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REVIEW

Determining the role of skewed X-chromosome inactivation in developing muscle symptoms in carriers of Duchenne muscular dystrophy

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Abstract Duchenne and Becker dystrophinopathies (DMD and BMD) are X-linked recessive disorders caused by mutations in the dystrophin gene that lead to absent or reduced expression of dystrophin in both skeletal and heart muscles. DMD/BMD female carriers are usually asymptomatic, although about 8 % may exhibit muscle or cardiac symptoms. Several mechanisms leading to a reduced dystrophin have been hypothesized to explain the clinical manifestations and, in particular, the role of the skewed XCI is questioned. In this review, the mechanism of XCI and its involvement in the phenotype of BMD/DMD carriers with both a normal karyotype or with X;autosome translocations with breakpoints at Xp21 (locus of the DMD gene) will be analyzed. We have previously observed that DMD carriers with moderate/severe muscle involvement, exhibit a moderate or extremely skewed XCI, in particular if presenting with an early onset of symptoms, while DMD carriers with mild muscle involvement present a random XCI. Moreover, we found that among 87.1 % of the carriers with X;autosome translocations involving the locus Xp21 who developed signs and symptoms of dystrophinopathy such as proximal muscle weakness, difficulty to run, jump and climb stairs, 95.2 % had a skewed XCI pattern in lymphocytes. These data support the hypothesis that skewed XCI is involved in the onset of phenotype in DMD carriers, the X chromosome carrying the normal DMD gene being preferentially inactivated and leading to a moderate–severe muscle involvement.

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Introduction

Duchenne and Becker dystrophinopathies (DMD, OMIM #310200 and Becker, BMD, OMIM #300376) are X-linked recessive disorders that affect, respectively, 1 in 3500 or 1:18,500 male births (Emery 1991). Both diseases are caused by mutations in the dystrophin (DMD) gene located on Xp21.2. Dystrophin plays an important role in the muscle structure, as it links the cytoskeleton to the extracellular matrix. In particular, the amino terminus of the dystrophin binds to F-actin in the cytoskeleton, while the carboxyl terminus binds to the dystrophin-associated protein complex (dystroglycans, sarcoglycans, integrins and caveolin) in the extracellular matrix (Nowak and Davies 2004). Dystrophin is expressed in striated skeletal and smooth muscles, heart and in the brain. Deletions and duplications are the most frequent mutations in the DMD gene and account for 70–75 % and 5–10 % of cases, respectively (Emery et al. 1991); the remaining cases are determined by single point mutations or small rearrangements (Pikó et al. 2009). According to the Haldane theory, one-third of cases are de novo mutations (Davie and Emery 1978). Mutations not leading to the production of dystrophin determine Duchenne muscular dystrophy phenotype (DMD; OMIM 300377), while mutations leading to a reduced amount or an altered size of the dystrophin protein cause Becker muscular dystrophy (BMD; OMIM 300376). In both cases, a destabilization of the sarcolemma is determined due to reduction of the dystrophin-associated protein complex (Matsumura et al. 1993; Nigro et al. 1995). In DMD patients, the lack of dystrophin causes a progressive proximal muscle weakness associated with respiratory involvement and/or cardiomyopathy that represent the most common causes of death. In BMD patients, the maintenance of some amount of dystrophin causes a less severe muscle

weakness and prolonged functional abilities, but cardiomyopathy is more frequent and represents the most common cause of death.

DMD/BMD female carriers are usually asymptomatic, although several studies report that about 2.5–7.8 % may manifest skeletal muscle symptoms. The age of onset of muscle symptoms is variable; however, an onset before 15 years usually leads to severe clinical manifestations, similar to those observed in males (Moser and Emery 1974; Nigro et al. 1995; Politano et al. 1996; Palmucci et al. 1999; Romero et al. 2001; Ceulemans et al. 2008). Moreover, BMD/DMD carriers may show myocardial involvement (18 %; 16 % in DMD and 7 % in BMD) and in particular dilated cardiomyopathy, characterized by significant left ventricular dilatation and decreased shortening fraction (7–8 % in DMD or 6.6 % in BMD carriers, (Politano et al. 1996; Hoogerwaard et al. 1999; Grain et al. 2001).

Myocardial involvement such as hypertrophy, arrhythmias and left ventricular dilatation increases with age and has the same occurrence in both DMD and BMD carriers, with a prevalence of dilated cardiomyopathy after the age of 50. (Hoogerwaard et al. 1999). DMD/BMD carriers can develop isolated muscle or cardiac involvement (Comi et al. 1992; Nigro et al. 1995; Politano et al. 1996; Nigro et al. 2004), or both. However, cardiac symptoms develop usually later than muscle symptoms (Politano et al. 1986). Several mechanisms have been hypothesized to explain the clinical manifestations in BMD/DMD carriers, such as: (1) DMD gene mutation on both Xp21 alleles (Fujii et al. 2009; Soltanzadeh et al. 2010); (2) loss of one X chromosome (Turner syndrome) (Chelly et al. 1986; Satre et al. 2004); (3) polymorphisms in other non-DMD genes, such as osteopontin, recently associated with an early loss of ambulation in DMD males (Kyriakides et al. 2011); (4) skewed X-chromosome inactivation (XCI), with preferential inactivation of the normal dystrophin allele (Azofeifa et al. 1995; Yoshioka et al. 1998; Soltanzadeh et al. 2010; Seemann et al. 2011; Viggiano et al. 2013a); (5) women carrying X;autosome translocations involving the DMD gene (Boyd et al. 1986; Viggiano et al. 2013b); (6) uniparental disomy (Quan et al. 1997). All these mechanisms determine as final effect a reduction of dystrophin expression in the skeletal and cardiac muscles. In the present review, we will analyze the mechanism of XCI and its involvement in determining the onset of symptoms in DMD carriers.

X-chromosome inactivation

XCI is an epigenetic mechanism that equalizes X-linked gene dosage between men and women through the inactivation of one X chromosome in women. At the end of the process, women are a mosaic of two cell types expressing the maternal or the paternal X chromosome.

A random XCI indicates that cells present an equal (50:50) inactivation of the maternal or the paternal X chromosome. On the other hand, a skewed XCI indicates an unequal (>50 %) inactivation of the maternal or the paternal X chromosome.

However, there is no agreement on the cutoff for XCI to be considered skewed, as some authors consider 65:35 (Orstavik et al. 1996; Satoh et al. 2008), while others 70:30 (Matthews et al. 1995; Amos-Landgraf et al. 2006) or 80:20 (Orstavik 2009). Usually, the term extremely skewed XCI indicates the preferential inactivation of one X chromosome in 90–95 % of cells (Orstavik 2009).

Mechanism of XCI

The mechanism of XCI is not completely known. Several studies in mice demonstrated that the X-chromosome inactivation center (Xic)—a single *cis*-acting control locus at Xq13.2—is necessary for: (1) initiation of XCI by counting the number of X chromosomes and choosing one X chromosome to be inactivated; (2) spreading the inactivation in *cis* the X chromosome; and (3) maintaining the inactive state (Gartler et al. 1992). The non-coding X inactive-specific transcript (Xist)—a non-coding RNA (ncRNA)—is transcribed by the Xic (Kind and van Steensel 2010). Xist has different functions: (1) gene silencing in *cis* with Xic (Brown et al. 1991; Brockdorff et al. 1992; Brown et al. 1992); (2) spatial reorganization of X chromosome inactivated (Xi) (Splinter et al. 2011; Nora et al. 2012); (3) initiation of chromatin changes (Csankovszki et al. 2001; Plath et al. 2003). The mechanism induced by Xist to silence genes is not clear. It is suggested that the upregulation of Xist in the Xi, forms a sub-nuclear compartment or domain where the RNA polymerase II and transcription factors are sequestered, so that the genes in this region are not transcribed (Okamoto et al. 2004; Chaumeil et al. 2006; Dixon et al. 2012). This domain presents dense regions of interspersed nuclear elements (LINE) suggested to be involved in the silencing and/or silencing maintenance (Chaumeil et al. 2006). A recent work on mouse embryonic fibroblasts, embryoid bodies and trophoblast stem cells shows that genes and LINES-dense regions are present in separate nuclear territories, in particular adjacent to the Xist gene, suggesting a role of the LINE dense regions in Xist mediating silencing, but not a colocalization between genes inactivated and LINES (Calabrese et al. 2012). The spatial reorganization of Xi consists in modifications of the interaction between genes in *cis* and/or in *trans* with genes on autosome chromosomes. In particular, the active X chromosome (Xa) presents more gene interactions both in *cis* and in *trans*, whereas Xist upregulation determines in the Xi few gene interactions in *cis* but not in *trans*. Only genes that escape the inactivation show interaction in *cis* or in

trans with other active genes (Splinter et al. 2011; Nora et al. 2012). Moreover, Xic presents several regulatory elements of Xist, in particular down-regulator elements (Tsix, Linx, Dxpas34 and Xite, Oct4, Sox2, Nanog and Rex1) and up-regulator elements (Ftx, Jpx, Rnf12) (Debrand et al. 1999; Lee 2005; Cohen et al. 2007; Barakat et al. 2011; Chureau et al. 2011; Masui et al. 2011; Gontan et al. 2012). About 15 % of human X-linked genes escape the silencing, and an additional 10 % of genes present a variable pattern of inactivation (Carrel and Willard 2005). Some of these genes are pseudo-autosomal regions (PARs), homologous sequences of nucleotides on the X and Y chromosome (Lyon 1961). In particular, all genes of the PAR1 escape the XCI, while only some genes of PAR2 are silenced (Ciccodicola et al. 2000). Alu repetitive elements and ncRNAs are enriched around genes that escape XCI (Wang et al. 2006a, b; Reinius et al. 2010). Few mechanisms have been suggested to explain skewed XCI and its correlation with age:

1. The *stochastic mechanism* (Gale et al. 1997), according to which the XCI pattern depends on the pattern of stem cell expressing the maternal or paternal X chromosome at the time of lyonization. With age, there is a stochastic reduction of stem cells as some of them will be lost through the terminal differentiation of both daughter cells.
2. The *genetic mechanisms* (Vickers et al. 2001; Kristiansen et al. 2005) Previous studies reported cases of familial skewed XCI, leading to severe symptoms (Bicocchi et al. 2005; Renault et al. 2007) or cases of discordant phenotype (Orstavik et al. 1999; Tanner et al. 1999). Furthermore, a mutation in the XIST was reported that causes a familial skewed XCI (Plenge et al. 1997), and linkage analysis studies on XCI phenotype in normal human families found a linkage to the Xq13 trait that contains the XIC locus including the XIST gene. The authors hypothesized two types of XIC alleles—strong and weak—and suggested that "strong" XIC alleles have a higher probability of staying active. In turn, only subjects carrying alleles of different 'strength' will present skewed XCI (Naumova et al. 1998). It is also postulated that some polymorphic X-linked genes can influence cell division, growth or apoptosis, and in turn the selection of cell population expressing the maternal or the paternal X chromosome. Furthermore, cells with high turnover, such as hematopoietic or skin cells, seem to have a higher probability of skewed XCI compared with cells with lower mitotic activity (Knudsen et al. 2007).
3. The *selection mechanism*, according to which skewed XCI derives in elderly women from a growth or survival advantage conferred by one of the parental X chromosomes. In general, selection will favor cells expressing the normal allele (Migeon 2006), thus explaining the limited number of symptomatic carriers of X-linked diseases. However, in some cases, as X;autosome translocations, the cells can also express the mutant allele.

Skewed XCI in normal women

Several studies analyzed the prevalence of skewed XCI in unaffected women in the general population with controversial results, probably due to the different (a) methods of analysis (Gale et al. 1991; Fey et al. 1992), (b) age of women, or (c) type of tissue analyzed. Amos-Landgraf et al. (2006) reported a normal distribution of XCI pattern in the vast majority of the general population of women and an extremely skewed XCI in only 5 % of them, data confirmed by other authors (Migeon 2007; Bolduc et al. 2008). A correlation of skewed XCI with age (Fey et al. 1994) and type of tissue (Azofeifa et al. 1995; Gale et al. 1997) has also been reported. An increase of extremely skewed XCI with age up to 100 years (Lanasa et al. 2001; Kristiansen et al. 2005) has been reported by studies on blood, using the PCR-based androgen receptor gene (AR) analysis. Skewed XCI occurs in about 16–37 % of women >60 years and in 49 % of centenarians, while it occurs in 14 % of women aged ≤25 years and in 4.9–14.2 % of newborns (Kristiansen et al. 2005; Christensen et al. 2000; Bolduc et al. 2008). An extremely skewed XCI occurs in about 16–27 % of women ≥60 years and in 18 % of centenarians (Busque et al. 1996; Christensen et al. 2000; Sharp et al. 2002), while it occurs in 7 % of women ≤25 years and in 0.7–2.7 % of newborns (Busque et al. 1996; Lanasa et al. 2001; Bolduc et al. 2008). However, a higher percentage of skewing (27.9 %) and extreme skewing (4.9 %) XCI has been reported in mothers compared to their newborns, suggesting that hematopoietic cells suffer from age-associated skewing in early adulthood (Bolduc et al. 2008). Moreover, XCI pattern correlates with age-associated skewing occurrence in both hematopoietic and non-hematopoietic cells (e.g., buccal epithelial cells or urine samples) (Knudsen et al. 2007; Bolduc et al. 2008). However, this correlation is lost after the age of 60 years, suggesting that while the occurrence of skewing in hematopoietic cells continues to increase, in non-hematopoietic cells there is a plateau.

Moreover, there is no agreement among researchers on the correlation of the XCI pattern in different tissues. In fact, some authors report a good correlation between blood and epithelial tissue of the same individual (Gale et al. 1991; Sharp et al. 2002; Knudsen et al. 2007; Bolduc et al. 2008), and others between thyroid gland and muscle, or leucocytes and muscle, suggesting that tissues deriving from the same embryogenic layer have the same XCI pattern (Azofeifa et al. 1995). However, Bittel et al. (2008)

reported a good correlation also between haematopoietic (blood and/or spleen) tissue and tissues deriving from different embryogenic layers, brain, skin, heart, lungs, muscles, kidneys, and gastrointestinal tract included.

X chromosome inactivation in X;autosome translocations

Studies in animal models demonstrated that the spread of inactivation in X;autosome translocations is discontinuous, so that the autosomal genes in *cis* to the Xic gene are inactivated less efficiently than the normal X-linked genes (Russell 1963; Cattanach 1974). This suggests that autosomal chromatin lacks important signals in the spread and/or maintenance of X inactivation (Bailey et al. 2000; Ross et al. 2005). The spread of inactivation on the autosome is high near the breakpoint, although some inactivation is also observed distant from it while the spread across the centromere is more limited than in euchromatic regions (Cotton et al. 2014).

It is suggested that the LINEs frequency at the breakpoint may influence the degree of inactivation spread in the autosomal chromosome (Stankiewicz et al. 2006).

Skewed XCI in DMD carriers

Previous studies demonstrated a correlation between skewed XCI and onset of symptoms in carriers of X-linked diseases such as hemophilia B (Espinós et al. 2000; Okumura et al. 2008), dyskeratosis congenita (Devriendt et al. 1997), Wiskott–Aldrich syndrome (Wenger et al. 1992), and focal dermal hypoplasia (Gorski 1991). No agreement exists on DMD muscular dystrophies, as some AA report a good correlation between symptomatic carriers and skewed XCI (Azofeifa et al. 1995; Satoh et al. 2008; Jonàs Juan-Mateu 2012; Sandra Mercier 2013; Viggiano et al. 2013a) while others deny this correlation. (Sumita et al. 1998; Soltanzadeh et al. 2010; Brioschi et al. 2012). Table 1 shows the pattern of XCI in symptomatic DMD carriers. Although the number of subjects for each phenotype (mild, moderate and severe) is limited, skewed XCI seems to better correlate with severe rather then mild phenotype. However, further studies on a larger number of carriers are necessary to confirm this observation. Only one study analyzed XCI analysis in symptomatic carriers at muscular vs cardiac level and showed the highest degree of XCw inactivation in carriers manifesting at the muscular level. Moreover, the AA found a correlation between the degree of XCw inactivation and onset of symptoms, because all subjects presenting a severe muscle phenotype and a higher skewed XCI were younger (<40 years) compared with carriers showing cardiomyopathy (Viggiano et al. 2013a).

Possible explanations for the conflicting data on the correlation between skewed XCI and clinical symptoms in DMD carriers are:

1. To have analyzed as a sole group not homogeneous BMD and DMD carriers, or symptomatic and asymptomatic carriers (Bushby et al. 1993; Sumita et al. 1998).
2. Patient age: to have considered only the younger carriers that may manifest symptoms later and neglected that XCI increases with age (Sharp et al. 2002).
3. The failure in identifying the allele that carries the wild or the mutant DMD gene (Bushby et al. 1993; Matthews et al. 1995; Seemann et al. 2011) and in turn which X chromosome is highly inactivated.
4. The recombination between the androgen receptor locus (used for the STR analysis) and the DMD loci that confuses the linkage analysis for the identification of the mutant allele.
5. The different cutoff value to define a skewed XCI: >65:35 (Yoon et al. 2011), >70:30 (Bushby et al. 1993; Pegoraro et al. 1994; Matthews et al. 1995; Azofeifa et al. 1995), or >80:20 (Soltanzadeh et al. 2010; Seemann et al. 2011; Brioschi et al. 2012; Viggiano et al. 2013a).
6. The presence of alleles not located on the X chromosome that may modify the phenotype (modifiers alleles): in this respect, only one study analyzed the polymorphism in the osteopontin promoter (SPP1) that is involved in a more rapid progression of DMD (Pegoraro et al. 1995; Kyriakides et al. 2011), but both authors did not find any statistically significant difference.
7. The type of mutation: only few studies on XCI analysis in DMD carriers mentioned the type of causative mutation; furthermore, the only study that performed a statistical analysis was unable to show significant results (Soltanzadeh et al. 2010).
8. The type of tissue analyzed: some researchers suggest that the XCI analyzed in blood does not reflect the XCI pattern observed in the muscle, and in turn the XCI performed in the blood does not correlate with the phenotype. However, only few studies analyzed the pattern of XCI in muscle cells, because muscle biopsy is not a diagnostic test for BMD/DMD carriers. Moreover, a concordance of the XCI pattern between lymphocytes and muscles, tissues of same embryonic origin (Fialkow 1973; Azofeifa et al. 1995), has been proven. In addition, symptoms in BMD/DMD carriers seem to correlate better with the XCI pattern in blood than in the muscle cells (Matthews et al. 1995; Pegoraro et al. 1995; Brioschi et al. 2012). Finally, the XCI analyzed in cells from a single muscle does not reflect the XCI

Table 1 XCI analysis in symptomatic and asymptomatic BMD/DMD carriers

Mutation	Age	DMD carriers analyzed (*n*)	Informative subjects (*n*)	Tissue analyzed	Allele identification	Symptomatic XCI	Asymptomatic		Mild muscle involvement		Moderate muscle involvement		Severe muscle involvement		Cardiac involvement		References
							Random (*n*)	Skewed (*n*)	Random (*n*)	Skewed (*n*)	Random (*n*)	Skewed (*n*)	Random (*n*)	Skewed (*n*)	Random (*n*)	Skewed (*n*)	
DMD	Adult	2	2	Blood	Yes		1	0	0	0	0	1	0	0	0	0	Lupski et al. (1991)
BMD DMD	Young Adult	8	4	Blood	No		0	0	1	0	0.	3	0	0	0	0	Bushby et al. (1993)
DMD	Young Adult	12	10	Muscle	No		5	0	2	0	1	1	1	0	0	0	Matthews et al. (1995)
			2	Blood			0	1	0	0	0	0	0	1	0	0	
DMD	Adult	2	2	Blood	Yes		1	0	0	0	1	0	0	0	0	0	Tihy et al. (1994)
DMD	Young Adult	34	16	Blood	Yes		0	1	7	2	0	2	1	3	0	1	Pegoraro et al. (1995)
				Muscle			0	1	7	1	1	1	2	3	0	0	
DMD	Young	23	21	Blood	Yes		8	4	2	2	0	5	0	0	0	0	Azofeifa et al. (1995)
BMD DMD	Young Adult		3	Blood	Yes		1	0	1	0	0	1	0	0	0	0	Hoffman et al. (1996)
DMD	Young Adult	9	8	Blood	Yes		4	0	0	0	0	4	0	0	0	0	Yoshioka et al. (1998)
BMD DMD	Young Adult	107	93	Blood	Yes		74	15	0	0	2	1	0	1	0	0	Sumita et al. (1998)
BMD DMD	Young Adult	2	2	Muscle	No		0	0	1	0	0	1	0	0	0	0	Doriguzzi et al. (1999)
BMD	Young	1	1	Blood Muscle	Yes		0	0	1	0	0	0	0	0	0	0	Ceulemans et al. (2008)
DMD	Young Adult	15	14	Blood	Yes		0	0	4	5	3	2	0	0	4	0	Soltanzadeh et al. (2010)
DMD	Young	2	2	Blood	Yes		1	0	0	0	0	1	0	0	0	0	Abbadi et al. (1994)
DMD	Adult	1	1	Blood	No		0	0	0	0	0	1	0	0	0	0	Yoon et al. (2011)
DMD	Young	9	7	Blood	No		0	0	1	6	0	0	0	0	0	0	Seemann et al. (2011)
DMD	Adult	24	22	Blood	Yes		0	0	4	0	5	5	0	3	1	1	Jonàs Juan-Mateu (2012)
DMD	Young	18	16	Muscle	Yes		6	5	3	0	1	1	0	0	0	0	Brioschi et al. (2012)
	Adult			Blood			1	1	0	0	0	0	0	0	0	0	

Table 1 continued

Mutation	Age	DMD carriers analyzed (*n*)	Informative subjects (*n*)	Tissue analyzed	Allele identification	Symptomatic XCI	Asymptomatic		Mild muscle involvement		Moderate muscle involvement		Severe muscle involvement		Cardiac involvement		References
							Random (*n*)	Skewed (*n*)	Random (*n*)	Skewed (*n*)	Random (*n*)	Skewed (*n*)	Random (*n*)	Skewed (*n*)	Random (*n*)	Skewed (*n*)	
DMD	Young Adult	44	26	Blood	Yes		17	0	0	0	0	1	0	3	2	3	Viggiano et al. (2013a)
BMD DMD	Young Adult	26	21	Blood	No		0	0	5	2	4	9	0	1	0	1	Sandra Mercier (2013)
DMD	Young Adult	8	8	Blood	Yes		2	0	2	0	0	3	0	3	1	1	Giliberto et al. (2014)

The subjects who were not informative or with chromosomal abnormalities were not included. Mild muscle involvement = minor proximal weakness (MCR >4), myalgia; moderate muscle involvement = proximal weakness (3 > MRC < 4), positive Gower's sign, difficulty climbing stairs; severe muscle involvement = major proximal weakness (MRC <3) or loss of deambulation at age 13–20 years. Cardiac involvement = dilated cardiomyopathy (left ventricular ejection fraction <50 %)

pattern of the total muscles, due to the mosaic pattern observed in carriers. In fact, studies in heterozygous mice for a null mutation of dystrophin gene (mdx/+) demonstrated a variable pattern of dystrophin expression between samples from different skeletal muscles, and between cardiac and skeletal muscles, suggesting a different pattern of XCI (Weller et al. 1991; Bittner et al. 1997). This result could rely on the mechanism of (a) biochemical and (b) genetic normalization. The first consists in the production of dystrophin in the area near a dystrophin-negative membrane, by nearby dystrophin-positive myonuclei (Watkins et al. 1989; Karpati et al. 1990); the second consists in the regeneration of myofibers by the activation of satellite dystrophin-positive cells (Moss and Leblond 1971; Segalés et al. 2014). We hypothesize that mild symptomatic carriers present both biochemical and genetic normalization, whereas carriers with moderate–severe muscle phenotype present only genetic normalization that leads to failure of dystrophin production in muscles, as previously reported (Pegoraro et al. 1995). This is also supported by the evidence that muscle normalization increases with age (Karpati et al. 1990; Bittner et al. 1997), and that carriers with random XCI usually do not show symptoms worsening with age or onset of a severe phenotype in old ages.

9. Finally, it is impossible to exclude that carriers with extremely XCI and a severe phenotype present a X;autosome chromosome translocation, because the karyotypes were not analyzed in all studies. In some papers, in fact, DMD carriers exhibit muscle involvement in addition to other symptoms (e.g., speech delay, behavioral anomalies, mental retardation, aggressiveness) (Pegoraro et al. 1994; Jonàs Juan-Mateu 2012) not common in DMD, suggesting that other genes or chromosome abnormalities could be involved.

Skewed XCI in women with balanced Xp21;autosome translocations

X;autosome translocations occur in about 1/30,000 live births and may show different breakpoints on the X chromosome (Gupta et al. 2006). The estimated risk for developing a clinical phenotype in cases with X;autosome translocations is approximately 6 % (Genesio et al. 2011).

In balanced X;autosome translocations, the normal X chromosome is usually, though not always, inactivated to prevent deleterious monosomy of autosomal genes (Mattei et al. 1981, 1982; Sharp et al. 2002). In unbalanced X;autosome translocations, there is a preferential inactivation of the derivative X chromosome that causes the lack of expression of the extra-autosomal genes and prevents the occurrence of deleterious trisomies of autosomal genes

Table 2 X chromosome inactivation analysis in women with X;autosome translocation involving the Xp21 locus

	Translocation	Age at reporting	Reported phenotype	Clinical features	XCI	XCI assay	References
1	t(X;1)(p21;p34)	2 years	Absent	Asymptomatic	86:14 (L)	BrdU	Laurent et al. (1975)
2	t(X;1)(p21.1;p34)	8 years	DMD	Delayed motor milestones, proximal muscle weakness, tendon Achilles contractures, wheelchair	100:0 (L)	BrdU	Lindenbaum et al. (1979), Meitinger et al. (1988)
3	t(X;1)(p21.2;p36.3)	Fetus	Absent	Normal muscle biopsy	n.d.	n.d.	Evans et al. (1993)
4	t(X;2)(p21;q14)	n.d.	DMD	n.d.	n.d.	n.d.	Zatz acc. (Boyd et al. 1986)
5	t(X;2)(p21;q32)	22 years	Mild DMD	Toe walking, abnormal gait, proximal muscle weakness, delayed speech	n.d.	n.d.	Seemann et al. (2011)
6	t(X;2)(p21.1;q37)	15 years	DMD	Hypotonia, delayed milestones, Gower's sign, enlarged calf muscles, marked proximal muscle weakness, microcephaly, mental retardation	n.d.	n.d.	Holden et al. (1986)
7	t(X;2)(p21.1;q37.3)	14 years	DMD	Unable to walk without assistance, mental retardation	n.d.	n.d.	MacLeod et al. (1983)
8	t(X;3)(p21;p24)	11 years	DMD	Delayed motor milestones, toe walking, Gower's sign, moderately waddling gait, impossibility to get up from the floor, enlarged calf muscles. Muscle biopsy: absence of dystrophin	72.5:27.5 (M) 100:0 (L)	HUMARA	Viggiano et al. (2013b)
9	t(X;3)(p21;q27)	n.d.	DMD	n.d.	n.d.	n.d.	Pearson and Ferguson-Smith, cited by Boyd et al. (1986)
10	t(X;3)(p21.1;q13)	6 years	DMD	Psychomotor and growth retardation, delayed speech, generalized atopic skin changes, recurrent respiratory tract infections and dysmorphic features, progressive lordosis and scoliosis of thoraco-lumbar spine, muscle weakness, waddling gait, tendo-Achilles contractures	Skewed (L)	n.d.	Obersztyn et al. (2008)
11	t(X;3)(p21.1;q13.3)	4 years	DMD	Mental retardation, dysmorphisms	95:5 (L)	BrdU	Canki et al. (1979), Meitinger et al. (1988)
12	t(X;4)(p21.1;q26)	5 years	Mild DMD	Walk at 14 months, enlarged calf muscles	100:0 (L)	BrdU	Saito et al. (1985), Kimura et al. (1986), Dubowitz (1986)
13	t(X;4)(p21.1;q31)	7 years	DMD	Enlarged calf muscles, myalgia, running at reduced speed, learning difficulties, cardiomyopathy. Muscle biopsy: reduced expression of dystrophin	Highly skewed (L); moderately skewed (M)	BrdU HUMARA	Trippe et al. (2014)
14	t(X;4)(p21.1;q33)	n.d.	n.d.	n.d.	100:0 (L)	X-replication studies	Waters et al. (2001)
15	t(X;4)(p21.1;q31.22)	3 years	DMD	Progressive muscle weakness, enlarged calf muscles, hyperlordosis of the lumbar spine, anserin walk, Gower's sign	n.d.	n.d.	Giacalone and Francke (1992)
16	t(X;4)(p21.2;q35)	4.5 years	DMD	Muscle weakness, no Gowers' sign, enlarged calf muscles	100:0 (A)	BrdU	Bodrug et al. (1990)

Table 2 continued

	Translocation	Age at reporting	Reported phenotype	Clinical features	XCI	XCI assay	References
17	t(X;5)(p21.1;q35.3)	9 years	DMD	Frequent falls, enlarged calf muscles, Gower's sign, moderate proximal muscle weakness, slight tightness of both Achilles' tendons, mild lumbar lordosis; mental retardation	100:0 (L)	BrdU	Jacobs et al. (1981), Meitinger et al. (1988)
18	t(X;5)(p21.1;q31.1)	16 years	DMD	Walk at 16 mounth, enlarged calf muscles, muscle weakness, Gower's sign. wheelchair, episodes of supraventricular tachycardia, mild mental retardation	100:0 (L)	BrdU	Nevin et al. (1986), van Bakel et al. (1995), Meitinger et al. (1988)
19	t(X;5)(p21.1;q31.1)	3 years	DMD	Walking on her toes, weakness of neck flexors, deltoids and quadriceps, enlarged calves, Gower's maneuver	n.d.	n.d.	Perez Vidal et al. (1983)
20	t(X;6)(p21;q16)	4 years	DMD	Typical features	n.d.	n.d.	Perez Vidal et al. (1983)
21	t(X;6)(p21;p24)	n.d.	n.d.	n.d.	n.d.	n.d.	Gaál and László (1977)
22	t(X;6)(p21.1;q21)	11 years	DMD	n.d.	99:1 (L)	BrdU	Zatz et al. (1981), Meitinger et al. (1988)
23	t(X;7)(p21.1;p15.1)	16 years	DMD	Difficulty with stairs, Gower's sign, enlarged calf muscles, anxiety, learning difficulties, delayed motor milestones	93:7 (L)	HUMARA	Seemann et al. (2011)
24	t(X;8)(p21.1;q24.3)	Not reported	Mild DMD	Not reported	80:20 (L)	BrdU	Narazaki et al. (1985), Meitinger et al. (1988)
25	t(X;9)(p21;?)	7 years	DMD	n.d.	n.d.	n.d.	Dubowitz (1995)
26	t(X;9)(p21;p21)	23 years	DMD	Turner syndrome, epilepsy, mental retardation	100:0 (L)	BrdU	Bjerglund Nielsen et al. (1983)
27	t(X;9)(p21;q22)	6 years	DMD	n.d.	n.d.	n.d.	Waters et al. (2001)
28	t(X;9)(p21;p22)	9 years	DMD	Walk at 16 months, difficulty in getting up from the floor, Gower's sign, enlarged calf muscles, proximal muscle weakness	98:2 (L)	BrdU	Emanuel et al. (1983)
29	t(X;9)(p21.1;p22.1)	2 years	DMD	Muscle weakness, no running, no jumping, Gower's sign	100:0 (L, M)	HUMARA	Brioschi et al. (2012)
30	t(X;9)(p21.2;q21.3)	n.d.	n.d.	n.d.	100:0 (L, F)	BrdU	Robinson et al. (1990)
31	t(X;9)(p21.2;q21.3)	2 years	DMD	Short stature, muscular dystrophy	100 (L)	BrdU	Waters et al. (2001)
32	t(X;11)(p21.1;q13.5)	16 years	DMD	n.d.	n.d.	n.d.	Greenstein et al. (1980)
33	t(X;11)(p21;q14)	16 years	n.d.	n.d.	n.d.	n.d.	Waters et al. (2001)
34	t(X;11)(p21.2;q23.3)	13 years	Mild DMD	Walk at 17 months, clumsy at walking upstairs, early fatigue, weak back muscles, increased lordosis	Skewed (L)	BrdU	Bjerglund Nielsen et al. (1983), Meitinger et al. (1988)
35	t(X;12)(p21.2;q24.33)	4 years	Absent	Mild muscle weakness. Muscle biopsy: absent of dystrophin	99:1 (L)	BrdU	Wenger et al. (1992)
36	t(X;15)(p21;q26)	9 years	DMD	Walk at about 12 months, unable to climb stairs, to rise from seated position, to run, or to walk a long distance; lordosis; enlarged calf muscles, shortening of the Achilles tendons, waddling gait, Gowers' sign, proximal muscle weakness	93:7 (L) 95:5 (F)	BrdU	Ribeiro et al. (1986)
37	t(X;17)(p21;q12/21)	7 years	DMD	n.d.	100:0 (L)	BrdU	Kalz-Füller (1999)

Table 2 continued

	Translocation	Age at reporting	Reported phenotype	Clinical features	XCI	XCI assay	References
38	t(X:21)(p21.1;p12)	20 years	Mild DMD	Enlarged calf muscles, waddling gait, lower and upper limb weakness, Gower's sign, all reflexes absent, still able to walk	99:1 (L) 95:5 (F)	BrdU	Verellen-Dumoulin et al. (1984), Meitinger et al. (1988)
39	t(X:22)(?:?)	13 years	DMD	Severe muscle weakness	100:0 (L)	HUMARA	Sumita et al. (1998)
40	t(X:22)(p21.1;q11.2)	5 years	DMD	Typical features	n.d.	n.d.	Fidzianska et al. (1995)
41	t(X:22)(p21;q13)	n.d.	DMD	n.d.	n.d.	n.d.	Zatz acc. (Boyd et al. 1986; Meitinger et al. 1988)
42	t(X:22)(p21;q13)	5 years	Absent	Congenital abnormalities and developmental delay	n.d.	n.d.	Waters et al. (2001)
43	t(X:22)(p21.2;q13.3	n.d.	Absent	n.d.	n.d.	n.d.	Fidzianska et al. (1995)

BrdU 5-bromodeoxyuridine labeling (Grzeschik et al. 1975; Willard and Latt 1976), *HUMARA* human androgen receptor assay (Allen 1993), *n.d.* not determined, *L* peripheral blood leukocytes, *M* muscle cells, *F* fibroblast cells, *A* amniotic fluid cells

(Hall et al. 2002; Gupta et al. 2006). However, several cases of a preferential inactivation of the normal X have been reported in unbalanced X;autosome translocations, probably to confer a survival advantage to the fetus (Palmer et al. 1980; Gupta et al. 2006). In the balanced X;autosome translocations disrupting the DMD gene, this is usually split into two parts and joined with a segment of the autosome; as a consequence, no functional dystrophin can be produced by the derivative X chromosome. Previous studies suggested that the part of the X chromosome on the autosome, separated from the Xic, will not be silenced. In addition, the inactivation of the derivative X chromosome will spread into the translocated autosomal sequences resulting in a silencing of the autosomal genes. The derivative monosomy of the autosome genes determines the apoptosis of the cells with X-derivative inactivated in early embryonic stages, resulting in the selection of the cells: only cells with the normal X chromosome inactivated survive.

In Table 2, we report 43 cases with X;autosome translocation involving the locus Xp21 so far published. All cases reported are de novo translocations. Thirty-four of them (79 %) showed a mild or moderate–severe DMD phenotype, while 5 (11 %) did not show symptoms and/or signs of muscle involvement. However, the age at reporting was <5 years in four of these cases. In five cases in which chromosomes 2, 3, 5 or 9 were involved in translocations, neurological symptoms, such as mental retardation and epilepsy, were also associated.

In the only one fetus with 46,X,t(X;l)(p21.2;p36) in which muscle biopsy was performed in utero, normal dystrophin immunostaining was found (Evans et al. 1993). The analysis of the autosomes involved in translocations shows that the highest percentage involved the chromosome 9 (16 %), followed by chromosome 4 and 22 (11.3 %), chromosomes 1, 2, 3 (9 %) and chromosomes 5, 6, 11 (6.8 %). Chromosomes 7, 8, 12, 15, 17 and 21 were involved in only one case, respectively, and never chromosomes 10, 13, 14, 16, 18 and 20.

The analysis of XCI, performed on 20/34 symptomatic patients, on lymphocytes, showed a skewed XCI pattern in all cases. In particular, the XCI was extremely skewed in 15/20, near to extremely skewed (93:7) in 2 subjects and skewed in 3. XCI was analyzed in both muscle and lymphocytes in three symptomatic DMD carriers, and only in one the pattern was the same in the two tissues. In two out of five asymptomatic cases of X(p21);autosome translocations, in which the XCI was performed, this was skewed and extremely skewed, respectively. However, it is impossible to exclude that these subjects will present muscle symptoms later, because of their young age (under 4 years) at the time of investigation. A summary of the phenotype—DMD, Mild DMD, Absent, or Not reported—associated with a skewed or extremely skewed XCI, is shown in Table 3.

Table 3 Association of skewed and extremely skewed XCI with phenotype in women carrying X;autosome translocation involving the Xp21 locus

Phenotype	DMD	mild DMD	Absent	n.d.
Subjects *n* (%)	29 (67.4)	5 (11.6)	5 (11.6)	4 (9.3)
XCI analysis (*n*)	17	4	2	2
XCI lymphocytes	16	4	2	2
Skewed (%)	3	2	1	0
Extremely skewed (%)	13	2	1	2

Discussion

The present review shows that skewed XCI is the main factor determining the appearance of symptoms in DMD carriers or in women with X;autosome translocations involving the locus Xp21, and in particular in women with an early onset of symptoms.

The data reported support the hypothesis that a random XCI can cause only mild symptoms (Pegoraro et al. 1995), while a skewed XCI plays an important role in the moderate–severe phenotypes. In the case of skewed XCI, the preferential inactivation of the X chromosome carrying the normal allele determines in muscles an aberrant dystrophin expression—likely, near to zero—avoiding any biochemical and/or genetic normalization and favoring the onset of a DMD-like phenotype. The mechanism that leads to a skewed XCI, including the cases of X;autosome translocations, is not completely clear. Several mechanisms have been suggested, including stochastic factors (Gale et al. 1997; Brown and Robinson 2000), genetic mechanisms (Vickers et al. 2001; Kristiansen et al. 2005) or developmental post-inactivation selection, particularly in cells with a high turnover (Knudsen et al. 2007). Though familial cases of skewed XCI presenting mutations in the XIST gene promoter have been reported (Plenge et al. 1997; Tomkins et al. 2002), however, the analysis of XIST promoter in DMD symptomatic carriers did not show any variant in this region (Jonàs Juan-Mateu 2012). Moreover, no correlation between the XCI pattern and mother–daughter pairs was found, suggesting that the pattern of XCI is not inherited (Abrams and Cotter 2004; Viggiano et al. 2013a, b). However, it is not possible to exclude a genetic control, because the paternal inheritance was not investigated. In cases of X;autosome translocations, the functional disomy for the segment of X chromosome translocated or the monosomy for the autosomal translocated segment play a crucial role; in fact only cells expressing the derivative X chromosome can survive (Schmidt and Du Sart 1992; Waters et al. 2001).

A limitation of this review is that the groups of DMD and BMD carriers in which the correlation between XCI and phenotype was analyzed in the different studies were not homogeneous. In fact, only few analyzed DMD versus BMD carriers, or symptomatic versus asymptomatic carriers, or identified correctly the mutant versus the wild allele and reported exactly the clinical symptoms. Moreover, the cases of X;autosome translocations so far published are usually symptomatic, and XCI analysis was performed only in 20 subjects, thus preventing to perform a statistical analysis to validate the data. However, the hypothesis that X;autosome translocation involving the Xp21 determines a skewed XCI associated with the clinical phenotype is supported by previous studies demonstrating that the normal X chromosome is preferentially inactivated in balanced X;autosome translocations to confer survival to the fetus (Emery 1991; Gupta et al. 2006; Giliberto et al. 2014). Our experience in the field of muscular dystrophies emphasizes the problem of a correct communication on the risk of a possible DMD phenotype in women prenatally diagnosed as DMD carriers or X;autosome translocated, with breakpoint at the Xp21 locus. In fact, such a possibility is usually underestimated in prenatal genetic counseling and families are not informed about this risk. In our opinion, information should be given, even in cases in which the mothers are asymptomatic carriers. However, further studies on a larger group of subjects are necessary to confirm this hypothesis.

In everyday clinical practice, XCI analysis should be recommended a) in the case of adult women with reporting muscle symptoms to exclude a skewed XCI and b) in young girls (<18 years of age)—symptomatic at the muscle level and related to DMD patients—to evaluate skewed XCI as the potential cause of their symptoms, without the use of genetic testing for carrier status.

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