Original Article

Molecular Syndromology

Mol Syndromol 2010;1:311–315
DOI: 10.1159/000331323

Accepted: July 23, 2011
by A. Rauch
Published online: September 14, 2011

Whole Xp Deletion in a Girl with Mental Retardation, Epilepsy, and Biochemical Features of OTC Deficiency

K. Joost[a, g] P. Tammur[e] R. Teek[e] O. Žilina[b, e] M. Peters[c] M. Kreile[h] B. Lace[h] R. Žordania[g] I. Talvik[d, f] K. Õunap[d, e]

[a]The Centre of Excellence for Translational Medicine, [b]Department of Biotechnology, Institute of Cell and Molecular Biology, [c]Department of Obstetrics and Gynecology, and [d]Department of Pediatrics, University of Tartu, [e]Department of Genetics, United Laboratories, and [f]Children's Clinic, Tartu University Hospital, Tartu, and [g]Tallinn Children's Hospital, Tallinn, Estonia; [h]Medical Genetics Clinic, Children Clinical University Hospital, Riga, Latvia

Key Words

Mental retardation · Ornithine transcarbamylase (OTC) deficiency · Skewed X-inactivation · Turner syndrome · Xp deletion

Abstract

Background: Females with a total or partial deletion of the short arm of the X chromosome have variable features of Turner syndrome, but mental retardation (MR) rarely occurs. The haploinsufficiency of deleted genes that escape X-inactivation may explain the occurrence of MR and autism. Ornithine transcarbamylase (OTC) deficiency is the most common urea cycle disorder and is inherited in an X-linked semidominant trait, and the *OTC* gene maps to Xp21. ***Methods:*** We report on a girl with MR, epilepsy and biochemical changes characteristic of OTC deficiency but no identifiable point mutation in the *OTC* gene. Standard G-banding cytogenetic analysis, whole genome karyotyping, and X-inactivation studies were performed to determine the genetic etiology of the OTC deficiency in the patient. ***Results:*** Cytogenetic analysis and molecular karyotyping using SNP array revealed a deletion of the whole short arm of the X chromosome (Xp22.33–p11.1). Inactivation studies also revealed a completely skewed X-inactivation. ***Conclusion:*** Our patient presented with MR, epilepsy, and some evidence of reduced OTC activity, but performed genetic studies gave no explanation for this phenotype. We hope that this case report contributes to the understanding of the underlying genetic factors of the manifestation of X-linked disorders in female patients.

The classic phenotype of Turner syndrome includes short stature, ovarian failure, and variable somatic stigmata including pigmented naevi. Females with a total or partial deletion of the short arm of the X chromosome have variable features of Turner syndrome [Lachlan et al., 2006]. The short stature and skeletal features of Turner syndrome are due to haploinsufficiency of the *SHOX* gene located in the pseudoautosomal region in Xp22 [Rao et al., 1997]. Mental retardation (MR) very rarely occurs in patients with complete or partial deletion of Xp [Schinzel, 2001]. Lachlan et al. [2006] have shown that the size of the deletion is related to the skewing of the X chromosome: if the breakpoint is situated proximal of Xp22.3, the aberrant X chromosome is preferentially in-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1661–8769/10/0016–0311$38.00/0

Accessible online at:
www.karger.com/msy

Kairit Joost
The Centre of Excellence for Translational Medicine
University of Tartu, 19 Ravila Street
EE–50411 Tartu (Estonia)
Tel. +372 5662 6204, E-Mail kairit.joost@ut.ee

activated. Usually, skewing has no biological consequences, but in rare cases, where skewing occurs toward a nonlethal mutated allele, it can expose a female carrier of an X-linked trait to the pathological phenotype [Bolduc et al., 2008].

Ornithine transcarbamylase (OTC) deficiency is the most common urea cycle disorder and is inherited in an X-linked semi-dominant trait. Symptoms in carrier females are variable; some are completely asymptomatic, while others have episodes of severe hyperammonemia that can lead to brain damage or even death [Yorifuji et al., 1998]. The *OTC* gene (MIM ID *300461) maps to Xp21 [Hata et al., 1988], and nearly 350 different mutations have been reported and found in about 80% of patients. It has been hypothesized that the remaining 20% of cases may be caused by mutations in the promoter region or introns of the *OTC* gene and by locus heterogeneity or copy number variants such as microdeletions [Yamaguchi et al., 2006].

We herein present an 8.5-year-old girl with MR, epilepsy, and some evidence of OTC deficiency and describe her clinical picture and genetic investigations in detail.

Case Report

The girl was born from the mother's 3rd pregnancy and 3rd delivery in the year 2000. One elder brother died at the age of 4 days due to cardiac anomaly. The girl's birth weight was 3,128 g (–1 SD), length 48 cm (–1.5 SD), and her Apgar score was 8/8. On day 2 she was admitted to the intensive care unit due to breathing difficulties and hypoglycemia, and meconium aspiration was diagnosed. Episodic hypoglycemia was observed within the first 3 weeks of life, but no specific metabolic investigations were performed in the newborn period. At the age of 2 months, during an episode of acute bronchitis, hepatomegaly was observed. EEG showed nonspecific paroxysmal activity, and treatment with phenobarbital was started. Ammonia was for the first time increased, i.e., 90 μmol/l (reference <48 μmol/l) at the age of 3 months. The first signs of developmental problems were noticed at the age of 6 months, and at 9 months, psychomotor retardation was diagnosed. Also at the age of 9 months, a high level of blood ammonia became apparent (297 μmol/l). Amino acid analysis showed no detectable citrulline in serum, and in urinary organic acid GC/MS analysis slightly elevated excretion of orotic acid (47.7 μmol/g creatinine; reference 1.7–35 μmol/g creatinine) was seen. Citrulline in serum was constantly low during the first 2 years of life (in range from 0–18 μmol/l; reference 8–52 μmol/l). The highest glutamine level in serum was 1,951 μmol/l (reference 350–720 μmol/l) at the age of 2 years. Thereafter, an allopurinol loading test was performed at the Charité-Virchow Klinikum (Berlin, Germany). This test showed a significant increase in the excretion of orotic acid (3–4 times over the upper normal limit 12–24 h after loading). Based on the metabolic investigations, the girl was suspected to be affected with an OTC deficiency (mild form). The treatment with oral citrulline (5 g/day) and a low-protein diet was initiated at the age of 2 years. Since that age, all metabolic characteristics (ammonia and amino acids) have been in normal range.

At the age of 12 months she had the first generalized seizures. EEG showed generalized complexes of spike waves. Brain MRI showed bilateral white matter damage in the periventricular region. A treatment with carbamazepine was started (20 mg/kg/day) and continued simultaneously to the low-protein diet and citrulline.

At the age of 7.5 years she was investigated at Tartu University Hospital, as despite the normal ammonia level and antiepileptic treatment, the patient's seizure control was poor. Our clinical evaluation revealed a short stature (height 111 cm (–3 SD), weight 19 kg (–2 SD), occipitofrontal head circumference 48.5 cm (–2 SD)), and developmental delay. In addition, she had slightly upslanting eyes, widely spaced teeth, a high-arched palate, low-set and posteriorly rotated dysmorphic ears, and many pigmented naevi and a large (10 × 10 cm) depigmented area in the upper spinal region. Anticonvulsive treatment was changed to lamotrigine (7 mg/kg/day), and in addition the treatment with sodium phenylbutyrate (Ammonaps) was initiated (395 mg/kg/day) for a period of 1 month, with a positive effect on seizure control.

One year later, at the age of 8.5 years, she was readmitted to the hospital. Her height remained low (–3 SD), and moderate MR was diagnosed. Neuropsychological evaluation with a Kaufmann ABC test revealed impaired abilities in all areas. She had a more pronounced attention deficit, difficulties in exercises demanding analysis and synthesis, and limited short memory. She could read simple words, but she did not know numbers. She was able to scope in daily activities like walking, dressing, and eating. In addition to her facial dysmorphy and skin pigmentation anomalies, brain MRI showed partial agenesis of the corpus callosum and bilateral white matter damage in the periventricular region. Regarding all results, this raised questions concerning the correctness of the diagnosis of OTC deficiency. Additional investigations to establish the genetic etiology were performed. According to those results, the deletion of the short arm of the X chromosome and a skewed X-inactivation was diagnosed in the patient.

Results

Cytogenetic and molecular investigations in the patient gave the following results:

(1) Bidirectional sequencing of all 10 coding exons of the *OTC* gene including intron-exon boundaries was performed using the ABI PRISM 3100 Genetic Analyzer, but no genetic variations within coding regions were identified.

(2) Standard G-banding cytogenetic analysis was performed on peripheral blood lymphocytes according to standard methods and showed an abnormal karyotype 46,X,del(X)(p11). Therefore, Turner syndrome due to the loss of the short arm of the X chromosome was diagnosed in the patient (fig. 1).

(3) Whole genome karyotyping analysis using Human CytoSNP-12 BeadChips (Illumina Inc., San Diego, Calif.,

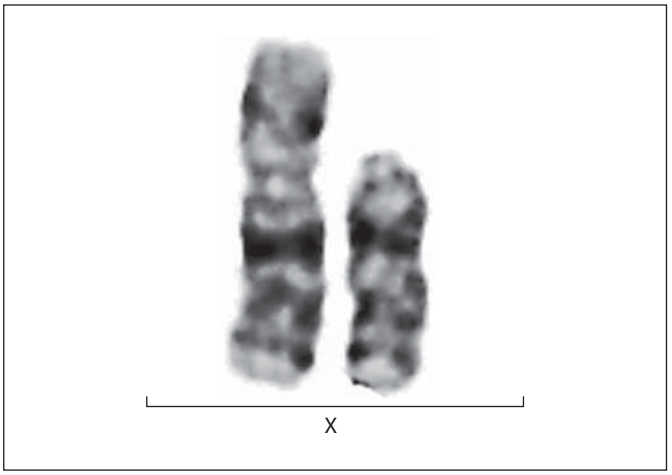

Fig. 1. X chromosomes of the patient with the karyotype 46,X,del(X)(p11).

USA) confirmed the deletion of the whole short arm of the X chromosome. The deletion involved the Xp22.33–p11.1 region, and the borders of the deletion were found to be the following: 1–56,975,659 bp (NCBI build 36). The data was analyzed using Illumina BeadStudio v3.1 and QuantiSNP v1.1 software [Colella et al., 2007].

(4) For the evaluation of the X-inactivation status, the androgen receptor *(AR)* (CAG)n and fragile X mental retardation 1 *(FMR1)* (CGG)n variable repeat region was examined. X-chromosome inactivation (XCI) pattern was determined by comparative quantitative detection of fluorescent-labeled PCR products using intact and methylation-sensitive restriction enzyme *Hpa*II-digested [Bolduc et al., 2008] and *Hha*I-digested DNA templates, respectively. *FMR1* PCR was performed using the *FMR1* sizing PCR Set (Abbott Molecular, Abbott Park, Ill., USA) according to the manufacturer's protocol. Amplicon size and inactivation ratios were determined by electrophoresis on an ABI 3130XL Genetic Analyzer (PE Applied Biosystems) and analyzed using GeneScan 4.0 software (PE Applied Biosystems). The XCI study showed that the aberrant X chromosome was completely skewed in our patient (fig. 2).

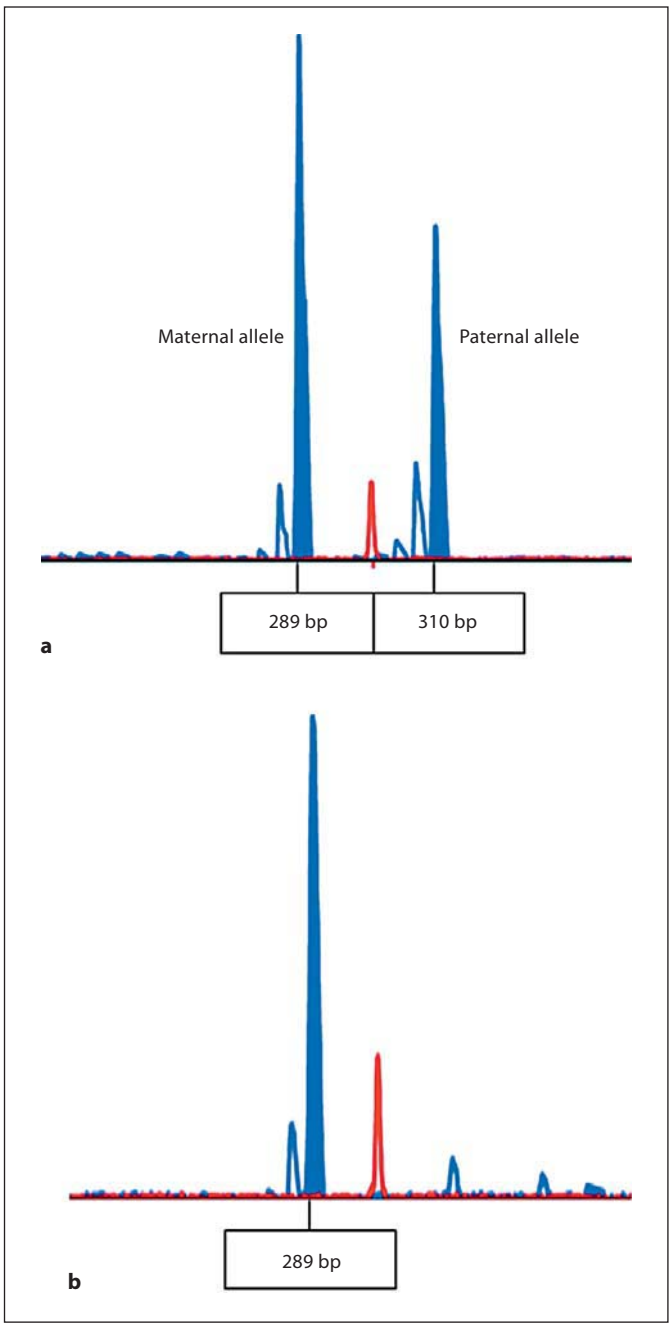

Fig. 2. Results of the *FMR1* methylation assay. **a** Undigested DNA of the patient. **b** DNA of the patient predigested with *Hha*I, showing an extremely skewed X-inactivation pattern.

Discussion

Our patient presented relatively nonspecific symptoms in the newborn period, but later she developed psychomotor retardation and seizures. Based on biochemical abnormalities, OTC deficiency was suspected and respective treatment was started. Sequencing of the *OTC* gene was performed for final confirmation of the diagnosis in the patient. However, no pathogenic change of the *OTC* gene was found, and therefore, further cytogenetic and molecular studies were indicated to explain the bio-

chemical changes observed in the patient. As a result, deletion of the whole short arm of the X chromosome and completely skewed X-inactivation were detected.

Females with partial deletions of the short arm of the X chromosome have variable phenotypes, but MR rarely occurs [Schinzel, 2001; Lachlan et al., 2006]. There are few cases reported with small deletions located in Xp22 who had MR or developmental delay and/or autistic features [Thomas and Huson, 2001; Chocholska et al., 2006; Lachlan et al., 2006; Puusepp et al., 2008; Shinawi et al., 2009]. Haploinsufficiency of deleted genes that escape X-inactivation may explain the occurrence of autism and developmental delay in the previously described cases [Shinawi et al., 2009].

Lachlan et al. [2006] have shown that if the breakpoint of an X chromosome deletion was proximal to Xp.22.3, there was a non-random X-inactivation, with the deleted X being preferentially inactivated. XCI study of our patient showed also a completely skewed X-inactivation. The variability in the phenotype associated with Xp deletions can be explained by different mechanisms. A mosaicism for a normal cell line in other tissues that are not routinely tested by cytogenetic methods can be present [Thomas and Huson, 2001]. Unfortunately, it was not possible to perform karyotyping of skin fibroblasts (normal skin and depigmented areas) from our patient. The degree of expression of genes that escape inactivation from the inactive X chromosome is variable among different females [Carrel and Willard, 2005], and the degree of skewed X-inactivation can be variable in different tissues [Sharp et al., 2000].

Our patient had some evidence of reduced OTC activity as expected in a heterozygous female, but genetic studies gave no explanation. The first cytological demonstration of cellular mosaicism in an obligate heterozygote of OTC was provided by Ricciuti et al. [1976]. The authors stated that the patchy distribution of OTC-positive cells within the liver provided an explanation both for the marked variation in OTC activity observed in different heterozygous females and for variable OTC activities in repeated biopsies from the same patient. The X-inactivation of a manifesting female with OTC deficiency has been previously studied by Yorifuji et al. [1998] who performed an *AR* assay using genomic DNA extracted from liver samples to study the skewing of the X chromosome. The results were then compared with residual enzyme activity. A close correlation was observed, and the authors stated that residual enzyme activity is actually determined by the X-inactivation status in the liver. Unfortunately, there was no liver sample available from our patient. We realize that our studies are insufficient for drawing a correlation between X-inactivation and the severity of clinical manifestations, but they shed some light on the complexity of the genetic mechanisms that are sometimes involved in the manifestation of genetic disorders.

As our patient has MR and epilepsy, the question arises whether the patient has any other symptoms of haploinsufficiency of the genes located in the deleted region. There are at least 22 genes related to MR, and some of these are also connected to seizures, according to the Online Mendelian Inheritance in Man (OMIM) database (http://www.ncbi.nlm.nih.gov/omim/). These genes may contribute to the cognitive problems and epilepsy in our patient. Another possible cause for her symptoms is an encephalopathy due to hyperammonemic crises in infancy and childhood.

The characterization of the precise nature of the genetic mechanisms involved in the presentation of the X-linked disorder in the patient made possible not only an accurate genetic consultation for the family, but also a better surveillance in the future. Due to Turner syndrome, a treatment with growth hormone is considered.

In conclusion, we hope that this case report will contribute to the understanding of the underlying genetic factors of the manifestation of X-linked disorders in female patients.

Acknowledgements

We would like to thank the family and the patient for their kind cooperation. This study was supported by Estonian Science Foundation grant GARLA 8175 and by Estonian Ministry of Education and Science grant no. 0180044s09. We are very grateful to Prof. E. Mönch from the Charité-Virchow Klinikum, Berlin, Germany for performing urinary organic acid analyses in the years 2001–2002.

References

Bolduc V, Chagnon P, Provost S, Dube MP, Belisle C, et al: No evidence that skewing of X chromosome inactivation patterns is transmitted to offspring in humans. J Clin Invest 118:333–341 (2008).

Carrel L, Willard HF: X-inactivation profile reveals extensive variability in X-linked gene expression in females. Nature 434:400–404 (2005).

Chocholska S, Rossier E, Barbi G, Kehrer-Sawatzki H: Molecular cytogenetic analysis of a familial interstitial deletion Xp22.2–22.3 with a highly variable phenotype in female carriers. Am J Med Genet A 140:604–610 (2006).

Colella S, Yau C, Taylor JM, Mirza G, Butler H, et al: QuantiSNP: An Objective Bayes Hidden-Markov Model to detect and accurately map copy number variation using SNP genotyping data. Nucleic Acids Res 35:2013–2025 (2007).

Hata A, Tsuzuki T, Shimada K, Takiguchi M, Mori M, Matsuda I: Structure of the human ornithine transcarbamylase gene. J Biochem 103:302–308 (1988).

James RS, Coppin B, Dalton P, Dennis NR, Mitchell C, et al: A study of females with deletions of the short arm of the X chromosome. Hum Genet 102:507–516 (1998).

Lachlan KL, Youings S, Costa T, Jacobs PA, Thomas NS: A clinical and molecular study of 26 females with Xp deletions with special emphasis on inherited deletions. Hum Genet 118:640–651 (2006).

Puusepp H, Zordania R, Paal M, Bartsch O, Ounap K: Girl with partial Turner syndrome and absence epilepsy. Pediatr Neurol 38: 289–292 (2008).

Rao E, Weiss B, Fukami M, Rump A, Niesler B, et al: Pseudoautosomal deletions encompassing a novel homeobox gene cause growth failure in idiopathic short stature and Turner syndrome. Nat Genet 16:54–63 (1997).

Ricciuti FC, Gelehrter TD, Rosenberg LE: X-chromosome inactivation in human liver: confirmation of X-linkage of ornithine transcarbamylase. Am J Hum Genet 28:332–338 (1976).

Schinzel A: Catalogue of Unbalanced Chromosome Aberrations in Man (Walter de Gruyter, Berlin 2001).

Sharp A, Robinson D, Jacobs P: Age- and tissue-specific variation of X chromosome inactivation ratios in normal women. Hum Genet 107:343–349 (2000).

Shinawi M, Patel A, Panichkul P, Zascavage R, Peters SU, Scaglia F: The Xp contiguous deletion syndrome and autism. Am J Med Genet A 149A:1138–1148 (2009).

Thomas NS, Huson SM: Atypical phenotype in a female with a large Xp deletion. Am J Med Genet 104:81–83 (2001).

Yamaguchi S, Brailey LL, Morizono H, Bale AE, Tuchman M: Mutations and polymorphisms in the human ornithine transcarbamylase *(OTC)* gene. Hum Mutat 27:626–632 (2006).

Yorifuji T, Muroi J, Uematsu A, Tanaka K, Kiwaki K, et al: X-inactivation pattern in the liver of a manifesting female with ornithine transcarbamylase (OTC) deficiency. Clin Genet 54:349–353 (1998).