Mental Retardation in a Boy with Congenital Adrenal Hypoplasia: A Clue to Contiguous Gene Syndrome Involving *DAX1* and *IL1RAPL*

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Abstract. We report on a 2 years and 9 months old Japanese boy with adrenal hypoplasia and mental retardation (MR) (developmental quotient ~60) which occurred in the absence of severe adrenal crisis and resultant brain damage. Cytogenetic and molecular studies were performed in this boy and his parents with normal phenotype, showing that the boy had a maternally derived ~2 Mb interstitial Xp deletion involving *DAX1* (DSS–AHC critical region on the X chromosome, gene 1) for adrenal hypoplasia congenita and disrupting *IL1RAPL* (interleukin-1 receptor accessory protein-like) for non-specific MR. The results explain the development of MR in this boy in terms of contiguous gene syndrome, and suggest the importance of *IL1RAPL* analysis in patients with adrenal hypoplasia and MR.

Key words: Mental retardation, Adrenal hypoplasia, *IL1RAPL*, *DAX1*, Contiguous gene syndrome

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CONTIGUOUS gene syndrome is a genomic disorder caused by a segmental chromosomal deletion involving plural disease genes [1, 2]. This condition occurs in a variety of chromosomal regions including the middle part of Xp which harbors several disease genes including *DAX1* (DSS [dosage dependent sex] reversal and AHC [adrenal hypoplasia congenita] critical region on the X chromosome, gene 1) [3] and *IL1RAPL* (interleukin-1 receptor accessory protein-like) [4]. *DAX1* is an orphan member of the nuclear hormone receptor superfamily, and its mutations are known to result in both AHC and hypogonadotropic hypo-

gonadism [3]. *IL1RAPL* is one of the multiple X-linked genes for non-specific mental retardation (MR) [5], and its mutations, though identified in only a few of patients with MR linked to the Xp21.3–22.1 region, have been reported to cause MR as the sole discernible phenotype [4].

Clinical features of contiguous gene syndrome are primarily determined by the combination of deleted disease genes. However, when mutations of a specific disease gene can result in a wide range of phenotype including that of abnormalities of its adjacent disease gene, clinical features would become similar between patients with contiguous gene syndrome and those with mutations of a single pleiotropic disease gene. In this context, as adrenal crisis can lead to persistent brain damage and resultant MR, phenotypic features could be similar between patients with contiguous gene syndrome involving *DAX1* and *IL1RAPL* and those with *DAX1* mutations who have experienced ad-

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renal crisis. By contrast, as AHC itself does not cause MR, the presence of unexplainable MR in patients with AHC would suggest the occurrence of contiguous gene syndrome involving *IL1RAPL*.

Here, we report on a boy with contiguous gene syndrome involving *DAX1* and *IL1RAPL*, and discuss on the importance of *IL1RAPL* analysis in patients with adrenal hypoplasia and MR.

Case Report

This Japanese boy was born to non-consanguineous parents at 41 weeks of gestation with a birth weight of 3.36 kg (+1.5 SD for Japanese). The 35-year-old mother was found to be phenotypically normal with pertinent social behavior and verbal communication, although her intelligence quotient was not measured. The 41-year-old father was also clinically normal.

At 2 months of age, the patient was hospitalized because of failure to thrive and mild hyperpigmentation. External genitalia were well developed with intrascrotal testes of 2 mL in volume. Biochemical and endocrine studies indicated mild adrenal insufficiency due to AHC: blood Na was 132 mEq/L, K 5.3 mEq/L, Cl 98 mEq/L, glucose 70 mg/dL, ACTH 360 pg/mL, 17 α -hydroxyprogesterone 0.6 μ g/L, dehydroepiandrosterone sulfate 42 ng/mL, cortisol 1.2 μ g/dL, renin activity 120 μ g/L/hr, aldosterone 88 ng/dL, urine 17-hydroxycorticosteroids <0.5 mg/day, and 17-ketosteroids <0.5 mg/day. Abdominal ultrasounds failed to detect the adrenal glands. Therefore, he was placed on adrenal steroid supplementation therapy. Subsequently, a GnRH test (100 μ g/m² bolus i.v.; blood sampling at 0, 30, 60, 90, and 120 min) was performed, indicating poor responses of FSH (1.5→2.8 mIU/mL) and LH (1.1→2.8 mIU/mL) characteristic of a *DAX1* mutation. To evaluate whether he had brain damage because of mild adrenal insufficiency, brain computed tomography (CT), magnetic resonance imaging, and single photon emission CT were performed at 9 months of age, showing normal brain structure and blood flow. In addition, he could respond to small sounds placed behind him, suggesting that he had normal hearing acuity. Nevertheless, he had developmental retardation: although he was able to control his head at 3 months of age and sit without support at 6 months, he walked without support at 18 months of age and spoke single words at 22 months of age. His

developmental quotient was estimated to be ~60 at 2 years and 8 months of age. At present, he is 2 years and 9 months old, measures 85.2 cm (–1.5 SD for Japanese), and weighs 12.0 kg (–0.4 SD for Japanese).

Conventional and Molecular Cytogenetic Studies

After obtaining informed consent, G-banding analysis was performed for 50 lymphocytes obtained from the boy and his parents. Fluorescence *in situ* hybridization (FISH) analysis was carried out for lymphocyte metaphase spreads of the three subjects, using a BAC probe covering the entire coding region of *DAX1* (MBC) and a PAC probe (127F18) covering exons 3–5 of *IL1RAPL* [4], together with a probe for *DXZI* (Vysis) used as an internal signal control. The probes for *DAX1* and *IL1RAPL* were labeled with digoxigenin and detected by rhodamine anti-digoxigenin, and the probe for *DXZI* was detected according to the manufacturer's protocol.

The karyotype was normal in the three subjects examined. FISH analysis showed that the sequence encompassing the *DAX1* coding region was absent from the boy and was deleted hemizygotously in the mother (Fig. 1), whereas the sequence spanning exons 3–5 of *IL1RAPL* was normally preserved in the boy and the mother. The father possessed both *DAX1* and *IL1RAPL* on the X chromosome.

Polymerase Chain Reaction (PCR) Analysis

Multiple loci/regions in the middle part of Xp were examined in this boy. In short, 0.5 μ g of leukocyte genomic DNA was amplified with primers defining each locus/region, and the PCR products were loaded onto a standard agarose gel. The primer sequences for *IL1RAPL* exon 10 are 5'-ACAATAAAGATTATGATGCATACTTATC-3' (forward) and 5'-TTCCAGTTGGGATTAAATCTCTAT-3' (reverse), and those for *IL1RAPL* exon 11 are 5'-CCATAGGCAATCAGCATACC-3' (forward) and 5'-CACCATATCACACTGGATATAC-3' (reverse). The primer sequences for the remaining loci have been reported in Genome Database (<http://www.gdb.org/>). For controls, leukocyte genomic DNA of a normal male was similarly analyzed with permission.

The data are summarized in Fig. 2, together with representative results. The boy had an interstitial Xp deletion with the distal breakpoint between intron 6

Fig. 1. FISH analysis for *DAX1* (arrow) and *DXZI* (arrowheads). In the boy, the signal for *DXZI* is detected, but no signal for *DAX1* is delineated. In the mother, only a single signal is found for *DAX1*, whereas two signals are identified for *DXZI*.

(*DXS7187*) and exon 10 of *IL1RAPL* and the proximal breakpoint between *DXS1018* and *DXS1029*.

X-inactivation Analysis

Methylation status of the *AR* gene (exon 1) was analyzed in the mother, according to the method of Allen *et al.* [6]. In brief, 0.3 μ g of leukocyte genomic DNA was amplified by PCR with a fluorescently labeled forward primer and an unlabeled reverse primer flanking the polymorphic CAG repeat and two methylation sensitive *HpaII* sites before and after *HpaII* digestion, and the PCR products were examined for marker size and area under curve on an ABI PRISM 310 auto-sequencer. The primer sequences were as described previously [6].

PCR amplification yielded 272 bp and 278 bp peaks in the mother, before and after the *HpaII* digestion. After compensation for unequal amplification of the two peaks caused by different product size and slippage phenomenon, the ratio of inactivation between two X chromosomes was calculated as 48%: 52%.

Discussion

The boy had a submicroscopic interstitial Xp deletion involving *DAX1* and disrupting *IL1RAPL*. According to the genetic map of the human X chromosome [7], the deletion is estimated to be ~2 Mb in physical length. With respect to the disruption of *IL1RAPL*, it is noteworthy that exons 1–6 were apparently preserved on the abnormal X chromosome, because an alternative small isoform may have been produced with a stop codon on exon 6 [4]. However, the truncated isoform is unlikely to have a significant biological function, because it is predicted to lack the N-terminal region of the extracellular domain and the whole transmembrane and cytoplasmic domains of *IL1RAPL* [4]. Thus, the results are consistent with adrenal hypoplasia in this boy, and explain why he had MR in the absence of severe adrenal crisis and resultant brain damage.

The mother heterozygous for the Xp deletion was apparently free from MR, under random X-inactivation. This is in contrast to mild to moderate MR of the previously reported mother and her daughter who had a heterozygous small Xp interstitial deletion between

Fig. 2.

Upper panel: Summary of the PCR examinations for multiple loci/regions in the middle part of Xp. *IL1RAPL* consists of 11 exons and contains previously known loci in its introns [4]. The plus and minus symbols represent the presence and absence of the examined loci/regions, respectively.

Lower panel: PCR analysis for *IL1RAPL*. Shown are PCR products obtained by concomitant amplification of intron 2 (*DXS1218*) and exon 11. The boy is positive for intron 2 (*DXS1218*) and negative for exon 11, indicating an intragenic *IL1RAPL* disruption.

DXS1022 and *DXS7182* involving *DAX1* and *IL1RAPL* and random X-inactivation [8]. In this context, it is known that X-inactivation pattern in leukocytes utilized for the X-inactivation analyses in both the present and the previous studies does not reflect that in other tissues [9]. It is likely, therefore, that females heterozygous for the *IL1RAPL* abnormality exhibit a wide range of mental development, probably depending on the X-inactivation pattern in target tissues such as the central nervous system, together with other genetic and environmental factors. It might be possible, however, that another gene for MR resides in a region between *IL1RAPL* and *DXS7182* that is deleted from the two females reported by Muroya *et al.* [8]

and yet preserved in the mother described in the present study. If so, reduced expression of the MR gene caused by random X-inactivation might be responsible for the mild to moderate MR in the carrier females described by Muroya *et al.* [8].

The results provide useful information on the development of MR, in terms of contiguous gene syndrome involving *DAX1* and *IL1RAPL*. In contrast to *DAX1* point mutations or tiny deletions involving *DAX1* as the sole disease gene, male patients with this contiguous gene syndrome should have MR even in the absence of adrenal crisis. Furthermore, carrier females may exhibit mild MR, and have a risk to produce children with MR. Thus, analysis of *IL1RAPL* is recom-

mended in patients with adrenal hypoplasia and MR, as well as in their family members.

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