

SHORT COMMUNICATION

Novel deletion at Xq24 including the *UBE2A* gene in a patient with X-linked mental retardation

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By using an in-house bacterial artificial chromosome-based X-tilling array, we detected a 0.4 Mb novel deletion at Xq24 that included *UBE2A* in a 4-year-old and 10-month-old boy with mental retardation and various other characteristics inherited from his mother; for example, marked developmental delay, synophrys, ocular hypertelorism, esotropia, low nasal bridge, marked generalized hirsutism and seizure. Although additional nine transcripts around *UBE2A* were also defective, a phenotypic similarity with a recently reported X-linked familial case involving a novel X-linked mental retardation syndrome and a nonsense mutation of *UBE2A* indicates a functional defect of *UBE2A* to be responsible for most of the abnormalities in these cases. Because some characteristics, such as congenital heart disease and proximal placement of the thumb, were not described in the family reported previously, suggesting genes other than *UBE2A* within the deleted region to be responsible for those abnormalities.

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X-linked gene defects have long been considered important causes of mental retardation (X-linked mental retardation, XLMR), given the observation that mental retardation is significantly more common in males than in females.¹ Approximately 90 genes involved in XLMR have now been identified through genetic linkage analysis and positional cloning, candidate gene analysis or molecular cytogenetic studies.^{2,3} Each of these accounts for only a small number of families with XLMR and, despite this success, the genes involved in most affected families still await identification.⁴

Recently, *UBE2A/HR6A*, one of the two human orthologs of *Saccharomyces cerevisiae RAD6/UBC2* encoding E2 conjugase, was identified as causative of a novel XLMR syndrome through a nonsense mutation.⁵ In the course of a program to screen possible patients with XLMR for copy-number aberrations by array-comparative genomic hybridization using a bacterial artificial chromosome (BAC)-based X-tilling array (MCG X-tilling array),⁶ we detected a novel 0.4 Mb deletion at Xq24 that included *UBE2A* in a 4-year-old and 10-month-old boy with mental retardation. Although additional nine transcripts around *UBE2A* were defective, phenotypic similarity between our patient and three males with a premature stop codon of this gene in a two-generation family⁵ indicates that a functional defect of *UBE2A* is responsible for a syndromic mental retardation.

The proband was born at 39 weeks of gestation by vaginal delivery as the first child of a 27-year-old father and a 27-year-old mother (Figure 1a). His birth height, weight and head circumference were 47 cm (10th percentile), 3330 g (85th percentile) and 32.5 cm (<50th percentile), respectively. A ventricular septal defect was found and surgically treated at 10 months of age. Developmental milestones were as follows: head control at 1 year and 6 months, sitting unaided at 4 years and severe speech impairment. At 1 year, he suffered a tonic-clonic seizure.

At 4 years and 10 months, he was referred to us for a clinical evaluation of developmental delay. His height and weight at age 4 years and 10 months were 99.5 cm (10th percentile) and 14.5 kg (10th percentile), respectively. He was noted to have the following craniofacial and other abnormalities: synophrys, ocular hypertelorism, esotropia, low nasal bridge, upslanted palpebral fissures, proximal placement of thumb and marked generalized hirsutism (Figure 1b). Brain magnetic resonance imaging showed white matter hypodensity and slight brain atrophy. Electroencephalography revealed sporadic spikes in the bilateral frontal and right central areas. Conventional chromosomal examination showed a normal male karyotype.

As the patient's mother was clinically unaffected and did not show any overt intellectual or adaptive impairment but the patient's brother,

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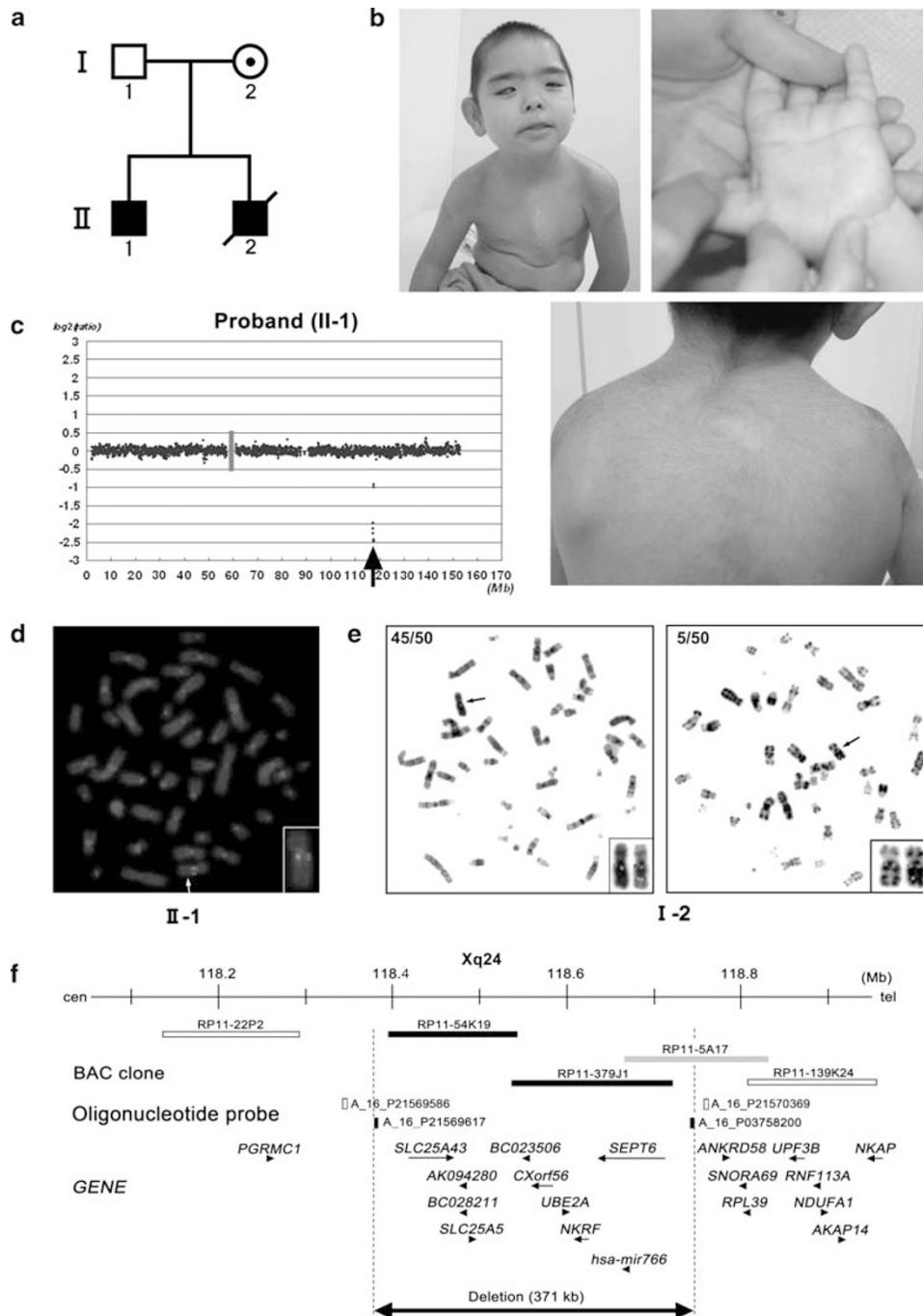


Figure 1 (a) Two-generation genealogy of the study family, showing the affected males (II-1, II-2) related through their clinically normal mother (I-2). (b) The patient (II-1) at age 4 years and 10 months showed synophris, whorls and marked hirsutism. (c) Profile of the copy-number ratio on chromosome X in patient II-1 detected with array-comparative genomic hybridization (aCGH) using an MCG X-tiling array. Clones are ordered according to the UCSC mapping position (<http://genome.ucsc.edu/>; assembly March 2006). Each spot represents the test/reference value after normalization and \log_2 transformation in each bacterial artificial chromosome (BAC) clone. An approximately 0.4 Mb deletion at Xq24 was detected based on the reduced ratios of four BAC clones (arrow). (d) Representative results of a fluorescence *in situ* hybridization (FISH) analysis in patient (II-1) using RP11-379J1 within the deleted region (green) at Xq24 and RP11-13M9, a reference clone at Xq13.2, (red) showed the complete deletion of RP11-379J1 on chromosome X (arrow). An enlarged image of chromosome X is shown in the lower right box. (e) Representative results of a late replication assay with FISH analysis in patient's mother (I-2) using the same clones in (c) showed the complete deletion of RP11-379J1 on chromosome X (arrow), indicating the deletion at Xq24 in patient (II-1) to be inherited from his mother. The del(X)(q24) showed late replicating pattern in 45/50 cells (90%, left), whereas normal X chromosome showed late replication pattern in 5/50 cells (10%, right). Arrows indicated inactivated chromosome X. An enlarged image of chromosome X is shown in the lower right box in each panel. (f) Schematic physical map of BAC clones and genes around the deleted region (closed arrow) in patient II-1 at Xq24. Color code of BACs: black, mapped within the deletion; gray, spanned the deletion breakpoint; white, located outside of the deletion. Color code of probes of the oligonucleotide-array (Human Genome CGH Microarray 244 K; Agilent Technologies): black, mapped within the deletion; white, located outside of the deletion. Genes with a 5'3' orientation around the deletion are indicated by arrows. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

who died suddenly at age 2 years and 4 months of unknown cause, was noted to have a characteristic phenotype similar to that in the patient, for example, marked developmental delay, synophrys, ocular hypertelorism, low nasal bridge, marked generalized hirsutism, seizure, proximal placement of thumb and heart diseases, including hypoplastic left heart syndrome and coarctation of the aorta, we assumed an X-linked pattern of inheritance for this family. Therefore, we performed array-comparative genomic hybridization using an MCG X-tilling array.⁶ This study has been approved by the ethics committees of the National Center of Neurology and Psychiatry, Japan and Medical Research Institute, Tokyo Medical and Dental University. All the subjects provided written informed consent for the use of their phenotypic and genetic data. A loss of genomic copy number between RP11-54K19 and RP11-379J1 on Xq24 with a deletion size at 0.4 Mb was identified (Figure 1c). Subsequently performed fluorescence *in situ* hybridization using BAC clones RP11-54K19, RP11-379J1 and RP11-5A11 (Figure 1d) and detailed oligonucleotide array-comparative genomic hybridization using Human Genome CGH Microarray 244 K (Agilent Technologies, Palo Alto, CA, USA; data not shown) narrowed down the deleted region to between positions 118377451 and 118748043. Although some copy-number variants were detected

on other regions simultaneously, all of them have been registered in Database of Genome Variation (<http://projects.tcag.ca/variation/>), suggesting these aberrations to be benign copy-number variants (Supplementary Table S1). Fluorescence *in situ* hybridization performed on the parents showed the same heterozygous deletion only in the mother (Figure 1d and data not shown), indicating a maternally inherited deletion in the proband. Our finding that the mother, a presumptive obligate carrier, had skewed X inactivation (del(X): X=45:5) in leukocytes, as shown by a late replication assay with fluorescence *in situ* hybridization⁶ (Figure 1e) supported our assumption that skewed X-chromosome inactivation appears to be characteristic of carriers of gene mutations involved in XLMR.⁷ The final diagnosis was arr Xq24(118377451–118748043)×0.

Because the deleted region contains *UBE2A*, a gene causative of a putative XLMR syndrome through a nonsense mutation,⁵ and his phenotype except the cardiovascular abnormalities and proximal placement of the thumb is almost concordant with that in reported cases of *UBE2A* nonsense mutations,⁵ for example, marked developmental delay, synophrys, ocular hypertelorism, low nasal bridge, marked generalized hirsutism and seizure (Table 1), the novel syndromic XLMR may be caused by a functional defect of *UBE2A* not

Table 1 Clinical characteristics of the patient and his younger brother, comparable to previously reported patients with *UBE2A* nonsense mutation (Nascimento *et al.*⁵)

Characteristic	Patient				
	Nascimento <i>et al.</i> ⁵			Our cases	
				II-1	II-2
Age at examination	46 years and 7 months	19 years and 11 months	5 years and 4 months	4 years and 10 months	2 years and 4 months
Birth weight (percentile)	50	90–97	>97	85	10
Height (percentile)	<3	10–25	10	10	<3
Weight (percentile)	>97	90	>97	10	<3
Head circumference (percentile)	>98	>98	50	3	NA
Hair whorls	+	+	+	+	NA
Wide face	–	+	+	+	+
Midface hypoplasia	–	+	+	+	+
Synophrys	+	+	+	+	+
Uplanted palpebral fissures	+	+	+	+	+
Ocular hypertelorism	–	+	–	+	+
Low nasal bridge	–	+	+	+	+
Large mouth with downturned corners and thin lips	+	+	+	+	+
Short, broad neck	+	+	+	+	+
Low posterior hairline	+	+	+	+	+
Widely spaced nipples	+	+	+	+	+
Small penis	+	+	+	+	+
Small, flat feet, with dorsum swelling	+	+	+	+	+
Onychodystrophy	+ ^a	+ ^a	–	–	+
Marked generalized hirsutism	+	+	+	+	+
Myxedematous appearance	+	+	+	+	+
Dry skin	+	+	+	+	+
Seizures	+	+	+	+	+
Severe speech impairment	+	+ ^b	+ ^b	+	+
White matter hypodensity ^c	NE	+	+	+	+
Congenital heart disease	–	–	–	+	+
Proximal placement of thumb	–	–	–	+	+

Note: +, present; –, absent; NA, not available; NE, not examined.

^aAfter puberty.

^bAbsent speech.

^cDetermined by magnetic resonance imaging.

only due to the mutation by base substitution⁵ but also due to the cryptic chromosomal deletion. Interestingly, he showed defects of an additional nine transcripts, including one microRNA and four predicted transcripts, around *UBE2A* (Figure 1f), suggesting genes other than *UBE2A* within the region to be responsible for some of the abnormalities, such as the congenital heart diseases, esotropia and proximal placement of the thumb, which were not described in the cases reported by Nascimento *et al.*,⁵ although none of these nine transcripts has been described as a disease-associated gene. Further collection of cases with similar characteristics and screening of deletion/mutations of *UBE2A* in syndromic as well as idiopathic XLMR-affected males, especially those mapped to areas encompassing *UBE2A*, will be needed to determine the significance and frequency of alterations of this gene as a causative gene for XLMR. Although, several mutations in ubiquitination and proteasome function-related genes, especially in E3 ligase genes, are involved in human neurological disorders, such as *UBE3A* (Angelman syndrome), *PARK2* (recessive juvenile Parkinson disease), *UBR1* (Johanson–Blizzard syndrome), *NHLRC1* (Lafora's disease) and *CUL4B*, *BRWD3* and *HUWE1* (XLMR),⁸ *UBE2A* is the only E2 gene known to be associated with a neurological disease. It will be also necessary to assess whether the mutation or deletion of E2 genes other than *UBE2A* leads to neurodevelopmental anomalies.

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