

## BRIEF COMMUNICATION

Molecular and Structural Analysis of Two Novel *STAR* Mutations in Patients with Lipoid Congenital Adrenal Hyperplasia

**Mutations in the gene encoding steroidogenic acute regulatory protein (StAR) cause lipoid congenital adrenal hyperplasia. We report a novel homozygous splice site mutation (IVS1 + 2T → G) in *STAR* in two sisters (46XY, 46XX) who presented with primary adrenal insufficiency at birth and a novel homozygous R182H missense mutation in the putative lipid transfer domain of StAR in a phenotypic female (46XY) with adrenal failure and a parotid tumor. These cases highlight the importance of StAR-dependent steroidogenesis during fetal development and early infancy and of the critical functional role of R182 in cholesterol transport.**

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**Key Words:** StAR; lipoid congenital adrenal hyperplasia; lipid transfer domain; adrenal; testis; parotid tumor.

Steroidogenic acute regulatory protein (StAR) (OMIM 600617) is a 30-kD protein that plays a crucial role in the transport of cholesterol from the cytoplasm into the inner mitochondrial membrane, the rate-limiting step in pregnenolone synthesis (1–4). Homozygous or compound heterozygous mutations in the gene encoding StAR (*STAR*) cause lipoid congenital adrenal hyperplasia (LCAH) (OMIM 201710) (2,5,6). Children with this condition typically present with primary adrenal failure during infancy. Their adrenal glands are usually enlarged and contain lipid deposits, reflecting cytoplasmic accumulation of cholesterol following adrenocorticotropin (ACTH) stimulation (5). Mutations in *STAR* also affect gonadal steroidogenesis. Impaired testosterone biosynthesis causes undervirilization of males (46XY), and the accumulation of cholesterol in Leydig cells leads to cellular toxicity. Females are often able to synthesize sufficient estrogen for pubertal development through StAR-independent

mechanisms, but ovulatory failure and arrested development ensue (5,7). Here we report two novel homozygous mutations in *STAR* among children who presented with severe primary adrenal failure in infancy. We show how these mutations have important effects on StAR protein structure.

## MATERIALS AND METHODS

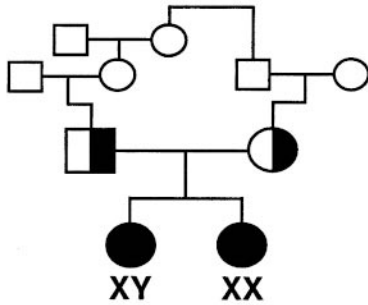
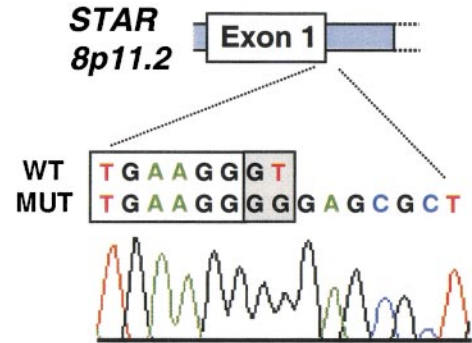
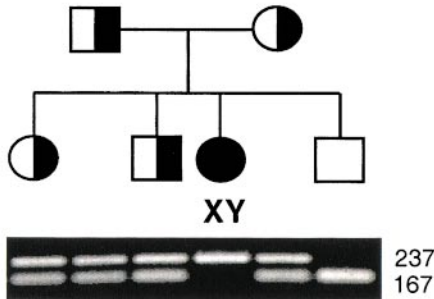
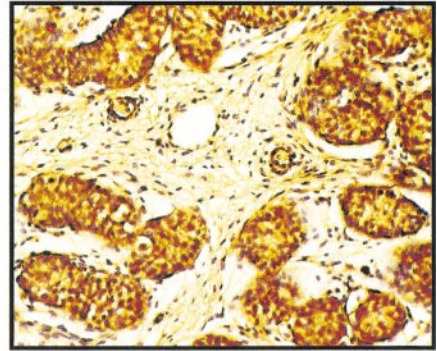
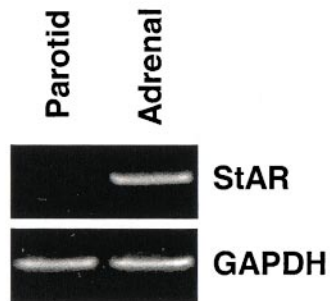
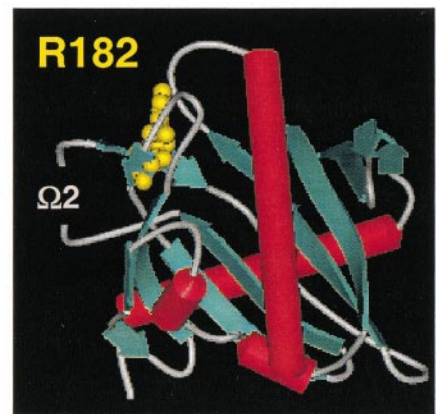
**Mutational analysis.** DNA was extracted from peripheral leukocytes, and all seven exons of the *STAR* gene were amplified by polymerase chain reaction (PCR) using primer pairs and conditions described previously (5). Direct sequencing was performed using a dRhodamine sequencing kit and an ABI 377 automated DNA sequencer (PE Applied Biosystems, Foster City, CA). Restriction enzyme digestion with *Mae*III (Roche Molecular Biochemicals, Mannheim, Germany) was used to confirm the mutation in Kindred II.

**Immunohistochemistry.** Immuno-detection of StAR expression was performed using 5- $\mu$ m sections of testis and a 1:100 dilution of rabbit anti-human StAR antibody as described previously (8).

**Structural modeling.** The position of arginine 182 was mapped onto the crystal structure of the StAR-related lipid transfer domain of MLN64 [protein database reference ID: 1EM2 (9)] using WebLab ViewerLite software (Molecular Simulations, San Diego).

## RESULTS

**Kindred I.** The pedigree of this consanguineous family from Libya is shown in Fig. 1A. The proband (IV.1), a phenotypic female, was hyperpigmented at birth and developed vomiting and diarrhea during

**A****Kindred I: IVS1+2T>G****B****C****Kindred II: R182H****D****E****F**

**FIG. 1.** (A) Kindred I. (B) A homozygous mutation was found in the splice donor site of exon 1 in both affected children. (C) Kindred II. The affected child has a homozygous R182H missense mutation in StAR. The CGT to CAT transversion eliminates a *Mae*III site from the DNA sequence. Restriction digestion was used to confirm the recessive mode of inheritance in this family. (D) Immunohistochemistry of the testis removed from the patient at 4 months of age. The interstitial cells do not show StAR expression. The seminiferous tubules are uncanalated and show weak staining with the anti-StAR antibody (250 $\times$ ). (E) RT-PCR of StAR in normal mouse parotid and adrenal. (F) Arginine 182 of StAR lies within  $\beta$ -sheet 6 of the putative lipid transfer domain and likely forms a critical hydrogen bond interaction with  $\Omega$ -loop 2. This model is based on the crystal structure of the START domain of the related protein, MLN64 (9).

the first days of life. Her karyotype was 46XY. Examination revealed a complete lack of virilization. A younger sibling (IV.2) was also found to be hyperpigmented at birth and developed similar symptoms. Her karyotype was 46XX. Investigations were consistent with a diagnosis of primary adrenal failure, and both children responded well to glucocorticoid and mineralocorticoid replacement therapy. Direct DNA sequencing of the *STAR* gene revealed a homozygous T to G transversion within the splice donor site of exon 1 (IVS1 + 2T > G, g.66) in both affected individuals (Fig. 1B).

*Kindred II.* The proband (II.3 in Fig. 1C) in this family from Qatar is a phenotypic female who presented with pallor and failure to thrive at 3 weeks of age. She was hyponatremic (122 mmol/L) and hyperkalemic (6.1 mmol/L). Cortisol was within the normal range (19  $\mu$ g/dl), but she had elevated ACTH (642 mg/dl) and plasma renin activity (5.7 mg/ml/h). Her karyotype was 46XY. Bilateral gonadectomy performed at 4 months of age revealed immature testes. At 8 years of age, she developed a mucoepidermoid adenocarcinoma of the right parotid gland. DNA sequencing revealed a homozygous R182H missense mutation in StAR in the proband, which was confirmed by *Mae*III digestion (Fig. 1C). Immunohistology of the testis showed low-level StAR expression in the uncanalated seminiferous tubules but no evidence of StAR expression in the interstitium (Fig. 1D). No StAR expression was detected in the parotid tumor by immunohistochemistry (data not shown) or in normal human or mouse parotid by RT-PCR (Fig. 1E).

## DISCUSSION

The association between *STAR* mutations and lipid CAH is well established. The incidence within the Japanese and Palestinian populations is particularly high (5,10). This report of novel splice site and missense mutations in StAR in two families from Libya and Qatar extends the geographic distribution of patients with this condition and provides additional insight into structural domains of the StAR protein.

Although two splice site mutations in *STAR* have been reported previously (11, 12), the exon 1 splice donor site mutation described here is the most amino-terminal of any mutation in StAR described to date. Consequently, this mutation is predicted to cause truncation of most of the StAR protein, includ-

ing much of the N-62 region and the entire lipid transfer domain (10). Consistent with this, both patients had a severe phenotype and evidence of adrenal insufficiency at birth.

Only seven different missense mutations in StAR have been reported to date. All of these changes are located within exons 5–7 of the *STAR* gene (10), and arginine to leucine mutations at codon 182 (R182L) have been described previously in unrelated families from Palestine and Japan (5,10). Recently, the crystal structure of the StAR-related lipid transfer (START) domain of MLN64 has been determined, and a putative lipid-binding tunnel has been identified that shuttles cholesterol through the intermembrane space of the mitochondria (9,13). All reported missense mutations lie within this region. The arginine at position 182 of StAR, which is highly conserved in the START domains of other proteins, lies within  $\beta$ -sheet 6 of this domain and likely forms a critical hydrogen bond interaction with  $\Omega$ -loop 2 (Fig. 1F) (9). Consistent with this, the R182L mutation in StAR impairs steroidogenesis in functional studies (5) and causes abnormal protein folding (14). Our finding of a novel amino acid change at codon 182 provides additional data that this arginine is critical for StAR-dependent steroidogenesis.

Examination of the testicular tissue from this patient revealed a lack of StAR expression in the interstitium. This finding is consistent with destruction of fetal Leydig cells following cholesterol accumulation *in utero* (5,7,8,15) and the complete lack of virilization in this patient. We were unable to show a link between the StAR mutation and development of a rare parotid tumor in this patient, although further reports of tumor development in patients with LCAH would be valuable. However, the description of carcinoma *in situ* in the gonad of an adolescent with a StAR mutation confirms the importance of gonadectomy in these patients (16).

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