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Simple virilising congenital adrenal hyperplasia in monozygotic twins: A rare report and review of previous cases

Wrodzony przerost nadnerczy z prostą wirylizacją u bliźniąt monozygotycznych: opis rzadkiego przypadku i przegląd wcześniejszych przypadków

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Abstract
Congenital adrenal hyperplasia (CAH) occurring in twins is extremely rare. Most of these cases are of classic salt-wasting CAH due to 21-hydroxylase enzyme deficiency. Only two cases of the simple virilising form of CAH have been reported previously, with variable clinical presentations. In this report, we describe a pair of monozygotic twins with classic simple virilising form of CAH, who had a simultaneous onset and similar severity of clinical manifestations. Genetic analysis of the *CYP21A2* gene in twin 1 showed the presence of two heterozygous pathogenic sequence variants, c.518T>A and c.955C>T in the *CYP21A2* gene, consistent with a diagnosis of CAH due to 21-hydroxylase deficiency. We also present a brief review of previous cases of twins with CAH.
Key words:
congenital adrenal hyperplasia, classic simple virilising form, disorders in twins, monozygotic twins.

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Introduction

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders due to a defect in the enzymes of the adrenal steroidogenesis pathway, which results in alterations in glucocorticoid, mineralocorticoid, and sex steroid production [1]. The most common enzyme defect is 21-hydroxylase (21-OH) deficiency due to mutations of the 21-OH gene (also known as *CYP21* or *CYP21A2* gene) accounting for almost 95% of all CAH cases [1]. Depending upon the residual 21-OH enzyme activity, the clinical presentations vary widely from neonatal salt wasting and atypical genitalia to hirsutism in adult women [1]. The 21-OH deficiency may present as a classic salt-wasting (SW) or simple virilising (SV) form and a non-classic form. The occurrence of CAH is rare in twins, with about 15 cases reported in the literature. The majority of the previously reported cases were of SW type. Only two cases of twins with SVCAH have been reported so far. Variability in clinical manifestations has been noted even in monozygotic twins with SVCAH. We report a pair of monozygotic twins with SVCAH, who had simultaneous-onset and similar severity of clinical manifestations, and present a brief literature review of the previous cases.

Case report

The 2.5-year-old twin girls presented with complaints of atypical genitalia noticed at birth. There was no history to suggest adrenal crisis or failure to thrive. There was no history of maternal drug intake during pregnancy or parental consanguinity. Antenatal ultrasonogram showed monochorionic diamniotic placentation. Both were born at the 33rd week of gestation by vaginal delivery and weighed 1500 g (twin 1) and 1700 g (twin 2).

Physical examination showed normal anthropometric parameters. Examination of genitalia showed enlarged clitoris and single urogenital opening and posterior labial fusion in both girls. The genitalia pigmentation was normal (Fig. 1). Systemic examination was unremarkable. Laboratory investigations showed normal serum levels of Na^+ (137 mEq/l), K^+ (4.6 mEq/l), and random blood glucose (120 mg/dl). The baseline serum 17-hydroxyprogesterone (17-OHP) values in twin 1 and 2 were 24.8 ng/ml and 18.4 ng/ml while their serum cortisol values were 137.6 and 115.1 nmol/l, respectively. The peak stimulated serum concentrations of 17-OHP after a standard dose (250 μg) short corticotropin stimulation test were 34.9 and 39.9 ng/ml in twin 1 and 2, respectively, with corresponding peak cortisol of 151.1 and 132.0 nmol/l, respectively. Both children had 46,XX karyotype. Radiographs of the left wrist and hand showed that bone age corresponded with the chronological age in both twins. An ultrasonogram showed normal adrenal glands, and Mullerian structures. Mutation analysis of the *CYP21A2* gene was carried out on the DNA extracted from the peripheral blood of twin 1. The *CYP21A2* and *CYP21A1P* gene regions and potential deletions or rearrangements in the region were screened for by using long PCR with four primer combinations followed by gel electrophoresis. Paired-end custom amplicon next-generation sequencing (NGS) of the *CYP21A2* gene (using long amplification followed by tagmentation to minimise interference with the *CYP21A1P* pseudogene) and subsequent bioinformatic analysis were used to detect the presence of small sequence variants. The NGS detected the presence of two heterozygous

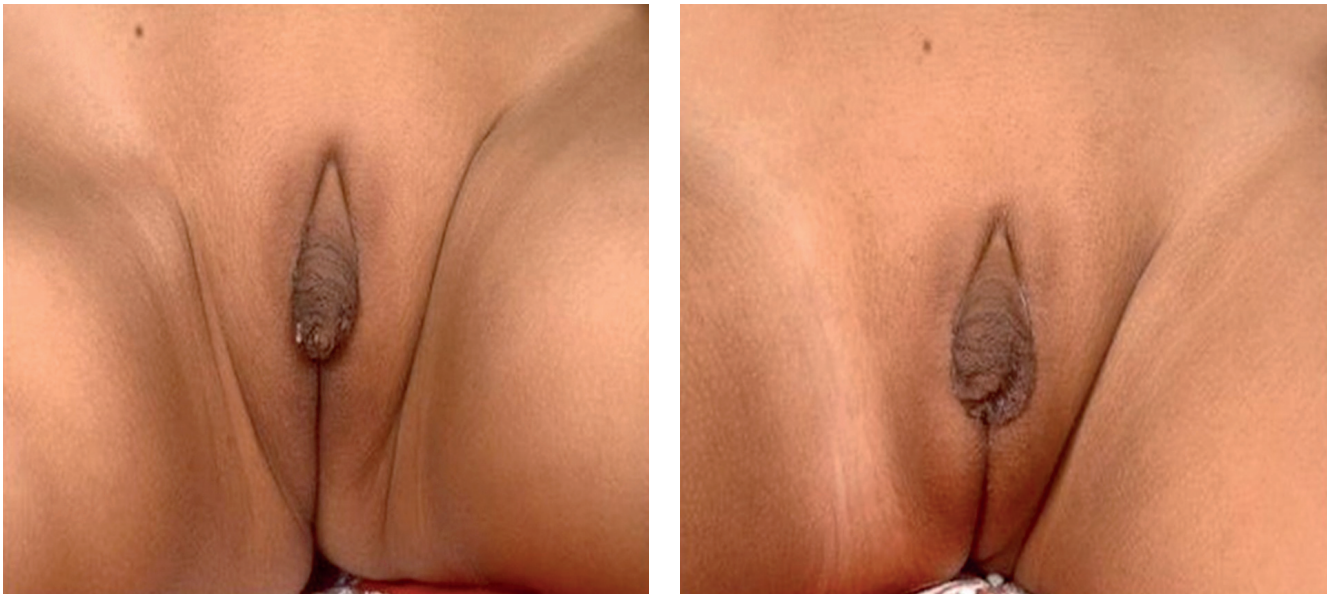

Figure 1. Clinical photograph of external genitalia showing clitoral enlargement in Twin 1 (a) and Twin 2 (B)

Table I. Previous reported cases of CAH twins

S. no.	Authors, year of report	Type of twins	Type of CAH	Genetic analysis	Treatment	Follow-up duration
1.	Wolff *et al.* 1954 [4]	MZ, girls	SW 21-OHD	Not done	Oral cortisone	12 months
2.	Schneeberg *et al.* 1959 [5]	MZ, girls	NC 21-OHD	Not done	IM cortisone or prednisone	3 years
3.	König *et al.* 1966 [6]	MZ, twins	Article in German; limited information available		Depot 6-α methylprednisolone	Long-term
4.	Moine *et al.* 1970 [7]	MZ, girls	SV	Article in French; limited information available		
5.	Stoica *et al.* 1970 [8]	NM, girls	Article in Romanian; details not available			
6.	Tze *et al.* 1972 [9]	DZ, twins	SW 21-OHD	Not done	Dexamethasone	NM
7.	Connors *et al.* 1976 [10]	MZ, boys	SW 21-OHD	Not done	Oral cortisone & fludrocortisone	2 years
8.	Kondo *et al.* 1983 [11]	DZ, twins	Article in Japanese; details not available			
9.	Kanjilal *et al.* 1989 [12]	MZ, girls	SV 21-OHD	Not done	Oral cortisone	NM
10.	Brian *et al.* 1991 [13]	DZ, Boy, girl	SW 21-OHD	Not done	Oral cortisone	NM
11.	Bromley *et al.* 1994 [14]	NM	11-β-OHD	Not done	Cortisone and fludrocortisone	NM
12.	Gunther *et al.* 1997 [15]	DZ, girls	SW 21-OHD	Complete deletion of the CYP21 gene (paternal) and an R356W mutation (maternal) in affected girl	Oral hydrocortisone, fludrocortisone and adrenalectomy	9 months
13.	AvRuskin *et al.* 2003 [16]	MZ, girls	SW 21-OHD	8bp deletion in exon 3 (maternal) and 1172N missense mutation (paternal)	Oral hydrocortisone and fludrocortisone	6 years
14.	Incorvaia *et al.* 2003 [17]	DZ, girls	NC 21-OHD	Not done	Ethinylestradiol and cyproterone	15 months
15.	Park *et al.* 2013 [18]	NM, girls	STAR protein deficiency	Mutation of p.R182C in exon 5 of the STAR gene	Oral hydrocortisone and fludrocortisone	14 months
16.	Index patient	MZ, girls	SV 21-OHD	Two heterozygous pathogenic variants c.518T>A and c.955C>T in the CYP21A2 gene	Hydrocortisone	6 months

CAH – congenital adrenal hyperplasia; STAR – steroidogenic acute regulatory protein; MZ – monozygotic; DZ – dizygotic; SW – salt-wasting; NC – non-classic; SV – simple virilising; OHD – hydroxylase deficiency; NM – not mentioned

sequence variants in the *CYP21A2* gene. The first variation, NM_000500.7:c.518T>A NP_000491.4:p.I173N (I172N by old nomenclature), a single nucleotide change, shifts the coded amino acid from isoleucine to asparagine and is reported in the Human Gene Mutation Database and ClinVar as "pathogenic" for CAH due to 21-OH deficiency. The second detected variant, NM_000500.7:c.955C>T NP_000491.4:p.Gln319Ter (Q318X by old nomenclature), results in a stop codon at position 318 in exon 8 of the *CYP21A2* gene and is reported as pathogenic in ClinVar. Individuals homozygous for this variant are reported to have SW type of CAH. The genetic analysis could not be performed in twin 2 or in the parents due to financial constraints. Both children were initiated on oral hydrocortisone.

Discussion

Classic CAH caused by the 21-OH deficiency may manifest either as a SW or SV form. The classic SW form usually manifests during the first few weeks of life with adrenal insufficiency whereas patients with SVCAH are usually diagnosed late with either clitoral enlargement or precocious puberty [2, 3]. In the index patients also, the diagnosis was delayed although genital ambiguity had been noticed by parents at birth. The delays in seeking medical advice by parents as well as the lack of a newborn screening program for CAH contribute significantly to the diagnostic delays in developing country setups like ours [3].

Our literature search revealed only 15 cases of twins with CAH, highlighting the rarity of this condition. Table I shows the cases of twins with CAH that have been reported so far [4–18]. The anticipated incidence is probably less than 1 in 23 million births [5]. For unknown reasons, the majority of such cases were due to SW type of 21-OH deficiency. Only two cases of SVCAH have been reported in twins born in USA and France [7, 12]. Another noteworthy feature was the occurrence of CAH predominantly in monozygotic twins, underlying the importance of the genetic basis of the disease. Although monozygotic twins are expected to have similar manifestations compared to dizygotic twins, variability in clinical presentation has been observed even in monozygotic twins, suggesting the role of non-genetic factors in virilisation [12]. The phenotypic variation in monozygotic twin may also occur as a result of epigenetic states, which are dynamic and potentially reversible marks involved in gene regulation, and they can be influenced by genetics, environment, and stochastic events. However, both our twins were found to have similar disease severity. The reports of cases of CAH twins predominantly in females probably indicate missed diagnoses in boys due to lack of genital ambiguity.

A majority of CAH patients (almost 95%) are due to 21-OH deficiency resulting from pathogenic sequence variants in the *CYP21A2* gene. As in our index patient, most affected individuals are compound heterozygotes, presenting different pathogenic variants on each allele rather than being homozygous for the same pathogenic variant [19]. Most of the heterozygotes and carriers remain asymptomatic, with some exceptions [19]. The pathogenic variants of the *CYP21A2* gene are associated with variable impairment of cortisol and aldosterone synthesis depending upon the severity of loss of function and the consequent degree of residual activity of the 21-OH enzyme. The decrease in serum concentrations of cortisol results in loss of negative feedback inhibition and a compensatory increase of corticotropin secretion that causes adrenal cortex hypertrophy and hyperpigmentation of genitalia. The steroid precursor molecules accumulate and lead to increased adrenal androgen production through the delta-5 pathway and CYP17A1 resulting in features of virilisation [19].

Only three previous cases of CAH twins had confirmation of diagnosis with molecular analysis [15, 16, 18]. The sequence variation observed in our patient was different from the previously reported cases and is more commonly found in the patients from the Asian continent [20]. To the best of our knowledge, ours is the only case reported from the Indian subcontinent of monozygotic twins with SVCAH.

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