



11 β -Hydroxylase Deficiency

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The authors have nothing to disclose

BACKGROUND

Deficiency of 11 β -hydroxylase is the second most frequent type of congenital adrenal hyperplasia (CAH).

Review of the Turkish patients with CAH showed that 13.5 % are 11 β -hydroxylase deficiency. This result showed that it is more common in Turkey than other populations.

OBJECTIVE AND HYPOTHESES

The purpose of this study is to examine the spectrum of CYP11B1 gene mutations in Turkish population and to evaluate genotype-phenotype relation in our population.

PATIENTS and METHODS

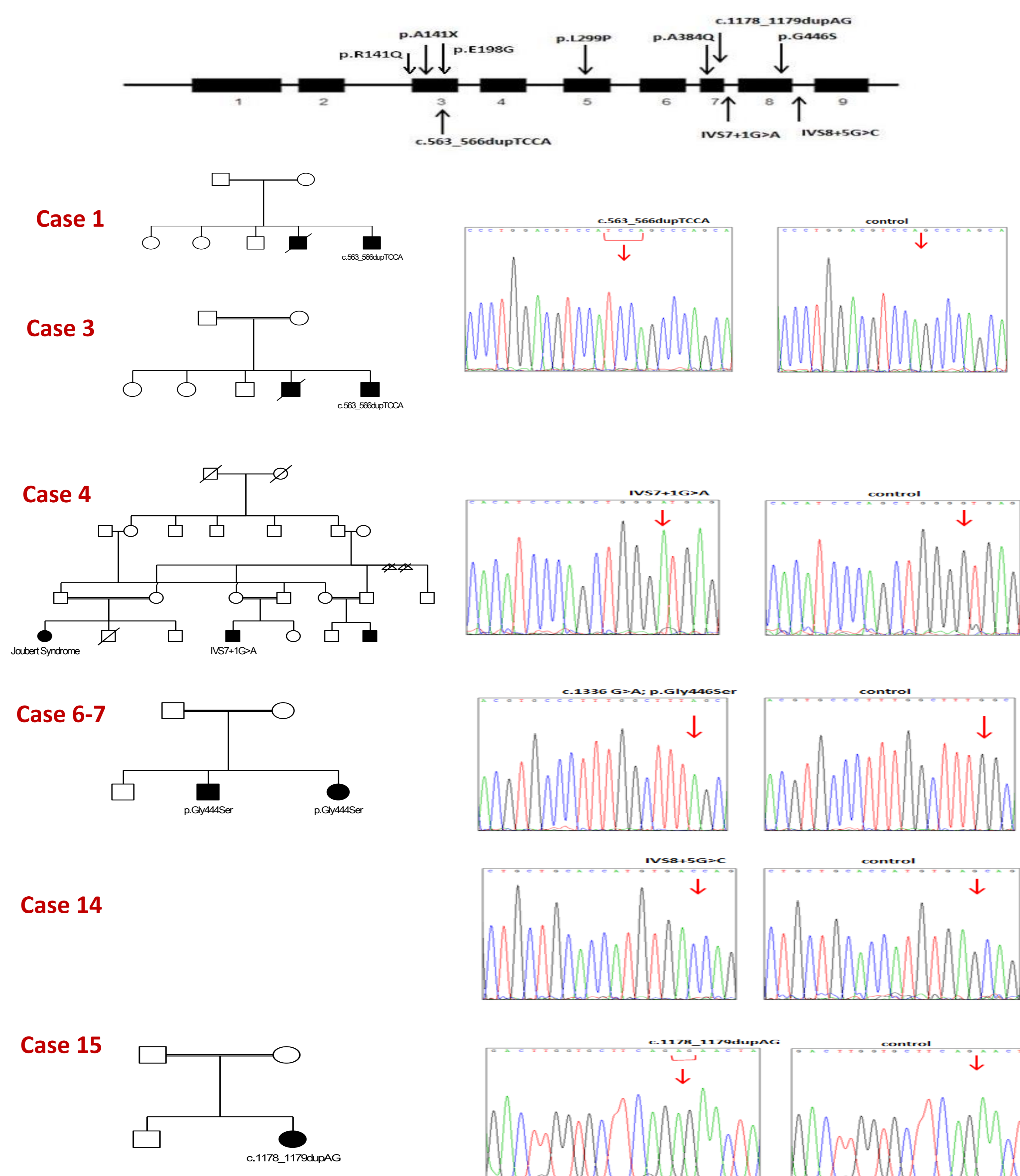
- 17 patients from 13 families are included in this study.
- Consanguinity was present in all families
- Diagnosis was based on virilisation and high levels of 11-deoxycortisol.
- 15 cases had classical and 2 cases had non-classical form. Patients with nonclassical form admitted at 7 and 4.9 years old with premature adrenarche and they are siblings.
- The karyotype of 9 cases out of 15 with classical form was 46,XX and the remaining was 46, XY
- Mutation screening of 9 exons in CYP11B1 gene was performed using direct DNA sequencing analysis.
- Amplification of exons 1–9 of the CYP11B1 gene was performed using the AccuTaq LA DNA Polymerase (Sigma, Germany). The CYP11B1 gene was specifically amplified in three fragments avoiding simultaneous amplification of homologous CYP11B2 sequences.
- Three pairs of primers were used: fragment I, comprising exons 1 and 2, sense 5'-TCC CTC TCG AAG GCA AGG CAC CA-3', antisense 5'-CTG CTC CCA GCT CTC AGC TCG C-3'; fragment II, comprising exons 3–5, sense 5'-CTT GCA GAA AAT CCC TCC CCC CTA-3', antisense 5'-GGA CAC GTG GGC GCC GTG TGA-3'; fragment III, comprising exons 6–9, sense 5'-TGA CCC TGC AGC TGT GTC TCC TG-3', antisense 5'-CCA TTT GTG CTG GGG CTG GTT AGA-3'.
- PCR fragments were purified with 96-well PCR filter plates (MinElute PCR purification kit, Qiagen Inc., Valencia, CA, USA) and mutation analysis was performed by direct sequencing of purified PCR products. Sequencing reactions were performed using the BigDye Terminator Cycle Sequencing kit (version 3.1) and analyzed on ABI 3130 automated DNA sequencer (Applied Biosystems, CA, USA).

RESULTS

- The age at diagnosis ranged 0,1-4,2 years in classical form of 11- hydroxylase.
- Two siblings with late onset form, presenting with premature adrenarche were diagnosed at 4,9 and 7 years
- 46,XX cases presented with severe virilisation (Prader genital stage IV and V). Four of 46,XX patients were reared as male at presentation and 3 of them remained male due to establishment of male gender identity.
- Overall mutation analyses revealed 8 homozygous and 1 compound heterozygote pathogenic mutations of four different types (two splicing, two duplication, two nonsense, four missense).
- The detected nucleotide changes in patients resulted in seven novel (c.1336G>A;p.G446S, c.563_566dupTCCA, IVS7+1G>A, IVS8+5G>C, c.1178_1179dupAG, c.442G>A;p.Arg141Gln and c.593 A>G;p.Glu198Gly) and three previously reported mutations (p.A141X, p.L299P, p.A384Q,) in the CYP11B1 gene. Mutation prediction software tools PolyPhen-2 and SIFT, both indicate that the novel mutations detected in this study are likely to be pathogenic.

Case	Age yr	Karyotype	Sex of rearing	External genitalia	11-DOC (ng/ml)	Mutation (Novel mut. indicated by red)
1	1.4	46, XY	Male	Male	163	c.563-566 dup TCCA
2	0.4	46,XX	Female	Prader 4	>25	c.421CT;p.Arg141stop
3	2.0	46,XY	Male	Male	>25	c.563-566 dup TCCA
4	1.9	46,XY	Male	Male	552	IVS7+1G>A
5	2.7	46,XX	Male	Prader 5	>25	c.896T>C;p.Leu299pro
6*	7,0	46,XY	Male	Male	147	c.1336GA;p.Gly446Ser
7*	4,9	46,XX	Female	Female	179	c.1336GA;p.Gly446Ser
8	4.2	46,XX	Male	Prader 5	229	c.421CT;p.Arg141stop
9	2.1	46,XX	Male	Prader 5	295	c.896T>C;p.Leu299pro
10	2	46,XY	Male	Male	>100	c.896T>C;p.Leu299pro
11	2,5	46,XX	Female	Prader 4	>25	c.442G>A;p.Arg141Gln c.593 A>G;p.Glu198Gly
12	0,1	46,XX	Female	Prader 4	289	c.421CT;p.Arg141stop
13	1,9	46,XY	Male	Male	392	c.421CT;p.Arg141stop
14	0,1	46,XX	Female	Prader 4	320	IVS8+5 G>C
15	0,5	46,XX	Female	Prader 5	>25	c.1178-1179dup AG
16	3,9	46,XY	Male	Male	>100	c.1151G>A;p.Arg384Gln
17	0,1	46,XX	Female	Prader 4	268	c.896T>C;p.Leu299pro

Novel mutations and the pedigrees are shown below



CONCLUSION

All detected mutations were scattered throughout the gene. The most common mutation in this group of Turkish patients was A141X with the allele frequency of 27%. All novel and known mutations in the classical form led to severe virilisation. The missense novel mutation c.1336GA;p.Gly446Ser caused late onset form of the disease.

REFERENCES

- Kandemir N, Yordam N. Congenital adrenal hyperplasia in Turkey: a review of 273 patients. Acta Paediatr. 1997 Jan;86(1):22-5
- Ben Charfeddine I, Riepe FG, Kahloul N, Kulle AE, Adala L, Mamai O, Amara A, Mili A, Amri F, Saad A, Holterhus PM, Gribaa M. Two novel CYP11B1 mutations in congenital adrenal hyperplasia due to steroid 11 β hydroxylase deficiency in a Tunisian family. Gen Comp Endocrinol. 2012;175(3):514