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BRIEF REPORT

A Novel Mutation L260P of the Steroidogenic Acute Regulatory Protein Gene in Three Unrelated Patients of Swiss Ancestry with Congenital Lipoid Adrenal Hyperplasia

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Context: Lipoid congenital adrenal hyperplasia (CAH) is the most severe form of CAH leading to impaired production of all adrenal and gonadal steroids. Mutations in the gene encoding steroidogenic acute regulatory protein (StAR) cause lipoid CAH.

Objective: We investigated three unrelated patients of Swiss ancestry who all carried novel mutations in the StAR gene. All three subjects were phenotypic females with absent Müllerian derivatives, 46,XY karyotype, and presented with adrenal failure.

Methods and Results: StAR gene analysis showed that one patient was homozygous and the other two were heterozygous for the novel missense mutation L260P. Of the heterozygote patients, one carried the novel missense mutation L157P and one had a novel frameshift mutation (629–630delCT) on the second allele. The functional ability of all three StAR mutations to promote pregnenolone production was severely attenuated in COS-1 cells transfected with the cholesterol side-chain cleavage system and mutant *vs.* wild-type StAR expression vectors.

Conclusions: These cases highlight the importance of StAR-dependent steroidogenesis during fetal development and early infancy; expand the geographic distribution of this condition; and finally establish a new, prevalent StAR mutation (L260P) for the Swiss population. **(*J Clin Endocrinol Metab* 90: 5304–5308, 2005)**

LIPOID CONGENITAL ADRENAL hyperplasia (CAH) was originally described in 1955 by Prader and Gurtner (1) in a Swiss 46, XY baby girl presenting with severe salt wasting due to adrenal insufficiency and female external genitalia but male gonads and adrenocortical hyperplasia on autopsy. Lipoid CAH is probably the severest form of CAH, impairing production of both adrenal and gonadal steroids (2), and results from mutations in the gene encoding steroidogenic acute regulatory protein (StAR) (3). StAR fosters the transport of cholesterol into the mitochondria, in which cholesterol is converted to pregnenolone by the P450 side-chain cleavage-adrenodoxin-adrenodoxin reductase system (2–4). Patients with lipoid CAH present with primary adrenal failure in infancy. Their adrenal glands are enlarged and contain lipoid deposits, reflecting cytoplasmic cholesterol accumulation of cholesterol after prolonged ACTH stimulation (2). In addition, impaired testosterone biosynthesis prevents virilization in karyotypic males (46, XY) (2).

To date, more than 35 StAR mutations have been described in more than 70 patients, predominantly from Japan, Korea, and Arabic countries (5–8). However, genetic diagnosis of the Swiss patient with lipoid CAH originally described by Prader was never made.

We report three patients of Swiss ancestry with lipoid CAH in whom we found three novel StAR mutations. One of them, identified in all patients, establishes a new, prevalent StAR mutation for the Swiss population.

Patients and Methods

Mutation analysis

After obtaining informed consent, DNA was extracted from peripheral blood leukocytes (9). Direct sequencing of the StAR gene was performed as described previously (ABI 373A DNA analyzer; PerkinElmer, Branchberg, NJ) (10).

Microsatellite analysis

Two microsatellites, D8S2331 and D8S536 (AFM265zf9), were used to search for a common ancestral allele carrying the L260P StAR mutation.

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Abbreviations: CAH, Congenital adrenal hyperplasia; StAR, steroidogenic acute regulatory protein; START, StAR-related lipid transfer.

Oligonucleotide sequences and PCR conditions were obtained from the CEPH genotype database (http://www.cephb.fr/cephdb/).

Functional assay

Vectors expressing cDNAs of wild-type StAR and the fusion protein P450 side-chain cleavage/adrenodoxin/adrenodoxin reductase (F2) were a generous gift of Walter L. Miller (Department of Pediatrics, University of California San Francisco, San Francisco, CA) (3, 11). Mutant StAR cDNA expression vectors were constructed by PCR-based, site-directed mutagenesis. All mutants were confirmed by direct sequencing. Functional analysis of the mutants was performed as described previously (3), using MultiCalc software (PerkinElmer).

Structural modeling

The positions of the two mutated leucines, L157 and L260, were mapped onto the crystal structure of the StAR-related lipid transfer (START) domain of human MLN64 [protein database reference ID: 1EM2 (12)] using DS ViewerPro 5.0 software (Accelrys Inc., San Diego, CA). Previously reported StAR missense mutations were included in the model for comparison.

Case reports

Patient 1. This 46, XY girl was a single child of a nonconsanguineous Swiss couple. She was delivered at term by cesarean section [birth weight 2360 g (−3.0 SD); length 47 cm (−2.0 SD)]. At 5 months of age, she was referred to the hospital with vomiting. She was dehydrated, and her weight was 5240 g (P3) and height 63.5 cm (P25). She had normal female external genitalia, without virilization, but was hyperpigmented. Inguinal gonads were palpable. Laboratory investigations revealed severe hypocortisolemia and grossly elevated ACTH and plasma renin activity consistent with primary adrenal insufficiency (Table 1). Plasma steroids remained low or undetectable after ACTH stimulation. Gonads were removed and laparoscopy showed no Müllerian structures. Treatment with hydrocortisone and fludrocortisone was started and resulted in normal growth and development. Estrogens were started at 12 yr of age to induce breast development. Pubic and axillary hair remained absent, reflecting impaired adrenal and gonadal androgen production. The patient is now 27 yr old and healthy. She has a heterosexual orientation.

Patient 2. This patient was a full-term 46, XY baby girl born to a nonconsanguineous Swiss couple. Her birth weight was 2930 g (−1.6 SD) and length 49 cm (−0.6 SD). At 2.5 months of age, she was admitted with failure to thrive. Physical examination revealed normal female external genitalia and hyperpigmentation. Laboratory investigations revealed severe salt wasting and low serum cortisol. Magnetic resonance imaging showed inguinal gonads but no Müllerian structures. The adrenals were of normal size. Hydrocortisone and fludrocortisone replacement were started. The girl is now 3.5 yr old and shows normal physical and mental development.

Patient 3. This 46, XY term baby was 3920 g (+2.0 SD) at birth and had normal female external genitalia. She was the third child of a nonconsanguineous French couple with Swiss ancestry. At the age of 3.5 months, she was admitted with vomiting and apathy during a mild viral infection. Hyperpigmentation of the skin-fold areas was noted. Ultrasound and computed tomography scan revealed gonads but no uterus. Laboratory investigations were consistent with severe primary adrenal insufficiency (Table 1). She recovered quickly after glucocorticoid and mineralocorticoid replacement and, at 9 yr of age, is developing normally.

Results

Mutation analysis

Because of severe, primary adrenal insufficiency, sex reversal with female external genitalia and impaired adrenal and gonadal steroid hormone production, all three 46, XY patients were analyzed for genetic defects in the StAR protein (Fig. 1).

Patient 1 harbored a novel homozygous missense mutation L260P in exon 7 (T779 to C) (Fig. 1B). In patient 2, the same L260P mutation was found on one allele, but the other allele had a L157P missense change (T470 to C) in exon 5. Her father is heterozygote for L260P and her mother heterozygote for L157P. Patient 3 also had a L260P mutation on one allele, combined with a 2-bp deletion in exon 5 (C629, T630) on the other allele, which leads to a frame shift and premature stop at amino acid 235 (Fig. 1B). This patient shared L260P with the father and 629–630delCT with the mother. All three StAR mutations are novel. The identification of a L260P change in all three patients indicates that this might be a common mutation in the Swiss population.

Microsatellite analysis

Because no intragenic polymorphisms of the StAR gene were identified in our population, two microsatellite markers were used to search for a common origin of the L260P StAR

TABLE 1. Biochemical characteristics of patients at diagnosis of adrenal insufficiency

	Normal range (1–6 months)	Patient 1	Patient 2	Patient 3
Age (months)		5.5	2.5	3.5
Sodium	129–143 mmol/liter	124	121	114
Potassium	3.7–5.8 mmol/liter	8.4	6.4	9.6
Glucose	50–88 mg/dl		22	31
	(2.8–4.9 mmol/liter)		(1.2)	(1.7)
ACTH	9–52 pg/ml	>2000		1362
	(2–11 pmol/liter)	(>440)		(300)
Cortisol	5–25 μg/dl	<1	2.8	
	(140–690 nmol/liter)	(22)	(78)	
17α-Hydroxyprogesterone	3.3–89 ng/dl		9.9	6.9
	(0.1–2.7 nmol/liter)		(0.3)	(0.21)
Progesterone	6.3–50 ng/dl		9.4	
	(0.2–1.6 nmol/liter)		(<0.3)	
Aldosterone	5–90 ng/dl		5.1	
	(140–2500 pmol/liter)		(141)	
DHEA	26–375 ng/dl		14.4	
	(0.09–1.3 nmol/liter)		(0.05)	
PRA	2.35–37 μg/liter·h	>33		
Urinary 17-ketosteroids		Undetectable		

All values are determined from plasma, except 17-ketosteroids from urine. DHEA, Dehydroepiandrosterone; PRA, plasma renin activity.

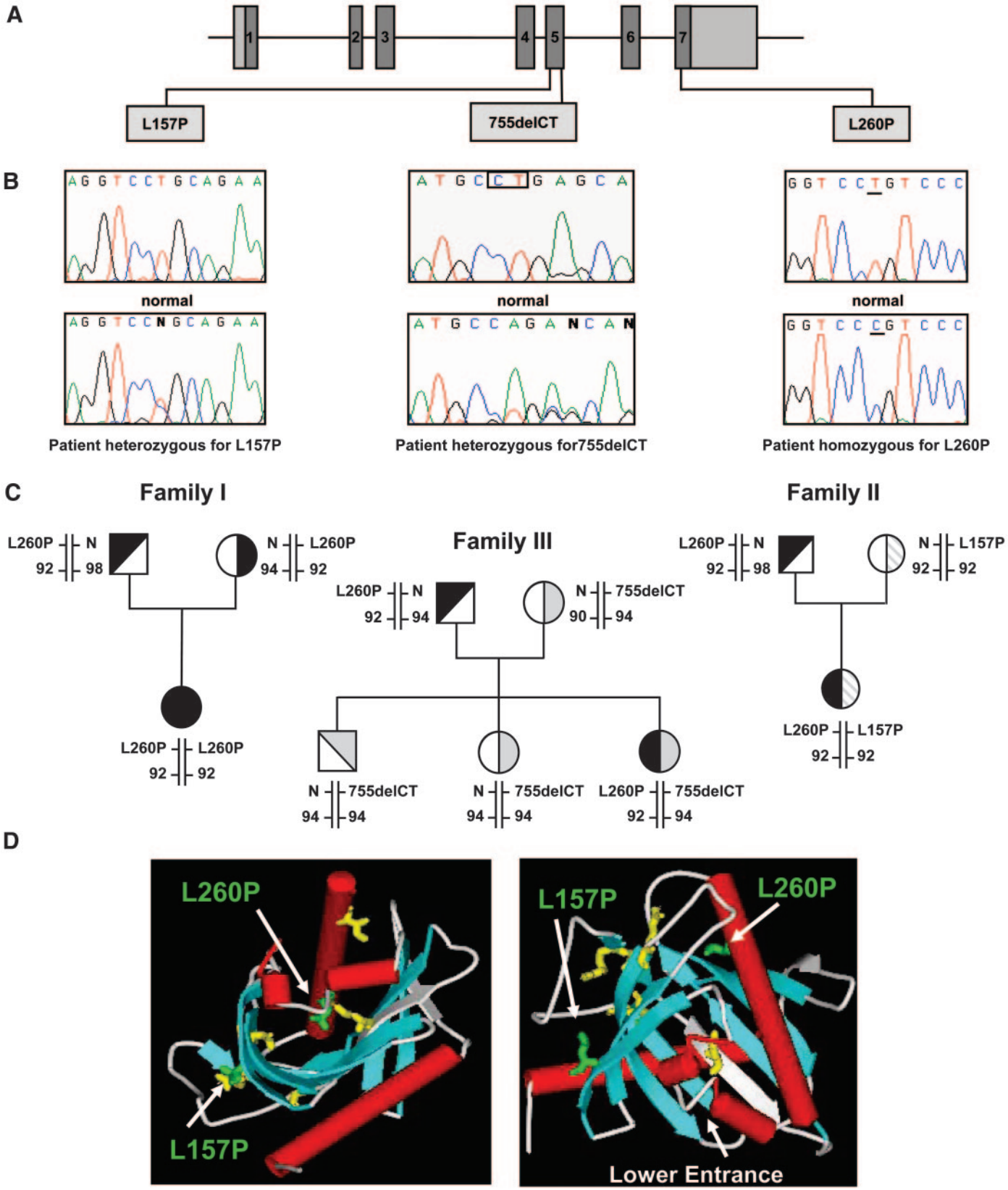

FIG. 1. Mutation analysis, microsatellite studies, and protein structure. A, Diagram of the StAR gene showing the location of newly identified mutations. B, Chromatograms showing the L157P, 629–630delCT, and L260P mutations identified from the three reported patients, compared with normals. C, Family trees with the mutations and the size of the associated D8S536 allele. D, Ribbon diagram of the StAR protein based

mutation in our three families (D8S2331, 41 kb upstream of the StAR gene; D8S536, 85 kb downstream). Only D8S536 varied in our three families (Fig. 1C). L260P was always associated with a D8S536 allele 92 bp in length. However, this 92-bp fragment was also identified with the normal and the L157P allele in family II and was found to be present in 31% (10 of 32 alleles) of the Swiss population. Nevertheless, the obligate association of the L260P mutation with the 92-bp allele of D8S536 indicates that the L260P mutation in all three families of Swiss ancestry might originate from a common ancestral allele.

Structural modeling of missense mutations

Based on the START domain of human MLN64 (12), the mutated leucine at position 157 is a conserved residue that lies within the β-sheet β4 and forms part of the β-barrel (Fig. 1D, *green*). The leucine at position 260 is a conserved residue in the helix α4, which is thought to contribute directly to the lipid tunnel harboring cholesterol. Together with previously reported missense mutations in StAR (L169, R182, D203, R217, A218, M225, L275) (Fig. 1D, *yellow*), mutations seem to cluster around this lipid tunnel.

Functional studies

To test the functional consequences of the newly identified StAR mutations, we built vectors expressing the mutant StAR proteins and compared their ability to promote pregnenolone production when transfecting nonsteroidogenic COS-1 cells with either wild-type or mutant StAR and an expression vector containing the fused cholesterol side chain cleavage system (3, 11). In this assay, pregnenolone production from cholesterol was only slightly higher with all three novel StAR mutations (L157P, L260P, 629–630delCT) than vector control (14.7–17.3 *vs.* 9.4%; $P \leq 0.05$) (Fig. 2). In contrast, wild-type StAR promoted pregnenolone production from endogenous cholesterol similar to StAR-independent pregnenolone production from exogenously added 22R-hydroxycholesterol, a nonphysiological substrate for the side chain cleavage system crossing the mitochondrial membrane independent of StAR (positive control). Thus, the StAR mutations identified (L157P, L260P, and 629–630delCT) are fully responsible for lipoid CAH in the reported patients.

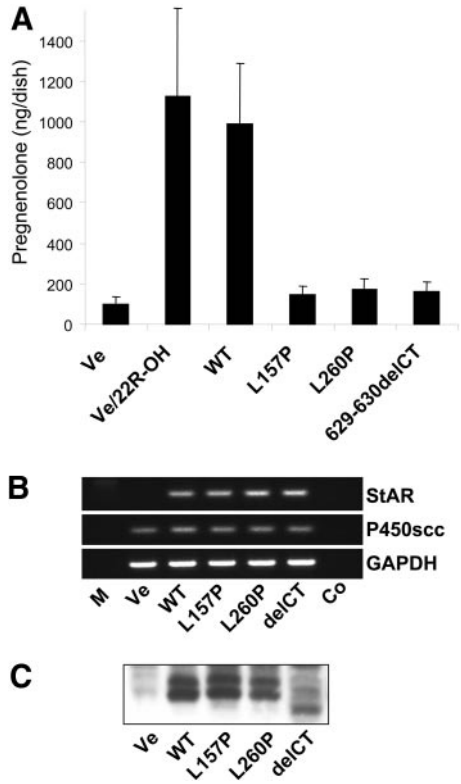

FIG. 2. Functional activity of mutant *vs.* wild-type StAR. A, Ability to produce pregnenolone from cholesterol was tested for wild-type (WT) and mutant StAR *in vitro*. COS-1 cells were transiently transfected with expression vectors for the side chain cleavage system (F2) and either wild-type or mutant StAR (3). Pregnenolone concentration was determined by RIA. Empty vector control (Ve) was used as a negative control, and the StAR-independent substrate 22(R)-hydroxycholesterol added to the cell culture media provided a positive control. B and C, Equal gene expression of wild-type *vs.* mutant StAR was assessed by semiquantitative RT-PCR using RNA extracted from COS-1 cells 36 h after transfection (B) and by Western blot using StAR antibody (C). Data represent the mean ± SEM from three independent experiments, each performed in triplicate. GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; P450scc, P450 side-chain cleavage. M, Marker; CO, negative control for PCR.

Discussion

The association between StAR mutations and lipoid congenital adrenal hyperplasia is well established (2). Defects in the StAR gene are rare and usually occur sporadically (5). The only consistent genetic clusters identified to date are the Q258X mutation in the Japanese and Korean populations (13, 14), the R182L mutation among Palestinian Arabs (2), and the R182H mutation in eastern Saudi Arabians (7). Our report of three unrelated patients with Swiss ancestry carrying either a homozygous or compound heterozygous L260P mutation expands the geographic distribution of this condition and establishes a new prevalent mutation for the Swiss population. Intriguingly, one may wonder whether Prader's original Swiss lipoid CAH patient also had this precise genetic defect (1). However, because this patient died more than 60 yr ago, we were neither able to get his DNA for analysis nor found family members who could have been investigated for heterozygosity. Nevertheless, our microsatellite studies favor a possible founder effect for the L260P mutation in the Swiss population.

Analysis of truncated StAR proteins have shown that the

on the START domain of human MLN64 [2.2 Å resolution, protein database reference ID:1EM2 (12)]. The four α-helices are shown in *red* and nine β-sheets are shown in *blue*. *Left*, Lipid binding tunnel of the START domain is formed by the U-shaped β-barrel and helix-α4. *Right*, Rotated image to show the lower entrance of the tunnel. The positions of the two mutated leucines in these patients (L157, L260) are shown in *green*. Leucine at position 157 is a conserved residue within β4 and forms part of the β-barrel. Leucine at position 260 is a conserved residue in helix-α4 and is thought to contribute directly to the lipid tunnel. For comparison, previously reported missense mutations in StAR (L169, R182, D203, R217, A218, M225, L275) are shown in *yellow*.

carboxyl-terminal domain is critical for biological function: deletion of only 10 carboxyl-terminal amino acids reduces StAR activity by half (15), and deletion of 28 carboxyl-terminal amino acids eliminates activity completely (3). By contrast, at least 62 amino-terminal amino acids can be removed without functional consequences (15). All missense mutations described to date are clustered in exons 4–7 and lie in the carboxyl-terminal half of the molecule (5, 16). In functional studies (3, 11), only the M225T mutation was found to have partial activity of 29%, whereas all other reported missense mutations had activities of less than 10% (13, 16). Thus, because StAR-independent pregnenolone production in this system is about 14% (2), all reported missense mutations but M225T lack StAR activity. Similarly, the L260P and L157P mutations described here both affect highly conserved residues of the carboxyl-terminal StAR domain and cause loss of StAR activity. This complete loss of functional activity of most StAR mutations *in vitro* contrasts with clinical variation in disease severity and age of presentation. Onset of symptoms in our patients ranged from 2.5 to 5.5 months but may occur from 1 to 14 months in patients carrying R182H (7). This poor correlation between clinical findings and *in vitro* studies of StAR mutations may be explained by limited sensitivity of the functional assay, interindividual variability of StAR-independent steroidogenesis, or variable expression of StAR-like molecules, such as MLN64 or Star D4–8 (7).

Recently the crystal structure of the StAR-related lipid transfer domain MLN64 has been determined, and a putative lipid-binding tunnel that shuttles cholesterol through the intermembranous space of the mitochondria has been identified(12, 17). This lipid-binding tunnel is formed by a U-shaped β-barrel and helix-α4 (Fig. 3). Using a modeling approach, we have shown that the leucine at position 157 is a conserved residue within β-sheet-β4 that forms part of the β-barrel, whereas the leucine at position 260 is a conserved residue in helix-α4 that is thought to contribute directly to the lipid tunnel (12). These missense mutations in StAR lie within the lipid-tunnel region and presumably impair function by causing abnormal protein folding and reduced cholesterol binding. Furthermore, these cases show how naturally occurring missense mutations found in patients with lipoid CAH can provide information about critical structural domains of the StAR protein.

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