Endocrine Journal 2014, **61** (6), 629-633

NOTE

Uniparental disomy of chromosome 8 leading to homozygosity of a *CYP11B1* mutation in a patient with congenital adrenal hyperplasia: Implication for a rare etiology of an autosomal recessive disorder

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Abstract. Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder that usually results from paternally and maternally transmitted mutations in genes for steroidogenic enzymes. Recent studies on steroid 21-hydroxylase deficiency, the most common form of CAH, have revealed that a small percentage of patients have a non-carrier parent; uniparental disomy (UPD) and *de novo* mutations were reported as disease-causing mechanisms in these patients. However, it remains unknown whether UPD and *de novo* mutations underlie other forms of CAH. Here, we report a male patient with steroid 11β-hydroxylase deficiency (11OHD) born to a non-carrier mother. The patient was identified by an elevated 17-hydroxyprogesterone level at a neonatal mass-screening test. His clinical features were comparable to those of previously reported patients with 11OHD. Direct sequencing of *CYP11B1* identified a homozygous IVS7+1G>A mutation in the patient, which was not shared by his mother. Comparative genomic hybridization of the patient detected UPD of chromosome 8 [UPD(8)]. Microsatellite analysis indicated non-maternal origin of the UPD(8) and confirmed parentage of other chromosomes. This study shows for the first time that 11OHD can be caused by UPD in the presence of a non-carrier parent. Awareness of such rare cases should improve the accuracy of genetic counseling for families with CAH. Our data support the importance of UPD as an underlying mechanism of autosomal recessive disorders.

Key words: Autosomal recessive disorder, Congenital adrenal hyperplasia, *CYP11B1*, Mutation, Uniparental disomy

CONGENITAL ADRENAL HYPERPLASIA (CAH) is an autosomal recessive disorder that usually results from paternally and maternally transmitted mutations in genes for steroidogenic enzymes [1]. Recent studies on steroid 21-hydroxylase deficiency (21OHD, OMIM #201910), the most common form of CAH, have revealed that a small percentage of patients have a non-carrier mother or father [2, 3]. Uniparental disomy (UPD) leading to unmasking of recessive mutations in *CYP21A2* and *de novo* mutations in the germline were reported as disease-causing mechanisms of such patients. However, it remains unknown whether UPD and *de novo* mutations underlie other forms of CAH.

Steroid 11β-hydroxylase deficiency (11OHD, OMIM #202010) is one form of CAH that accounts for ~1.7% of Japanese patients (Data from the Study Group for Intractable Diseases; http://www.pediatric-world.com/asahikawa/fukujin/) and ~5 – ~8% of patients of other ethnic origin [1, 4]. To date, several mutations in *CYP11B1* on 8q24.3 encoding steroid 11β-hydroxylase

Submitted Nov. 28, 2013; Accepted Feb. 24, 2014 as EJ13-0509
Released online in J-STAGE as advance publication Mar. 13, 2014

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Abbreviations: 11OHD, steroid 11β-hydroxylase deficiency; 21OHD, steroid 21-hydroxylse deficiency; CAH, congenital adrenal hyperplasia; CGH, comparative genomic hybridization; SNP, single nucleotide polymorphism; UPD, uniparental disomy

have been identified in homozygous or compound heterozygous states in patients and in a heterozygous state in the parents, although mutation analysis of the parental samples remained fragmentally [1, 4]. Here, we report a Japanese male patient with 11OHD born to a non-carrier mother.

Subjects and Methods

Case report

The male patient was born to non-consanguineous parents at 37 weeks gestation after uncomplicated pregnancy. The 30-year-old mother and father were clinically normal and did not undergo assisted reproduction. At birth, weight and length of the patient were 2980 g (-0.5 SD) and 47.5 cm (-1.1 SD), respectively. He showed normal male-type external genitalia without skin pigmentation. A neonatal mass-screening test showed an elevated 17-hydroxyprogesterone level (27.7 ng/mL; cut-off value, 8.0 ng/mL). Laboratory examinations at two weeks of age revealed increased levels of steroid metabolites including 17-hydroxyprogesterone and 11-deoxycortisol (Table 1). Blood ACTH and plasma renin activity were also elevated, while serum electrolytes and urine osmolarity were within the reference range. He was normotensive with a systolic blood pressure of 100 mmHg and had no symptoms of adrenal insufficiency. He showed tall stature (+3.0 SD) at 3.5 years of age. Thus, he was clinically diagnosed as having classic CAH and treated with hydrocortisone from 3.6 years of age. On the latest visit at 7.1 years of age, he showed no clinical abnormalities except for advanced bone age (11.2 years of age).

Molecular analyses

This study was approved by the Institutional Review Board Committee at the National Center for Child Health and Development and performed after obtaining informed consent. We analyzed genomic DNA samples obtained from the patient and his mother. The paternal sample was not available for genetic testing. Direct sequencing was performed for coding exons and their flanking regions of *CYP11B1*. Comparative genomic hybridization (CGH) was carried out using a catalog microarray (human CGH + single nucleotide polymorphism [SNP] array, Sureprint G3, catalog number G4890, 4×180K format, Agilent Technologies, Palo Alto, CA) containing ~120,000 CGH probes and ~60,000 SNP probes. PCR-based genotyping of 18 microsatellite loci was performed using fluorescently labeled forward primers and unlabeled reverse primers. The sequences of primers used in the present study are available upon request.

Results

Molecular analyses

Direct sequencing identified a homozygous nucleotide substitution in *CYP11B1* intron 7 (IVS7+1G>A) in the patient. This substitution was absent from the mother (Fig. 1A). CGH of the patient revealed loss of heterozygosity of SNPs on chromosome 8, indicating complete uniparental isodisomy of this chromosome (Fig. 1B). Microsatellite analysis of the patient and his mother suggested non-maternal origin of chromosome 8 and confirmed parentage of other chromosomes (Table 2).

Table 1 Laboratory findings of the patient

Age at examination	2 weeks	1.5 years	3.5 years
Blood examination			
Na (mEq/L)	136 (134-146)	139 (139-146)	141 (138-145)
K (mEq/L)	4.3 (4.3-7.6)	4.3 (3.0-6.0)	3.4 (3.5-5.6)
Cl (mEq/L)	105 (97-110)	107 (98-106)	**110** (98-106)
ACTH (pg/mL)	**205** (5-46)	**1230** (5-46)	**1530** (5-46)
Plasma renin activity (ng/mL/hr)	**18.8** (0.1-14.8)	< 0.1 (< 0.1-4.3)	< 0.1 (< 0.1-3.74)
17-hydroxyprogesterone (ng/dL)	**1495** (11-173)	**1297** (4-114)	40 (4-114)
11-deoxycortisol (ng/dL)	**26,401** (10-200)	**55,986** (7-210)	**19,377** (7-210)
Cortisol (μg/dL)	**27.0** (3.0-21.0)	**27.6** (5.7-25.0)	12.9 (5.7-25.0)
Aldosterone (ng/dL)	1.7 (2.2-129.6)	0.007 (2.2-25.3)	0.04 (2.2-25.3)
Testosterone (ng/dL)	98.0 (0.58-500.6)	**135.4** (2.0-25.9)	**161.4** (2.0-25.9)

Hormone values above the reference range (shown in parenthesis) are boldfaced, and those below the reference range are underlined.

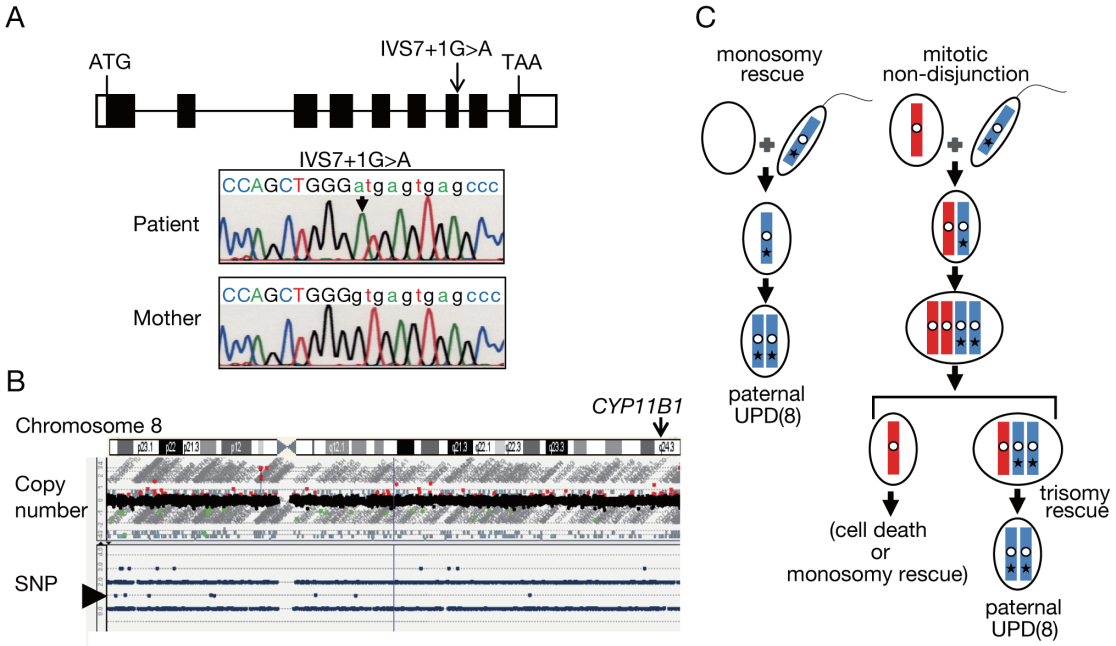

Fig. 1 A. Genomic structure of *CYP11B1* and the position of the mutation identified in the present study. The black and white boxes indicate the coding and non-cording regions, respectively. The patient is homozygous for a nucleotide substitution at the splice donor site in intron 7 (IVS7+1G>A, indicated by an arrow). The mutation is absent from the mother.
B. Comparative genomic hybridization analysis of the patient. No copy-number alteration was identified. Loss of heterozygosity of almost all SNPs on chromosome 8 (an arrowhead) indicates complete uniparental isodisomy.
C. Predicted underlying mechanisms of 11OHD in the patient. The blue and red lines depict the paternally- and maternally-transmitted chromosomes, respectively. The star symbols indicate paternally inherited or *de novo* mutations in *CYP11B1*. UPD(8): uniparental disomy of chromosome 8.

Table 2 Representative results of microsatellite analysis

Locus	Chromosomal position[a]	Mother[b]	Patient[b]	Assessment
D8S264	8p23.3	129	133	isodisomy[c]
D8S260	8q12.2	199/201	195	isodisomy[c]
D8S550	8p23.1	252/272	268	isodisomy[c]
D8S532	8p11.21	238/240	242	isodisomy[c]
D8S1705	8q21.13	192	192	N.I.
D8S1769	8p12	243/255	253	isodisomy[c]
D8S1836	8q24.3	135/151	135	N.I.
D8S256	8q24.22	222/226	222	N.I.
D8S1778	8q22.2	206/208	210	isodisomy[c]
D8S1799	8q24.13	258	254	isodisomy[c]
D15S541	15q11.2	138/148	138	N.I.
D15S542	15q11.2	141/149	135/141	biparental inheritance
D15S1035	15q11.2	236/248	230/236	biparental inheritance
D15S128	15q12	199	199/205	biparental inheritance
D15S117	15q14	131/145	131	N.I.
D15S131	15q23	239/241	239/265	biparental inheritance
D15S205	15q25.3	157	157/159	biparental inheritance
D15S642	15q26.3	206/208	206/210	biparental inheritance

[a], The chromosomal positions are based on Ensembl Genome Browser (http://www.ensembl.org);
[b], The numbers indicate the PCR sizes in bp; [c], Uniparental isodisomy of non-maternal origin.
N.I.: Not informative

Discussion

The patient carried a homozygous IVS7+1G>A mutation in *CYP11B1* and UPD of chromosome 8 [UPD(8)]. The IVS7+1G>A mutation affects the consensus splice donor site of exon 7 and therefore is likely to be a pathogenic mutation. Although IVS7+1G>A has not been described previously, multiple splice-site mutations in *CYP11B1* have been identified in patients with CAH [1, 4]. The results of molecular analyses indicate that CAH in this patient was caused by paternal UPD(8) that unmasked a paternally inherited or *de novo* mutation in *CYP11B1*. Paternal UPD(8) of the patient can be ascribed to monosomy rescue, a condition in which paternal chromosome 8 is replicated in a zygote formed by fertilization of a nullisomic oocyte (Fig. 1C, left panel) [5]. Alternatively, UPD(8) can occur by mitotic non-disjunction; when a segregation error of paternal chromosome 8 takes place during mitosis after normal fertilization, loss of maternal chromosome (trisomy rescue) and subsequent paternal UPD(8) could theoretically occur in one-third of cases (Fig. 1C, right panel) [5]. In this context, although advanced maternal age at childbirth has been suggested as a predisposing factor for oocyte aneuploidy and subsequent UPD [6, 7], this was not applicable to the present case. Thus, hitherto unknown factors may have played a role in the development of paternal UPD(8) in our patient.

Despite carrying UPD(8), the patient manifested no clinical features other than steroidogenic defects. Indeed, hormone values of the patient were comparable to those of previously reported patients with *CYP11B1* mutations [4], and his tall stature is consistent with androgen excess due to 11OHD [4]. These findings, together with a previous report of apparently normal phenotype except for unmasked autosomal recessive lipoprotein lipase deficiency in a patient with paternal UPD(8) [8], indicate that paternal UPD(8) would not result in imprinting defects in disease-causing genes. However, the phenotype of maternal UPD(8) remains to be clarified [9, 10].

Recent studies have revealed that UPD is a fairly common phenomenon which occurs in ~1/3500 live births [5]. Although UPD can be a clinically neutral event, it can also lead to genetic diseases by unmasking recessive mutations or dysregulating imprinted genes [2, 5]. UPD of various chromosomes, together with germline *de novo* mutations, have been implicated in several autosomal recessive disorders [2, 5]. Finkielstain *et al*. identified UPD and *de novo* mutations in 0.9% and 1.9% of patients with 21OHD, respectively [3]. These findings argue against the classical concept that the parents of patients with autosomal recessive disorders are carriers of the disease-causing mutations [2]. Our data provide a novel example of autosomal recessive disorders resulting from UPD. Given the rise in the number of births associated with advanced maternal age that represents a possible predisposing factor of oocyte aneuploidy and UPD [6, 7, 11], UPD should be regarded as an important cause of congenital disorders. Indeed, carrier status of the parents should be examined before genetic counseling of families with autosomal recessive disorders including CAH, because the recurrence risk of such disorders is 25% in the majority of cases, and low or negligible when the disease is associated with UPD or *de novo* mutations.

In summary, the present study shows for the first time that 11OHD can be caused by UPD in the presence of a non-carrier parent. Awareness of such rare cases should improve the accuracy of genetic counseling for families with CAH. Furthermore, our data suggest the importance of UPD as an underlying mechanism of autosomal recessive disorders.

Acknowledgements

This work was supported by grants from the Ministry of Health, Labor and Welfare, Takeda Science Foundation, from the Japan Society for the Promotion of Science, from the Ministry of Education, Culture, Sports, Science and Technology, and from National Center for Child Health and Development.

Disclosure

None of the authors have any potential conflict of interest associated with this research.

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