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Alu Sx repeat-induced homozygous deletion of the StAR gene causes lipoid congenital adrenal hyperplasia

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ABSTRACT

Lipoid congenital adrenal hyperplasia (Lipoid CAH) is the most severe form of the autosomal recessive disorder CAH. A general loss of the steroid biosynthetic activity caused by defects in the StAR gene manifests as life-threatening primary adrenal insufficiency. We report a case of Lipoid CAH caused by a so far not described homozygous deletion of the complete StAR gene and provide diagnostic results based on a GC–MS steroid metabolomics and molecular genetic analysis. The patient presented with postnatal hypoglycemia, vomiting, adynamia, increasing pigmentation and hyponatremia. The constellation of urinary steroid metabolites suggested Lipoid CAH and ruled out all other forms of CAH or defects of aldosterone biosynthesis. After treatment with sodium supplementation, hydrocortisone and fludrocortisone the child fully recovered. Molecular genetic analysis demonstrated a homozygous 12.1 kb deletion in the StAR gene locus. The breakpoints of the deletion are embedded into two typical genomic repetitive Alu Sx elements upstream and downstream of the gene leading to the loss of all exons and regulatory elements. We established deletion-specific and intact allele-specific PCR methods and determined the StAR gene status of all available family members over three generations. This analysis revealed that one of the siblings, who died a few weeks after birth, carried the same genetic defect. Since several Alu repeats at the StAR gene locus increase the probability of deletions, patients with typical symptoms of lipoid CAH lacking evidence for the presence of both StAR alleles should be analyzed carefully for this kind of disorder.

1. Introduction

In the process of human steroidogenesis all metabolites are derived from pregnenolone, a product of the cholesterol side-chain cleavage reaction catalyzed by cytochrome CYP11A1 at the inner mitochondrial membrane (IMM). Pregnenolone relocates to the endoplasmic reticulum and there serves as a precursor for the synthesis of all groups of steroid hormones. The CYP11A1 reaction relies on the transport of the substrate cholesterol through the cytosol to the outer mitochondrial membrane (OMM) and on the transport from the OMM to the IMM [1]. The latter process represents the rate-limiting step in steroidogenesis depending on the steroidogenic acute regulatory (StAR) protein. The rapid synthesis of this protein accompanying the acute steroidogenic response in steroidogenic cells in case of e.g. stress or injury leds to its designation [2–6]. Thus StAR synthesis supports the acute steroidogenesis on the level of cholesterol transport and as a consequence on the level of substrate access to CYP11A1 [7].

Loss-of-function StAR mutations described in patients cause a general deficiency of the adrenal and gonadal steroid biosynthesis [8] as can be observed in serum and urinary samples. This life-threatening deficiency typically manifests itself in the perinatal period as the most severe form of congenital adrenal hyperplasia, classical lipoid CAH. Patient symptoms include low body weight, severe dehydration, skin hyperpigmentation, respiratory distress and vomiting and are accompanied by massively enlarged adrenal glands filled up with droplets of cholesterol esters. Patients with a 46, XY karyotype show phenotypically normal female genitalia. After birth, adrenal insufficiency occurs within the first 2 months of life in the majority of patients [9].

Abbreviations: StAR, steroidogenic acute regulatory protein; Lipoid CAH, lipoid congenital adrenal hyperplasia; IMM, inner mitochondrial membrane; OMM, outer mitochondrial membrane; GC–MS, gas chromatography–mass spectrometry; Steroid nomenclature, steroid abbreviations are listed in Section 2.3.

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Nonclassic lipoid CAH is an attenuated disorder caused by partial loss-of-function StAR mutations that retain about 10–25% of normal StAR activity [10,11]. Affected individuals typically develop weak symptoms of adrenal insufficiency within the first 2 years and show only mildly disordered sexual development or normal development with hypergonadotropic hypogonadism [12].

So far several complete or incomplete loss-of-function StAR mutations have been described, resulting in a variable presentation of the disorder spanning from classical to nonclassical lipoid CAH. In this study we describe the first case of a family carrying a deletion of the complete StAR gene. Two family members with the homozygous deletion developed symptoms of classical lipoid CAH. One of the siblings died a few weeks after birth, for the other a detailed phenotypic, metabolic and molecular genetic characterization is presented.

2. Materials and methods

2.1. Case report

The female patient is the third child of consanguineous Turkish parents (1. grade cousins). Her mother's parents are 1. grade cousins, too. She was delivered at 38 + 4 weeks of gestation after an uneventful pregnancy through planned cesarean section because of previous cesarean section. Her birth weight was 3580 g (75th centile), her length was 51 cm (50th centile). On physical examination immediately after birth she was in good clinical condition (APGAR score 9/9/10, pH 7.3), her skin showed relatively dark pigmentation, and presented normal external genitalia. However, 5 h after birth her condition was noticed to deteriorate, she developed muscular hypotonia and needed oxygen. Finally, a blood glucose of 7 mg/dL led to referral to the neonatal intensive care unit where she was started on i.v. glucose. Lab tests for signs of infection were negative, an ultrasound examination of her heart showed a small atrial septum defect and slight stenosis of the pulmonary artery. However, both entities were of no hemodynamic relevance. Hyperbilirubinaemia (max. 18.6 mg/dL) led to phototherapy for 9 days. Full nutrition – without any i.v. glucose – by oral feeding was accomplished by the fourth day. No further hypoglycaemic events were noticed.

While oral feeding did not present a problem, the child showed sporadic episodes of vomiting during the second week while staying rather adynamic. On day 11, a serum sodium level of 123 mmol/L (normal range 130–145) and a serum potassium level of 6.6 mmol/L (normal range 3.2–5.0) were noted. Skin pigmentation increased and there was slight enlargement of the liver. The latter was confirmed by abdominal ultrasound which furthermore showed normal uterus and ovaries and enlarged adrenal glands. Hormonal investigations revealed an increased serum ACTH of >1250 pg/L (normal range 5–26) and a low serum cortisol of 2.92 μg/dL (normal range 5–25). A 12-h urinary specimen for GC–MS steroid analysis was obtained. However, during the last few hours of urine collection, sodium, hydrocortisone and fludrocortisone were supplemented due to the worsening clinical condition of the patient. Under this therapeutic regimen the child fully recovered and her skin pigmentation decreased.

In the meantime the child is 3 years old and is taking part in regular medical check-ups. Since then, she has not had any major health problems and her neurological development has been adequate.

The patient has a healthy 7-year-old brother. Three years before her birth, another sibling, born after 30 weeks of gestation, developed sepsis and died in an Addisonian crisis at the age of 4 weeks. The phenotype was female, while the karyotype was 46, XY. This sibling had a Pierre-Robin like dysmorphic face, cleft palate, premature closure of sutures and muscular hypotonia.

2.2. GC–MS urinary steroid metabolomics

Urinary steroid metabolomics applying gas chromatography–mass spectrometry (GC–MS) was performed as described previously [13]. Free and conjugated urinary steroids were extracted by solid phase extraction (Sep-Pak C18 cartridge, Waters Associates, Milford, MA), and the conjugates were enzymatically hydrolyzed (type I powdered Helix pomatia, Sigma–Aldrich Corp., St. Louis, MO). The hydrolyzed steroids were recovered by Sep-Pak extraction. Known amounts of three internal standards (5α-androstane-3α,17α-diol, stigmasterol, and cholesteryl butyrate) were added to a portion of each extract before formation of methyloxime–trimethylsilyl ethers. GC was performed using an Optima-1 fused silica column (Macherey-Nagel, Dueren, Germany). Helium was used as carrier gas at a flow rate of 1 mL/min. The gas chromatograph (Agilent 6890 series GC, Agilent 7683 Series Injector, Agilent Technologies, Waldbronn, Germany) was directly interfaced to a mass selective detector (Agilent 5973N MSD, Agilent Technologies) operated in the selected ion monitoring mode. The injections took place with an 80 °C (2 min) GC oven; the temperature was then increased by 20 °C/min to 190 °C (1 min). For separation of steroids, it was increased by 2.5 °C/min to 272 °C. Values for the excretion of individual steroids were determined by measuring the selected ion peak areas against the internal standards.

2.3. Steroid nomenclature (trivial name (abbreviation; systematic name))

2.3.1. Fetal zone steroids

5-Androstene-3β,17α-diol (A^5-3β,17α; 5-androstene-3β,17α-diol); 16α-OH-dehydroepiandrosterone (16α-OH-DHEA; 5-androstene -3β,16α-diol-17-one); 16β-OH-dehydroepiandrosterone (16β-OH-DHEA; 5-androstene-3β,16β-diol-17-one); androstentriol-16α (A^5T-16α; 5-androstene-3β,16α,17β-triol); 15β,16α-OH-dehydroepiandrosterone (15β,16α-OH-DHEA; 5-androstene-3β,15β,16α-triol-17-one); 16-O-androstendiol (16-O-A^5D; 5-androstene-3β,17β-diol-16-one); 16α,18-OH-dehydroepiandrosterone (16α,18-OH-DHEA; 5-androstene-3β,16α,18-triol-17-one); 15β,17α-OH-pregnenolone (15β,17α-OH-P^5-olone; 5-pregnene-3β,15β,17α-triol-20-one); 16α-OH-pregnenolone (16α-OH-P^5-olone; 5-pregnene-3β,16α-diol-20-one); androstenetetroles (A^5-tetrole 1; 5-androstene-3β,16α,17β,18-tetrole and A^5-tetrole 2; 5-androstene-3β,15β,16α,17β-tetrole); 15β-OH-pregnenetetrol (15β-OH-P^5-tetrol; 5-pregnene-3β,15β,17α,20α-tetrol); 21-OH-pregnenolone (21-OH-P^5-olone; 5-pregnene-3β,21-diol-20-one); 3β,20α,21-pregnenetriol (P^5-3β,20α,21-triol; 5-pregnene-3β,20α,21-triol); P^5-tetrole (P^5-tetrole; 5-pregnene-3β,16α,20α,21-tetrole).

2.3.2. Cortisol metabolites

Allotetrahydrocortisol (aTHF; 5α-pregnane-3α,11β,17α,21-tetrol-20-one); tetrahydrocortisone (THE; 5β-pregnane-3α,17α,21-triol-11,20-dione); 1β-OH-tetrahydrocortisone (1β-OH-THE; 5β-pregnane-1β,3α,17α,21-tretrol-11,20-dione); 6α-OH-tetrahydrocortisone (6α-OH-THE; 5β-pregnane-3α,6α,17α,21-tetrol-11,20-dione); α-cortolone (α-CL; 5β-pregnane-3α,17α,20α,21-tetrol-11-one); 6α-OH-α-cortolone (6α-OH-α-CL; 5β-pregnane-3α,6α,17α,20α,21-pentol-11-one); β-cortolone (β-CL; 5β-pregnane-3α,17α,20β,21-tetrol-11-one); 1β-OH-β-cortolone (1β-OH-β-CL; 5β-pregnane-1β,3α,17α,20β,21-pentol-11-one); 6α-OH-β-cortolone (6α-OH-β-CL; 5β-pregnane-3α,6α,17α,20β,21-pentol-11-one).

2.3.3. Diagnostic key steroids

Tetrahydro-11-dehydrocorticosterone (THA; 5β-pregnane-3α,21-triol-11,20-dione); pregnenetriol (P^5T-17α; 5-pregnene-3β,17α,20α-triol); pregnanetriolone(PT-one; 5β-pregnane-3α,17α,20α-triol-11-one).

2.4. PCR amplification

PCR was applied to amplify the protein-coding regions of CYP11A1 and StAR, as well as to detect the region of the StAR deletion and also to characterize in detail the recombination sequence. Used primer pairs are listed in Tables 1 and 2. The genomic DNA was isolated from blood using QIAamp DNA Blood Midi Kit (Quiagen, Hilden, Germany). All PCR assays were performed in a 50 μL volume containing 200 ng of genomic DNA, 1.75 mM MgCl, 0.3 mM dNTPs, 0.5 μM of forward and reverse primers and 1 U of *KAPA Long Range* DNA Polymerase (KAPA Biosystems, Boston, MA, United States) in the appropriate buffer. After the denaturation step at 94 °C for 2 min, the amplification was performed for 30 cycles at 94 °C for 30 s, at the appropriate annealing temperature for 30 s, and at 72 °C for 1 min, ending with a final extension step at 72 °C for 10 min in the PTC-200 DNA Engine Cycler (BioRad, Hercules, Kalifornien, USA). Amplicons were run on 1% agarose gels in 1× TBE (Tris–acetate–borate–EDTA) buffer.

2.5. Sequence analysis

For the sequencing reactions the amplicons were isolated by electrophoresis on a 1% agarose gel and then purified using PCR clean-up gel extraction kit (Macherey-Nagel, Germany). Fragments were sequenced by Eurofins MWG (Germany) with appropriate primers (Tables 1 and 2), and alignments were performed with reference sequences ENSEMBL StAR (ENSG00000147465) and CYP11A1 (ENSG00000140459) using ClustalW2 (EBI, European Bioinformatics Institute). RepeatMasker software (Institute for Systems biology) was applied to characterize repeated elements.

3. Results

3.1. Urinary steroid metabolome

In the patient, a main finding of the GC–MS steroid metabolomics analysis was the absence of 3β-hydroxy-5-ene-steroids (fetal zone steroids). Furthermore, the absence of aldosterone precursor steroids reflected inadequate aldosterone synthesis. Likewise, important diagnostic metabolites of enzyme defects affecting 17-hydroxylase/lyase, aldosterone synthase, 3β-hydroxysteroid dehydrogenase, 21-hydroxylase or 11-hydroxylase had either clearly subnormal or undetectable concentrations. Due to hydrocortisone treatment, cortisol metabolites were present in normal amounts (Table 3). Both parents and the brother had normal steroid excretion profiles.

3.2. Genomic characterization of CYP11A1 and StAR

Since genetic defects in CYP11A1 cause a rare autosomal recessive disorder that is clinically indistinguishable from StAR defects, our initial strategy was to screen the genomic DNA of the patient for putative mutations in the StAR as well as in the CYP11A1 gene. Eight fragments of the CYP11A1 gene were amplified by PCR, comprising the nine protein-coding exons. Sequencing of the products showed no mutations of CYP11A1 (data not shown). All attempts to amplify any fragment of the StAR gene failed for the patient, but not for the healthy control. Therefore, we assumed the presence of a large genomic deletion extending the whole StAR gene.

3.3. Characterization of the detected deletion

To determine the extent of the deletion, we performed PCR-based DNA walking using primer pairs located 10, 5, 3.5 and 3.0 kb up- and downstream of the StAR gene (Table 2). PCR amplification

Table 1
Oligonucleotides for amplification and sequencing of CYP11A1 and StAR genes and for allele-specific amplicons.

Amplified fragment	Exon	Name	Sequence	Amplicon size (bp)
StAR gene				
A	1	481-F	TGTGTGCCTTCATCTAAGCTGCCCC	567
		1048-R	GGAAGAAACTTGCCCAGGTTCACCC	
B	2, 3	2774-F (P1)	CTAGTTCAGGTCCCTGCTAGAATCC	908
		3682-R (P2)	ACTCTCCAGGGTGTCTTTTATGGGG	
Ex4	4	Ex4S	TGCTGGGATTATAGGCGTGAAC	322
		Ex4AS	GCTAGGGGTCCTCTCTTTGATACAG	
S3	5, 6	S3	GTGAGCAAAGTCCAGGTGCAG	2069
		AS1	ATGAGCGTGTGTACCAGTGCAG	
D	7	6190-F	ATGAGCTGGTAACTGTGCCAACTGC	1361
		7551_R	GTGTCATACTCTAAACACGAACCCC	
Cyp11A1				
1	1	SCC 1-F	TCCAGACTGAACCTTCATACCGAG	586
		SCC 1-R	GGAGTTTGGAACCAGAGAACAGCC	
2	2	SCC 2-F	CAGCAAGTTGTGGAACTGTGAGGC	402
		SCC 2-R	TTTAGGGAGGCAGGAAAATGACGG	
3	3	SCC 3-F	CTGGATGCTGTGAGCTGTGACTCA	430
		SCC 3-R	ATCCCAGAAGTCAGTCTCAATGCC	
4	4	SCC 4-F	AGGGGCAAGAGATCCTTTGTGCTG	394
		SCC 4-R	CATTGACATAGCGTGGGACAAAGG	
5	5	SCC 5-F	CCACCTTTCACCACAGACTGCCCT	330
		SCC 5-R	ACAACAGGGTTTCAGACAACGAGGG	
6/7	6, 7	SCC 6/7-F	TTCGGGGAGAAAGAGGTTCTTGG	690
		SCC 6/7-R	AATTCCAGCCTGCCCCAGCCCTCT	
8	8	SCC 8-F	AAGGGGGTTTTGTGCTCAGGGCTG	355
		SCC 8-R	GCTCTGGACAGAGATAGGAGGAGG	
9	9	SCC 9-F	TATGGGTGAATTGGCCTGGCTAGG	441
		SCC 9-R	GAGTCTGATGGAACGCAAGACACC	
Deletion				
		U2484-F (P3)	TCCAGAAGACCAGGATGG	1382
		D5KF-R (P4)	GGTGACAGTGTTCAGAAATGTAAGCAG	

Table 2
Oligonucleotides for gene walking.

Amplified region	Primer name	Sequence	Amplicon size (bp)
StAR upstream			
10 kb	U10KF2	TTATACAAATACTTATTACTGCCTATAATCC	971
	U10KR2	CCTGTAAACTCTAATTTGGGATATG	
5 kb	U5KF2	ATCAAGATACAGAATATTTTCATCACTCC	373
	U5KR	CCTGGCTGCCATCTAGCAGTCAATA	
3,5 kb	U3738-F	AAGGACTGTTGAATGAATGG	1292
	U2465-R	CTCCAGGAAACCTTCCTAG	
3 kb	U2484-F	TCCAGAAGACCAGGATGG	1145
	U1358-R	ACCCTTCCTCACACTTTCC	
StAR downstream			
10 kb	D10KF	CTTAACGAGCATGCTGCCTTCAAGC	912
	D10KR	CTCACGTTTCTTACTAAAGGCCTACTGAA	
5 kb	D5KF	CAGCTACTCAGGAGGCTGAGG	894
	D5KR	CCTGGCTGCCATCTAGCAGTCAATA	
3 kb	D9617-F	TAAGATATTCAGAAGAGCCACAGGC	1368
	D5KF-R	GGTGACAGTGTTCAGAAATGTAAGCAG	

Table 3
Essential findings from gas chromatographic-mass spectrometric urinary steroid metabolomics analysis in the 2-week-old patient with StAR defect.

Diagnostic parameter [μg/L]	Value	Reference range median (range); $n = 47$	Clinical significance
Σ of fetal zone steroids	132	8460 (1563–30,828)	Absence of adrenal fetal zone
Σ of cortisol metabolites (Fs)	2409	1029 (447–2691)	Contamination from i.v. hydrocortisone
Σ of aldosterone precursors	3	66 (30–258)	Absent aldosterone biosynthesis
Tetrahydro-11-dehydrocorticosterone	3	56 (20–175)	Exclusion of 17-hydroxylase defect and exclusion of aldosterone synthase defect
Pregnenetriol	0	31 (0–146)	Exclusion of 3β-HSD defect
Pregnanetriolone	0	3 (0–27)	Exclusion of 21-hydroxylase defect
Tetrahydro-substance S	0	24 (0–115)	Exclusion of 11-hydroxylase defect

Legend: To assess overall cortisol secretion, the following major neonatal urinary glucocorticoid metabolites were quantified and summed: aTHF, THE, β-CL, 6α-OH-THE, 1β-OH-THE, 6α-OH-α-CL, 6α-OH-β-CL, 1β-OH-β-CL. The following 3β-hydroxy-5-ene-steroids – steroid sulfates – reflect the production rate of fetal zone steroids: A^5-3β,17α, 16α-OH-DHEA, 16β-OH-DHEA, A^5T-16α, 15β,16α-OH-DHEA, 16-O-A^5D, 16α,18-OH-DHEA, 15β,17α-OH-P^5-olone, 16α-OH-P^5-olone, A^5tetrole 1 and 2, 15β-OH-P^5-tetrole, 21-OH-P^5-olone, P^5-3β,20α,21-triol, P^5-tetrole. For systematic steroid nomenclature see in Section 2.3. Steroid nomenclature. Σ, sum; 3β-HSD defect, 3β-hydroxysteroiddehydrogenase defect.

using primers binding in a distance less than 3.5 kb to the StAR gene did not generate any fragments for the patient. This result implied the residence of the deletion boundaries within this region. A PCR with a pair of primers (U2484-F (P3), D5KF-R (P4)) binding within the obtained amplicons upstream and downstream adjacent to this region generated a product of 1382 bp for the patient, while the reaction with the control DNA did not produce any fragment under the applied conditions, as expected (the PCR product of the healthy control would be about 13 kb) (Fig. 1). 807 bp at the 5′ end of the patient-specific PCR product are identical to the genomic sequence up to 1230 bp upstream of exon 1 and 582 bp at the 3′ end of the PCR product correspond to the genomic sequence starting 2670 nucleotides downstream of Exon 7 (Fig. 1). This result clearly indicates that a fragment of 12.1 kb is missing at the StAR gene locus of the patient, resulting in a deletion of all regulatory elements as well as all exons.

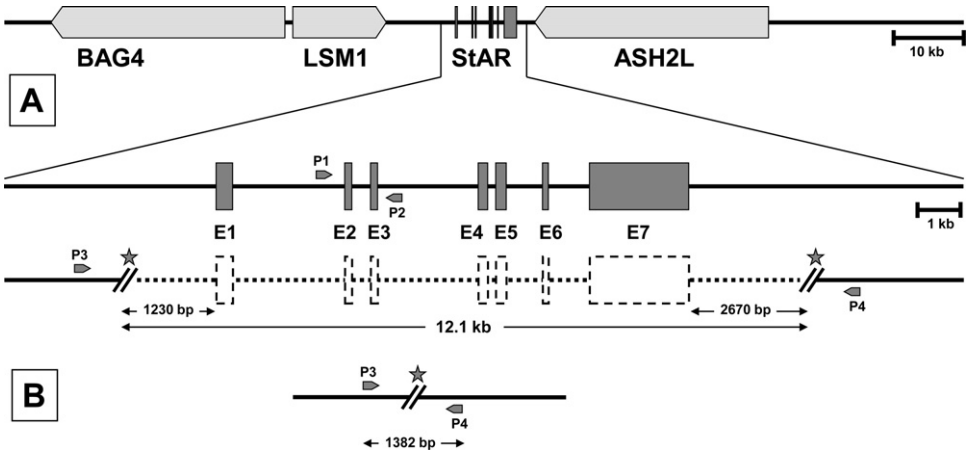

Fig. 1. Schematic representation of the deletion at the StAR gene locus. (A) The region of the StAR gene is magnified. Exons are labeled with E1–E7, primer binding sites for the allele-specific and deletion-specific PCR with P1–P4 and the positions of Alu Sx repeats flanking the deletion site are indicated with asterisks. The allele-specific PCR product of 908 bp obtained using primers P1 and P2 is depicted. Dashed lines represent the 12.1 kb spanning deleted part of the gene locus. The distances between the upstream recombination sequence and exon 1 and the distance between exon 7 and the downstream recombination sequence are indicated being 1230 and 2670 bp, respectively. (B) The sequence of the recombination junction contains the resulting Alu Sx repeat indicated with an asterisk. The deletion-specific PCR product of 1382 bp obtained using primers P3 and P4 is depicted.

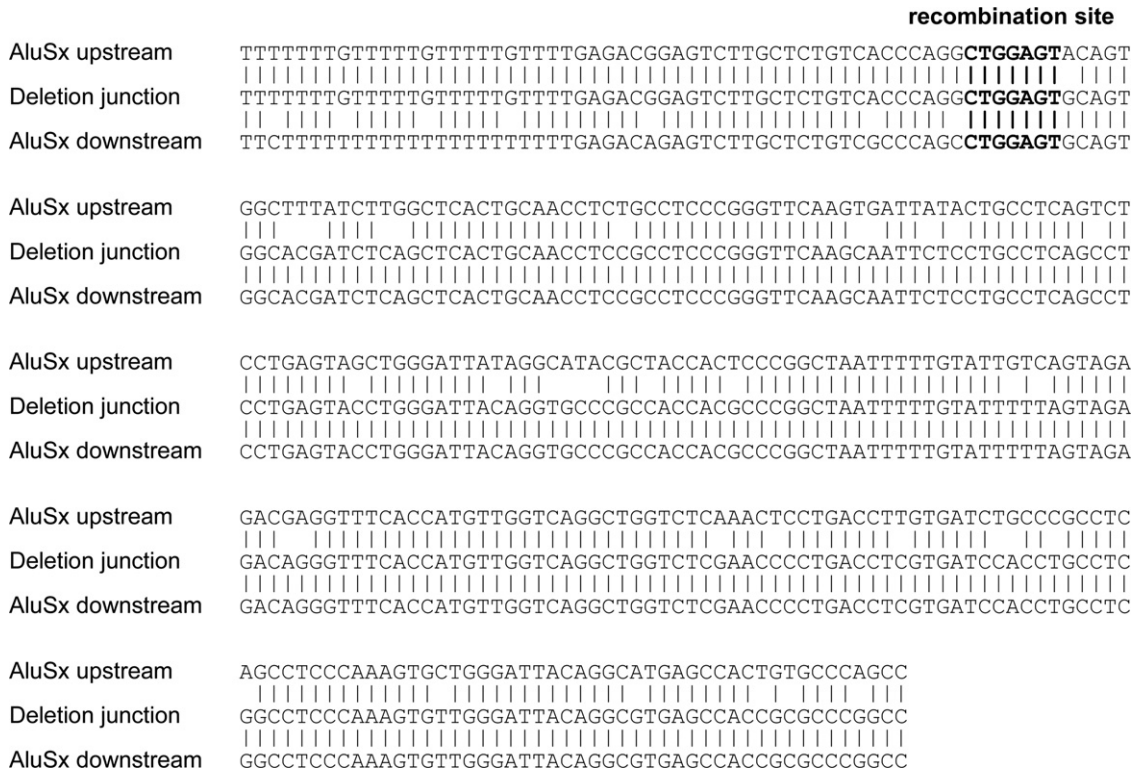

Fig. 2. Alignment of the Alu Sx sequences flanking the StAR gene locus in intact alleles with the sequence at the deletion junction on the defective allel. The sequence of the Alu repeat-induced homologous recombination site has been defined as a 7-bp sequence (CTGGAGT) indicated in bold. This sequence is identical to both Alu Sx-repeat regions, whereas the sequences upstream and downstream of the breakpoint show only 100% identity to one of the two Alu Sx-repeats involved.

3.4. Alu element recombination causes the StAR gene deletion

Analyses of the sequence around the recombination sequence defined a DNA element of 304 bp showing homology to the regions up- and downstream of the StAR gene spanning the deletion breakpoints. Further examination of this sequence identified them as Alu sequences of the Alu Sx subfamily representing a homology of 82%, oriented in the same direction. The precise site of the recombination event has been deduced from the Alu Sx sequence alignment as a 7-bp sequence (CTGGAGT), which shows 100% homology in the two Alu Sx-repeat regions involved (Fig. 2).

3.5. Family pedigree for the StAR gene locus

The genetic status of the StAR locus was analyzed for all family members using two specific PCR amplifications. The first reaction generates the StAR gene-specific fragment B using primers 2774-F (P1) and 3682-R (P2) (Table 1, Figs. 1 and 3A). An amplicon of 908 bp indicates the presence of at least one unaffected allele of the StAR gene. The second PCR with primers U2484-F (P3) and D5KF-R (P4) generates a deletion-specific fragment spanning the deletion gap and creating a 1382 bp amplicon if at least one allele is bearing the StAR gene deletion (Figs. 1 and 3B).

When both amplicons are generated, the family member is heterozygous at the StAR locus. This is shown in Fig. 3 for grandfather (1), aunt II (4), parents (5, 6) and brother I (7). Samples of grandmother (2) and aunt I (3) of the index show only the StAR gene-specific fragment of 908 bp, indicating the existence of two intact alleles of the StAR gene. PCR of the index (9) and the sibling (8) who died at the age of four weeks, generated only the deletion-specific amplicon, which confirms a homozygous deletion for the StAR gene as a reason for the steroid deficiency.

4. Discussion

Due to her worsening clinical condition, the patient received hydrocortisone during the last hours of urine collection. In this context, it has to be emphasized, that for the diagnosis of adrenal enzyme deficiencies spot urinary specimens are sufficient. On the one hand, cumbersome collection of urine can thus be avoided, especially in neonates and young children. On the other hand, spot urinary samples can be taken instead of timely integrated urine samples because the steroid profile of neonates and young infants is not subject to circadian rhythm.

Since hydrocortisone had only been given during the last few hours of the urine collection period, a significant suppressive effect on endogenous steroid biosynthesis could not be expected. Thus, important conclusions could still be drawn from the urinary steroid metabolomics analysis, a noninvasive diagnostic technique with uniquely high diagnostic potential [14]. Adrenal enzyme defects of cortisol biosynthesis such as 17-hydroxylase deficiency, 3β-hydroxysteroid dehydrogenase deficiency, 21-hydroxylase deficiency or 11β-hydroxylase deficiency could clearly be ruled out by absent elevations of their respective indicator steroids [15,16]. Their consistently subnormal levels rather pointed to an overall grossly decreased adrenal steroidogenesis. This finding was further enhanced by the absence of fetal zone steroids – which usually dominate the steroid profile – and the lack of aldosterone precursors.

At this stage, in a female neonate the differential diagnosis would either comprise entities such as severe hypopituitary insufficiency, CYP11A1 or StAR deficiency. In context with the sonographic and NMR findings of enlarged adrenals, excessively elevated plasma levels of ACTH and renin, lipoid congenital adrenal hyperplasia was suspected.

The clinical observation prompted us to screen both the CYP11A1 and the StAR gene for defects. Whereas no mutations

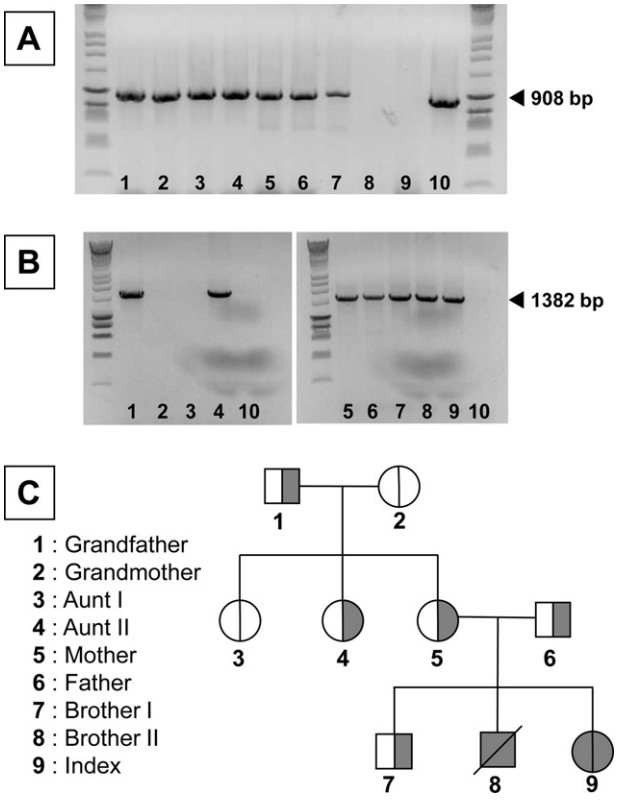

Fig. 3. Analysis of the StAR gene status of a family transmitting an allele with a StAR gene deletion. (A) represents an electrophoretic separation of StAR-specific PCR products amplified using primers P1 and P2 as indicated in Fig. 1. Occurrence of a 908 bp product corresponds to the presence of an intact StAR allele. (B) shows products of a deletion-specific PCR performed with primers P3 and P4. A 1382 bp amplicon occurs only in case of an allele bearing the deletion. The family pedigree of the analyzed index (sample 9) is shown in (C).

were detected in the exons of CYP11A1, a homologous deletion of 12.1 kb at the StAR gene locus was demonstrated by PCR-based DNA walking and sequencing of a PCR amplicon containing the deletion breakpoints. This analysis implies a homologous recombination of two Alu Sx sequences present at the breakpoints resulting in the loss of the complete StAR gene. Alu element linked defects occur frequently because these elements belong to the group of human mobile elements accounting for nearly 11% of the genome with about 1.1 million copies [17]. This large number of Alu elements provides abundant opportunities for insertion mutations and homologous recombination events, which are discussed to cause 0.4% of human genetic disorders [18]. In case of the StAR gene locus (12474 bp) we identified in total 15 Alu elements, representing 30.17% of the DNA in this region. Since this value is significant disproportionate to the 11% in the whole human genome, we can expect an increased probability of Alu element induced recombination events in the StAR gene locus. Therefore, this kind of defect has to be considered and investigated in case of molecular genetic analysis of patients with typical symptoms of lipoid CAH. In order to analyze the molecular genetics of this kind of defect we established deletion-specific and intact allele-specific PCR methods and determined the StAR gene status of all available family members over three generations (Fig. 3). The resulting consanguineous pedigree confirmed that this new mutation manifested as homozygous defect in the index patient and her sibling, whereas all other family members carry at least one intact allele of the StAR gene.

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