

A novel compound heterozygous mutation in the CYP11B2 gene, including an intron 7 splice site, is responsible for aldosterone synthase deficiency type II

Jianfang Zhu, Fangyuan Zhao, Hong Chen, Liqiong Jiang, Chunlin Wang, Yanlan Fang, Yuanmei Kong, Kana Wang, Li Liang
Department of Pediatrics, the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310003, China

Objective

To investigate the clinical and molecular characteristics of a girl with aldosterone synthase deficiency type II (ASDII). We also identified the consequences of a novel splice site mutation in the CYP11B2 gene.

Materials and Methods

A 4-month-old girl presented with vomiting, diarrhea, and failure to thrive. Her 17 α -OHP, cortisol, renin, and aldosterone were all in the normal range, and the ACTH stimulation test suggested that the adrenal cortex responded well. Sequencing was performed using genomic DNA. An in vitro analysis was performed using a minigene splicing assay based on the pSPL3 exon trapping vector. The wild-type (pSPL3-WT) and mutant (pSPL3-Mut) plasmids, containing exon 7, intron 7, exon 8, a partial sequence of intron 6 and intron 8, were separately cloned into the pSPL3 vector. The wild-type and mutant constructs were transiently expressed in COS-7 cells. Cellular RNA was extracted for reverse transcription, and the PCR products were sequenced for further identification. Structural simulation of the two novel mutations was conducted.

Results

The patient's clinical presentation shows in Table 1. The mutation analysis identified the patient with compound heterozygosity [c.1342C>T/p.R448C; c.1200+1G>A/p.L375Cfs*1] (Figure 1). The minigene construction analysis revealed that the mutation resulted in aberrant splicing, in which exons 7 and 8 were skipped, resulting in the deletion of exons 7 and 8 and the premature formation of a stop codon in exon 9 (Figure 2).

Table 1: Laboratory findings at admission

Items	Patient	Age related reference range
Plasma sodium	128	135~145 mmol/L
Plasma potassium	5.69	3.5~5.5 mmol/L
Plasma chloride	91	98~107mmol/L
pH	7.42	7.35~7.45
SBE	-3.1	\pm 2~3mmol/L
HCO ₃ ⁻	20.6	21~24mmol/L
Plasma aldosterone	0.204	0.01~0.45nmol/L
Plasma renin	2.99	1.2~2.8 ug/L/h
PRA	1.182	0.13~1.94 ng/ml/h
Plasma cortisol (8am)	9.48	5.7~16.6 ug/dL
Cortisol (poststimulation)	24.7	
17-OH-Progesterone	0.1	<30.00nmol/L
ACTH (8am)	5.5	1.1~12.1pmol/L

SBE: standard base excess; PRA: plasma renin activity; ACTH, adrenocorticotropic hormone

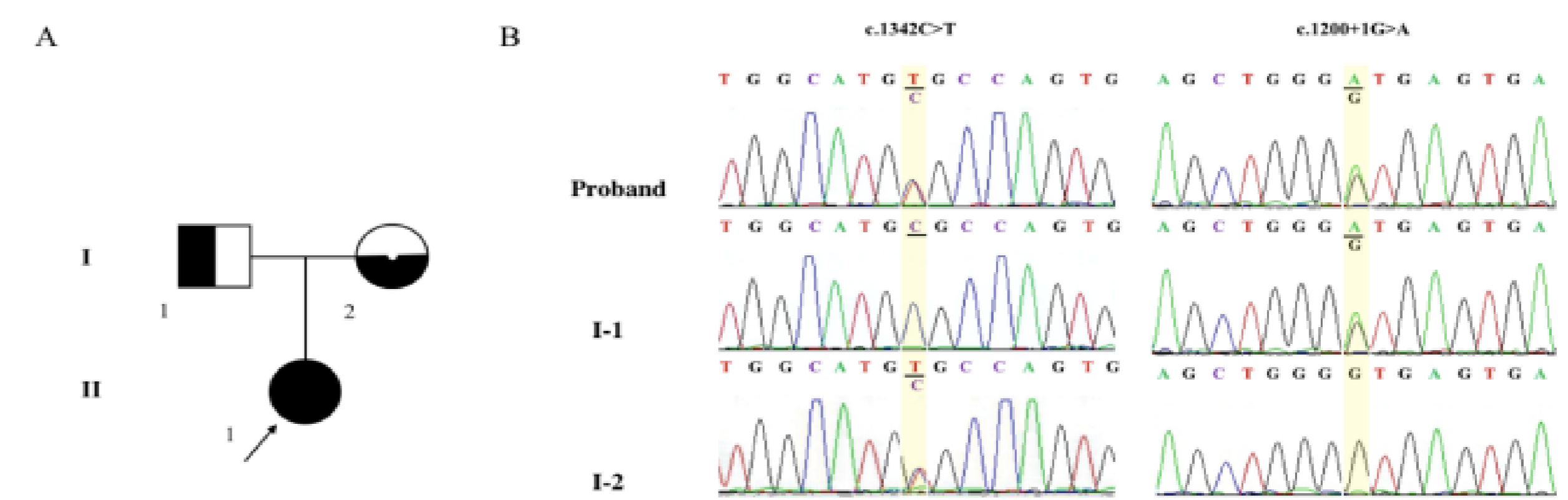


Figure 1: A: Family pedigree. The proband is indicated by an arrow. The affected individual is shown as a filled black symbol. The half-filled symbols are clinically unaffected subjects harboring a heterozygous mutation. B: Mutation analysis by direct DNA sequencing. The CYP11B2 gene of the proband contained a compound heterozygous mutation: the missense mutation c.1342C>T/p.R448C and the splice site mutation c.1200+1G>A. I-1: The proband's father carried the missense mutation in the CYP11B2 gene: c.1342C>T/p.R448C (heterozygous). I-2: The proband's mother carried the splice site mutation in the CYP11B2 gene c.1200+1G>A (heterozygous).

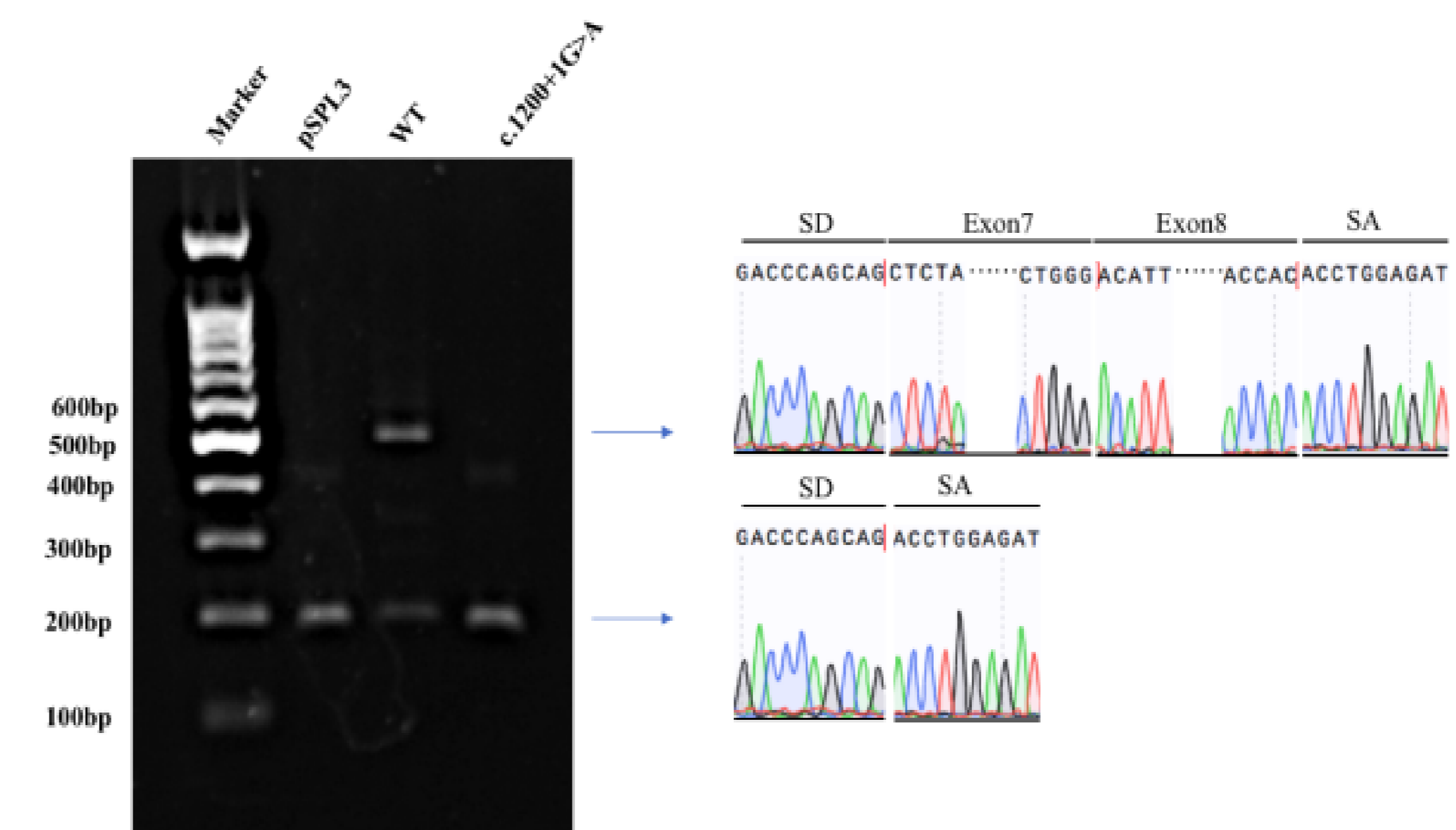


Figure 2: Agarose gel electrophoresis of the PCR products. The forward and reverse primers were designed based on the SD and SA sequences and designed for RT-PCR amplification of the cDNA sequence of interest. Lane 1: Marker; Lane 2: empty vector; Lane 3: WT, for which the sequencing of the PCR product revealed that it included the exons SD and SA of pSPL3 and exons 7 and 8 of CYP11B2; and Lane 4: The splice site mutation c.1200+1G>A disrupted the normal splicing, resulting in the deletion of exons 7 and 8. The PCR band is identical to lane 2 (pSPL3 empty vector).

Conclusion

In summary, we report two novel CYP11B2 mutations in a Chinese family with ASD type II, and we identified one splice mutation (c.1200+1G>A/p.L375Cfs*1). The description of the metabolic phenotype of the proband is important for the clinician and genetics. Genetic testing contributes to the diagnosis and differential diagnosis of ASD. Our study not only enhances the understanding of CYP11B2 gene mutations, but also demonstrates that gene sequencing can be an effective means of diagnosis of clinically complex diseases.

References:

- Nomoto S, Massa G, Mitani F, et al. CMO I deficiency caused by a point mutation in exon 8 of the human CYP11B2 gene encoding steroid 18-hydroxylase (P450C18). *Biochem Biophys Res Commun.* 1997;234(2):382-385.
- Bassett MH, White PC, Rainey WE. The regulation of aldosterone synthase expression. *Mol Cell Endocrinol.* 2004;217(1-2):67-74.
- White PC. Aldosterone synthase deficiency and related disorders. *Mol Cell Endocrinol.* 2004;217(1-2):81-87.
- Holloway CD, MacKenzie SM, Fraser R, et al. Effects of genetic variation in the aldosterone synthase (CYP11B2) gene on enzyme function. *Clin Endocrinol (Oxf).* 2009;70(3):363-371.

