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Delineation of Clinical Manifestations of the Inherited Xq24 Microdeletion Segregating with sXCI in Mothers: Two Novel Cases with Distinct Phenotypes Ranging from UBE2A Deficiency Syndrome to Recurrent Pregnancy Loss

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Keywords
Recurrent pregnancy loss · Skewed X-chromosome inactivation · UBE2A deficiency syndrome · Xq24 microdeletion

Abstract
Chromosomal microdeletion syndromes present with a wide spectrum of clinical phenotypes that depend on the size and gene content of the affected region. In a healthy carrier, epigenetic mechanisms may compensate for the same microdeletion, which may segregate through several generations without any clinical symptoms until the epigenetic modifications no longer function. We report 2 novel cases of Xq24 microdeletions inherited from mothers with extremely skewed X-chromosome inactivation (sXCI). The first case is a boy presenting with X-linked mental retardation, Nascimento type, due to a 168-kb Xq24 microdeletion involving 5 genes (*CXorf56, UBE2A, NKRF, SEPT6*, and *MIR766*) inherited from a healthy mother and grandmother with sXCI. In the second family, the presence of a 239-kb Xq24 microdeletion involving 3 additional genes (*SLC25A43, SLC25A5-AS1*, and *SLC25A5*) was detected in a woman with sXCI and a history of recurrent pregnancy loss with a maternal family history without reproductive wastages or products of conception. These cases provide evidence that women with an Xq24 microdeletion and sXCI may be at risk for having a child with intellectual disability or for experiencing a pregnancy loss due to the ontogenetic pleiotropy of a chromosomal microdeletion and its incomplete penetrance modified by sXCI.

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Fig. 1. Clinical features of a boy with an Xq24 microdeletion from Family 1. **A** Macrocephaly, broad forehead, very light and sparse hair, widely spaced and deep-set eyes, bluish sclera, wide and depressed nasal bridge, short nose with a broad tip and underdeveloped alae nasi, anteverted nares, long and deep philtrum, macrostomia, open mouth, thin vermillion of the upper lip, full cheeks, and macrotia are shown. **B** Low-set dysplastic soft ears with an angular antihelix and an underdeveloped spiral and antilobium on the left ear, longitudinal incisions on both lobes, short neck as well as **C** mesoaxial polydactyly of the left foot and onychodystrophy are shown.

Xq24 chromosomal microdeletions, affecting the *UBE2A* gene, have been recently associated with a rare X-linked mental retardation syndrome, Nascimento type (MIM 300860) [Thunstrom et al., 2015]. The *UBE2A* gene encodes the RAD6 ubiquitin-conjugating enzyme. This enzyme is involved in the ubiquitination pathway. Ubiquitination is a type of posttranslational protein modification that is catalyzed by a 3 sequentially acting enzymes, including E1 ubiquitin activation enzyme, E2 ubiquitin-conjugating enzyme, and E3 ubiquitin-protein ligase [Weissman, 2001]. Ubiquitination plays an important role in protein function and can affect it in many ways: mark them for degradation, influence their cellular localization and activity. RAD6 is an E2 ubiquitin-conjugating enzyme that has been recently shown to be involved in histone modifications that control gene expression [Wojcik et al., 2018]. Recent studies have shown that the repressive histone modification H2AK119Ub is one of the earliest chromatin alterations in the process of X-chromosome inactivation (XCI) [Żylicz et al., 2019]. In humans, RAD6 is encoded by 2 genes, *UBE2A* and *UBE2B*, which have 95% homology. *UBE2A* is located in the Xq24 region; therefore, UBE2A deficiency syndrome appears only in men who are hemizygous carriers of the mutation, while women are not affected but always have extremely skewed X-chromosome inactivation (sXCI) [Czeschik et al., 2013; Thunstrom et al., 2015]. Currently, several structural variants affecting this gene are described, from point mutations to microdeletions and microduplications in the Xq24 region, and the clinical features in patients vary widely and can be presented as intellectual deficiency and congenital malformations [Takenouchi et al., 2012; Thunstrom et al., 2015]. However, there are no reports about the association of the Xq24 microdeletion with abnormal embryo development or recurrent pregnancy loss.

In the present study, we analyzed the clinical features and gene content of the Xq24 microdeletions inherited from mothers with extremely sXCI in 2 unrelated families. In the first family, an affected boy has intellectual disability and dysmorphic features corresponding to the UBE2A deficiency syndrome, Nascimento type (MIM 300860). In the second family, we report for the first time a woman with an Xq24 microdeletion and a history of recurrent pregnancy loss.

Case Presentations

Family 1

A 2.5-year-old boy (Fig. 1) was referred to the genetic clinic of the Research Institute of Medical Genetics because of intellectual disability, diffuse muscular hypotension, and hypersalivation. The boy's characteristics were measured: height 80 cm (<3d centile), weight 12.5 kg (25th centile), and head circumference 51 cm (90–95th centile). The first pregnancy of the patient's mother was interrupted due to multiple congenital malformations in the fetus (Fig. 2A; V-3), including gastroschisis and wrist deformity during the 13th week of pregnancy. The embryo had not been cytogenetically investigated. The patient was born from a second pregnancy (Fig. 2A; V-4) at 37–38 weeks of gestation with mild anemia. Childbirth was complicated by a prolonged anhydrous period and secondary weakness of labor. The condition of the

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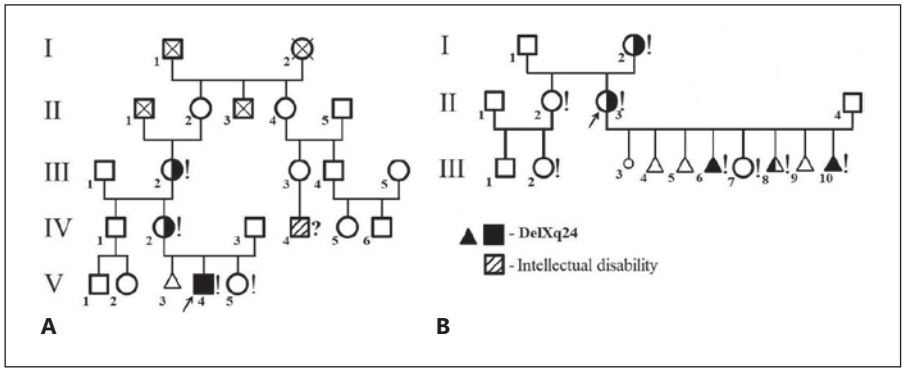

Fig. 2. Pedigrees of 2 families with the Xq24 microdeletion. **A** Family 1. **B** Family 2.

newborn was satisfactory. Apgar scores were 8 at 1 min and 9 at 5 min. The boy's weight was 4,050 g (75th centile), height was 55 cm (97th centile), head circumference was 34 cm (10–25th centile), and chest circumference was 35 cm (75th centile). A congenital heart disease was diagnosed, namely, a major ventricular septal defect with signs of pulmonary hypertension and pulmonary hyperemia as well as an abnormal origin of the right coronary artery from the pulmonary trunk. A radical correction of the heart defects was carried out at the age of 3 months. The milestones of his development were severely delayed: he was unable to hold his head up until 5 months, began sitting at 1 year and 1 month, and cannot sit on his own; at the age of 2, the boy tried to get on all fours and did not stand or walk. He could not speak meaningful words and only made sounds. It was difficult for the child to serve himself. Neurosonography was used to detect the dilation of the lateral and third ventricles of the brain. The neurologist's diagnosis was encephalopathy of mixed genesis, a delay of psychomotor and speech development, and myotonic syndrome. A dysmorphic facial appearance included relative macrocephaly, open large fontanel, broad forehead, very light and sparse hair, widely spaced eyes, almond-shaped palpebral fissure, low-set dysplastic ears, antilobium on the left ear, wide and depressed nasal bridge, short nose with a broad tip and underdeveloped alae nasi, anteverted nares, long and deep philtrum, macrostomia, high palate, micro-retrognathia, macrotia, open mouth, short neck, bilateral transverse palmar crease, long fingers and toes, mesoaxial polydactyly of the left foot, onychodystrophy, left-sided cryptorchidism, and reduced penis (Table 1). The 20-year-old first cousin of the patient's mother (Fig. 2A; IV-4) and the uncle of her affected son are also intellectually disabled.

During the examination of the proband, the mother of the boy became pregnant for the third time. A transabdominal aspiration at the 11th week of gestation of the chorionic villi showed a female fetus with a balanced karyotype, according to array-based comparative genomic hybridization (aCGH), without an Xq24 microdeletion and random XCI (Fig. 2A; V-5). The newborn girl (the proband's sister) is phenotypically normal.

Family 2

A 29-year-old woman (Fig. 2B; II-3) had 8 pregnancies in anamnesis, one of which was terminated as an induced abortion; another 4 were missed abortions, and 2 were terminated by spontaneous abortions at 5–7 weeks of pregnancy. The woman gave birth to an apparently healthy girl (Fig. 2B; III-7). The pregnancy proceeded with the threat of interruption due to cervical insufficiency and ended with cervical surgery. There were cardiovascular diseases and thrombotic episodes among the patient's relatives.

Materials and Methods

Cytogenetic Analyses and Array-Based Comparative Genomic Hybridization

Conventional cytogenetic analysis for extraembryonic tissues of spontaneous abortions was performed based on GTG-banded metaphases at a 400-band resolution.

aCGH was performed using the SurePrint G3 Human CGH+SNP 4×180K Microarray Kit (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's recommendations. Labeling and hybridization of the patient's and reference DNA (#5190-3796, Human Reference DNA, Agilent Technologies) were performed using enzymatic labeling and hybridization protocols (v. 7.5 Agilent Technologies). Array images were acquired with an Agilent SureScan Microarray Scanner (Agilent Technologies). Data analysis was performed using Cytogenomics Software (v. 3.0.6.6) (Agilent Technologies) and the publicly available DGV and DECIPHER databases. Annotations of the genes located within the region of genomic imbalance were retrieved from the NCBI Gene Database, OMIM, and the literature.

Genomic DNA Preparation and Confirmation of CNV Using Quantitative Real-Time PCR

Genomic DNA was isolated from blood, endometrial samples, and extraembryonic tissues using phenol-chloroform extraction. The isolation of DNA from buccal epithelium was performed using a diaGene KIT (#3322.0050, DIA-M, Russia).

Target sequences within the delXq24 and specific amplification primers for quantitative real-time PCR assays were selected using Primer 3 software. The presence of Xq24 microdeletions was tested using genomic DNA from peripheral blood lymphocytes from the women as well as in noncultured extraembryonic tissues of miscarriages using the AriaMX Real-Time PCR System (Agilent Technologies). Control genomic DNA was obtained from the peripheral blood lymphocytes of a healthy donor. The control gene was *HEXB*, which encodes the beta subunit of hexosaminidase and is located at 5q13.3.

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Table 1. Comparison of the clinical features of the patient from Family 1 with data from the literature

Clinical features	Literature data**	Index patient
Delayed psychomotor and speech development	10/10	+
Muscle hypotonia	8/10	+
Seizures	7/10	+
Visual and hearing impairments	6/10	–
Microcephaly	2/10	–
Macrocephaly	8/10	+
Open large fontanel	5/10	+
Broad forehead*	6/10	+
Sparse hair	3/10	+
Synophrys	6/10	–
Widely spaced eyes	7/10	+
Deep-set eyes	2/10	+
Alternating divergent strabismus	3/10	–
Almond-shaped palpebral fissures	9/10	+
Wide and depressed nasal bridge	10/10	+
Short nose with broad tip*	8/10	+
Underdeveloped alae nasi*	5/10	+
Anteverted nares	5/10	+
Long philtrum	8/10	+
Thin vermillion of the upper lip	8/10	+
Macrostomy	8/10	+
Microretrognathia*	2/10	+
Macrotia	8/10	+
Dysplastic soft ears with angulated antihelix and underdeveloped helix*	2/10	+
Longitudinal incisions on both earlobes*	2/10	+
Short broad neck	6/10	+
Long fingers	2/10	+
Polydactyly	0/10	+
Onychodystrophy	2/10	+
Camptodactyly	4/10	–
Unilateral single palmar crease	5/10	+
Long toes	5/10	+
Broad first toes	5/10	+
Knee joint contracture	0/10	+
Impaired posture	9/10	–
Congenital heart defect	8/10	+
Brain defect	9/10	–
Cryptorchidism	8/10	+
Reduced penis	7/10	+

* Some patients have clinical symptoms in the photographs; however, in the articles themselves, the authors do not mention these symptoms. ** According to de Leeuw et al. [2010], Honda et al. [2010], Czeschik et al. [2013], Vandewalle et al. [2013], and Thunstrom et al. [2015].

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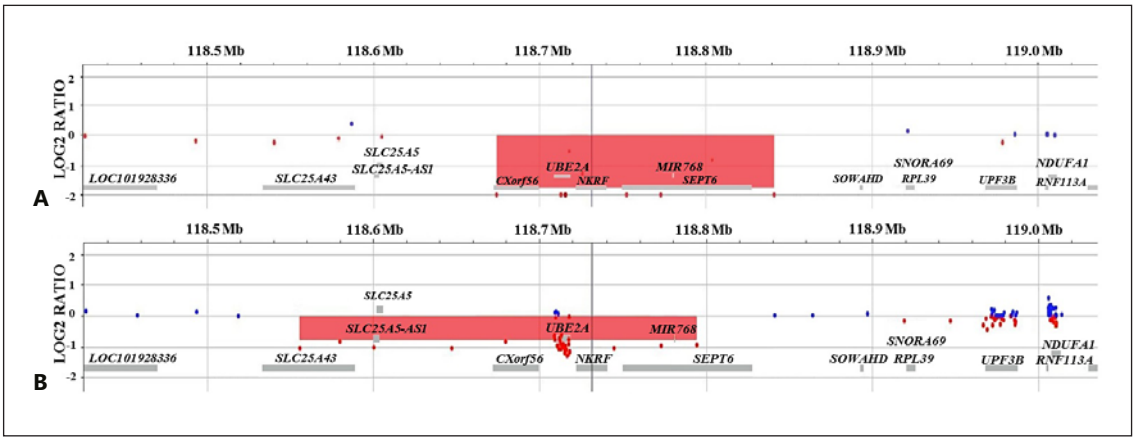

Fig. 3. aCGH image of the Xq24 region for the patient from Family 1 (**A**) and the patient from Family 2 (**B**).

Assessment of Skewed X-Chromosome Inactivation

The XCI status was determined using a method based on the amplification of high polymorphic CAG repeats in exon 1 of the androgen receptor gene (*AR*) [Allen et al., 1992]. Samples of native DNA and DNA that was previously enzymatically digested with methylation-sensitive *Hpa*II endonuclease were used as templates. The degree of XCI was calculated according to Lau et al. [1997].

The molecular cytogenetic and molecular genetic analyses were performed in the "Medical Genomics" Core Facility of the Tomsk National Research Medical Center of the Russian Academy of Sciences using the resources of the biocollection "Biobank of population of Northern Eurasia."

Results

aCGH revealed a 168-kb deletion, arr[hg19] Xq24(118673728_118841277)×0, in a boy with intellectual deficiency (Fig. 2A; V-4) and in his healthy mother, arr[hg19] Xq24(118673728_118841277)×1 (Fig. 2A; IV-2) in Family 1. The genes located in the deleted region were *CXorf56, UBE2A, NKRF, SEPT6,* and *MIR766* (Fig. 3A). The deletion was inherited from an apparently healthy mother and grandmother (Fig. 2A; III-2). The presence of the deletion was confirmed by real-time PCR (Fig. 4). The mother and grandmother demonstrated sXCI in blood lymphocytes (98 and 100%, respectively) and buccal epithelium (95 and 100%, respectively).

The larger Xq24 microdeletion was 239 kb in size and involved 8 genes (*SLC25A43*, *SLC25A5-AS1*, *SLC25A5*, *CXorf56*, *UBE2A*, *NKRF*, *SEPT6*, and *MIR766*); this deletion was identified in the woman (Fig. 2B; II-3) from Family 2 by aCGH, arr[hg19] Xq24(118555586_118794279)×1 (Fig. 3B) and was confirmed by real-time PCR (Fig. 4). sXCI levels in the patient's peripheral blood lymphocytes, buccal epithelium and endometrium were 100, 95 and 98%, respectively. This microdeletion was inherited from her mother (Fig. 2B; I-2), who also had 100% sXCI in her blood lymphocytes. delXq24 was also detected in 2 male missed abortions (Fig. 2B; III-6, III-10) by aCGH and real-time PCR (Fig. 4). Another spontaneous abortion with a 46,XX karyotype (Fig. 2B; III-8) had an Xq24 microdeletion, but the analysis of its extraembryonic mesoderm revealed a random pattern of XCI (60/40). The 2-year-old healthy daughter (Fig. 2B; III-7) of the patient did not have the Xq24 microdeletion, according to real-time PCR. The X inactivation in her blood leukocytes and buccal epithelium was random (50/50). The woman's sister (Fig. 2B; II-2) did not have an Xq24 microdeletion, and XCI in her blood leukocytes was within the accepted normal variation (70/30). She has 2 healthy children: a boy and a girl. Therefore, the segregation of the Xq24 microdeletion in both reported families was accompanied by an extremely skewed X inactivation in at least 2 generations.

Discussion/Conclusion

delXq24 is associated with a syndromic X-linked intellectual disability (XLID) named UBE2A deficiency syndrome or mental retardation, X-linked, syndromic, Nascimento type (MIM 300860). This disease is characterized by a moderate-to-severe intellectual disability and various facial dysmorphic features. This is a very rare X-linked disease. To date, only 10 patients with different

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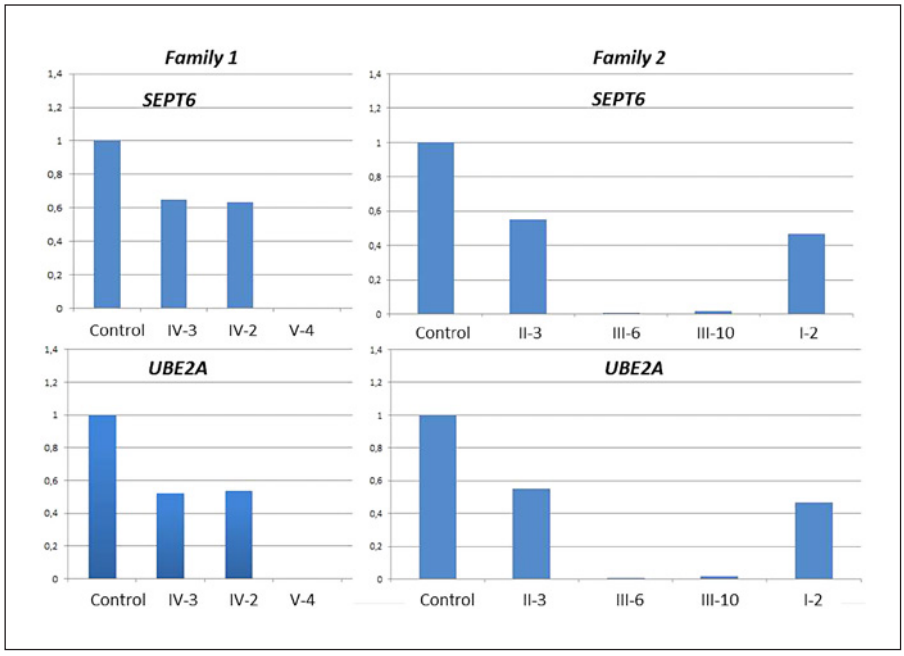

Fig. 4. The results of quantitative real-time PCR analyses in Family 1 and Family 2.

UBE2A mutations and 8 cases of Xq24 microdeletions with *UBE2A* involvement have been reported [Nascimento et al., 2006; Budny et al., 2010; de Leeuw et al., 2010; Honda et al., 2010; Czeschik et al., 2013; Haddad et al., 2013; Tucker et al., 2013; Thunstrom et al., 2015; Tsurusaki et al., 2017]. In addition, one patient with an Xq24 microduplication with *UBE2A* involvement [Takenouchi et al., 2012] and 2 deletion variants not affecting this gene [Vandewalle et al., 2013] have also been reported. In families with mutations affecting the *UBE2A* gene, all men with a mutant X chromosome were affected, while female carriers were always healthy but had 98–100% sXCI [Czeschik et al., 2013; Thunstrom et al., 2015].

All patients with UBE2A deficiency syndrome can be divided into 2 groups: (I) those with intragenic mutations (either point mutations or small deletions) and (II) those with chromosomal microdeletions that also include other genes in addition to *UBE2A*. Patients with microdeletions usually had a higher prevalence of brain white matter defect (BWMD) (6/7) and urogenital malformations (UM) (8/9) than patients with intragenic deletions (0/1 BWMD and 1/3 UM), missense mutations (1/6 BWMD and 6/10 UM) or truncating mutations (2/4 BWMD and 4/6 UM). Moreover, heart defects were also more common among patients from group II [Thunstrom et al., 2015].

The patient from Family 1 had one of the smallest microdeletions at Xq24 reported in the literature. The microdeletion included 5 genes: *UBE2A*, *CXorf56*, *NKRF*, *SEPT6*, and *MIR766*. This region is a part of larger reported Xq24 microdeletions (Fig. 5). Our proband has the following features common with other patients from the literature: delayed psychomotor and speech development, muscle hypotonia, seizures, a congenital heart defect (defect of the interventricular septum), anomalies of the genital organs and others (Table 1). At the same time, the index patient did not have the following symptoms described in the literature: visual and hearing impairments, impaired posture, synophrys, alternating divergent strabismus, camptodactyly, anomalies of other internal organs (Table 1). The unique symptoms in our patient were relative macrocephaly, protruding frontal tubercles, deep-set eyes, longitudinal incisions on both earlobes, mesoaxial polydactyly on the left foot, and right knee joint contracture (Fig. 1; Table 1).

All patients described in the literature with a hemizygous mutation of *UBE2A* have moderate-to-severe intellectual deficiency with no or very poor speech [Thunstrom et al., 2015]. However, the Xq24 microdeletion in Family 2 reported here was not related to any phenotype associated with intellectual deficiency. Here, for the first time, we report a carrier of a Xq24 microdeletion with recurrent pregnancy loss. The Xq24 microdeletion in this family affects 8 genes: *SLC25A43*, *SLC25A5-AS1*, *SLC25A5*, *CXorf56*, *UBE2A*, *NKRF, SEPT6,* and *MIR766.* Significantly, the size and location of the Xq24 microdeletion in this woman do not differ from those of previously

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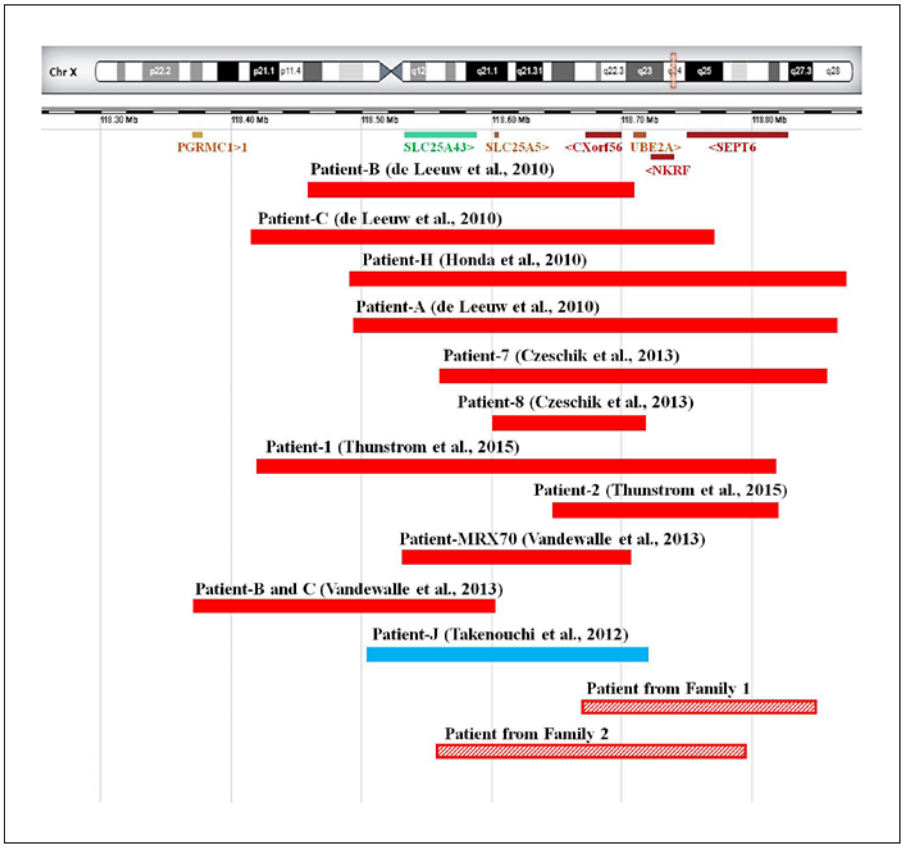

Fig. 5. A schematic scale-fitted presentation of the deleted regions of the previously reported patients with UBE2A deficiency syndrome (red bars), Xq24 microduplication (blue bar), and Xq24 microdeletions in patients from Families 1 and 2 (striped bars).

described deletions in viable patients with intellectual disability, and the microdeletion includes the same list of genes, providing evidence that this microdeletion may have different manifestations at different stages of pre- and postnatal human development, a phenomenon known as developmental or ontogenetic pleiotropy [Paaby and Rockman, 2013].

The affected genes are involved in cell metabolism, and some are directly related to cell proliferation. RAD6 protein encoded by *UBE2A* gene acts as an E2 ubiquitin-conjugating enzyme that binds certain E3 ubiquitin ligases in order to transfer ubiquitin to target proteins. Recently, several RAD6 interacting E3 ubiquitin ligases and respective targeted proteins have been identified. Interaction of RAD6 with Parkin targets certain mitochondrial proteins for degradation and facilitates death of dysfunctional mitochondria in cells [Haddad et al., 2013]. Another important process where RAD6 interacting with E3 ubiquitin ligases RNF20/40 or RAD18 was shown to be involved is histone ubiquitination and DNA damage repair [Hoege et al., 2002; Wojcik et al., 2018].

We did not find other reports on reproductive problems in obligate carriers of a mutation in the *UBE2A* gene or chromosomal microdeletions affecting the Xq24 region. However, it was previously shown that knockout mice for the gene *Mhr6a*, which is the mouse homologue of human *UBE2A*, have significant reproductive problems [Roest et al., 2004]. Homozygous $Mhr6a^{-/-}$ females cannot produce offspring, despite normal ovarian histology and ovulation. The absence of the *Mhr6a* product in oocytes stopped the development beyond the embryonic 2-cell stage, but did not disrupt the pattern of histone H3 methylation at this early stage of mouse embryonic development. $Mhr6a^{+/-}$ females were fertile, but the number of $Mhr6a^{-/y}$ males born was substantially less than the number of $Mhr6a^{+/y}$ males ($p < 0.001$). It is likely that a deficiency in the product of this gene can negatively affect the viability of the embryo. In addition to participation in histone H2B modifications, the *UBE2A* gene controls the processing of the protein p53, which plays a key role in cell proliferation [Chen et al., 2012].

The *MIR766* gene encodes a microRNA, which is also an important regulator of the p53 signaling pathway. This

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microRNA inhibits one of the key inhibitors of the p53 protein MDM4 [Wang et al., 2017].

The SLC25 family consists of mitochondrial proteins, which are carriers of soluble substances that are located in the inner mitochondrial membrane. These proteins mediate the transfer of a variety of intermediate metabolic products (ATP/ADP, carboxylic and amino acids, and ions) between the cytoplasm and mitochondria. *SLC25A43* participates in mitochondrial control of cell cycle progression [Tina et al., 2012; Gabrielson and Tina, 2013]. Cell entry into the G1 phase of the cell cycle is associated with a burst of mitochondrial activity; the G1-S transition also increases the consumption of mitochondrial oxygen. Knockdown of the *SLC25A43* gene in immortalized noncancerous breast epithelial cells of MCF10A significantly inhibited the progression of the cell cycle during the G1-S transition, which significantly reduced the proliferation rate and the proportion of Ki-67-positive MCF10A cells. In contrast, the suppressed expression of *SLC25A43* in the HER2-positive breast cancer cells BT-474 resulted in a significant increase in the proliferation rate along with an improved G1-S transition. This resulted in an increase in the proportion of positive Ki-67 cells and a lower level of nuclear p21. Thus, the role of *SLC25A43* as a regulator of the cycle progression was demonstrated.

SLC25A5 is a highly conserved gene that is ubiquitously expressed at a high level in the cortex and hippocampus and has a presumed role in the mitochondrial exchange of ADP/ATP. This gene is involved in memory formation or establishment, which could add mitochondrial processes to the wide array of pathways that regulate normal cognitive functions.

It is also important to note that both genes have been implicated in the apoptotic pathway. The morphogenesis of the digits from the pad-like limb bud structure relies on the apoptotic process to remove the interdigital membrane. Thus, excessive apoptotic signaling within the interdigital membrane may lead to split-hand malformation [Takenouchi et al., 2012].

Both genes are expressed in the brain. Moreover, *SLC25A5* is one of the genes associated with nonsyndromic intellectual disability [Vandewalle et al., 2013]. Men with deletions of this gene had intellectual deficiency, whereas women carriers of deletions were normal but had extremely high sXCI.

The functions of the *CXorf56* gene have been poorly investigated. Endogenous CXorf56 protein expression has been identified in the mouse brain cortex and cerebellar neurons in the nucleus and cell soma. Recently, it was shown that insertion into the *CXorf56* gene, leading to the appearance of a premature stop codon and nonsense mRNA, led to the development of a familial form of intellectual disability (OMIM 301013). This mutation was described in 3 generations of a large Dutch family, in which 5 men and 1 woman had weak or moderate intellectual disability. Two men had serious behavioral problems, such as ubiquitous developmental disorder, attention deficit, hyperactivity and transgressive behavior. Patients had different facial dysmorphic features. In this case, similar to UBE2A deficiency syndrome, all male mutation carriers are affected, and female carriers have sXCI [Verkerk et al., 2018].

The product of the *NKRF* gene encodes a transcriptional repressor that interacts with specific negative regulatory elements to mediate the transcriptional repression of certain nuclear factor kappa B-responsive genes.

The *SEPT6* gene encodes one of the septins that belong to the group of GTP-binding proteins or G-proteins that are involved in many processes, including cell division, cytoskeleton organization, and cytokinesis. The dysregulation of septins leads to neurodegenerative diseases [Neubauer and Zieger, 2017].

In contrast to intellectual deficiency, it was not possible to determine "the causative gene" for embryo developmental defects among the genes involved in the Xq24 microdeletion in 2 male spontaneous abortions (III-6 and III-10) and a female miscarriage with random X inactivation (III-8) from Family 2 in our study because it was not possible to compensate for the deleterious effect of the Xq24 microdeletion. However, it is known that UBE2A deficiency syndrome, in addition to intellectual deficiency, is also characterized by multiple dysmorphic features. Most likely, mechanisms leading to developmental defects can affect the viability of the embryo, as is the case for Barth syndrome (MIM 302060), which is also associated with sXCI [Orstavik et al., 1998]. Barth syndrome is a rare X-linked genetic disease characterized by cardiomyopathy, skeletal myopathy, growth delay, neutropenia, and increased urinary excretion of 3-methylglutaconic acid (3-MGCA). The causative mutation affects the *TAZ* gene, leading to a knockdown of its expression. For one-third of families, this syndrome is characterized by a high proportion of prenatal (approximately 18 weeks of gestation) or postnatal deaths of males carrying the mutation [Clarke et al., 2013]. It is supposed that the cause of embryo death is cardiomyopathy.

Interestingly, sXCI is a common feature in carriers of many XLIDs [Plenge et al., 2002; Gieldon et al., 2017]. Obviously, this can be explained by the fact that muta-

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tions in X-linked genes affect the viability of cells. Genes responsible for syndromic XLID are expressed in various tissues, and their products are regulators of transcription, cell proliferation, and development [Plenge et al., 2002]. One can suggest that the disturbance of cell proliferation can be the cause of not only sXCI and XLID but also early embryo or fetal death, which is the reason for recurrent pregnancy loss for a carrier of the Xq24 microdeletion. However, the relationship between sXCI and mutations, deletions, or duplications of XLID genes may be random and arise from the fact that there are many X-linked genes responsible for brain development.

In conclusion, for the first time, the 2 presented cases indicate that women with the Xq24 microdeletion and sXCI may be in a risk group for having a child with intellectual disability or for experiencing pregnancy loss. Our data highlight the enlargement of the boundaries of the ontogenetic pleiotropy of recurrent Xq24 chromosomal microdeletions and their incomplete penetrance modified by sXCI.

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Statement of Ethics

The study was approved by the Ethics Committee of the Research Institute of Medical Genetics, Tomsk National Research Medical Center of the Russian Academy of Sciences. Informed consent for research was obtained from the parents in both families.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

E.N. Tolmacheva performed the integrated analysis of all clinical and molecular cytogenetic data, investigated the X-chromosome inactivation status in both families, wrote the text of the article, and read and approved the submitted version of the manuscript. A.A. Kashevarova wrote the text of the article, took part in the discussion of results, read and approved the submitted version of the manuscript. L.P. Nazarenko performed the clinical research management and read and approved the submitted version of the manuscript. L.I. Minaycheva performed the clinical investigation of patients and read and approved the submitted version of the manuscript. N.A. Skryabin performed the array-based comparative genomic hybridization and read and approved the submitted version of the manuscript. M.E. Lopatkina performed the confirmation of array-based comparative genomic hybridization results by quantitative real-time PCR and read and approved the submitted version of the manuscript. T.V. Nikitina performed the cytogenetic analyses and read and approved the submitted version of the manuscript. E.A. Sazhenova performed the cytogenetic analyses and read and approved the submitted version of the manuscript. E.O. Belyaeva performed the clinical investigation of patients and read and approved the submitted version of the manuscript. E.A. Fonova investigated the X-chromosome inactivation status in both families and read and approved the submitted version of the manuscript. O.A. Salyukova performed the clinical investigation of patients and read and approved the submitted version of the manuscript. V.S. Tarabykin wrote the text of the article, took part in the discussion of results, read and approved the submitted version of the manuscript. I.N. Lebedev performed the planning of the study and conception of the research, discussed the results, read and approved the submitted version of the manuscript.

All authors agree to take responsibility for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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