CLINICAL STUDY

Long-term clinical data and molecular defects in the *STAR* gene in five Greek patients

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Abstract

Context: Steroidogenic acute regulatory (*STAR*) gene mutations lead to adrenal and gonadal failure. Interesting, though as yet unexplained, features are the formation of ovarian cysts and the potential presence of CNS findings.

Objective: To report biochemical, genetic, and long-term clinical data in five Greek patients from four different families with *STAR* gene defects (three 46,XX and two 46,XY).

Methods and results: All patients presented in early infancy with adrenal insufficiency. The *STAR* gene mutation c.834del11bp, detected in three of our patients, completely alters the carboxyl end of the *STAR* protein and has not thus far been described in other population groups. These three patients belong to three separate families, possibly genetically related, as they live in different villages located in a small region of a Greek island. However, their interrelationship has not been proven. A second mutation, p.W250X, detected in our fourth family, was previously described only in two Serbian patients. Ovarian cysts were detected ultrasonographically in our 46,XX patients and seemed to respond to a low dose of a contraceptive. The histology of an excised ovarian cyst was diagnosed as a corpus luteum (CL) cyst. In two out of the four patients who had undergone brain magnetic resonance imaging, asymptomatic Chiari-1 malformation was observed.

Conclusions: The occurrence of *STAR* gene mutation c.834del11bp in three families living in a restricted geographic region could indicate either a founder effect or simply reflect a spread of this defect in a highly related population. The ovarian histological findings suggest that ovarian cysts detected ultrasonographically in 46,XX individuals with *STAR* gene defects may be CL cysts. The Chiari-1 malformation in two of our patients may be part of the *STAR* gene mutation phenotype. Nevertheless, more data are needed to confirm or disprove the existence of specific CNS pathology in patients with *STAR* gene mutations.

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Introduction

Steroidogenic acute regulatory (*STAR*) protein mediates the transfer of cholesterol from the outer to the inner mitochondrial membrane. This process provides the substrate for the initiation of steroidogenesis and constitutes a rate-limiting step in the acute response of steroidogenic cells of the adrenal cortex and the gonads to the corresponding tropic hormones. The gene encoding the *STAR* protein mapped on chromosomal locus 8p11.2 consists of seven exons and six introns and is expressed in the adrenal cortex and the gonads but not in the placenta (1, 2, 3, 4). Interestingly, *STAR* is also expressed in other tissues, including the brain (5, 6). Most mutations of the *STAR* gene are located in exons 5–7, affecting the *STAR*-related lipid transfer domain (*START*), resulting in

a drastic reduction of *STAR* cholesterol transfer activity, while deletion of 28 carboxyl terminal residues suppresses all activity (7, 8, 9).

Mutations in the *STAR* gene lead to a defect at an early step of steroidogenesis, resulting in severe adrenal insufficiency and gonadal failure (congenital lipoid adrenal hyperplasia (CLAH)), usually manifested in infancy (3, 4, 9). There have been cases, however, characterized by late onset of adrenal failure and normal male genitalia (10, 11) or by a phenotype that could be misinterpreted as familial glucocorticoid deficiency (12, 13).

Adrenal insufficiency does not differ in 46,XY and 46,XX individuals with *STAR* gene mutations, while the gonadal defect shows gender dimorphism. Thus, testicular failure is manifested antenatally, leading to sex reversal in most cases, whereas ovarian function

is normal for many years, leading to normal puberty and menarche but premature menopause (14, 15, 16, 17, 18). We herein present long-term data of five Greek patients with *STAR* gene mutations.

Materials and methods

Patients

Six patients belonging to four families were admitted to our center in infancy with vomiting, failure to thrive, electrolyte abnormalities, low cortisol and androgen values, and high corresponding ACTH values, with initial diagnosis being primary adrenal insufficiency. Clinical and biochemical characteristics of all patients are shown in Tables 1 and 2.

Family 1 Patient 1, a female, now aged 30 years, was examined at the age of 8 months because of vomiting and failure to thrive since the age of 4 months and was diagnosed with adrenal insufficiency (Table 1). The first child of the family had presented with vomiting and dehydration and died at the age of 3 months. Two other siblings are healthy. On replacement therapy with hydrocortisone and 9 α -fludrocortisone, the patient grew well, reaching a final height of 160 cm (SDS, -0.6; target height (TH) SDS, -0.2). Breast Tanner II stage was noted at 9 years and menarche at 12 years. She married at the age of 16 years. Up to the age of 24 years, she could not conceive. An ovarian cyst was detected sonographically at the age of 20 years during a workup for infertility (Fig. 1A) and was excised before the *STAR* gene mutation diagnosis (see histology in the Results section). At the age of 24 years, molecular genetic analysis revealed the presence of *STAR* gene mutation. As the same mutation was detected in her husband in the heterozygous state, she underwent IVF and preimplantation diagnosis and gave birth to a normal girl (19). Following her pregnancy, menses were irregular and ovarian cysts were again detected on ultrasonography (U/S). On half dose of a contraceptive (estrogen content 20 μ g), the ovarian cysts disappeared and reappeared whenever the patient herself interrupted treatment.

Family 2 Patient 2, a female, now aged 28 years, was first examined at the age of 2 months and diagnosed with adrenal insufficiency (Table 1). On replacement therapy with hydrocortisone and 9 α -fludrocortisone, she grew well and reached a final height of 162 cm (SDS, -0.2; TH SDS, -1). Breast development started at age 11 years and menarche occurred at age 13 years. Pelvic U/S at age 5 years showed normal-for-age ovarian size. Ovarian cysts were first detected at 19.5 years during a workup for menstrual irregularities. Molecular genetic analysis revealed the presence of *STAR* gene mutation. Patient 3, a phenotypic female, was diagnosed with adrenal insufficiency and microcephaly at the age of 3 months and replacement therapy for the adrenal failure was initiated. Nevertheless, the infant died at the age of 14 months at home. Unfortunately, no details are available; no autopsy or molecular genetic analysis was carried out, but a *STAR* gene mutation is strongly suspected. Four other children in this family (three males and one female) are healthy, but they declined the molecular genetic analysis.

Family 3 Patient 4, a female, now aged 19.5 years, was first examined on the 20th day of life and diagnosed with adrenal insufficiency (Table 1). On replacement therapy with hydrocortisone and 9 α -fludrocortisone, she has been well and reached a final height of 154.5 cm (SDS, -1.7; TH SDS, -1.8). At the age of 6.6 years, as well as at 12.1 years, the ovaries were sonographically normal. Thelarche and pubarche Tanner stage II were noted at the age of 13 years, at which time ovarian cysts were first identified (Fig. 1B). She was then started on a contraceptive regimen (estrogen content 20 μ g), and 1 month later, the ovaries were of normal size and architecture. After 1 month, the contraceptive pill was discontinued and an ovarian cyst was detected on the left ovary. Normal ovaries were seen soon after resumption of the contraceptive pill. At the age of 14.5 years, half dose of the contraceptive regimen (estrogen content 20 μ g) was initiated, and during 6 years on this regimen, no cysts have developed, while she has regular vaginal bleeding. A brain magnetic resonance imaging (MRI) at age 19 years disclosed Chiari-1 malformation. No neurological deficit has been

Table 1 Biochemical, hormonal, and sonographic data of the patients with *STAR* gene mutations at diagnosis. Normal values are shown in parenthesis.

	Patient 1	Patient 2	Patient 4	Patient 5	Patient 6
Age at diagnosis (months)	8	2	0.7	0.8	0.5
Serum Na (mmol/l) (138–150)	115	135	115	106	127
Serum K (mmol/l) (3.5–5.5)	8.0	7.5	7.3	6.9	7.7
Cortisol baseline (nmol/l) (138–552)	Undetectable	74.5	300.8	350	187.7
Cortisol post ACTH (nmol/l) (> 500)	NA	36	NA	NA	NA
ACTH (pmol/l) (1.1–13.2)	NA	NA	455	> 341	> 330
17OH progesterone (nmol/l)	6.9 (0.7–4.7)	NA	0.9 (2.5–12.6)	1.5 (2.5–12.6)	1.5 (2.5–12.6)
Adrenal sonography	Not visualized	NA	NA	'Normal'	'Normal'

NA, not available.

Table 2 Clinical, hormonal, histological, and genetic characteristics of the patients with CLAH.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Parents' relation	Third cousins	Second cousins	Second cousins	Second cousins	None	None
Pregnancy/delivery	Uneventful	Uneventful	Uneventful	Uneventful	Uneventful	Cesarean section
Birth weight (g)	3000	3000	3000	4100	3140	3300
External genitalia	Female	Female	Female	Female	Female	Female
Karyotype	46,XX	46,XX	NA	46,XX	46,XY	46,XY
Initial symptoms	Vomiting and FT	Vomiting and diarrhea	FT	Vomiting, anorexia, and hyperpigmentation	Vomiting and FT	Vomiting and hyperpigmentation
Age first symptomatic (months)	4	1.7	3	0.3	0.7	0.3
Age at diagnosis (months)	8	2	3	0.7	0.8	0.5
Molecular defect	C834del11bp	C834del11bp	–	C834del11bp	W250X	W250X
Sonography of gonads	20 years ovarian cysts	5 years: normal ovaries 19 years: cyst	NA	6, 8, 5, 12 years: normal 13 years: cysts (breast Tanner stage II)	Small testes in inguinal canal	Small testes in inguinal canal
Age at gonadectomy (years)	–	–	–	–	10	8
Menarcheal age (years)	12	13	–	13.5	–	–
LH/FSH (age in years)	22/6.6 (23)	17.5/6.3 (22)	–	3.6/4.8 (11.5)	1.6/5.7 (8)	0.5/14.8 (1.75)
Gonadal histology	39.5/8.6 (24) Corpus luteum cyst	27/4.5 (23)	–	12/8 (13), 15/77 (13.5)	–	0.3/14.2 (2.3)
Current status (age in years)	Well. Has one child (30)	Well. Married (28)	Died at age 14 months	Well (19.5)	Sertoli cells and rare germ cells (see text) Well (15.5)	Sertoli cells and rare germ cells (see text) Well (14)
Mental development	Normal. Finished first grade of high school	Normal. Poor school progress	Retardation and microcephaly	Normal	Normal	Normal
Brain MRI	Normal	NA	–	Chiari-1 malformation	Nonspecific spotted alterations in the white matter of the R hemisphere Chiari-1 malformation	Normal

NA, not available; FT, failure to thrive.

Figure 1 (A) Ovarian ultrasonography of patient 1 showing multiple cysts in both ovaries at the age of 20 years. (B) Ovarian ultrasonography of patient 4 showing a cystic allantoic structure across the entire length of the right ovary.

detected, but her school progress has been poor. Molecular genetic analysis revealed the presence of *STAR* gene mutation. One older male sibling is healthy but declined molecular genetic analysis.

Family 4 Patient 5 was admitted at the age of 25 days and diagnosed with adrenal insufficiency (Table 1). The external genitalia had normal female appearance. However, the pelvic U/S revealed gonads in the inguinal canal, which resembled testes, and no uterus was identified. The karyotype was 46,XY. At the age of 12 months, a human chorionic gonadotrophin test demonstrated no response of testosterone and its precursors, revealing an additional defect in testicular steroidogenesis. At the age of 10 years, gonadectomy was carried out (see histology in the Results section). The patient, now aged 15.5 years, is well and growing normally on replacement therapy with hydrocortisone and 9 α -fludrocortisone. A brain MRI at the age of 12 years disclosed a Chiari-1 malformation and nonspecific spotted alterations in the white matter of the right cerebral hemisphere. Clinically, the patient had no neurological deficits and the school progress has reportedly been average.

Patient 6, a sibling of patient 5, was admitted at the age of 16 days and diagnosed with adrenal insufficiency

(Table 1). Although a phenotypic female, pelvic ultrasonography disclosed no uterus, while gonads resembling testes (0.1 cm³) were present in the inguinal canal. The karyotype was 46,XY. Gonadectomy was carried out at the age of 8 years. The patient, now aged 14 years, has been doing well on replacement therapy with hydrocortisone and 9 α -fludrocortisone. A brain MRI carried out at age 10 years revealed no pathological findings and the school progress was reported as good. Molecular genetic analysis revealed a *STAR* gene mutation in both patients 5 and 6.

Methods

DNA sequencing analysis DNA was extracted from peripheral blood leucocytes using the QIAamp DNA Blood mini kit (Qiagen) after informed consent was obtained from the parents that genetic analysis be carried out. The seven exons and their flanking intronic sequences of the *STAR* gene (Ensembl Transcript ID, ENST00000276449) were PCR amplified and directly sequenced on an automated sequencer (ABI 3100 Avant, Applied Biosystems). The PCR and sequencing primers were designed using the Primer 3 (v.0.4.0) program and are available on request.

Immunohistochemical studies Tissue sections from the ovarian cysts and the testes, formalin fixed and paraffin embedded, were stained with hematoxylin–eosin. The Bond Polymer Detection Kit (Vision Biosystems, Norwell, MA, USA) was used on paraffin sections for the detection of inhibin α subunit (Biocare Medical, Concord, CA, USA, 1/100), calretinin (Novocastra (Newcastle, UK), 1/150), placenta-like alkaline phosphatase (PLAP; DAKO (Glostrup, Denmark), 1/50), progesterone receptor (Spring Bioscience (Pleasanton, CA, USA), SP2 1/40), and Melan-A (Novocastra, 1/50). Melan-A, a product of the *MART1 (MLANA)* gene, is an antigen recognized by antibody A103, and although its primary utility is in the identification of melanoma cells, it also stains steroid-producing cells. Control experiments with anti-peptides were conducted to show specificity of the antibodies.

Hormonal studies LH, FSH, cortisol, and ACTH were measured by electrochemiluminescent immunoassays on a Cobas e411 automatic analyzer (Roche Diagnostics GmbH (Mannheim, Germany); precision CV: LH 1.1%, FSH 1.7%, cortisol 1.2%, and ACTH 2.3%). Testosterone was measured by chemiluminescent immunoassay on an Immulite 2000 automatic analyzer (Siemens Healthcare Diagnostics, Tarrytown, NY, USA; precision CV: 9.5%). 17OHP was determined by RIA employing reagents from ICN Pharmaceuticals, Inc. (Costa Mesa, CA, USA; precision CV: 9.1%).

Results

Hormonal data are depicted in Table 1. Patients 1, 2, and 4 (Families 1, 2, and 3) were shown to be homozygotes and their parents were heterozygotes for the deletion c.834del11bp (p.K236delsX43) in exon 6 of the *STAR* gene (Table 2, Fig. 2). This 11 bp deletion results in a frameshift, which alters the carboxyl end of the protein, from amino acid 235 onward. It also creates a new stop codon at amino acid 278, making the protein shorter than the wild type by seven amino acids (Fig. 2). The three families that harbor the 11 bp deletion reside in different villages located in a small region of the island of Crete. In all three families, the parents were second- or third-degree relatives. However, the interrelationships between the three families are not known.

In both 46,XY siblings of Family 4 (patients 5 and 6), the mutation p.W250X was detected (Table 2). The parents, heterozygotes for the p.W250X mutation,

Figure 2 Family trees and partial sequence chromatograms of *STAR* gene. (A) F1, family 1, patient 1; F2, family 2, patients 2 and 3; F3, family 3, patient 4. Black symbols and arrows indicate the affected probands. (B) Partial sequence chromatograms of exon 6 of the *STAR* gene. (i) wild type; (ii) homozygote for the 11 bp deletion c.834del11bp; and (iii) heterozygote for the 11 bp deletion c.834del11bp. The arrows indicate the nucleotide at which the deletion starts; red for wild type, black for homozygote, and blue for heterozygote.

Figure 3 Histology of the excised ovarian cyst of patient 1. (A) Cytoplasmic focal distribution of Melan-A, (B) cytoplasmic focal distribution of inhibin α subunit, and (C) immunostaining of progesterone receptors in a moderate number of cells of the cyst and the stroma, Envision $\times 200$. These sections represent typical findings from the wall of the cyst.

originated from the island of Rhodes but mentioned no consanguinity.

The ovarian cyst excised from patient 1 (Family 1) was shown to be a corpus luteum (CL) cyst. Specifically, the immunohistochemical analysis disclosed: i) cytoplasmic focal distribution of Melan-A (a marker of steroid-producing cells); ii) cytoplasmic focal distribution of inhibin α subunit indicating the presence of granulosa cells; and iii) immunostaining of progesterone receptors in a moderate number of cells of the cyst and the stroma (Fig. 3 A, B and C).

The histological studies of the testes removed from patient 5 (Fig. 4 A, B and C) revealed prepubertal testes at a static stage of development, consisting of testicular tubules with Sertoli cells and rare germ cells some of which were large. The Sertoli cells had a focal vesicular appearance. A significant number of Leydig cells were observed in the interstitial tissue with microvesicular appearance. The immunohistochemical study showed positive staining for inhibin α subunit as well as Melan-A and calretinin expression (Melan-A and Calretinin are markers expressed in Leydig cells; calretinin data not shown). PLAP was not identified in the isolated spermatogonia. There was no evidence of intratubular germ cell neoplasia. The only difference observed between patient 6 and his sibling (patient 5; Family 4) was the lower number of Leydig cells in the interstitial stroma containing microvesicular vacuoles. The MRI results are depicted in Table 2 and in patients' descriptions.

Discussion

The majority of reported *STAR* gene mutations are located in exons 5–7. Various studies have shown that the C-terminal region of the *STAR* protein is important for cholesterol transfer and, hence, for steroid

Figure 4 Histology of the testes of patient 5 at the age of 10 years. (A) Spermatid tubules of prepubertal testis with Sertoli cells and vesicular Leydig cells possibly due to lipid droplets or degeneration (hematoxylin–eosin $\times 200$), (B) immunohistochemical expression of inhibin α subunit in the Sertoli cells (streptavidin–biotin–HRP $\times 200$), and (C) immunohistochemical expression of Melan-A in vesicular Leydig cell (streptavidin–biotin–HRP $\times 200$).

biosynthesis (1, 2, 3, 20, 21, 22). The 11 bp deletion (c.834del11bp, p.K236delfsX43) detected in our three 46,XX patients has not thus far been identified in other population groups. These patients belong to three separate families, reportedly not related and living in different villages located in a small geographic region of the island of Crete. These data possibly indicate either a founder effect or simply a spread of this molecular defect in a highly related population. The mutation p.W250X, detected in the two 46,XY siblings (patients 5 and 6), was previously reported in two 46,XY phenotypically female Serbian patients (11, 23). The parents of patients 5 and 6 originated from the island of Rhodes but no consanguinity was reported.

In general, mutations in the *STAR* gene usually lead to a classic form of the disease characterized by early-onset adrenal insufficiency and gonadal failure, the latter showing gender dimorphism both in severity and in time of onset. Most affected 46,XY patients fail to masculinise, whereas the affected 46,XX individuals reported thus far have had normal pubertal development and menarche at the appropriate age (14, 15, 16).

Large cysts of the ovaries have been reported in 46,XX individuals with *STAR* gene defects (15, 16). In our two older patients, ovarian cysts were first identified around the age of 20 years during a workup for either irregular menses or infertility before the specific molecular diagnosis. In one of them (patient 2), earlier ovarian sonography at the age of 5 years was normal. In the younger patient of our cohort (patient 4), with an early diagnosis of *STAR* gene mutation, ovarian sonography was carried out at a younger age and at frequent intervals. In this patient, an ovarian cyst was first detected at 13 years and at pubertal Tanner stage II. An analogous observation of normal ovaries at prepubertal stage was reported in a girl, aged 8 years, with CLAH (24). These findings indicate that cysts

might not be present before puberty but appear with the rise of gonadotropins, i.e. at a stage of higher functional demands when initiation of follicle recruitment occurs.

The mechanism of ovarian cyst formation in girls with *STAR* gene mutations remains an enigma (25), and especially the proneness to recurrence, as was illustrated in patient 4. The mechanism of an increased LH/FSH ratio (25, 26), also detected in our 46,XX patients, and its relation to cyst formation is not clear. In a failing ovary, a high FSH/LH ratio is found, whereas an increased LH/FSH ratio is detected in women with polycystic ovary syndrome (27). The analogy between the latter entity and the 46,XX women with *STAR* gene mutations regarding the LH/FSH ratio is not apparent. It must be mentioned, however, that targeted overexpression of LH in transgenic mice leads to infertility, ovarian cyst formation, and enlarged ovaries (28).

The histological data of the ovarian cyst, surgically excised from patient 1 at the age of 20 years, indicate CL cyst. The CL is a 'temporary endocrine organ' producing steroids for which the *STAR* gene is required (29, 30). In an infertile cycle, the CL undergoes a process of regression known as luteolysis. We may thus assume that in 46,XX women with *STAR* gene mutations, ovulation takes place but the function of the CL is impaired, this possibly leading to cyst formation, as well as to low progesterone synthesis, and infertility. Only one previous report described histological findings from the ovary of a 22-year-old woman, which, however, had undergone torsion. In that particular case, the expected decrease in steroidogenesis was demonstrated by histochemical studies. No CL was detected, while corpus albicans, a regressed form of CL, was present. In *STAR* knockout mice, no CL was found (31).

The role of ovarian cysts in causing 'mechanical damage' to the ovary is not known, but such an effect is theoretically expected. We must emphasize that ovarian torsion has been reported in 46,XX subjects with *STAR* gene mutations (26, 32). Hence, early recognition and management of ovarian cysts is imperative. Most likely, by prompt suppressive therapy of gonadotropin secretion, depletion of normal follicles and the presumed mechanical damage from ovarian cysts could be prevented and thus normal ovarian architecture and potential for fertility may be preserved for longer periods. It is quite possible that half dose of the low estrogen pill might prove effective, as demonstrated by the course of events in our patients 1 and 4. A limited number of successful pregnancies of patients with *STAR* gene mutations have been reported (19, 33).

The synthesis of data related to testicular histology in *STAR* patients, both from the literature (34, 35, 36, 37, 38, 39) and those of our patients, indicate that at an early development stage (fetal and early postnatal life), the testes have normal architecture and a normal number of germ cells, whereas Leydig cells are either scattered or absent. It appears that germ cells are normal prenatally and in early postnatal life but

decrease in number and become large later on. This observation indicates that although germ cells follow a different lineage than steroidogenic cells of the testis, endogenous testosterone production is required for normal germ cell evolution and maintenance. It must be mentioned that testicular biopsy in a 36-year-old male with mild *STAR* gene mutation, investigated for infertility, showed partial atrophy with interstitial fibrosis, many Reincke crystalloids (confirming the presence of Leydig cells), and reduced spermatogenesis (12). In a 15-year-old patient (23), a carcinoma *in situ* was detected in the excised testis. Moreover, in a 1-year-old patient, the excised testes showed positive PLAP and octamer binding transcription factor staining, indicative of neoplastic potential (39). This information is of considerable interest and should be considered when deciding the timing of gonadectomy.

The CNS findings in two of our patients are intriguing. *STAR* protein, a key factor in steroidogenesis, is widely expressed in the brain and specifically in neurons, glial cells, and proliferating precursors (5, 6, 8) and therefore *STAR* gene defects could lead to CNS malfunction. Nevertheless, thus far, convincing evidence for the existence of impaired brain function or structure in CLAH patients is lacking (20, 39). A possible interpretation could be the following: i) brain steroidogenesis is not essential for brain formation and/or function in humans; and ii) a search for pertinent brain pathology has not been systematically carried out to uncover possible defects. Specifically, brain MRI scans or cognitive and other related studies have not been performed systematically in an adequately large number of CLAH patients to offer reliable data.

To our knowledge, of the few children with *STAR* gene mutations in whom an MRI was performed, Chiari-1 malformation was detected in one. In two of our four patients in whom a brain MRI was carried out, Chiari-1 malformation was detected. Other CNS abnormalities have also been detected in such patients, like frontal and temporal lobe atrophy, supratentorial white matter lesions, mental retardation, and hemiatrophy of the right cerebrum, which, however, could have been caused by electrolyte abnormalities in early postnatal life.

The exact prevalence of Chiari-1 malformation in the general population remains unknown. In a study carried out in a general population sample of individuals younger than 20 years ($n = 5248$), the prevalence of Chiari-1 malformation was 0.7/10,000, whereas in subjects who had an MRI for CNS symptoms, it was 1% (40). Of note is the report that 37% of the subjects with Chiari-1 younger than 20 years were asymptomatic at the time of diagnosis, as were our two patients with Chiari-1 malformation. Thus, the existence of CNS pathology (functional or morphologic) in this nosologic entity remains uncertain. Obviously, data from a systematic study with brain MRI scans even in asymptomatic patients and pertinent functional studies in a significant number of patients with *STAR* gene

mutations might offer a satisfactory answer to the questions raised above.

In conclusion, the mutation c.834del11bp has thus far been identified only in our three patients who belong to different families living in a restricted geographic region. The p.W250X mutation detected in two 46,XY siblings from the island of Rhodes has previously been reported only in two Serbian patients. The Chiari-1 malformation in two of our patients may be part of the *STAR* gene mutation phenotype. Nevertheless, further studies are needed to confirm or disprove CNS pathology in patients with *STAR* gene mutations. The histological findings of an excised ovarian cyst, indicating a CL cyst, represent a novel observation with implications for the elucidation of the pathophysiology involved in ovarian cyst formation in this entity. An estrogen–progesterone regimen at half the usual dose, initiated at puberty, may preserve normal ovarian architecture and fertility for longer periods.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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