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SHORT COMMUNICATION

A locus for familial skewed X chromosome inactivation maps to chromosome Xq25 in a family with a female manifesting Lowe syndrome

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Abstract In mammals, X-linked gene products can be dosage compensated between males and females by inactivation of one of the two X chromosomes in the developing female embryos. X inactivation choice is usually random in embryo mammals, but several mechanisms can influence the choice determining skewed X inactivation. As a consequence, females heterozygous for X-linked recessive disease can manifest the full phenotype. Herein, we report a family with extremely skewed X inactivation that produced the full phenotype of Lowe syndrome, a recessive X-linked disease, in a female. The X chromosome inactivation studies detected an extremely skewed inactivation pattern with a ratio of 100:0 in the propositus as well as in five out of seven unaffected female relatives in four generations. The OCRL1 ''de novo'' mutation resides in the active paternally inherited X chromosome. X chromosome haplotype analysis suggests the presence of a locus for the familial skewed X inactivation in chromosome Xq25 most likely controlling X chromosome choice in X inactivation or cell proliferation. The description of this case adds Lowe syndrome to the list of X-linked disorders which may manifest the full phenotype in females because of the skewed X inactivation.

Keywords Lowe syndrome · Skewed X inactivation · X chromosome inactivation · Family study

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Introduction

In mammals, X-linked gene products can be dosage compensated between males and females by inactivation of one of the two X chromosomes in the developing female embryos. The early events in X inactivation are under the control of the X inactivation center or *Xic* that contains several elements thought to have a role in X inactivation. One of these, *Xist*, encodes a large untranslated transcript that is required and sufficient for X inactivation. At the beginning of the X inactivation process, one of the X chromosomes is chosen to remain active and *Xist* expression is stabilized on the future inactive X and accumulates in cis (Avner and Heard 2001). The *Xist* RNA expression is associated with different chromatin modifications, including histone deacetylation, methylation, and DNA methylation that render and maintain the inactivation state through direct or indirect recruitment of several proteins (Heard 2005). X inactivation choice is

usually random in embryo mammals, and genetic studies have revealed that the randomness behaves as a complex trait under the control of multiple genes (Clerc and Avner 2006). In mice, this choice has been shown to be under the control of X controlling element *Xce*, such that X inactivation is skewed toward the X chromosome possessing the weaker *Xce* allele, and of *Xist* gene and its negative regulator, antisense RNA *Tsix* (Gribnau et al. 2005). In humans, there is evidence that mutations in *XIST* promoter result in familial skewing X inactivation. Both mutations, C(-43)A and C(–43)G (Plenge et al. 1997), affect the CTCF binding efficiency and show the possibility that the choice of X chromosome inactivation is due to the stabilization of a higher order of chromatin conformation based on CTCF-*XIST* promoter complex (Clerc and Avner 2006). Although there is existence of strong evidence of the involvement of *XIC* locus in skewed X inactivation, few reports suggest the possibility that different loci in X chromosome could contain genes implicated in the skewed X inactivation (Naumova et al. 1998).

In this paper, we report a family in which six out of eight females show a completely skewed X inactivation transmitted as an X-linked dominant trait in four generations, and in one of these females this had led to a manifestation of the full typical picture of Lowe syndrome The oculocerebrorenal syndrome of Lowe (OCRL) (MIM:309000) is a multisystemic disorder affecting the eyes, nervous system and kidney due to mutations in OCRL1 gene locus at Xq26.1 (Nussbaum et al. 1997). Lowe syndrome is transmitted as an X-linked recessive trait with a high rate of new mutations, which are responsible for about one-third of the cases (Lin et al. 1997; Addis et al. 2004). However, several females showing the complete typical clinical manifestations of the syndrome have been described (Scholten 1960; Svore et al. 1967; Harris et al. 1979; Sagel et al. 1970; Yamashina 1981; Hodgson et al. 1986; Müller et al. 1991). There are several causes for the development of the complete syndrome phenotype of X-linked recessive disorders in females: cytogenetic abnormalities such as reciprocal translocations involving the X chromosome; the presence of the 45, X karyotype; uniparental disomy; and skewed X inactivation. After the exclusion of cytogenetic abnormalities and uniparental disomy, extremely skewed X inactivation was ascertained to be the cause of this phenotype in the female. This extremely skewed X inactivation was also present in five out of seven phenotypically normal females. X chromosome haplotype analysis showed that in this family the skewed X inactivation co-segregates with the chromosomal region Xq25.

Materials and methods

Family

We have examined 12 subjects belonging to a large four-generation family in which one female manifested Lowe syndrome, a rare X-linked recessive disorder (Fig. 1). DNA was extracted by standard method from peripheral blood of all the family members except the subjects I-1 and II-2 whose DNA was obtained from lymphoblastoid cell lines because at the time of the analysis they were dead. The propositus (III-8), a 22-year-old girl, is the third child of non-consanguineous marriage. Family history was negative for OCRL or eye abnormalities. Her mother had three spontaneous abortions and died at the age of 40 from disseminated lymphoma. At the time of her birth, her father (II-3) was 33 years old. All the family members are healthy. She was born at term and, soon after birth, congenital cataract and severe hypotonia was noted. At 1 year of age, the development of the renal Fanconi Syndrome, severe psychomotor retardation and the typical somatic characteristics of Lowe syndrome led to the making of the diagnosis. At that time failure to thrive was evident. Biochemical blood analysis showed low serum phosphorus, normal calcium and PTH in the low normal range. Urine analysis detected low osmolarity, hypercalciuria, hyperphosphaturia, generalized hyperaminoaciduria and low molecular weight proteinuria. The renal ultrasound examination showed small, diffusely hyperechogenic kidneys. Cytogenetic analysis detected a normal female karyotype.

OCRL1 sequencing

DNA was extracted by standard methods. The 23 coding exons and their flanking intronic sequences of OCRL1 gene were amplified from genomic DNA using forward and reverse primers designed according to nucleotide sequences (Addis et al. 2004).

XIST sequencing

The highly conserved minimal promoter of the *XIST* gene located in Xq13 was amplified using primers previously described (Plenge et al. 1997) and sequenced.

Determination of X inactivation pattern

In order to study the X-chromosome inactivation pattern, we used the androgen receptor assay on peripheral blood of all available female family members and

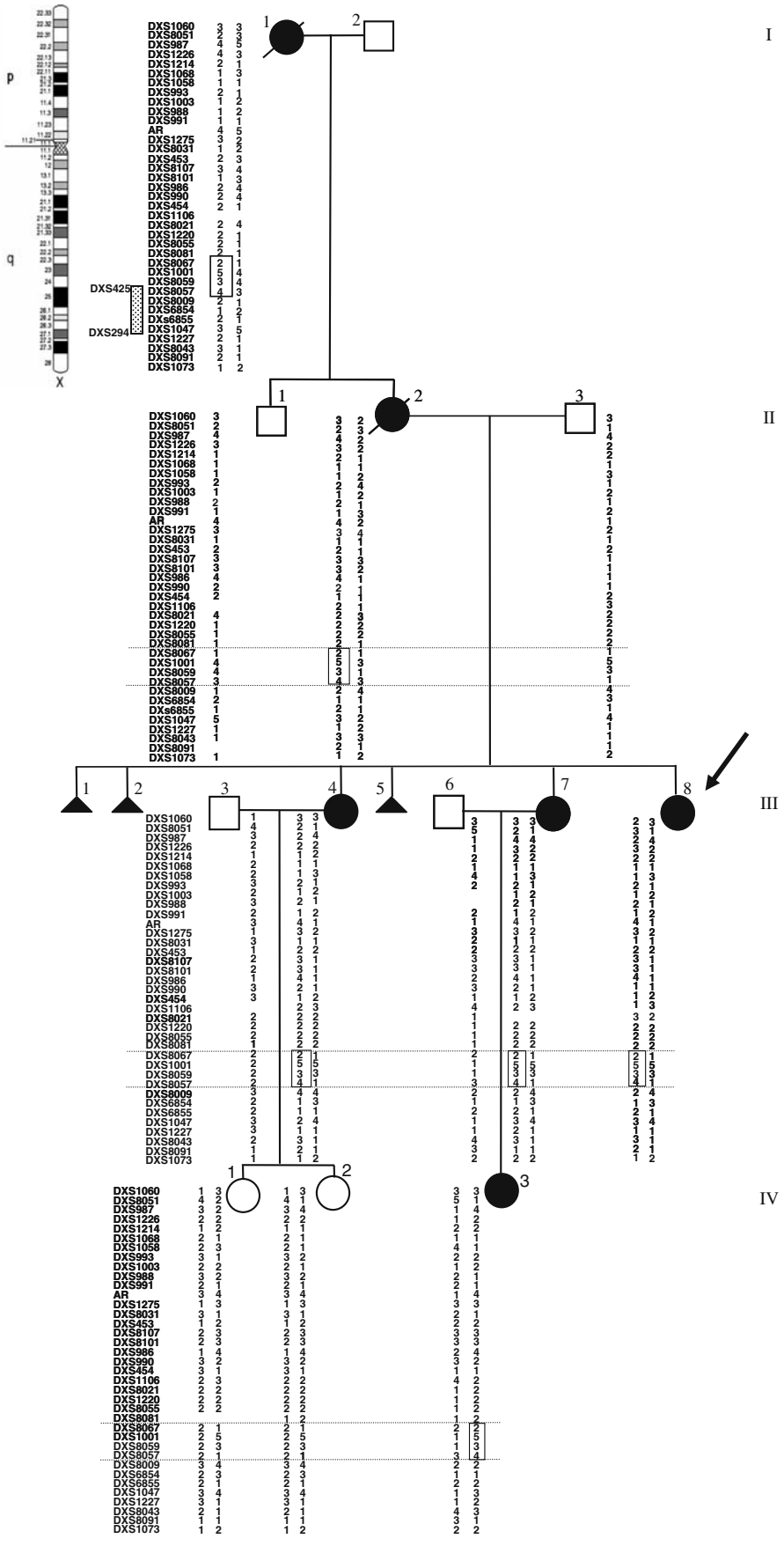

Fig. 1 Pedigree with haplotypes obtained using 37 microsatellite markers of X chromosome. Marker order was determined from the GeneLoc integrated map. *Boxes* indicate the chromosome region shared by females with skewed X inactivation (*black symbols*). Patient with Lowe syndrome is marked by the *arrow*. The *shaded bar* indicates the interval region suggestive of linkage with X inactivation trait observed by Naumova et al. (1998)

controls and on lymphoblastoid cell lines of dead females (I-1, II-2) as previously described (Allen et al. 1992). The XCIP were classified as random (ratios 50:50: <80:20), skewed (≥80:20) or extremely skewed (≥95:5).

Molecular cytogenetics analysis

Molecular karyotyping was performed by array-CGH with the Agilent kit in the patient (III-1). This platform is a 60-mer oligonucleotide-based microarray that allows genome-wide survey and molecular profiling of genomic aberrations with a resolution of ~75 kb (kit 44B). DNA (7 μg) of the patient and a female control (Promega) were double-digested with RsaI and AluI for 4 h at 37°C. Two microgram of each digested sample were purified and labeled by random priming (Invitrogen) for 2 h using Cy5-dUTP for the patient DNA and Cy3-dUTP for the control DNA. Labeled products were column purified. After denaturation of the probes and pre-annealing with 50 μg of Cot-1 DNA, hybridization was performed at 65°C with rotation for 40 h. Following two washing steps, the array was analyzed with the Agilent scanner and the Feature Extraction software (v.8.0). Graphical overview was obtained using the CGH analytics software (v.3.2.32).

X chromosome haplotypes

The X chromosome scan included 37 markers (ABI Prism Linkage Mapping Set, version 2.5-MD10; HD5) spanning the entire X chromosome. These markers were PCR amplified under standard conditions and loaded on ABI Prism 3100 Sequencer (Applied Biosystems). The data were analyzed with Applied Biosystems Genotyper software.

Statistical analysis

Two-point linkage analysis was performed by the MLINK routine of Fastlink package v.4.1P. Analysis assumed an X-linked dominant transmission with a disease-allele frequency of 0.0001 and equal allele frequencies at marker loci. Multipoint linkage analysis was carried out by the LINKMAP routine of Fastlink, with the markers showing non-zero genetic values using the Marshfield sex averaged map distances between the markers.

Results

Direct sequence analysis of the 23 coding exons and the exon–intron boundaries of the OCRL1 gene of the proband (III-8) revealed a T>G mutation at heterozygous state, in the invariable dinucleotide of the splice donor site in intron 14 (IVS14+2T>G). This novel mutation predicts an abnormal splicing. The mutation was absent in all the investigated family members (II-2, II-3, III-4, III-7).

By haplotype analysis using *DXS6854* and *DXS6855* microsatellites closely linked to the *OCRL1* locus, the *OCRL1* mutation resulted contained in the paternally transmitted X-chromosome.

To assess the pattern of X inactivation (XCIP) in this family, we used the androgen receptor (AR) X inactivation assay (Fig. 2). The propositus as well as five out of seven unaffected females belonging to the family showed a completely skewed X inactivation with a ratio of 100:0. DNAs of two females (I-1 and

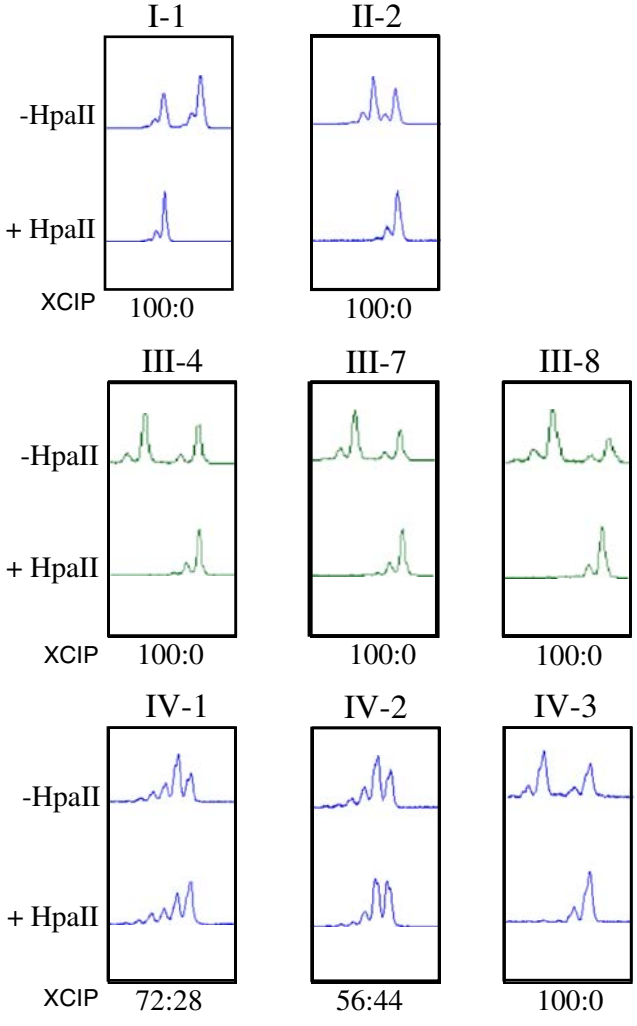

Fig. 2 Analysis of methylation of HpaII site at the human androgen receptor locus (HUMARA). *XCIP* = X chromosome inactivation pattern; *–HpaII* = undigested DNA; *+HpaII* = HpaII digested DNA. The same results were obtained using HinpI restriction enzyme (data not shown)

II-2) were extracted from lymphoblastoid cell lines and gave the same results (XCIP 100:0). Since the skewed X chromosome in these women was that transmitted to the female relatives showing the same trait, this observation is supposed not be due to an artefact introduced by EBV transformation. All the family members showing skewed inactivation inherited the active X from their fathers. In the propositus this X chromosome contains the *OCRL1* mutation, thereby explaining the development of Lowe syndrome in a female. The analysis of the minimal *XIST* promoter shows completely normal DNA sequences. Array-CGH revealed a normal profile of all chromosomes including the X. A decrease in the ratio of signal intensity, lower than the expected for a true deletion (0.78 instead than 0.5) was detected at 126 Mb distance from Xpter (between 126.030 and 126.911) (Fig. 3).

Although this region is not annotated among the genomic variants (http://www.projects.tcag.ca/variation/) it encloses a single gene (*ACTRT1* encoding for an actin-related protein T1) that is contained within a region with high homology to several other genomic portions thus explaining a variation in the values of the signal intensity. After genotyping all the family members with 37 polymorphic markers spanning the whole X-chromosome (Fig. 1), the data were analyzed by linkage analysis. The maximum two-point LOD score of 1.81 was achieved with the markers *DXS8067* and *DXS8057* at a recombination fraction of θ=0.0. Multipoint analysis supported linkage to this region, suggesting that the most likely localization for this locus was in an interval of approximately 3.4 cM between *DXS8067* and *DXS8057* in Xq25, where we observed a peak multipoint LOD score of 2.11 (Fig. 4). Traditionally a LOD score >2 indicates significant X linkage, hence parametric analysis pointed out the presence in this region of a locus controlling the X inactivation pattern.This region corresponds to a physical map distance of 4.2 Mb , partly overlapping with that already found by Naumova et al. (1998). Multipoint LOD scores

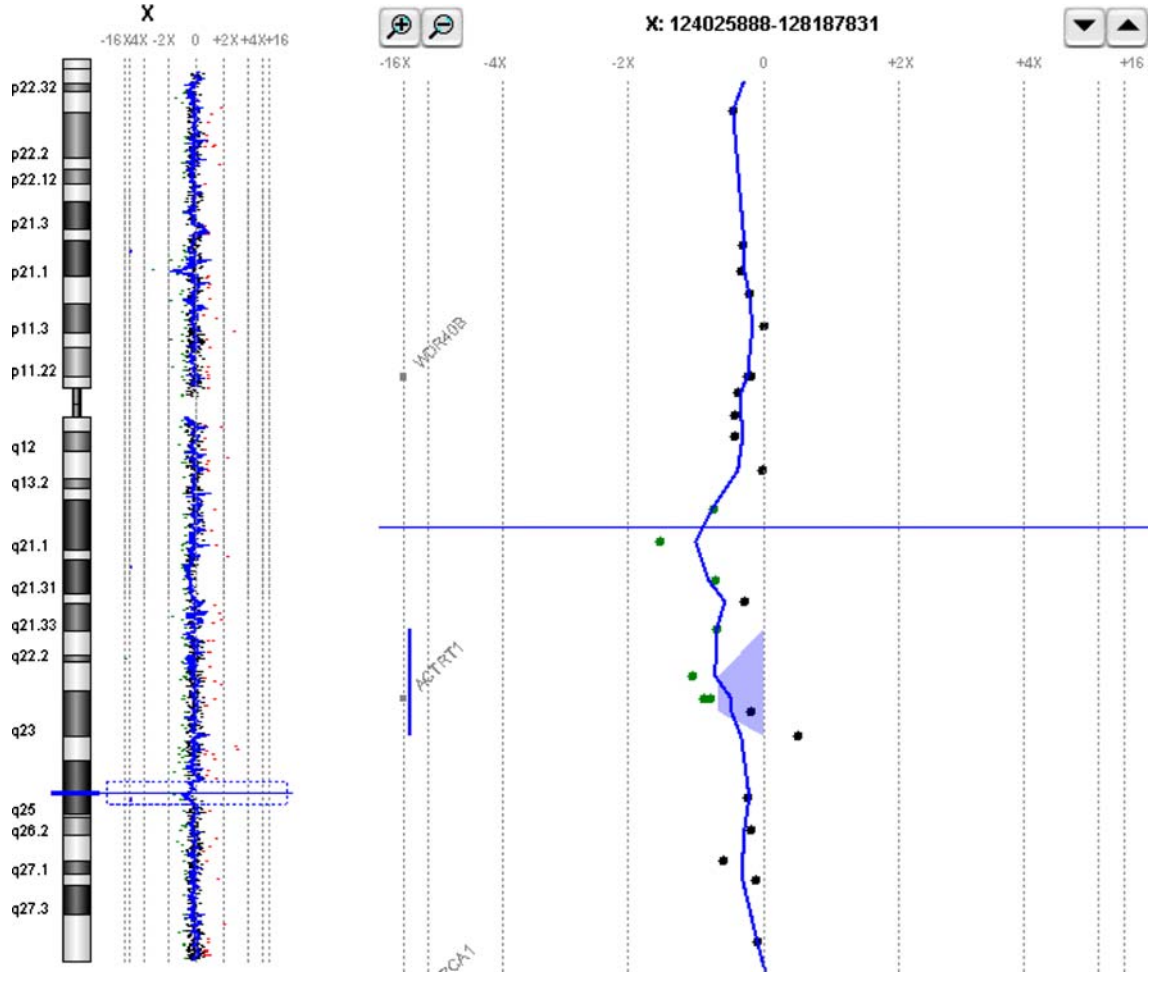

Fig. 3 Array-CGH at 75 Kb resolution: chromosome X shows a normal profile. On the right the enlargement of the possible variant at the ACTRT1 gene

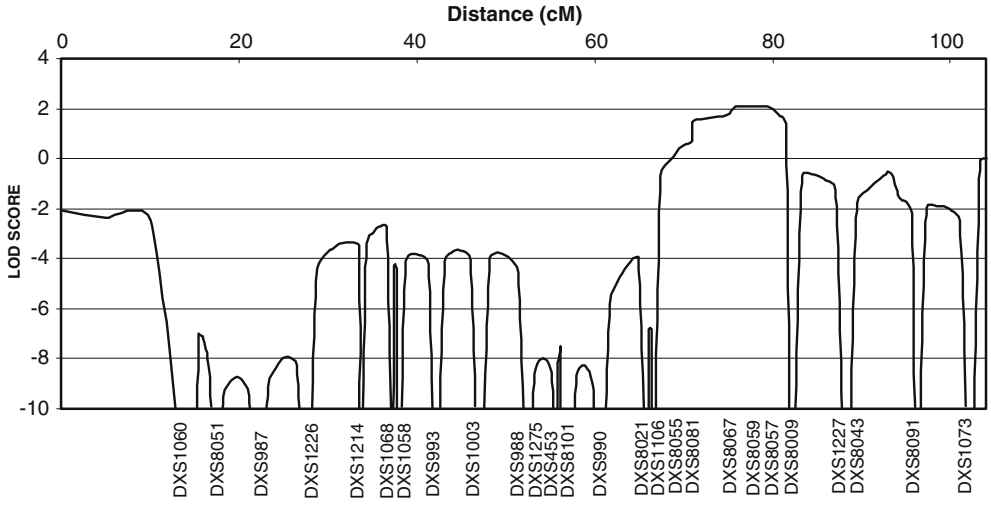

Fig. 4 Evidence for linkage on X chromosome of the family with skewed X chromosome inactivation. Parametric multipoint likelihood of the odds (*LOD*) scores are plotted as a function of chromosomal position of all the analyzed markers. Genetic distances are in centimorgans starting from the *p* telomere of the chromosome. The highest LOD score (2.11) is in the region between markers DXS8067 and DXS8057. All the interval centromeric to DXS1106 shows LOD scores lower than –2, therefore the locus can be excluded from being in this region

were less than –2 centromeric to *DXS1106*, excluding linkage with the *XIST* locus that resides in Xq13.2.

Discussion

In this paper, we report a family in which six out of eight females show a completely skewed X inactivation transmitted as X-linked dominant trait in four generations, that in one of these females having led to the manifestation of the full typical picture of Lowe syndrome (Lowe et al. 1952) due to a novel mutation ((IVS14+2T>G) in the*OCRL1* gene most likely arisen in the paternal germ-line. The extremely skewed X inactivation found in the blood cells determined the development of the Lowe syndrome phenotype in this female, while cytogenetic abnormalities and uniparental disomy have been excluded.

Extreme skewing of X inactivation occurs frequently in females carrying mutations in genes affecting the cell proliferation, maturation or survival. The skewed pattern of X inactivation in these cases is related to a post-inactivation growth disadvantage for those cells with an active X chromosome bearing the mutation. It should be noted that even a relatively mild selection advantage can lead to marked skewing in blood cells, which have a short life span and high turnover (Willard 2001).

Examples of this skewed inactivation in blood cells are the totally asymptomatic female carriers of HPRT deficiency, X-linked α thalassemia with mental retardation, incontinentia pigmenti type 2, focal dermal hypoplasia, X-linked dyskeratosis congenita, X-linked agammaglobulinemia (in B cells), X-linked severe immunodeficiency (in B, T and NK cells) or Wiskott Aldrich syndrome (in all the hemopoietic lineages) and X-linked mental retardation. However, in our family, all the females showing the extremely skewed inactivation and had the paternally inherited X-chromosome active. This finding excludes the possibility that the observed skewing is the result of a direct negative selection process. An alternative possibility, which we cannot exclude, is a positive selection. However, this phenomenon is extremely rare, since it has been described solely in X-linked adrenal-leukodystrophy (Willard 2001).

The presence of a concordant skewed X inactivation in females belonging to the same family could reflect a stochastic variation expected from a random event. However, the probability of finding by chance six females in four generations with the same chromosome inactivated is exceedingly rare. These observations suggested that the skewed X inactivation in this family may be related to the inheritance of an X-linked trait involved in the choice of X chromosome to be inactivated or in cell proliferation transmitted in a dominant fashion.

The early events in X inactivation are under control of the X inactivation center or *Xic* that contains several elements thought to have a role in X inactivation. So far the only gene that has been described in humans to determine the initial choice of which X chromosome should remain active and which X-chromosome is going to be inactivated is the *XIST* (Avner and Heard

2001). A number of families in which multiple females display a significant skewed X inactivation pattern, that is most likely unrelated to stochastic variation or negative cell selection, have been described (Willard 2001). In two such families, a promoter mutation in the *XIST* gene did indeed lead to the preferential inactivation of the X chromosome carrying the mutation (Plenge et al. 1997). In other families, haplotype analysis demonstrated co-segregation of a totally skewed pattern of X inactivation and a region at Xq28 (Pegoraro et al.1997), one at Xq25-26 and one at Xq13 (Naumova et al. 1996; Bicocchi et al. 2005).

In our family, no linkage was found in Xq13.2 where the *XIST* locus resides, and the sequencing of the minimal promoter of the *XIST* gene did not show any nucleotide variation. These data are in accordance with the observation that the *XIST* gene should be functional in the inactive chromosome. The extremely skewed X chromosome inactivation co-segregates with the X chromosomal region Xq25, partially overlapping with that already found by Naumova et al. (1998) between the markers *DXS425* and *DXS8057* (Fig. 1).

Database researches revealed that more than 38 possible genes map within this interval *DXS8067* and *DXS8057*. On the basis of the physiological and biological information on these genes, several should be considered as candidate genes for familial extremely skewed X inactivation.

This is the first report of a family with a completely skewed inactivation (100:0) in phenotypically normal females, inherited as X-linked dominant trait in four generations. The skewing is always in favor of the same X chromosome.

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