCI [2,3].

More than 80% (95% in severe cases) of cells show preferred inactivation of one X chromosome in skewed XCI [4]. So that, these heterozygote women mainly will have a single cell population, i.e. paternal or maternal active X [5,6]. Almost 10% of skewed XCI is by chance, meaning that, for example, a heterozygous woman for hemophilia has the least amount of factor VIII and shows typical form of the disease similar to homozygous women for the mutant allele [6,7] (Figure 2). Moreover, chromosomal abnormalities and mutations that give a

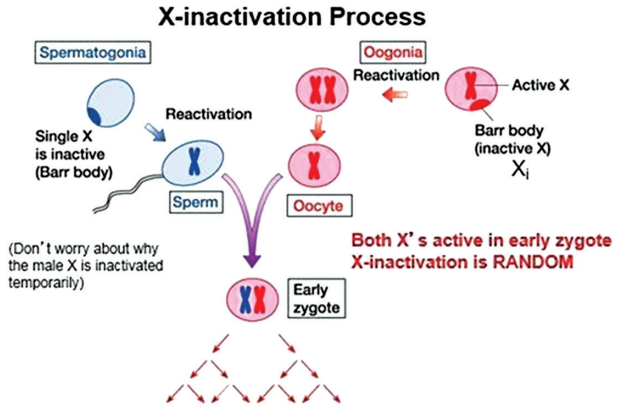

Figure 1. X chromosome inactivation. Reproduced from https://www.studyblue.com.

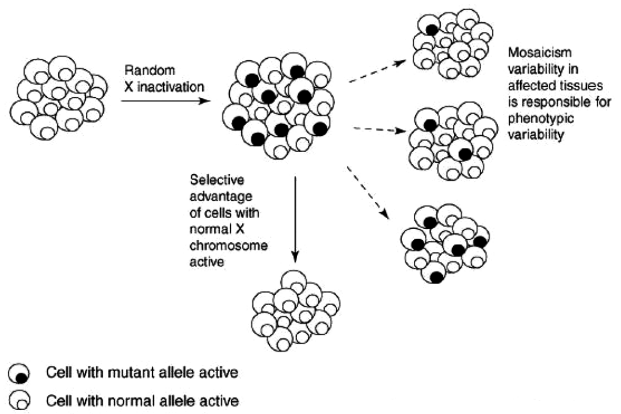

Figure 2. Schematic representation of XCI in female somatic cells [15].

cell selective advantage or negative attributes may play a role in this skewness. For instance, if one of the X chromosomes carry a mutant allele, which opposes cell survival or inhibits the growth of cells, the cells will inactivate that X chromosome in order to provide cell survival and growth [8]. Also, mutations in the XIC (X inactivation center), which is a 13 Mb area in Xq chromosome, mutation in Xist (X-inactive specific transcript) promotor [8,9] or Tsix gene (a gene antisense to Xist) deletion [10] may all cause skewed XCI. Promoter single nucleotide polymorphisms or mutations affecting the start of transcription of Tsix, Xist and Xite also can change start of a correct XCI. For instance, single nucleotide polymorphism in the second binding protein of CTCF in Xist causes skewed XCI in order to inactivate the other polymorphic allele [9-11]. It should be noted that in most cases, the reason for this skewing is unknown.

This skewing may occur during inactivating, which is called primary skewing, or it may be the result of selection after the XCI which is called secondary skewing. For example, the slower proliferation of cells expressing a mutant allele is considered as a secondary skewed XCI [3,5]. Cross of different strains of mice Shows Xce (X-controlling element) locus to be the cause of a primary, but not complete, skewed XCI and suggests that these alleles cannot disrupt inactivation process but they can disrupt the randomness of the beginning phases of inactivation process [12]. There are not any evidence to confirm the presence of Xce locus in humans [13]. As mentioned before, in heterozygous women with a skewed XCI, a single cell population with similar active X is observed. Thus, the mutant gene on that chromosome will be expressed in all cells and the normal allele will be covered on the inactivated X. Hence, these heterozygous women can show some diseases which are normally presented only in men. This phenomenon is known as manifesting heterozygote [7,14].

Despite some previous reports of familial skewed XCI, such evidences are insufficient to confirm the heritable pattern of skewing in humans [16]. In carriers of X-linked diseases, degree of skewing of XCI pattern, can affect the severity of disease. In general, investigating the primary phases of XCI is very difficult the spread of XCI in human studies early events is very difficult. A delayed replication is a good cytological marker for studying XCI process. In X:autosome translocations, XCI can spread to autosomal areas; however, this spread seems less efficient compared to spreading on X chromosome [17]. In women with Balanced X:autosome translocation, the normal X will be inactivated completely, but not persistently, to avoid partial autosomal monosomy or X disomy [3,18]. This selective inactivation of one of the X chromosomes will cause an X-linked disease in carrier women [4,16,19]. In imbalanced X:autosome translocations, the abnormal X will preferably be inactivated [3]. Investigating the spreading of XCI into autosomal areas will provide analysis of the DNA elements involved in the XCI signal propagation. In some cases of XCI spreading, the translocation stops in the breaking sites and cannot spread to autosomal areas [20], while, it will spread incompletely to that areas [21]. Study of spread of Xist RNA into autosomal areas has shown the variable abilities of Xist to cover autosomal areas [22]. Moreover, gene expression investigations have shown that most genes in a X:autosome translocation escape inactivation [3]. The incomplete spreading of XCI to autosomal areas suggests the presence of some functional areas that contribute to the spread of XCI and are more frequent on X chromosome compared to autosomal chromosomes [23]. Comparing the abilities of translocation in spreading XCI raised the "repeat hypothesis" in which the LINE-1 retrotransposons, which are frequent on X

chromosome, act as inactivation propagation elements [24]. Bioinformatics studies have shown that LINEs are frequent around transcription start sites of the genes that are incurred XCI [25,26]. Repeats of polymorphic CAG in exon 1 of the androgen receptor gene, which is located on the Xq 11.2, and methylation of active and inactive X chromosomes can be used to investigate the XCI pattern. Figure 3 shows the detection method of inactivated X chromosome by two polymorphic repeats (a and b). The PCR is done after the effect of methylation-sensitive enzymes, such as HpaII, which do not break inactive X chromosome because they are methylated before [4, 27].

Skewed XCI and X-linked diseases

Although gene expression studies are an important source of data in investigating neurodegenerative disorders, lists of differentially expressed genes are rarely helpful in understanding the underlying mechanisms of these diseases [29]. Hence, investigating single chromosomes with a high role in developing neuro-developmental disorders

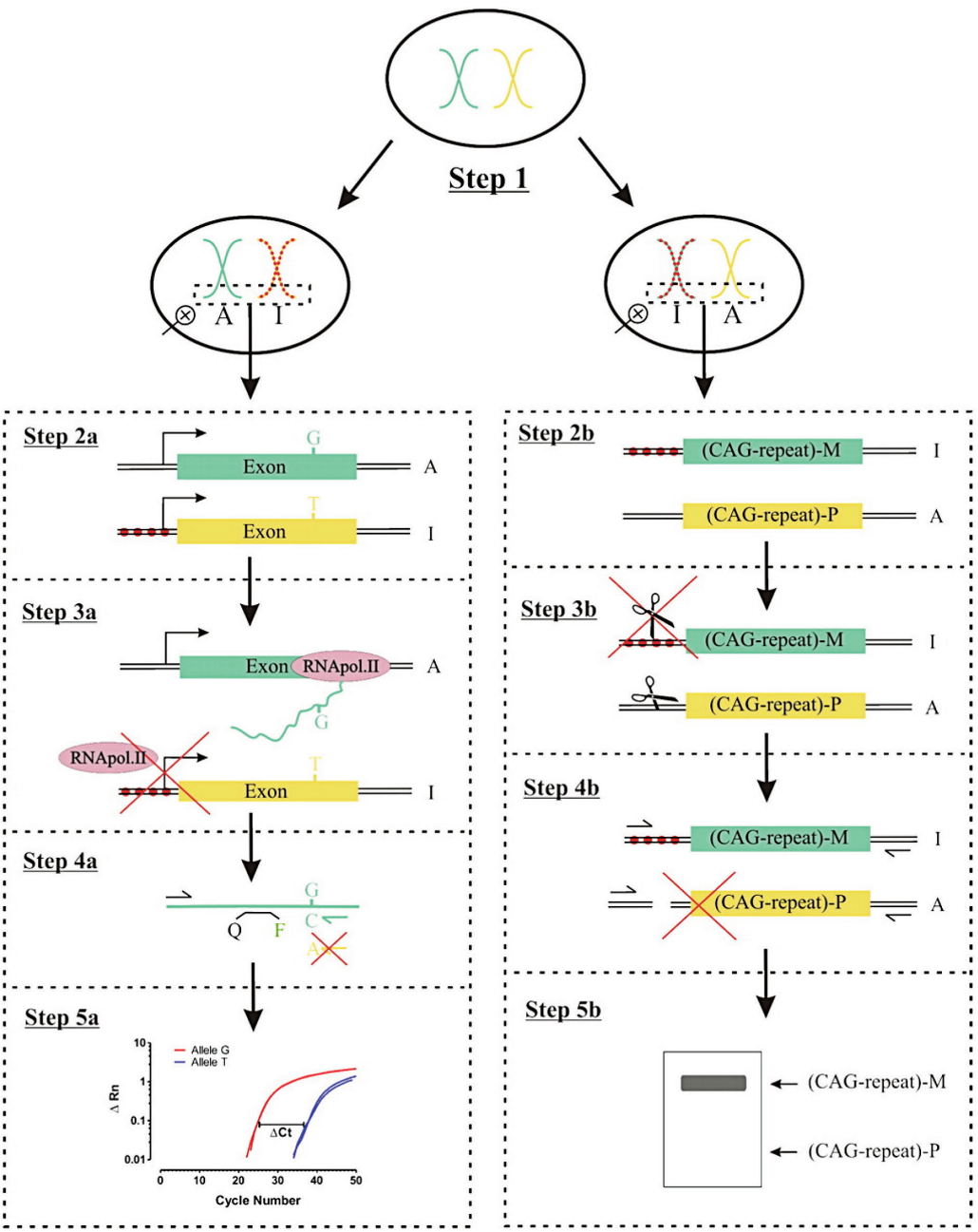

Figure 3. Method of identifying inactive X chromosome [28].

is more helpful. The X-chromosome contains a large number of genes that are essential for brain development and function [30]. The expression of chromosome X is generally higher in brain compared to other tissues [31]. This higher expression is linked to "X dosage compensation" mechanism; a mechanism that associates the expression of X-linked genes with the expression of genes on autosomal chromosomes [32-35]. Several brain disorders are also associated with mutations of genes on X chromosome [36-38]. Heterozygous women show skewed XCI in several X-linked diseases such as Wiskott-Aldrich, Lesch–Nyhan, Barth, Muscular dystrophy Duchesne, hemophilia, and some immune deficiency syndrome [39-41]. In these cases, a higher prevalence of skewed aging is observed by aging [6,7]. The skewed XCI causes variable manifestations of many neurological disorders including autism, Rett syndrome, X-linked adreno-leukodystrophy, X-linked mental retardation and etc [8,27,42-44].

Cell removal is the most common phenomenon occurred in heterozygote women with two cell populations and can yield many advantages for them [40]. For example, in heterozygotes older than 10 years who carry the Lesch-Nyhan syndrome gene, the mutant cells could not be detected in their blood and they are completely removed due to presence of cells expressing normal allele [7]. Although the presence of the cells with normal allele may not be dominant, it is sufficient to preclude the mutant allele phenotype.

In heterozygous women, dermal cells with normal allele compete well with Lesch-Nyhan mutation. Because, normal cells, transmit inosinic acid, which is the product of hypoxanthine guanine phosphoribosyl transferase (HGPRT) enzyme, and is absent in this disease. Indeed, heterozygous women have channels that pass molecules such as inosinic acid and so, with the removal of cells with mutant alleles that do not have these channels, deficiency in HGPRT is compensated [7].

X-linked adrenoleukodystrophy

X-linked adreno-leukodystrophy (X-ALD) was first described by Haberfeld and Spieler in 1910. However, Siemerling and Creutzfeld described combination of adrenocortical atrophy, cerebral demyelination and lymphocytic infiltration in a case of what is now considered the first true report of X-ALD. The name X-ALD was first introduced in 1970 [45]. In this disorder, the characteristic accumulation of very long chain fatty acids (VLCFA) is observed which was first observed as the presence of lipid inclusions in adrenal cells of X-ALD. Now, detection of the accumulation of VLCFAs in blood, red blood cells, fibroblasts and amniocytes with new assays enables better and earlier diagnosis of X-ALD. The adult form of X-ALD was named adrenomyeloneuropathy (AMN) by Griffin et al. in 1977 [46]. As our knowledge about X-ALD increases, the diagnosis of this disorder will be more probable and consequently its incidence will increase. Like other X-linked disorders, referring the X-ALD as an X-chromosomal recessive disorder is inappropriate and it should be simply called as an X-linked disorder [47]. It is estimated that about half of the heterozygous X-ALD females will develop adrenomyeloneuropathy-like syndrome [48]. Thus, heterozygote X-ALD is more prevalent than homozygote [49]. Thus, X-ALD is both the most frequent peroxisomal disorder and also the most frequent monogenetically inherited demyelinating disorder. This monogenic inherit is now considered to be related to the mutations in the ABCD1 gene [50] (Figure 4).

The inherited defect in X-ALD is linked to a mutation in G-6-PD gene which is located in the Xq28 [51] with polymorphic markers [52,53]. Using positional cloning, the G-6-PD was cloned and after that it was termed adreno-leukodystrophy gene. As this gene encodes a peroxisomal transmembrane protein with a structure similar to the ATP-binding cassette transporter, the gene was renamed to ATP-Binding Cassette transporter subfamily D member1 gene (ABCD1). However, the protein is still termed as adreno-leukodystrophy protein (ALDP). Until now, 431 different mutant ABCD1 alleles have been reported. Of these mutations, 221 are missense mutations, 53 nonsense mutations, 27 amino acid insertions and deletions, 113 frame shift mutations and 17 deletions of one or more exons. These mutations are equally distributed among the entire coding region of the ABCD1 gene, however, investigating the 221 missense

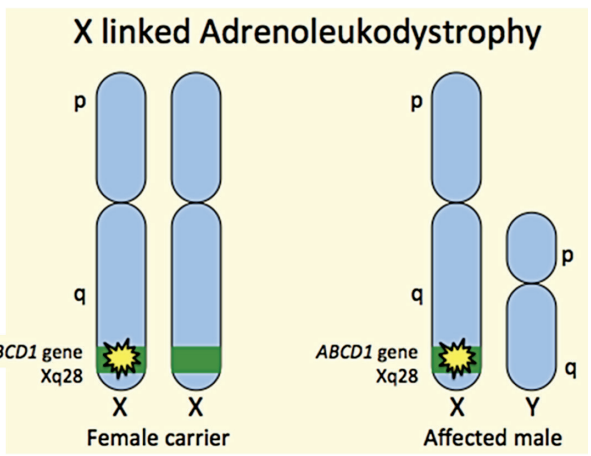

Figure 4. X-linked adreno-leukodystrophy genetic. Reproduced from http://genetics4medics.com

mutations showed that there is no disease-associated mutation within the first 88 N-terminal amino acids and in the last 45 C-terminal amino acids. Interestingly two single base pair substitutions in exon 1 is reported in one of X-ALD cases which causes amino acid exchanges (N13T and K217E). Previous studies have shown that K217E amino acid exchange was the only ineffective exchange in restoration of defected β-oxidation in X-ALD fibroblasts [54]. However, ALDP function is not affected by the N13T amino acid exchange which is consistent with the hypothesis that a reduced functional importance in the first 66 N-terminal amino acids of ALDP is present. No correlation is observed ABCD1 gene mutation type and the disease clinical presentation. This report is consistent with previous studies [55-58]. These studies showed that clinical presentation of X-ALD occur within the same nuclear family [55], mutations causing complete loss of ALDP are associated with various clinical presentations [56], a similar deletion in exon 5 leads to a wide spectrum of X-ALD [56,57] and finally, monozygotic twins present completely different clinical presentations [58]. However, these studies do not lower the possibility of the preventive role of residual ALDP in development of inflammatory cerebral form of X-ALD and its consequent milder phenotype. In the p-glycoprotein multidrug resistance transporter, some mutations can lower the transport rate. ABCA4 (ABCR) gene may also has residual functional activity in age-related macular degeneration and Stargardt disease [59]. Thus, although a general genotype–phenotype association cannot be observed, rare cases with this association may exist.

Besides the presence of functional ABCD1 gene on Xq28, several autosomal pseudogenes are detectable of different chromosomes. ABCD1 pseudogenes are located in 1, 2, 20, 22, and possibly 16 chromosomes which can be detected by PCR analysis of human monochromosomal mapping panel [56]. The pseudogenes on several different chromosomes harden the mutation analysis [60] and may show non-homologous interchromosomal exchange pericentromeric plasticity [61].

X-Linked Mental Retardation

Mental retardation (MR) is known as the impairment of cognitive function which affects about 3% of the population in the US. This disorder is often manifested before adulthood and 20–30% of MR may be inherited or have a genetic background by mutations on the X chromosome X-linked mental retardation (XLMR). Based on their clinical presentation, XLMRs are considered as two types of non-syndromic and syndromic. However, since there are several mutations responsible for these clinical presentation, the difference between these two types is now less notable. About 73 dysfunction of PAK are considered to be involved in the incidence of both types of XLMR. Several X-linked mutations causing non-syndromic MR (MRX) are detected on the X chromosome. This type of MR is clinically similar but they are genetically diverse. Now, more than 65 MRX pedigrees are detected on different loci on the X chromosome and it is estimated that these pedigrees represent about 10 genetic loci [62,63]. Among these loci, probably about two MRX genes have the key roles in the mentioned signaling. The first gene is named GDI1 which encodes a GDP-dissociation inhibitor for Rab3a and regulates vesicular transport. Hence, GDI1 mutations changes the exocytic activity which is in part related to the synaptic transmission. Another gene which is mutated in MRX encodes a protein which includes a GTPase activation domain (GAP) for Rho GTPases and is called oligophrenin. Since these proteins induce the GTPase activity, inactivation of them leads to permanent activity of the corresponding G protein. Oligophrenin have Gap activity for the signals that control actin cytoskeletal organization and cell shape [64]. One of these proteins is Rho GTPase which directly controls the axon and dendritic shape and activity [65-66]. The role of a Rho GAP in human mental retardation implicates its involvement in neural plasticity. Moreover, PAK3 is reported to be isolated from Xq22 in MRX families [67]. The PAK3 gene is mainly expressed in the brain, and it is known as an important downstream effector of Rho GTPases through actin cytoskeleton and MAPK cascades. Two diseases are reported to have mutations in MRX pedigrees, one of them includes a nonsense [68] and another a missense mutation [69] which implicate the association between PAK3 and pathogenesis of MRX.

Considering the role of PAK in fragile X syndrome (FXS), recent studies have shown a defective activation of synaptic RAC/PAK signaling in the mouse model of FXS [70]. Expectantly, inhibiting PAK activity reduces various cellular and behavioral deficits, including FXS-related abnormalities [71]. These results implicate the role of PAK signaling in the FXS pathogenesis.

Fragile X syndrome is the most common inherited form of mental retardation [72,73]. Although the pathogenesis of FXS is not well understood, it is probably the result of the expansion of the CGG repeat in the 5-untranslated region of the fragile X mental retardation 1 (FMR1) gene which is located on the X chromosome [74]. This expansion silences the transcription of FMR1 gene and leads to the

FXS phenotype. The resultant change of the length of the CGG is the main factor in determining FXS disease or its carrier form. Having more than 200 CGG repeats causes FXS-associated cognitive deficits and abnormal cortical dendritic spines [75].

FMR1 protein (FMRP) plays an important role in regulation of mRNA translation, transport and stability [76,77]. In the brain, FMRP can regulate translation of a group of mRNAs at synapses which are essential for learning and intellectual development. Consequently, in the absence of FMRP, reduced mRNA translation causes the defect in synaptic function and synaptic plasticity [76,78,79]. FMR1 Knocked-out mice show behavioral defects similar to FXS patients. These behavioral disturbances include hyper-reactivity to auditory stimuli, anxiety, impaired spatial learning and impairment in motor coordination [80-82]. Hence, the association between PAK and FMRP [71] or defected synaptic RAC/PAK signaling implicate the reduced synaptic plasticity in patients with FXS. Thus, targeting PAK signaling may be a potential therapeutic strategy in formulating new drugs to treat FXS.

In summary, previous data show that normal memory and leaning is achieved by functional PAK and pathways which are disturbed in FXS and similar disorders with developmental cognitive deficits such as dementia of aging (AD) and Huntington disease (HD). Inhibition of PAK seems to be an effective approach in treating FXS, AD and HD. Inhibitors of PAK likely have important effects on improving cognition by improving dendritic spine morphology and/or synaptic plasticity. This hypothesis considers PAK activation as a contributing factor in the incidence of various neurological disorders and probably suggest a common treatment for them based on the correcting PAK dysregulation.

Rett Syndrome

Rett syndrome (RTS) is considered as a progressive disorder affecting neuro-development in girls; however, some of the features of the disease appears slowing are not prominent at initial stages of the disease. These patients develop normally up to 6-18 months of age. During this development, patients will have a normal milestones including walking and saying some words. One of the early signs of neuro-developmental involvement is microcephaly which is the result of the deceleration of head growth between ages one and two and is associated with growth retardation and muscular hypotonia. Progression of the disease causes a lost purposeful use of hands which interrupts normal hand functions. The patient will be withdrawn socially and inability to speech become more apparent by aging which turns the patient irritable. The patients is also hypersensitive to surrounding sounds, cannot make an eye-to-eye contact and is unresponsiveness to social cues [83].

Genetic Basis of RTS

Considering that most of the RTS patients are female, early studies have hypothesized an X-linked dominant model of inheritance in RTS. However, almost all of the RTS cases are sporadic and mapping the generic inheritance of the disease was difficult. Using data from rare familial cases, Xq28 was identified as the candidate region and subsequently, mutations in MECP2 were identified in RTS patients [84] (Figure 5).

These mutations can be detected in about 96% of RTS cases and arise de novo in the paternal germline with a C to T transition at CpG dinucleotides [85]. These mutations include missense, nonsense, and frameshift mutation types with over 300 unique pathogenic nucleotide changes described [86] as well as deletions [87,88]. Eight missense and nonsense mutations are responsible for more than 75% of all of the mutations in RTS. C-terminal deletions causes 10% and complex rearrangements are responsible for about 6% of all mutations in RTS. Missense mutations cause a more mild phenotype than mutations affecting the NLS of MeCP2; Similarly, C-terminal deletions are associated with milder phenotypes [89]. Moreover, a mild phenotype is present in the R133C mutation [90,91].

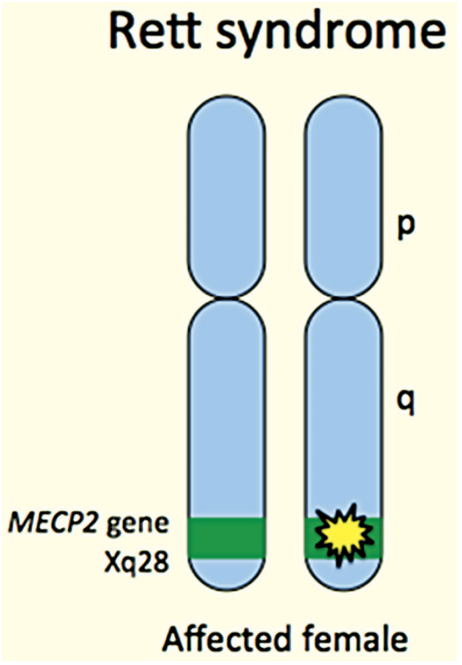

Figure 5. Rett syndrome genetic. Reproduced from http://genetics4medics.com.

Autism

The diagnosis of autism is based on the presence of abnormality in a triad of behavioral domains: social development, communication, and repetitive behavior/ obsessive interests. While autism can occur at any point on the intelligence quotient (IQ) continuum, IQ can predict the outcome of autism. The disease is associated with a language delay. Autism has a spectrum which Asperger syndrome is a subgroup of it. Patients with this syndrome have similar features of common autism; however, they have no history of language delay and moreover, they have an average or higher than average IQ. The features of autism spectrum is highly probable to be caused by genetic factors. The risk of presence of autism in the sibling of a patient is 4.5% which emphasizes a genetic inheritance [92] (Figure 6).

In a study of same sex autistic twins, while no dizygotic twins were concordant for autism, concordance was present in about 60% of monozygotic pairs [93]. This concordance in monozygotic twins shows a high degree of genetic inheritance of autism. Different genetic studies are done to determine the candidate regions involved in autism. Although these regions are not fully understood, two regions are the most probable candidates. These regions are 15q11-13, near the GABAAb3 receptor subunit gene (GABRB3) and 17q11.2, near the serotonin transporter gene (SLC6A4). The second region is under high investigations because of the previous reports about the increased serotonin levels of platelets in autism. Also, the involvement of serotonin in autism is highly probable because it innervates the limbic system which has known roles in emotion recognition and empathy. At least four loci on the X chromosome are also detected to be involved in autism and are high interest due to their ability to clarify sex differences in autism. These genes are the neuroligin (NLGN3, NLGN4), FMR1 (which causes fragile X syndrome), and MECP2. Despite there are candidate regions, specific genes for autism have not been detected yet. Further research is needed to detect these specific genes and also to clarify their function and ultimately the relation between different influential factors in autism [92].

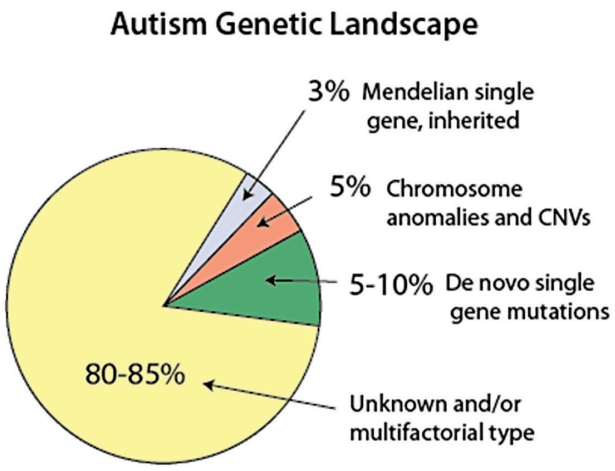

Figure 6. Autism genetic prospect [92].

Charcot-Marie-Tooth

Charcot-Marie-Tooth (CMT) which is known as hereditary motor and sensory neuropathy consists of clinically and pathologically heterogeneous group of disorders. This disease is considered as a common form of peripheral inherited neuropathy in humans. The disease includes slowly progressive atrophy; weakness of the distal muscles; sensory loss in the feet, lower legs, and hands; and reduced tendon reflexes [94]. CMT is classified into types 1 and 2 based on the histopathology and nerve conduction studies [95]. Although some X-linked and autosomal recessive forms of the disease are reported, the most common form of CMT is inherited autosomal dominant [96]. CMT 1A is a demyelinating neuropathy related to a duplication of piece of gene on 17p11 chromosome which encodes myelin protein [97]. It is the most common form of the disease inherited as an autosomal dominant disease. The clinical expression of this disease depends on the age of the patient and the average age of the onset of clinical features is 12.2+7.3 years. At least three loci are included in CMT1: the CMT1A locus maps to human chromosome 17 and the CMT1B locus maps to human chromosome 1 (region q23-q25) [98]. Since the clinical phenotypes of CMT1 syndromes cannot be distinguished, unless in cases of male to male inheritance, three genes must be sequenced which include connexin gene, po gene and PMP22 gene for X-linked CMT, CMT1B and CMT1A respectively [99]. The X-linked CMT due to the connexin 32 (Cx32) gene mutation is the second most common form of CMT [100] (Figure 7).

This gene is expressed in several tissues including both peripheral nerve axons and CNS glia and neurons. Families with dominant inheritance, which is revealed by a male-to-male inheritance, PMP22 and po genes must be screened for mutations. Families with no male-to-male inheritance or CMT1A may be screened for connexin 32 gene first and then Po and PMP22 [102]. In an X-linked pattern of inheritance, male are more severely affected and not father-to-son transmission is seen. Females who are heterozygous and may be asymptomatic. Intermediate

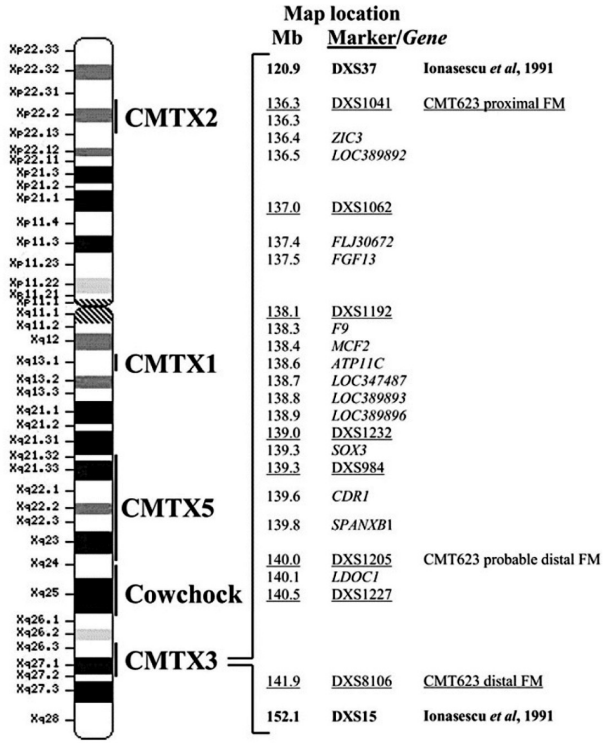

Figure 7. Genetic heterogeneity in X-linked Charcot–Marie–Tooth disease [101].

range motor conduction velocities is found in X-linked CMT. Accordingly, prolonged brainstem auditory evoked potentials (BAEPs) may be used as a distinguishing feature X-linked CMT in males and females due to XCI in females [103].

DISCLOSURE STATEMENT

The authors have nothing to disclose.

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