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ORIGINAL ARTICLE

A novel mutation in *DAX1* (*NR0B1*) causing X-linked adrenal hypoplasia congenita: clinical, hormonal and genetic analysis

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Abstract The association of primary adrenal insufficiency and hypogonadotropic hypogonadism is extremely infrequent in daily clinical practice. Differential diagnosis includes X-linked adrenal hypoplasia congenita, a genetic disease characterized by an alteration in the formation of the adrenal glands and the hypothalamus–pituitary–gonadal axis. The gene responsible is *DAX1* (*NR0B1*). The most common form of clinical presentation is neonatal primary adrenal insufficiency and complete hypogonadotropic hypogonadism. Members of a single family often present the same clinical form, although there may be relatives affected with different clinical symptoms. The aim of this study is to characterize clinically and genetically a family affected by different forms of hypogonadotropic hypogonadism and/or primary adrenal insufficiency. We describe a family with three members affected, two adults and a neonate. The way of presentation of the adults was neonatal primary adrenal insufficiency and hypogonadotropic hypogonadism (one complete and another presenting as interrupted puberty). The genetic study revealed a new mutation in *DAX1*, p.Q76X gene (c.C226T), resulting in a truncated protein of 76 amino acids, the same in all three affected male patients and in the asymptomatic women of the family. These cases further expand the number of *DAX1* mutations reported, as well as the description of infrequent forms of presentation of this disease as interrupted puberty.

Keywords Hypogonadism · Adrenal insufficiency · Hypoplasia · DAX-1 protein · X-linked genetic diseases

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Introduction

The association of primary adrenal insufficiency and hypogonadotropic hypogonadism is extremely infrequent in daily clinical practice [1]. The aetiologies of congenital primary adrenal insufficiency include X-linked adrenal hypoplasia congenita (AHC), a genetic condition due to a mutation of *DAX1* (*Dosage-sensitive sex reversal, Adrenal hypoplasia critical region, on chromosome X, gene 1*) [2]. The official symbol of this gene is *NR0B1* (*Nuclear Receptor Subfamily 0, group B, member 1*). This syndrome is characterized by an alteration in the formation of adrenal glands and the hypothalamus–pituitary–gonadal axis [3]. This entity constrains primary adrenal insufficiency and

hypogonadotropic hypogonadism. Whilst onset of adrenal insufficiency in the neonatal period and the development of hypogonadotropic hypogonadism occur frequently [4], other less frequent clinical forms have been described, such as adrenal insufficiency appearing during infancy [5], partial forms detected in adulthood [6, 7] or cases of neonatal adrenal crisis not requiring replacement treatment during a limited period of time [8]. As for hypogonadotropic hypogonadism, the failure is most often in the hypothalamic or pituitary glands or in both and the clinical spectrum is also quite varied, from complete forms of hypogonadotropic hypogonadism [9], incomplete forms [10], interrupted puberty [11, 12] and precocious puberty [13]. AHC has been associated with glycerol-kinase deficit and Duchene's muscular dystrophy, when deletions occur in the region where *DAX1* is located and including adjacent genes, the alteration of which causes these illnesses [14]. Although it is more frequent for the members of a single family affected to present the same clinical conditions, there are occasions when each member of a family, affected by the same mutation, may suffer the condition with different clinical forms [15], possibly due to the existence of factors modulating the effect of the mutation.

We report a family comprising three males affected by X-linked AHC, diagnosed by means of clinical, analytical and genetic studies. One of them shows the classic presentation consisting in adrenal insufficiency in the neonatal period and complete hypogonadotropic hypogonadism. His brother is characterized by suffering the same form of presentation of neonatal adrenal insufficiency but shows interrupted puberty. The third person affected is the nephew of both these patients, who presented adrenal insufficiency in the neonatal period.

Materials and methods

Biochemistry and hormonal parameter measurement methods

Plasma steroids were measured in a situation of raised ACTH, in order to confirm the diagnosis of cortisol deficit and to exclude enzymatic defects of steroidogenesis. Raised ACTH was achieved through a short-lasting withdrawal of hormone replacement treatment, supervised by medical personnel. ACTH was measured by means of chemiluminescent immunoassay (IMMULITE®, Siemens, UK; Intra-assay variation coefficient, IVC, 3.1–9.6%). Levels of 17-OHP were determined by means of enzyme immunoassay (ELISA, IBL, Hamburg, Germany; IVC 2.8–4.9%). FSH, LH, testosterone, cortisol and dehydroepiandrosterone sulfate (DHEAS) were measured with chemiluminescent immunoassay (CMIA, *Chemiluminescent Microparticle Immunoassay*) (ARCHITECT®, Abbott, Germany; the IVC values are FSH 3.2–4.6%, LH 2.4–4.1%, testosterone 3.1-8.0%, cortisol 2.5–6.2% and DHEAS 2.6–7.4%). Normal values for each of the foregoing parameters are given in Table 1.

Table 1 Baseline hormonal study

Parameter	Case 1	Case 2	Normal range
ACTH (ng/l)	>1200	>1200	5–50
Cortisol (nmol/l)	<5	< 5	220–660
LH (UI/l)	1.6	0.4	1–10
FSH (UI/l)	2.6	4.2	1–14
17-OHP (nmol/l)	0.3	0.2	0.1–4.8
Total testosterone (nmol/l)	3.1	1.2	7–30
DHEAS (nmol/l)	<25	<25	28–3000

Functional tests

Test with GnRH analogue (Luforan®): intravenous administration of 100 μg of GnRH with measurement of baseline FSH and LH, then after 60 and 120 min. A post-stimulus level of LH in excess of 7 U/l was considered normal.

Test with human chorionic gonadotropin (hCG) (hCG Lepori®): intramuscular administration of 5000 IU of HCG with measurement of baseline testosterone and after 72 h. A post-stimulus level of testosterone in excess of 10.4 nmol/l was considered normal.

DNA extraction and detection of mutations

Genomic DNA was extracted from peripheral blood leukocytes using the Chemagic system (Chemagen). The promoter, the exons 1 and 2 of the *DAX1* gene and the exon–intron boundaries (about 50 bp for each end) were amplified by the polymerase chain reaction (PCR), using specific oligonucleotide primer pairs (listed in Table 2) and the enzyme HotStarTaq DNA Polymerase (Qiagen). PCR was performed for 45 amplification cycles (95°C for 30 s, 62°C for 30 s and 72°C for 1 min) after an initial denaturation at 95°C for 15 min and a final extension at 72°C for 5 min. Direct sequencing of PCR products was performed using the enzyme Big Dye Terminator v3.1 Cycle sequencing kit and the sequencer 3730 DNA Analyzer (Applied Biosystems), following the manufacturer's instructions. The analysis of the amplified fragments to determine the nucleotide sequence and the detection of the mutation was done with the computer programmes SeqScape and Sequencing Analysis (Applied Biosystems).

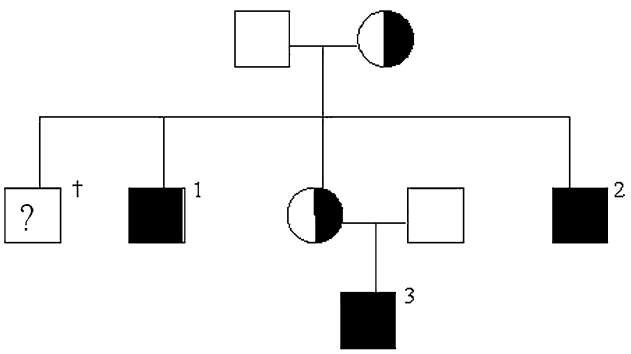
Table 2 Primers used for the amplification of the promoter and the exons of the *DAX1* gene

Promoter	5′-GAGCCTGAGATTGGGCATTT-3′	Promoter	5′-GTAGCCCAGTTCTGCCCAGT-3′
E1	5′-GAGGTCATGGGCGAACACAC-3′	E1_i1	5′-CACGTGCGTTTGCTTTGA-3′
E1_i2	5′-TGTTCGTGCGGCTCTGAT-3′	E1_i2	5′-ACCTGCGTTTGCTTTGA-3′
E1_i3	5′-GCGCTCAAGAGTCCACAGGT-3′	E1	5′-TTAGTCACGATTTCTTCACCTTTGC-3′
E2	5′-TTGGGTCTTGTTTAATTGGGATG-3′	E2	5′-ATGCTACCTGTTGGCAAATGTC-3′
Forward		Reverse	

Results

Patients

Case 1: Male aged 17 years and 11 months referred for a study of delayed puberty. Family history: mother with primary hypothyroidism and father with type 2 diabetes mellitus. Without any family history of delayed puberty. One brother deceased during the neonatal period due to unspecified causes, another brother has adrenal insufficiency and he has a healthy sister. The family tree is represented in Fig. 1, showing the segregation of the illness. The patient was diagnosed as having adrenal insufficiency of unspecified origin in the neonatal period and since then has followed treatment with hydrocortisone (dosage from 8 to 10 mg/m^2/day) and fludrocortisone (dosage from 0.05 to 0.25 mg/day). The patient had a normal infancy, with appropriate development of psychomotor functions, although he sometimes omitted doses of hydrocortisone and fludrocortisone. On physical examination: weight = 61.5 kg (percentile (p) = 10–25); height = 176 cm (p = 25–50); body mass index (BMI) = 19.85 kg/m^2; blood pressure = 110/70 mmHg, sexual characteristics at Tanner's stage III, testicular volume 4 ml of soft consistency, with left varicocele. No auditory or olfactory alterations. The baseline hormonal study (performed during a 24-h withdrawal period in his treatment with hydrocortisone and fludrocortisone, supervised by medical personnel) and the study after stimulations tests are given in Tables 1 and 3, respectively. His



†Exitus. [1] Case 1. [2] Case 2. [3] Case 3.

Fig. 1 Family tree

Table 3 GnRH analogue and hCG stimulation test

Stimulation test	Hormone measured	Case 1	Case 2
GnRH analogue	LH at 60 or 120 min	7.6 UI/l	0.7 UI/l
hCG	Testosterone at 72 h	19.4 nmol/l	1.3 nmol/l

hematimetry and biochemistry results were normal, including sodium and potassium. The X-ray of his carpus showed a bone age corresponding to 15 years and 6 months. A magnetic resonance imaging of the hypothalamus and pituitary was performed with normal results. Following a diagnosis of simple delayed puberty, probably influenced by the poor metabolic control of the adrenal insufficiency, it was decided to carry out an induction of puberty with testosterone enantate 50 mg/im/month for 6 months. Two months after the last injection, the hormone study was repeated and undetectable levels of FSH, LH and testosterone were found. This led to a diagnosis of definitive hypogonadotropic hypogonadism with interrupted puberty and its was decided to administer testosterone enantate intramuscularly at a dose of 250 mg per month, which achieved an adequate degree of androgenization with a final height of 182 cm (p = 75). The seminogram revealed azoospermia.

Case 2: Brother of the patient described above. Male aged 16 years and 7 months with a history of primary adrenal insufficiency, also diagnosed during the neonatal period; during infancy, he followed a treatment with hydrocortisone and fludrocortisone at normal doses, although he sometimes omitted doses, like his brother. His weight and height developed normally (p = 50–75) until 14 years of age, when a slight decrease in growth began, associated with scant progression of secondary sexual characteristics and bone maturity. At the moment of assessment, his height was 170 cm (p = 10–25), his weight 76.6 kg (p = 75), BMI = 26.50 kg/m^2, with a bone age of 15 years and sexual characteristics at Tanner's stages I–II with a testicular volume of 1–2 ml. The hormonal studies performed (shown in Tables 1 and 3) are compatible with hypogonadotropic hypogonadism.

Case 3: Neonatal male, the nephew of the previous patients reported. Healthy mother. Adrenal crisis at 33 days of life. The patient was taken to the emergency room because of vomiting, dehydration, lack of thriving

and poor general condition, and the urgent analysis detected sodium figures of 115 mmol/l, potassium 7.9 mmol/l, chlorine 86 mmol/l, pH 7.36 and bicarbonate 20.2 mmol/l. The hormonal study revealed: ACTH 349 ng/l (normal range 5–50), cortisol 469 nmol/l (220–660) and 17-hydroxyprogesterone (17-OHP) 36.9 nmol/l (<75).

Mutational analysis

Due to the association of primary adrenal insufficiency and hypogonadotropic hypogonadism in the two adult males in the family and the onset of an adrenal crisis in the neonatal period of the nephew of the previous patients, it was decided to conduct a genetic study in search of X-linked AHC, a condition with a very low prevalence caused by mutations in *DAX1*, clinically following the course described in the preceding cases. The study of *DAX1* was performed on all the males affected and on the females in the family. The study of this gene detected the mutation p.Q76X (c.C226T), consisting in the change of the nucleotide at position 226 and implying the appearance of an early stop codon at position 76 in the protein. This mutation in the females has not caused any symptoms, although they are carriers and transmitters of the condition.

Discussion

AHC is a hereditary disease characterized by the lack of development of the cortical layer of the adrenal gland during the embryonic period. This syndrome was first described by Sikl [16]. This first publication of an isolated case of adrenal hypoplasia was followed by other descriptions of families affected and the combination of the adrenal failure with different forms of hypogonadism, giving rise to the adrenal hypoplasia syndrome as we know it today. The histological study shows vacuolate adrenal cells [17]. This syndrome, an X-linked inheritance, is distinguished from recessive autosomal adrenal hypoplasia, which gives rise to very small adrenal cells (miniature form) [18].

Adrenal hypoplasia and hyperplasia are different conditions that must be clearly distinguished. Congenital adrenal hypoplasia is not due to any enzyme deficit of steroidogenesis. On the contrary, adrenal insufficiency due to congenital adrenal hyperplasia is due to enzymatic deficits at the various stages of adrenal steroidogenesis, constituting the most frequent cause of neonatal adrenal insufficiency [1]. Nonetheless, adrenal hypoplasia is much less frequent and is due to a mutation in *DAX1*. The level of 17-OHP is the basis for the differential diagnosis between adrenal insufficiency due to congenital adrenal hyperplasia and congenital adrenal hypoplasia, as occurred in the cases described. In the cases reported here, plasma 17-OHP was low, despite an increase in the ACTH levels. This finding discards the presence of a deficit of 21-hydroxylase and points the diagnosis towards adrenal hypoplasia rather than hyperplasia.

The localization of *DAX1* is the short arm of the X-chromosome, in band 21, between sub-bands 2 and 3 (Xp21.3-p21.2). This gene is expressed in adrenal glands, gonads and the hypothalamus–pituitary region [9], and it encodes the protein DAX1, a nuclear receptor of 470 amino acids belonging to the super-family of orphan receptors [19], so-called because their specific ligand is as yet unknown.

DAX1 is involved in the normal development processes of the adrenal glands and the hypothalamus–pituitary–gonadal axis, as a transcriptional repressor of the genes involved in steroidogenesis. It also takes part in sexual determination, insofar as the duplication of the gene *DAX1* provokes 46, XY sex-reversed patients. Thus, it has been shown that, when *DAX1* is duplicated, XY individuals develop phenotypically as females. On the other hand, its involvement has been described in spermatogenesis, as it is expressed in Sertoli cells [20].

There are almost 200 known mutations of *DAX1* to date [21]. They are deletions (involving only the *NR0B1* gene or contiguous genes as glycerol-kinase deficiency and Duchenne muscular dystrophy), nonsense mutations, frameshift and missense mutations. The truncated protein formed lacks the carboxy-terminal end that binds to the DNA domain; therefore, it does not exercise its function. The mutations in the early aminoterminal extreme are associated with a milder phenotype, due to an alternatively translated isoform of the protein [7]. Although a strong correlation between genotype and phenotype is not always the case, the late-onset forms seem to have a correlation between the phenotype and the function of the protein. Also, there is a tendency for boys within the same family either to present early or to present late.

The new mutation that we have detected in this family produces a short protein truncated at position 76 (p.Q76X) (c.C226T). This mutation is likely to produce a severe phenotype, because the lack of function of this protein. In the case of the adrenal insufficiency, its onset was in the neonatal period in the three cases, with an acute adrenal failure. However, marked clinical differences were noted in the development of hypogonadism between Case 1 (less affected) and Case 2 (more affected). The explanation for this condition is not clear, although it has been suggested that in some cases, *DAX1* could be partially functional [7], resulting in a less severe condition. However, the two cases have the same mutation and only Case 1 develops a mild form of hypogonadism.

The most frequent clinical presentation of AHC is adrenal insufficiency in the neonatal period and hypogonadotropic hypogonadism in puberty. In this family, the two brothers present neonatal adrenal insufficiency but one of them is associated with complete hypogonadotropic hypogonadism, whereas the other presents interrupted puberty. This form of presentation as interrupted puberty is among the least frequent, with few cases reported in the scientific literature [10, 11]. Two very frequent causes of delayed puberty are constitutional delays in growth and development (physiological variant of normality) and delays in maturity due to poor general health. In the first two cases presented here, the existence of poorly controlled adrenal insufficiency due to the frequent skipping of treatment schedules, made the diagnosis of congenital disease particularly difficult. In Case 1, baseline testosterone, and especially the response to stimulus with GnRH and hCG, indicated a start of puberty. However, despite the induction of puberty with low doses of testosterone enantate, it was not possible to maintain this. On the contrary, this process stopped, with undetectable levels of gonadotropins and testosterone presenting after treatment with intramuscular testosterone. In Case 2, there was no start of puberty at any time, with the patient displaying complete hypogonadotropic hypogonadism. In this case, the GnRH and hCG tests performed showed a null responsiveness, unlike his brother. The responses observed in the two patients are consistent with the clinical manifestations, and similar than those described in the literature [12].

Since the germ line is affected in these syndromes, hypogonadism was treated directly with testosterone and not with hCG. Seminara et al. [20] attempted to achieve spermatogenesis in a patient affected by adrenal hypoplasia through the administration of hCG, without success.

Due to the low incidence of this syndrome, the search for a mutation at the level of *DAX1* in adult patients with hypogonadotropic hypogonadism should be reserved for those patients with a personal or family history of adrenal insufficiency or perinatal deaths. On the contrary, the search of mutations in *DAX1* in children or adolescents with unexplained adrenal insufficiency, regardless of family antecedents, is useful. In fact, Lin et al. [21] detected mutations in *DAX1* in 37 of 64 patients (58%) with a history of primary adrenal insufficiency of unknown aetiology and in 8 of 8 patients (100%) with hypogonadotropic hypogonadism and a family history of primary adrenal insufficiency. Nonetheless, Achermann et al. [22] did not detect a single case of *DAX1* mutation in 100 patients with isolated hypogonadotropic hypogonadism. Therefore, the genetic study of the patients with the characteristics exposed above is considered to be of interest, not only for the adequate diagnosis but to provide adequate genetic counselling.

In conclusion, we have described in this paper three males from a single family affected by congenital adrenal hypoplasia who are carriers of a new mutation causing an early stop codon. The patients present two different forms of involvement of the hypothalamus–pituitary–gonadal axis, one with extremely infrequent interrupted puberty.

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References

1. D.I. Shulman, M.R. Palmert, S.F. Kemp, Lawson Wilkins Drug and Therapeutics Committee, Adrenal insufficiency: still a cause of morbidity and death in childhood. Pediatrics **119**, 484–494 (2007)
2. F. Muscatelli, T.M. Strom, A.P. Walker, E. Zanaria, D. Récan, A. Meindl, B. Bardoni, S. Guioli, G. Zehetner, W. Rabl, H.P. Schwarz, J.C. Kaplan, G. Camerino, T. Meitinger, A.P. Monaco, Mutations in the *DAX-1* gene give rise to both X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. Nature **372**, 672–676 (1994)
3. J.K. Phelan, E.R. McCabe, Mutations in NR0B1 (DAX1) and NR5A1 (SF1) responsible for adrenal hypoplasia congenita. Hum. Mutat. **18**, 472–487 (2001)
4. R.L. Habiby, P. Boepple, L. Nachtigall, P.M. Sluss, W.F. Crowley Jr, J.L. Jameson, Adrenal hypoplasia congenita with hypogonadotropic hypogonadism: evidence that DAX-1 mutations lead to combined hypothalmic and pituitary defects in gonadotropin production. J. Clin. Invest. **98**, 1055–1062 (1996)
5. A.T. Reutens, J.C. Achermann, M. Ito, M. Ito, W.X. Gu, R.L. Habiby, P.A. Donohoue, S. Panq, P.C. Hindmarsh, J.L. Jameson, Clinical and functional effects of mutations in the *DAX-1* gene in patients with adrenal hypoplasia congenita. J. Clin. Endocrinol. Metab. **84**, 504–511 (1999)
6. G. Mantovani, G. Ozisik, J.C. Achermann, R. Romoli, G. Borretta, L. Persani, A. Spada, J.L. Jameson, P. Beck-Peccoz, Hypogonadotropic hypogonadism as a presenting feature of late-onset X-linked adrenal hypoplasia congenita. J. Clin. Endocrinol. Metab. **87**, 44–48 (2002)
7. G. Ozisik, G. Mantovani, J.C. Achermann, L. Persani, A. Spada, J. Weiss, P. Beck-Peccoz, J.L. Jameson, An alternate translation initiation site circumvents an amino-terminal DAX1 nonsense mutation leading to a mild form of X-linked adrenal hypoplasia congenita. J. Clin. Endocrinol. Metab. **88**, 417–423 (2003)
8. R. Salvi, F. Gomez, M. Fiaux, D. Schorderet, J.L. Jameson, J.C. Achermann, R.C. Gaillard, F.P. Pralong, Progressive onset of adrenal insufficiency and hypogonadism of pituitary origin caused by a complex genetic rearrangement within DAX-1. J. Clin. Endocrinol. Metab. **87**, 4094–4100 (2002)
9. J.C. Achermann, J.J. Meeks, J.L. Jameson, Phenotypic spectrum of mutations in DAX-1 and SF-1. Mol. Cell. Endocrinol. **185**, 17–25 (2001)
10. A. Tabarin, J.C. Achermann, D. Recan, V. Bex, X. Bertagna, S. Christin-Maitre, M. Ito, J.L. Jameson, P. Bouchard, A novel mutation in DAX1 causes delayed onset adrenal insufficiency and incomplete *Hypogonadotropic hypogonadism*. J. Clin. Invest. **105**, 321–328 (2000)
11. J.H. Bassett, D.J. O'Halloran, G.R. Williams, C.G. Beardwell, S.M. Shalet, R.V. Thakker, Novel DAX1 mutations in X-linked

adrenal hypoplasia congenita and hypogonadotrophic hypogonadism. Clin. Endocrinol. **50**, 69–75 (1999)
12. K. Okuhara, S. Abe, T. Kondo, K. Fujita, N. Koda, H. Mochizuki, K. Fujieda, T. Tajima, Four Japanese patients with adrenal hypoplasia congenita and hypogonadotropic hypogonadism causedby DAX-1 gene mutations: mutant DAX-1 failed to repress steroidogenic acute regulatory protein (StAR) and luteinizing hormone beta-subunit gene promoter activity. Endocr. J. **55**, 97–103 (2008)
13. S. Domenice, A.C. Latronico, V.N. Brito, I.J. Arnhold, F. Kok, B.B. Mendonca, Adrenocorticotropin-dependent precocious puberty of testicular origin in a boy with X-linked adrenal hypoplasia congenita due to a novel mutation in the DAX1 gene. J. Clin. Endocrinol. Metab. **86**, 4068–4071 (2001)
14. J.H. Choi, Y.L. Shin, G.H. Kim, Y. Kim, S. Park, J.Y. Park, C. Oh, H.W. Yoo, Identification of novel mutations of the *DAX-1* gene in patients with X-linked adrenal hypoplasia congenita. Horm. Res. **63**, 200–205 (2005)
15. V. Calvari, M.G. Alpigiani, E. Poggi, B. Podesta, G. Camerino, R. Lorini, X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism: report on new mutation of the *DAX-1* gene in two siblings. J. Endocrinol. Invest. **29**, 41–47 (2006)
16. H. Sikl, Addison's disease due to congenital adrenal hypoplasia of the adrenals in an infant aged 33 days. J. Pathol. Bacteriol. **60**, 323–326 (1948)
17. I.D. Hay, P.J. Smail, C.C. Forsyth, Familial cytomegalic adrenocortical hypoplasia: an X-linked syndrome of pubertal failure. Arch. Dis. Child. **56**, 715–721 (1981)
18. B.A. Burke, M.R. Wick, R. King, T. Thompson, J. Hansen, B.T. Darrae, U. Francke, W.K. Seltzer, E.R. McCabe, B.W. Scheithauer, Congenital adrenal hypoplasia and selective absence of pituitary luteinizing hormone: a new autosomal recessive syndrome. Am. J. Med. Genet. **31**, 75–97 (1998)
19. E. Zanaria, F. Muscatelli, B. Bardoni, T.M. Strom, S. Guioli, W. Guo, E. Lalli, C. Moser, A.P. Walker, E.R. McCabe, T. Meitinger, A.P. Monaco, P. Sassone-Corsi, G. Camerino, An unusual member of the nuclear hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenita. Nature **372**, 635–641 (1994)
20. S.B. Seminara, J.C. Achermann, M. Genel, J.L. Jameson, W.F. Crowley, X-linked adrenal hypoplasia congenita: a mutation in DAX1 expands the phenotypic spectrum in males and females. J. Clin. Endocrinol. Metab. **84**, 4501–4509 (1999)
21. L. Lin, W.X. Gu, G. Ozisik, W.S. To, C.J. Owen, J.L. Jameson, J.C. Achermann, Analysis of DAX1 (NR0B1) and steroidogenic factor-1 (NR5A1) in children and adults with primary adrenal failure: ten years' experience. J. Clin. Endocrinol. Metab. **91**, 3048–3054 (2006)
22. J.C. Achermann, W.X. Gu, T.J. Kotlar, J.J. Meeks, L.P. Sabacan, S.B. Seminara, R.L. Habiby, P.C. Hindmarsh, D.P. Bick, R.J. Sherins, W.F. Crowley, R.C. Layman, J.L. Jameson, Mutational analysis of DAX1 in patients with hypogonadotropic hypogonadism or pubertal delay. J Clin Endocrinol Metab **84**, 4497–4500 (1999)