www.jpnim.com Open Access eISSN: 2281-0692
Journal of Pediatric and Neonatal Individualized Medicine 2022;11(1):e110108
doi: 10.7363/110108
Received: 2020 Dec 07; revised: 2021 Jan 15; accepted: 2021 Jan 31; published online: 2021 Nov 26

Case report

Neonatal adrenal insufficiency – beyond the common causes

Inês Pires Duro[1], Sara Mosca[1], Graça Araújo[2], Joana Lorenzo[1], Catarina Figueiredo[3], Joana Freitas[4], Maria João Oliveira[4], Teresa Borges[4]

[1]Department of Pediatrics, Centro Materno-Infantil do Norte – Centro Hospitalar Universitário do Porto, Porto, Portugal

[2]Department of Pediatrics, Hospital Central do Funchal, Funchal, Portugal

[3]Department of Pediatrics, Centro Hospitalar de Entre o Douro e Vouga, Santa Maria da Feira, Portugal

[4]Department of Pediatric Endocrinology, Centro Materno-Infantil do Norte – Centro Hospitalar Universitário do Porto, Porto, Portugal

Abstract

Background: Primary adrenal insufficiency (PAI) can be caused by multiple etiologies. One of the rarest is X-linked adrenal hypoplasia congenita (AHC), a disorder of adrenal development that results from mutations in the *NR0B1/DAX1* gene and in which half of the affected males present with salt loss and glucocorticoid insufficiency at birth or in early infancy. In adolescence, hypogonadotropic hypogonadism with absent or arrested pubertal development occurs. Pharmacological intervention is mandatory.

Case presentation/discussion: We report 2 male brothers that presented in early infancy with a salt-wasting crisis. Congenital adrenal hyperplasia (CAH) was the presumptive diagnosis, and treatment was instituted accordingly. However, 17-hydroxyprogesterone levels were normal, requiring the search for alternative causes of adrenal insufficiency. In case 2, treatment was discontinued, and he lost follow-up, but he remained well. When he was evaluated due to generalized skin hyperpigmentation, the association of PAI in 2 male brothers raised the suspicion of X-linked AHC and allowed the definitive diagnosis.

Conclusions: These 2 cases highlight the importance of a high index of suspicion when facing a male newborn with PAI in which CAH was excluded in order to allow appropriate management. Despite similar initial presentations and carrying the same mutation, these brothers had different clinical courses, which emphasizes the phenotypic heterogeneity and inherent genotype-phenotype correlation difficulties of X-linked AHC. Careful monitoring and regular follow-up should be warranted, due to phenotypic characteristics that can appear in later stages and may benefit from therapeutic interventions, such as delayed/precocious puberty. Genetic counseling is essential in order to detect female heterozygotes and offer prenatal testing.

Keywords

Primary adrenal insufficiency, neonatal adrenal insufficiency, X-linked adrenal hypoplasia congenita, hypogonadotropic hypogonadism.

Corresponding author

Dr. Inês Pires Duro, Department of Pediatrics, Centro Materno-Infantil do Norte – Centro Hospitalar e Universitário do Porto, Porto, Portugal; address: Largo da Maternidade de Júlio Dinis, 4050-651 Porto, Portugal; phone number: (00351) 919352148; email: ipiresduro@gmail.com.

How to cite

Pires Duro I, Mosca S, Araújo G, Lorenzo J, Figueiredo C, Freitas J, Oliveira MJ, Borges T. Neonatal adrenal insufficiency – beyond the common causes. J Pediatr Neonat Individual Med. 2022;11(1):e110108. doi: 10.7363/110108.

Introduction

Primary adrenal insufficiency (PAI) is a potentially life-threatening condition that can be caused by multiple etiologies, congenital adrenal hyperplasia (CAH) being the most common cause. Nonetheless, when facing a child with PAI, other causes must also be considered, including autoimmune disorders, as well as inherited developmental and metabolic causes. Reaching the correct diagnosis is critical to assess potentially associated comorbidities, initiate long-term management, and provide genetic counseling. One of the less common causes is X-linked adrenal hypoplasia congenita (AHC), a rare disorder of adrenal development that results from mutations in the *NR0B1/DAX1* gene on the short arm of the X chromosome, which plays a critical role in the embryological development of multiple organic tissues and is expressed in the hypothalamus, pituitary gland, adrenals, and gonads. Mutations in this gene lead to the non-development of the outer definitive zone of the fetal adrenal gland. Clinically, about half of the affected males present with salt loss and glucocorticoid insufficiency at birth or in early infancy, while the remaining have a more insidious course with chronic adrenal insufficiency throughout childhood [1]. In adolescence, hypogonadotropic hypogonadism (HH) with absent or arrested pubertal development occurs, due to gonadotropin synthesis impairment and release [2]. An underlying defect in spermatogenesis may also be present. Pharmacological intervention with glucocorticoid and mineralocorticoid replacement is mandatory, as well as treatment with testosterone throughout adolescence. On the other hand, female carriers are generally unaffected. The authors report 2 male brothers with a similar early presentation but different clinical courses.

Case descriptions

Case 1

A 14-day-old term male newborn was admitted to the Neonatal Intensive Care Unit with vomiting, lethargy and failure to thrive associated with hyponatremic (115 mmol/L) and hyperkalemic (8.5 mmol/L) dehydration. External genitalia were normal, and his testes assumed a normal scrotal position. His endocrine work-up revealed normal glucose (77 mg/dL), low serum aldosterone (< 7 pg/mL; normal values: 10-160 pg/mL), high plasma renin activity (2,310.8 pg/mL; normal values: 1.1-16.5 pg/mL) along with low serum cortisol levels (8.6 mcg/dL; normal values: < 18 mcg/dL) and high levels of adrenocorticotropic hormone (ACTH) (908 pg/mL; normal values: 9-52 pg/mL). Considering CAH as the most frequent cause of salt wasting, a treatment with fludrocortisone (100 mcg/day), hydrocortisone (20 mg/m^2/day) and oral sodium supplementation was started, and a favorable clinical evolution was achieved. Afterward, newborn screening results revealed normal levels of 17-hydroxyprogesterone. The abdominal ultrasonography was normal. Genetic testing for CYP11B2 mutations was negative. Fludrocortisone was maintained and hydrocortisone was reduced gradually, in order to reassess the response. However, after its suspension, at 9 months old, laboratory evaluation revealed low cortisol levels (0.1 mcg/dL; normal values: 6.2-19.4 mcg/dL), and the ACTH stimulation test (standard dose, 250 mcg/m^2) revealed no post-stimulation cortisol changes, confirming an adrenal insufficiency.

Case 2

The second case pertains to the older brother, who presented to the Emergency Department at 32 days old with failure to thrive, hyponatremic (123 mmol/L) and hyperkalemic (6.7 mmol/L) dehydration and metabolic acidosis (pH 7.24, HCO_3^- 16.5 mmol/L), consistent with a salt wasting crisis. Like his brother, his external genitalia and testes' location were normal. The endocrine assessment disclosed high levels of ACTH (317

pg/mL; normal values: 9-52 pg/mL), normal serum aldosterone (137 pg/mL; normal values: 10-160 pg/mL), high plasma renin activity (229.1 pg/mL; normal values: 1.1-16.5 pg/mL) along with low serum cortisol levels (6.7 mcg/dL; normal values: < 18 mcg/dL) and normal glucose (61 mg/dL). Abdominal ultrasound had no abnormal findings. Similarly to the first case, he began treatment with fludrocortisone (100 mcg/day), hydrocortisone (20 mg/m^2/day) and oral sodium supplementation. At the age of 2 months, after knowing the results of 17-hydroxyprogesterone, treatment was gradually reduced and suspended, and he maintained a normal endocrinological and metabolic evaluation. However, he missed the following endocrinological appointments. Throughout regular pediatric follow-up, he presented feeding difficulties until 4 months of age, persistently increased levels of creatine kinase (CK) (296-526 U/L) but no muscle weakness and length above the percentile 95th. At 5 years old, he was assessed at the endocrinology unit due to generalized skin hyperpigmentation, revealing an increased ACTH (7,107 pg/mL; normal values: 9-52 pg/mL), low serum aldosterone (64 pg/mL; normal values: 42-202 pg/mL), high plasma renin activity (73.2 pg/mL; normal values: 1.1-16.5 pg/mL) and low serum cortisol levels (3.7 mcg/dL; normal values: 6.2-19.14. mcg/dL), which prompted new treatment with fludrocortisone (50 mcg/day) and hydrocortisone (10 mg/m^2/day). Total testosterone levels were low (0.025 ng/mL; normal values: 2.8-8.0 ng/mL), as well as 17-hydroxyprogesterone (0.2 ng/mL; normal values: 0.59-3.44 ng/mL) and 3-alpha-androstenediol (0.1 ng/mL; normal values: 2.5-20.9 ng/mL) levels. He also presented with increased CK (281 U/L).

Diagnosis

Based on the clinical presentation and the biochemical profile of both brothers, genetic testing for *DAX1/NR0B1* was pursued initially in case 2, confirming the diagnosis of X-linked AHC. A Polymerase Chain Reaction (PCR) was used to amplify the DNA region of interest, and Sanger sequencing of all coding exons of the *NR0B1* gene and their flanking intronic sequences revealed a hemizygous mutation (c.543del [p.Gly183Valfs*81]) on the gene *NR0B1*. Additionally, given the presence of elevated CK levels and the possibility of a defect in the *NR0B1* gene involving deletion of neighboring genes (including Duchenne Muscular Dystrophy), mutations related to the Duchenne/Becker Muscular Dystrophy gene were also searched. Two techniques were used, PCR and Multiplex Ligation-dependent Probe Amplification (MLPA), to detect duplications and deletions in the Duchenne/Becker Muscular Dystrophy gene, which were not identified. After the older brother's diagnosis (case 2), the same genetic mutation was searched in the younger brother, confirming the presence of the familiar variant c.543del (p.Gly183Valfs*81). Further genetic study of the family members is still ongoing.

Case 2, currently at 8 years old, maintains fludrocortisone (50 mcg/day) and hydrocortisone (11 mg/m^2/day), and there are no reports of episodes of acute adrenal insufficiency. Case 1 maintained oral sodium supplementation until the age of 14 months and currently, at 5 years old, maintains treatment with oral fludrocortisone (75 mcg/day) and hydrocortisone (11 mg/m^2/day), also with no reports of episodes of acute adrenal insufficiency.

Discussion

X-linked AHC is a rare disorder of adrenal development whose incidence is estimated to be between 1:140,000 and 1:200,000 [1]. Presentation is typically with adrenal insufficiency during infancy or childhood. Both our patients had a similar presentation with a salt wasting crisis at a very early stage, which is consistent with current literature. Approximately 60% of the boys with *DAX1* mutations have an early onset of primary adrenal failure presenting with salt wasting in the first 2 months of life that can be misdiagnosed as CAH, as described in our patients. The presence of adrenal insufficiency with low cortisol and high ACTH levels suggests a PAI. Normal/low levels of 17-hydroxyprogesterone narrow the causes to AHC, IMAGe syndrome and rare causes of familial PAI such as melanocortin 2 receptor, melanocortin 2 receptor-associated protein, and nicotinamide nucleotide transhydrogenase mutations [3]. This highlights the importance of considering other causes of PAI after exclusion of CAH, especially in male newborns. In case 1, the serum levels of 17-hydroxyprogesterone and other steroids were normal, whereas ACTH level was increased, indicating cortisol deficiency, not related to 21-hydroxylase or other adrenal enzyme deficiencies. Salt-wasting crisis with high renin levels denoted aldosterone deficiency. Despite additional investigation, including serum levels of very-long-chain fatty acids, abdominal

ultrasound and genetic testing for CYP11B2 mutations, no definitive diagnosis was achieved. The evaluation of the older brother (case 2) was prompted by the hyperpigmentation of the skin, which is caused by increased pituitary production of proopiomelanocortin in the context of adrenal insufficiency. His laboratory results were similar to case 1, and consequently, the association of these findings in 2 male brothers raised the suspicion of an X-linked AHC and allowed the definitive diagnosis. Following the recommendations of the Endocrine Society [4], case 2 started treatment immediately, as high ACTH (> 300 pg/mL) and low cortisol (< 18 mcg/dL) levels, associated with cutaneous hyperpigmentation, were highly suggestive of PAI. Additionally, despite similar initial presentations, their clinical courses differed. In case 2, despite the lack of follow-up and absence of medication over a period of almost 5 years, he remained well, which highlights the possibility of a milder clinical phenotype compared to his sibling. Several defects in the *NR0B1* gene have been described in association with AHC: point mutations, partial or complete deletion of the gene or as part of a contiguous gene deletion syndrome (including *NR0B1* and neighboring genes). This diversity of defects dictates a phenotypic heterogeneity that can vary between a classical and an atypical presentation. It is possible that some mutations of the *NR0B1* gene may show atypical phenotypes, i.e., normal testis development and normal puberty [5, 6] or even gonadotropin independent precocious sexual development [7]. Studies *in vitro* also illustrated a possible correlation between the mutant *DAX1* activity and the severity of the clinical phenotype, namely the onset and severity of adrenal insufficiency [8, 9]. Moreover, there are also different symptoms described among patients with the same mutation [6]. Thus, genotype-phenotype correlation is difficult and uncertain in these patients. This heterogeneity can explain the differences between the clinical course of our 2 patients. This phenotypic heterogeneity associated with X-linked AHC was also corroborated by previous studies [10]. We also want to highlight the possibility of a defect in the *NR0B1* gene as part of a contiguous gene deletion syndrome. This was previously described involving deletion of the *NR0B1* and neighboring genes, including Duchenne muscular dystrophy [11, 12]. It was this knowledge, along with elevated CK levels, that prompted further genetic testing for mutations related to the Duchenne/Becker Muscular Dystrophy gene in case 2, that were not identified. Despite this, the diagnosis of dystrophinopathy cannot be definitely excluded, as rarer and subtle mutations were not screened. These findings emphasize the importance of a close follow-up and a regular neurological assessment. Long-term follow-up is crucial in these patients and should include a multidisciplinary team and frequent appointments. Moreover, after the completion of tissue development, *DAX1* continues to play a role in the regulation of hormone production. HH is a characteristic feature of AHC and can occur some time after the diagnosis [13]. It typically manifests as delayed puberty and less commonly as arrested puberty. In these cases, full development of secondary sexual characteristics is unlikely and replacement treatment with testosterone is required [14]. Rarely, the initial manifestation occurs in early adulthood with infertility [15]. It can result from hypothalamic and/or pituitary dysfunction, but it can also result from a local independent alteration of the testicular germinal epithelium that affects spermatogenesis [16]. Azoospermia is typically present in patients with X-linked AHC, and the mechanism is gonadotropin-independent, since it was found that gonadotropin therapy was unsuccessful in the induction of spermatogenesis [5]. Additionally, hormonal therapy may not reverse infertility, but it may benefit those patients with persistent short stature due to a lack of pubertal growth spurt [16]. These findings reinforce the need for a regular follow-up in our patients, with close monitoring of pubertal development and growth. Regarding the genetic testing, the mutation found was the familiar variant c.543del (p.Gly183Valfs*81). It is a known variant associated with AHC and described as pathogenic [17]. It comprises a deletion at position 543 that results in a frameshift and premature stop codon at position 183, causing loss of protein expression or originating a truncated protein, affecting protein function severely. The mutational analysis of *DAX1* is important not only for the provision of genetic counseling but also for the appropriate management of these patients. The presence of the same mutation in both brothers suggests a hereditary pattern. Although female carriers are generally unaffected, manifestations of adrenal insufficiency or HH can occasionally occur in homozygous or heterozygous female carriers [18, 19]. The genetic studying of the mother is still ongoing.

Conclusions

These 2 cases highlight the importance of a high index of suspicion when facing a male newborn

with PAI in which CAH was excluded, in order to allow appropriate management. Despite similar initial presentations and carrying the same mutation, these brothers had different clinical courses, which emphasizes the phenotypic heterogeneity and inherent genotype-phenotype correlation difficulties of X-linked AHC. Careful monitoring and regular follow-up should be warranted, due to phenotypic characteristics that can appear in later stages and may benefit from therapeutic interventions, such as delayed/precocious puberty. Regular neurological assessments are also crucial, especially in cases like case 2. Genetic counseling is also essential in order to detect female heterozygotes and offer prenatal testing.

Declaration of interest

The Authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The Authors state that there was no funding or research grants received in the course of study, research or assembly of the manuscript.

References

1. Lin L, Gu W-X, Ozisik G, To SW, Owen CJ, Jameson JL, Achermann JC. Analysis of DAX1 (NR0B1) and Steroidogenic factor-1 (NR5A1) in Children and Adults With Primary Adrenal Failure: Ten Years' Experience. Clin Endocrinol Metab. 2006;91(8):3048-54.
2. Zanaria E, Muscatelli F, Bardoni B, Strom T, Guioli S, Guo W, Lalli E, Moser C, Walker A, McCabe E. An Unusual Member of the Nuclear Hormone Receptor Superfamily Responsible for X-linked Adrenal Hypoplasia Congenita. Nature. 1994;372(6507):635-41.
3. Hochberg Z. Practical algorithms in pediatric endocrinology (3rd ed). Basel: Karger, 2007.
4. Bornstein SR, Allolio B, Arlt W, Barthel A, Don-Wauchope A, Hammer GD, Husebye ES, Merke DP, Murad MH, Stratakis CA, Torpy DJ. Diagnosis and treatment of primary adrenal insufficiency: An Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2016;101(2):364-89.
5. Rojek A, Flader M, Malecka E, Niedziela M. A novel mutation in the NR0B1 (DAX1) gene in a large family with two boys affected by congenital adrenal hypoplasia. Hormones. 2014;13(3):413-9.
6. Choi J-H, Shin Y-L, Kim G-H, Kim Y, Park S, Park J-Y, Oh C, Yoo H-W. Identification of Novel Mutations of the DAX-1 Gene in Patients with X-Linked Adrenal Hypoplasia Congenita. Horm Res Paediatr. 2005;63(4):200-5.
7. Landau Z, Hanukoglu A, Sack J, Goldstein N, Weintrob N, Eliakim A, Gillis D, Sagi M, Shomrat R, Kosinovsky E, Anikster Y. Clinical and genetic heterogeneity of congenital adrenal hypoplasia due to NR0B1 gene mutations. Clin Endocrinol. 2010;72(4):448-54.
8. Mantovani G, Ozisik G, Achermann J, Romoli R, Borretta G, Persani L, Spada A, Jameson J, Beck-Peccoz P. Hypogonadotropic Hypogonadism as a Presenting Feature of Late-Onset X-Linked Adrenal Hypoplasia Congenita. J Clin Endocrinol Metab. 2002;87(1):44-8.
9. Achermann J, Meeks J, Jameson J. Phenotypic spectrum of mutations in DAX-1 and SF-1. Mol Cell Endocrinol. 2002;185:17-25.
10. Rojek A, Krawczynski MR, Jamsheer A, Sowinska-Seidler A, Iwaniszewska B, Malunowicz E, Niedziela M. X-Linked Adrenal Hypoplasia Congenita in a Boy due to a Novel Deletion of the Entire NR0B1 (DAX1) and MAGEB1-4 Genes. Int J Endocrinol. 2016;2016:5178953.
11. Muscatelli F, Strom T, Walker A, Zanaria E, Recan D, Meindl A, Bardoni B, Guioli S, Zehetner G, Rabl W. Mutations in the DAX-1 Gene Give Rise to Both X-linked Adrenal Hypoplasia Congenita and Hypogonadotropic Hypogonadism. Nature. 1994;372(6507):672-6.
12. Peter M, Viemann M, Partsch CJ, Sippell WG. Congenital adrenal hypoplasia: Clinical spectrum, experience with hormonal diagnosis, and report on new point mutations of the DAX-1 gene. J Clin Endocrinol Metab. 1998;83(8):2666-74.
13. Kyriakakis N, Shonibare T, Kyaw-Tun J, Lynch J, Lagos C, Achermann J, Murray R. Late-onset X-linked adrenal hypoplasia (DAX-1, NR0B1): two new adult-onset cases from a single center. Pituitary. 2017;20:585-93.
14. Achermann JC, Vilain EJ. NR0B1-Related Adrenal Hypoplasia Congenita. In: Adam MP, Ardinger HH, Pagon RA (Eds.). GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle, 1993-2021. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1431/, date of publication: 20 November 2001, last update: 25 January 2018, last access: 13 January 2021.
15. Tabarin A, Achermann JC, Recan D, Bex V, Bertagna X, Christin-Maitre S, Ito M, Jameson JL, Bouchard P. A novel mutation in DAX1 causes delayed-onset adrenal insufficiency and incomplete hypogonadotropic hypogonadism. J Clin Invest. 2019;105(3):321-8.
16. Gupta S, Joshi K, Zaidi G, Sarangi A, Mandal K, Bhavani N, Pavithran P, Pillai M, Singh S, Godbole T, Bhatia V, Bhatia E. Novel mutations and spectrum of the disease of NR0B1 (DAX1)-related adrenal insufficiency in Indian children. J Pediatr Endocrinol Metab. 2019;32(8):863-9.
17. Zhang YH, Guo W, Wagner RL, Huang BL, McCabe L, Vilain E, Burris TP, Anyane-Yeboa K, Burghes AHM, Chitayat D, Chudley AE, Genel M, Gertner JM, Klingensmith GJ, Levine SN, Nakamoto J, New MI, Pagon RA, Pappas JG, McCabe ERB. DAX1 mutations map to putative structural domains in a deduced three-dimensional model. Am J Hum Genet. 1998;62(4):855-64.
18. Merke DP, Tajima T, Baron J, Cutler GB. Hypogonadotropic Hypogonadism in a Female Caused by an X-Linked Recessive Mutation in the DAX1 Gene. N Engl J Med. 1999;340(16):1248-52.
19. Seminara SB, Achermann JC, Genel M, Jameson JL, Crowley WF. X-linked adrenal hypoplasia congenita: A mutation in DAX1 expands the phenotypic spectrum in males and females. J Clin Endocrinol Metab. 1999;84(12):4501-9.