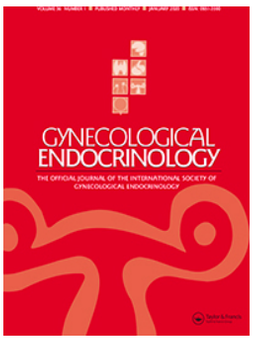

Gynecological Endocrinology

ISSN: 0951-3590 (Print) 1473-0766 (Online) Journal homepage: https://www.tandfonline.com/loi/igye20

# A report of congenital adrenal hyperplasia due to 17α-hydroxylase deficiency in two 46,XX sisters

Fernando Espinosa-Herrera, Estefanía Espín, Ana M. Tito-Álvarez, Leonardo-J Beltrán, Diego Gómez-Correa, German Burgos, Arianne Llamos, Camilo Zurita, Samantha Rojas, Iván Dueñas-Espín, Kenny Cueva-Ludeña, Jorge Salazar-Vega & Jorge Pinto-Basto

**To cite this article:** Fernando Espinosa-Herrera, Estefanía Espín, Ana M. Tito-Álvarez, Leonardo-J Beltrán, Diego Gómez-Correa, German Burgos, Arianne Llamos, Camilo Zurita, Samantha Rojas, Iván Dueñas-Espín, Kenny Cueva-Ludeña, Jorge Salazar-Vega & Jorge Pinto-Basto (2020) A report of congenital adrenal hyperplasia due to 17α-hydroxylase deficiency in two 46,XX sisters, Gynecological Endocrinology, 36:1, 24-29, DOI: 10.1080/09513590.2019.1650342

**To link to this article:** https://doi.org/10.1080/09513590.2019.1650342



Published online: 29 Aug 2019.

GYNECOLOGICAL ENDOCRINOLOGY
2020, VOL. 36, NO. 1, 24–29
https://doi.org/10.1080/09513590.2019.1650342





# A report of congenital adrenal hyperplasia due to 17α-hydroxylase deficiency in two 46,XX sisters

Fernando Espinosa-Herrera[a,b], Estefanía Espín[a], Ana M. Tito-Álvarez[c], Leonardo-J Beltrán[a], Diego Gómez-Correa[a], German Burgos[a], Arianne Llamos[d], Camilo Zurita[e], Samantha Rojas[f], Iván Dueñas-Espín[g], Kenny Cueva-Ludeña[h], Jorge Salazar-Vega[a,i] and Jorge Pinto-Basto[j]

[a]Escuela de Medicina, Facultad de Ciencias de la Salud, Universidad de las Américas (UDLA), Quito, Ecuador; [b]Sociedad Ecuatoriana de Medicina Familiar (SEMF), Hospital Vozandes Quito, Quito, Ecuador; [c]Escuela de Enfermería, Facultad de Ciencias de la Salud, Universidad de las Américas (UDLA), Quito, Ecuador; [d]Facultad de Ciencias Médicas, de la Salud y de La Vida, Escuela de Odontología, Universidad Internacional del Ecuador (UIDE), Quito, Ecuador; [e]Unidad de Investigaciones en Biomedicina, Zurita & Zurita Laboratorios, Cátedra de Inmunología, Facultad de Medicina, Universidad Central del Ecuador (UCE), Quito, Ecuador; [f]Hospital Isidro Ayora de Loja, Ministerio de Salud Pública del Ecuador, Quito, Ecuador; [g]Instituto de Salud Pública, Facultad de Medicina, Pontificia Universidad Católica del Ecuador, Quito, Ecuador; [h]Hospital General Docente de Calderón, Ministerio de Salud Pública del Ecuador, Quito, Ecuador; [i]Hospital Eugenio Espejo, Ministerio de Salud Pública del Ecuador, Quito, Ecuador; [j]Molecular Diagnostics and Clinical Genomics Laboratories, CGC Genetics, Porto, Portugal

**ABSTRACT**

Congenital adrenal hyperplasia (CAH) is a group of rare orphan disorders caused by mutations in seven different enzymes that impair cortisol biosynthesis. The 17α-hydroxylase deficiency (17OHD) is one of the less common forms of CAH, corresponding to approximately 1% of the cases, with an estimated annual incidence of 1 in 50,000 newborns. *Cases description* – two phenotypically female Ecuadorian sisters, both with primary amenorrhea, absence of secondary sexual characteristics, and osteoporosis. High blood pressure was present in the older sister. Hypergonadotropic hypogonadism profile was observed: decreased cortisol and dehydroepiandrosterone sulfate (DHEAS), increased adrenocorticotropic hormone (ACTH) and normal levels of 17-hydroxyprogesterone, extremely high deoxycorticosterone (DOC) levels, and a tomography showed bilateral adrenal hyperplasia in both sisters. Consanguinity was evident in their ancestors. Furthermore, in the exon 7, the variant c.1216T > C, p.Trp406Arg was detected in homozygosis in the *CYP17A1* gene of both sisters. We report a homozygous missense mutation in the *CYP17A1* gene causing 17OHD in two sisters from Loja, Ecuador. According to the authors, this is the first time such deficiency and mutation are described in two members of the same family in Ecuador.

**ARTICLE HISTORY**
Received 26 April 2019
Revised 9 July 2019
Accepted 23 July 2019
Published online 29 August 2019

**KEYWORDS**
Congenital adrenal hyperplasia; 17 alpha hydroxylase deficiency; sisters; consanguineous family

## Introduction

Congenital adrenal hyperplasia (CAH) is a group of disorders, considered as rare orphan diseases, caused by mutations in seven different enzymes that impair adrenal corticosteroid biosynthesis [1]. CAH are monogenetic diseases inherited in autosomal recessive fashion, with significant morbidity and mortality due to metabolic disorders, as a consequence of the imbalance of glucocorticoids and mineralocorticoids [1]. The diagnosis and treatment of CAH is challenging because of the rarity and variability of its clinical manifestations, depending on the type and severity of the enzyme deficiency [2]. Around 95% of CAH cases are caused by 21-hydroxylase deficiency, and the resting 5–8% are mainly due to 11β-hydroxylase deficiency [3]. 17α-Hydroxylase deficiency (17OHD) is one of the less common forms of CAH, corresponding to approximately 1% of the cases, with an estimated annual incidence of about 1 in 50,000 newborns [4].

The 17α-hydroxylase enzyme, which is expressed in gonads and adrenals, acts at two levels: (i) it catalyzes the 17α-hydroxylation of pregnenolone and progesterone, and (ii) its lyase activity cleaves their products at 17,20 side chain [2,3]. 17α-Hydroxylase, also known as P450c17, is a 508 amino acids protein, encoded by the *CYP17A1* gene, which is located on chromosome 10 (10q24.32-q25) and is composed of eight exons [3]. Approximately, 120 different mutations of *CYP17A1* have been reported [2,3,5]. These mutations impair the enzyme activity partially or completely, resulting in adrenal and gonadal sex steroid deficiency [3,4]. 17OHD causes cortisol synthesis blockage; that leads to adrenocorticotropic hormone (ACTH) accumulation and further activation of the 17-deoxy pathway of the zona fasciculata producing overstimulation of this pathway and increasing progesterone, corticosterone and deoxycorticosterone (DOC) synthesis [3]. Importantly, the excessive mineralocorticoid activity reduces renin activity and decreases aldosterone levels [2].

The hallmarks of 17OHD are hypokalemic hypertension – as a result of accumulation of cortisol precursors with mineralocorticoid activity – absence of secondary sexual characteristics,

**CONTACT** Fernando Espinosa fernando.espinosa@udla.edu.ec Escuela de Medicina, Facultad de Ciencias de la Salud, Universidad de las Américas, Quito, Ecuador

**Results from this work were presented in abstract and poster forms at**: XXX Jornadas Vozandes, Quito – Ecuador, March 2018, I Congreso Internacional de Medicina Familiar, Quito – Ecuador, April 2018, and I Congreso Internacional de Enfermedades Raras en Ecuador, Quito – Ecuador, March 2019.

**Table 1.** Clinical characteristics of the two sisters.

| Characteristics | Sister 1 | Sister 2 |
|---|---|---|
| Age (years) | 22 years | 24 years |
| Chief complaints | Primary amenorrhea, absence of secondary sexual characteristics | Primary amenorrhea, absence of secondary sexual characteristics |
| Weight (kg) | 63.2 | 44 |
| Height (m) | 1.68 | 1.60 |
| Body mass index (kg/m$^2$) | 22.3 | 17.2 |
| Blood pressure (average, in mmHg) | 128/82 | 150/96 |
| Tanner stage for breast/pubic hair | 2/1 | 2/1 |
| Gynecologic examination | Lack of pubic hair, no adenopathies, external infantile female genitalia, absence of labia minora fusion and clitoral index of 14 mm$^2$ | Lack of pubic hair, no adenopathies, external infantile female genitalia, absence of labia minora fusion and clitoral index of 12 mm$^2$ |
| Imaging study (US & CT scan) | Infantile uterus, small ovaries (incipient) and no tumors | Infantile uterus, small ovaries (incipient) and no tumors |
| Left hip bone mineral density *Z*-score | −3.0 | −3.2 |
| Phenotype | Female | Female |

US: ultrasonography; CT scan: computed tomography.

female phenotype (46,XX or 46,XY), osteopenia/osteoporosis, hypergonadotropic hypogonadism, elevated gonadotropins and increased progesterone [2,3]. Nonetheless, these clinical features are not always simultaneously present; even in patients harboring the same mutation [1,2]. Additionally, 46,XX cases are less common [5].

Herein, we report the rare cases of two 46,XX sisters of 22 and 24 years old, respectively, with clinical manifestations of hypergonadotropic hypogonadism and genetically confirmed 17OHD.

## Patients and methods

Two phenotypic female sisters, aged 22 (sister 1) and 24 (sister 2), complained of primary amenorrhea and absence of secondary sexual characteristics. They were born in Loja-Ecuador, in a consanguineous family. Their parents are a double first-cousin marriage; nevertheless, the rest of the family members are apparently healthy. Four years before this report, the sisters were misdiagnosed and treated as mosaic Turner Syndrome. Before we evaluated the patients, they were inconsistently taking alendronate and gestodene/ethinylestradiol (sister 1); and alendronate, gestodene/ethinylestradiol, enalapril and amiloride/hydrochlorothiazide (sister 2).

### *Biochemical analyses*

All the tests were performed in a quality certified clinical laboratory (Zurita&Zurita) in Quito, Ecuador. ACTH, growth hormone (GH), prolactin and renin were measured by chemiluminescence immunoassay; cortisol, estrogen, follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone and testosterone levels were detected by electrochemoluminiscence immunoassay; DOC was measured by radioimmunoassay and the rest of hormones were measured by ELISA. Importantly, none of the sisters received hormonal treatment during biochemical analyses.

### *Genetic analysis*

Peripheral blood samples of the patients were used for karyotyping. DNA from the sisters and their parents was isolated from peripheral leukocytes by a silica-based membrane technology in columns (GeneJET Genomic DNA Purification Kit, Thermo Fisher Scientific, Waltham, Massachusetts). We analyzed *SRY* gene presence by sequence tagged sites (STS) multiplex PCR amplification.

CGC Genetics laboratories, in Portugal, processed the DNA samples. All eight coding regions and flanking intron regions (±8 bp) of the *CYP17A1* gene (RefSeq: NM_000102.3) were amplified by multiplex PCR (primer sequences available upon request). The library was prepared by tagging (QXT, Agilent Technologies, Santa Clara, California). The library was prepared by tagging (SureSelect QXT, Agilent Technologies), according to manufacturer instructions. PCR products were directly sequenced using Next Generation Sequencing (NGS) (MiSeq, Illumina, San Diego, California), 2 × 150 cycles flowcell. Sequence alignment and base calling were performed in NextGENe (SoftGenetics®) software. The average read depth obtained for each sample was >200× for the targeted regions. Regions whose base coverage was insufficient were sequenced by Sanger. In addition, the reported variants were independently confirmed by Sanger. The variants detected in each case were annotated and classification was performed according to ACMG guidelines [6].

### *Ethical considerations*

The present case report was approved by the Institutional Ethics Committee of Universidad de Las Américas, Quito, Ecuador. Informed consent was signed by the subjects of study.

## Results

Clinical characteristics of both sisters are depicted in Tables 1 and 2. Both sisters had: (i) primary amenorrhea, (ii) external infantile female genitalia, (iii) absence of labia minora fusion, (iv) a Tanner stage for breast/pubic hair of 2/1 (Figure 1), (v) infantile uterus and small ovaries, according to imaging studies and (vi) osteoporosis, according to the left hip bone mineral density (*Z*-score of −3.0 for sister 1 and −3.2 for sister 2) (Table 1). Remarkably, sister 1 was normotensive (with an average blood pressure of 125/82 mmHg), while sister 2 had hypertension (with an average blood pressure of 150/96 mmHg) and hypokalemia (Table 2).

Regarding the results of the analytics (Table 2), hormonal levels of both sisters demonstrated a hypergonadotropic hypogonadal state, accompanied by a deficit of androgenic sex hormones, reduced levels of dehydroepiandrosterone (DHEA) and testosterone, and hypocortisolism. Interestingly, DOC, FSH and LH levels were remarkably higher in sister 2.

**Table 2.** Laboratory and genetic analyses of the two sisters.

| Characteristics (units) | Sister 1 | Sister 2 | Reference values |
|---|---|---|---|
| Karyotype/SRY presence | 46,XX/no | 46,XX/no | – |
| Potassium (mmol/L) | 4.4 | 3.3 (↓) | 3.5 a 5.1 |
| Follicle stimulating hormone (IU/L) | 25.28 (↑) | 116.40 (↑) | Follicular phase 3.5–12.5<br>Ovulatory phase 4.7–21.5<br>Luteal phase 1.7–7.7<br>Postmenopausal 25.8–134.8 |
| Luteinizing hormone (IU/L) | 22.33 (↑) | 71.83 (↑) | Follicular phase 2.4–12.6<br>Ovulatory phase 14.0–95.6<br>Luteal phase 1.0–11.4<br>Postmenopausal 7.7–58.5 |
| Dehydroepiandrosterone (nmol/L) | 0.31 (N) | 0.38 (N) | 0.21–42.47 |
| Dehydroepiandrosterone-S (μmol/L) | 0.0027 (↓) | 0.004 (↓) | 0.01–0.1 |
| Testosterone (nmol/L) | <0.001 (↓) | <0.001 (↓) | 0.003–0.017 |
| 17 β Estradiol (pmol/L) | <18.36 (↓) | <18.36 (↓) | Follicular phase 45.89–609.39<br>Ovulatory phase 313.87–1828.16<br>Luteal phase 160.79–774.58 |
| Progesterone (nmol/L) | 30.08 (↑) | 42.39 (↑) | Follicular phase 0.18–2.84<br>Ovulatory phase 0.38–38.16<br>Luteal phase 5.82–76<br>Postmenopausal <0.16–0.4 |
| 17-Hydroxyprogesterone (nmol/L) | 0.51 (N) | 1.4 (N) | Follicular phase 0.3–2.42<br>Luteal phase 1.82–6.96<br>Ovulatory phase 0.91–4.24<br>Postmenopausal 0.39–1.54 |
| Renine (pmol/L) | – | 0.28 (N) | Upright: 0.06–0.66<br>Lying down: 0.04–0.57 |
| Cortisol AM (nmol/L) | 28.69 (↓) | 25.38 (↓) | 171.05–535.21 |
| Aldosterone (pmol/L) | 3.50 (N) | 4.36 (N) | Upright: 1.11–8.60<br>Lying down: 0.28–4.44 |
| Deoxycorticosterone (nmol/L) | 0.06 (↑) | 0.41 (↑) | 0.001–0.01 |
| Adrenocorticotropic hormone (pmol/L) | 30.14 (↑) | 10.74 (↑) | 0–10.12 |
| Growth hormone (μg/L) | 8.0 (N) | 7.96 (N) | 0–8 |
| Prolactin (pmol/L) | 0.42 (N) | 0.37 (N) | 0.21–1.01 |
| Thyrotropin (mIU/L) | 0.0024 (N) | 0.0038 (N) | 0.0003–0.0042 |

SRY: sex-determining region Y; N: normal levels; ↓: below normal; ↑: above normal. All values in SI units.

### *Genetic analysis*

Both sisters had a 46,XX karyotype, discarding an initial clinical suspicion of mosaic Turner Syndrome. Absence of *SRY* gene was confirmed. Sequencing of the *CYP17A1* gene of both sisters detected homozygosis for the variant c.1216T > C in the exon 7; which results in a substitution from tryptophan to arginine p.Trp406Arg (Figure 2). Their parents were heterozygotes for the same genetic variant.

## Discussion

The sisters we studied are homozygotes for the missense mutation c.1216T > C, p.Trp406Arg in the exon 7 of *CYP17A1* gene, which codes for P450c17 enzyme with 17α-hydroxylase and 17,20-lyase activity. Their parents exhibited the same mutation as asymptomatic heterozygotes, confirming the homozygosity found in the sisters. The p.Trp406Arg mutation has also been reported in 14 subjects derived from 11 different Brazilian consanguineous kindreds, with Spanish ancestry [5,7,8].

The sisters in our study belong to the mestizo ethnic group from Ecuador, which has an admixed ancestry of Native Americans and a larger European component [9]. Additionally, ancestry-specific analyses using principal components analyses (PCA) reported that the European ancestry in South American Latinos is from the Iberian Peninsula [10,11]. Thus, it is possible that the sisters have a common Spanish ancestry with the reported in the Brazilian patients. On the other hand, a haplotype study of two Spanish siblings with the p.Trp406Arg mutation suggests no common Spanish ancestor with three Brazilian patients, and points out these nucleotides are prone to mutation, as another possible cause for this mutation [12]. Thus, further genetic studies should be performed, to confirm our patients' ancestry.

Besides, rarer autosomal recessive genetic diseases have been detected in the Ecuadorian population of Loja province; alluding a probable inbreeding depression attributable to the small size of the population and its endogamy practices [13]. This finding is in accordance with a Mexican study, wherein three identified cases of 17OHD originate from small, rural communities with consanguinity manifestations [6].

Further genetic studies should be conducted in Loja to identify endogamous populations with genetic risk. The latter is of particular importance from a public health perspective, in order to encourage physicians to recognize and counsel couples at risk of having an affected child. Although there are concerns about patients' religious and social values, and attitudes towards the risk of consanguinity [14], public policy makers should consider strategies to reduce consanguinity and its inherent risks.

Regarding the clinical presentation of both sisters, they manifested sexual infantilism and puberty failure due to the hypogonadism evidenced in the hormonal analysis, and an increased pituitary stimulation by high levels of ACTH and hypergonadotropism. Additionally, the deficit of androgenic sex hormones, the reduced levels of DHEA and testosterone, the hypocortisolism and the increase of DOC, in the patients, suggested an impairment of 17α-hydroxylase [13].

According to the 2015 ACMG guidelines for variant classification [6], the detected c.1216T > C variant was classified as pathogenic, causing 17OHD [7]; which may explain the phenotype of both sisters. Furthermore, a human structural model of P450c17, demonstrated that p.Trp406Arg mutation could seriously

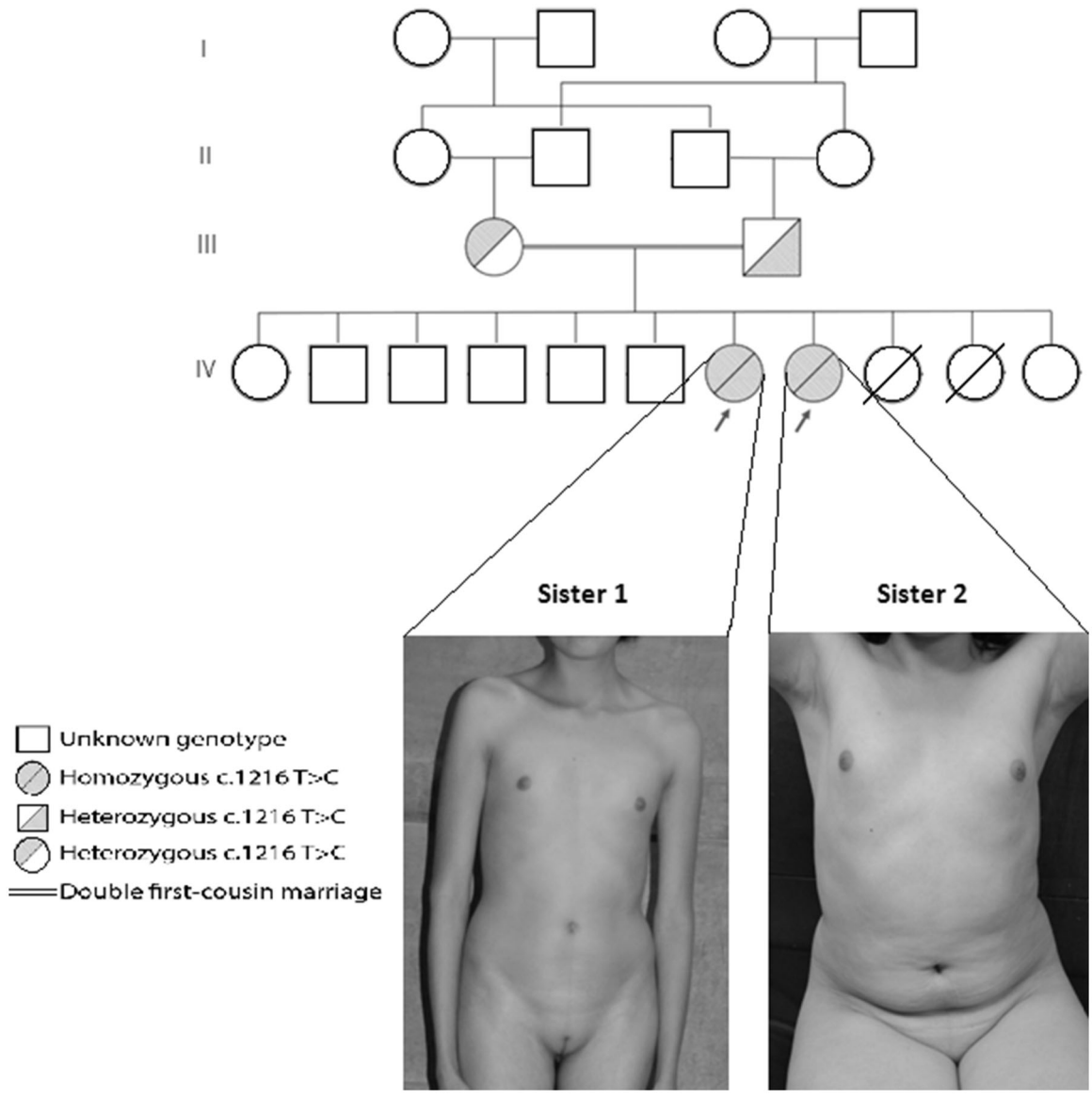


**Figure 1.** Genogram and physical traits of the two sisters with 17OHD. The sisters' progenitors and their siblings are apparently healthy, except for two infant sisters, who died from unknown causes. Not all the members are represented in the genogram in order to illustrate the consanguinity and allele inheritance of the patients. Both patients showed absence of breast development and axillary or pubic hair. Their clitoral index did not show clitoromegaly.

interrupt the heme binding, and affect the core structure integrity of P450c17, impairing both enzyme activities [15], which was confirmed in COS-7 or HEK-293 cells and in yeast assays in another study [7].

Phenotype and genotype correlation is still unknown in patients with 17OHD, and a remarkable variation in the severity of the disorder was noted even in patients with the same mutation in the *CYP17A1* gene [16,17]. In our study, sister 1 was normotensive, but sister 2 had hypertension and hypokalemia, which is consistent with the fact that corticosterone and DOC – also higher in sister 2 – lead to a mineralocorticoid effect. Accordingly, high levels of circulating DOC saturate the mineralocorticoid receptor, causing variation in the severity of clinical features and the age onset of hypertension and hypokalemia in patients harboring the same mutation [16,17].

The same variation in blood pressure was reported in Spanish and Brazilian patients, with the p.Trp406Arg mutation [7,12], and in Turkish and Chinese siblings with similar mutations producing a P450c17 completely inactive protein [16,18,19]. In addition to the reasons above mentioned, it has been reported that 10–15% of patients with 17OHD are normotensive at diagnosis [18]; strengthening the idea that other factors besides P450c17 activity, may be involved in blood pressure regulation.

Since our patients grew up under identical environmental and dietary conditions, it would not be possible to attribute the phenotype heterogeneity to environmental factors; thus, the reasons for this remain unknown. Three theoretical possible causes are: (i) differences in target tissue sensitivity to cortisol precursors with mineralocorticoid activity; (ii) enhancement of a minimal activity of mutant P450c17 enzyme by intracellular factors despite the absence of apparent activity [18] and (iii) a complex interaction of epigenetically regulated transcription factors of *CYP17A1* gene [20].

Some inquiries about these patients require further clinical explanation. First, 17OHD patients are not glucocorticoid deficient, since corticosterone, a glucocorticoid agonist, accumulates

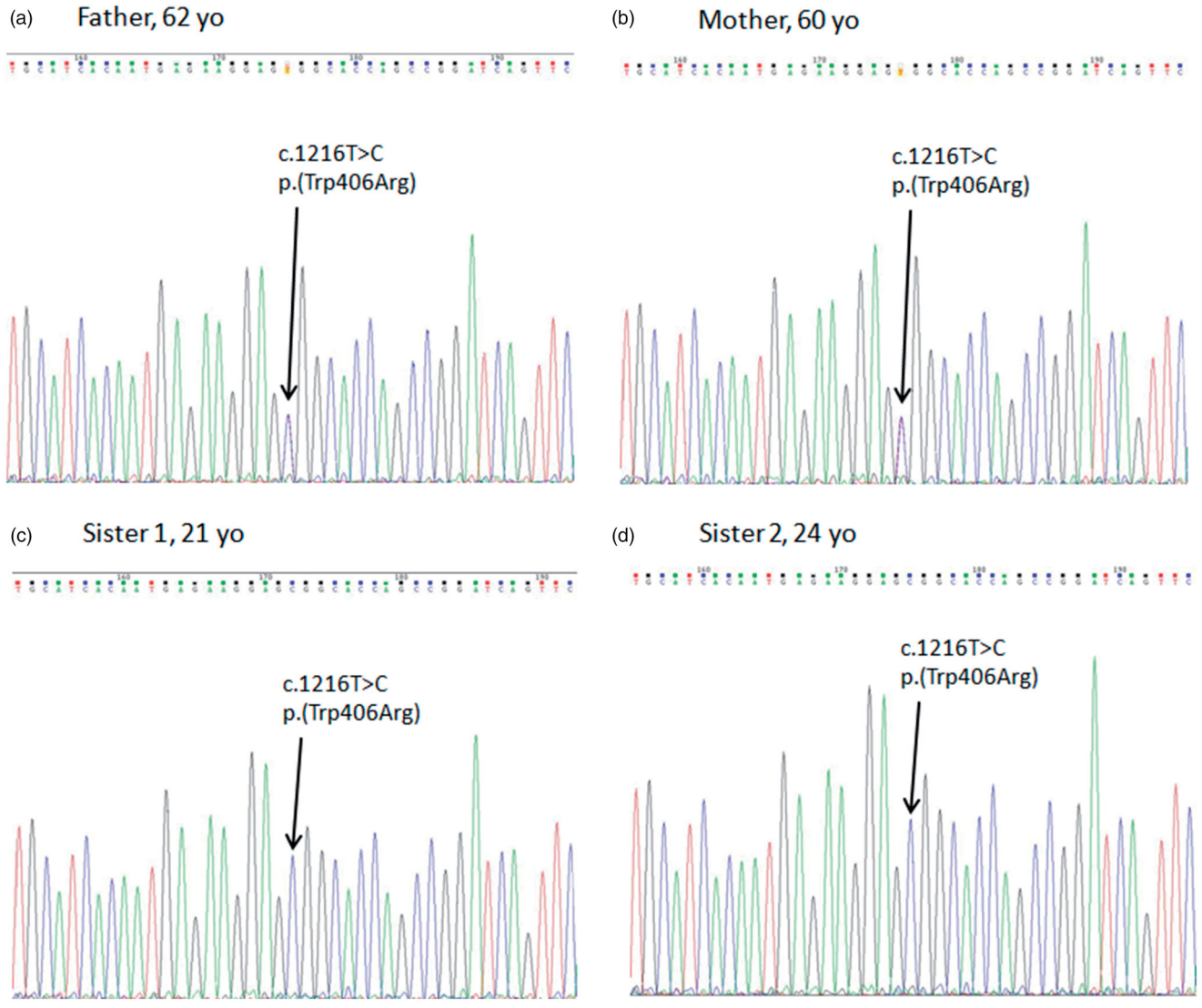


**Figure 2.** Parents exhibit the same mutation as asymptomatic heterozygotes panels (a) and (b), thereby confirming the homozygosity found in the sisters' panels (c) and (d).

and compensates the cortisol deficit; thus adrenal crisis are infrequent and children elude diagnosis until adolescence [21,22]. It would have been enlightening to know the result of an ACTH stimulation test, but the logistical limitations for performing the test in Ecuador precluded it. Second, ACTH concentration was high, but not as high as expected in the absence of cortisol production (especially for sister 2); in that regard, corticosterone – produced by the CYP11B1 enzyme – also modulates the secretion of corticotropin and could explain the modest increase in ACTH [22]. Third, in these patients the 17-hydroprogesterone level was normal, which could be explained by the irregular sexual hormone supplementation they received for hypogonadism [23], previous to our approach.

Despite the inappropriate management of both patients for a prolonged period prior to our approach, we started them on pharmacologic treatment, in order to mitigate the effects of mineralocorticoid excess, prevent glucocorticoid deficiency, restore desired secondary sexual characteristics and improved bone mineral density. Sex steroids were also required to induce cyclic withdrawal bleeding and to prevent endometrial hyperplasia [22]. Importantly, for sister 2, spironolactone was the drug of choice; considering it blocks the mineralocorticoid receptor, even though it requires close monitoring of blood pressure and electrolytes, in order for proper titration [22]. Current treatment has been initiated in both sisters and close follow up is performed by specialists.

Finally, high progesterone levels accompanied by hypergonadotropic hypogonadism and hypertension (with or without hypokalemia) are hallmarks of 17OHD [5,8]. Biochemical and molecular diagnosis of 17OHD is difficult, especially in low-income countries, making a timely clinical suspicion imperative for an accurate diagnosis and treatment, to improve the outcomes for the patients.

## Acknowledgements

We thank our patients who accepted to participate in this study. We also thank Dr. Luis Freire S, the ophthalmologist who approached the patients, and Dr. Esteban Peghini, the radiologist who supported in the interpretation and selection of the images. We thank Melissa Hidalgo, Pamela Benavídes and Alexis Guijarro, Universidad de las Américas (UDLA) Medical students who supported in logistics aspects during medical appointments of the sisters. A special acknowledgment to Tannya Lozada, PhD, Chief of Research Department in UDLA, who supported us throughout the whole

process. Finally, we thank María Eugenia Sánchez, a researcher, who performed the initial karyotype in the Biomedical Sciences Institute at UDLA.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## Funding

This work was supported by Universidad de las Américas (Grant number MED.FE.17.03).

## ORCID

Fernando Espinosa-Herrera http://orcid.org/0000-0002-7762-5688

## References

[1] Oh YK, Ryoo U, Kim D, et al. 17α-hydroxlyase/17, 20-lyase deficiency in three siblings with primary amenorrhea and absence of secondary sexual development. J Pediatr Adolesc Gynecol. 2012;25:e103–e105.

[2] Fontenele R, Costa-Santos M, Kater CE. 17α-hydroxylase deficiency is an underdiagnosed disease. High frequency of misdiagnoses in a large cohort of Brazilian patients. Endocr Pract. 2017;24:170–178.

[3] Hannah-Shmouni F, Chen W, Merke DP. Genetics of congenital adrenal hyperplasia. Endocrinol Metab Clin North Am. 2017;46:435–458.

[4] Grumbach M, Hughes I, Conte F. Williams textbook of endocrinology. In Larsen PR, Kronenb HM, Melmed S, editors. Williams Textbook of Endocrinology. 10th ed. Philadelphia: Saunders; 2003. p. 842–1002.

[5] Carvalho LC, de Brito VN, Martin RM, et al. Clinical, hormonal, ovarian, and genetic aspects of 46,XX patients with congenital adrenal hyperplasia due to CYP17A1 defects. Fertil Steril. 2016;105:1612–1619.

[6] Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–424.

[7] Costa-Santos M, Kater CE, Auchus RJ. Two prevalent CYP17 mutations and genotype-phenotype correlations in 24 Brazilian patients with 17-hydroxylase deficiency. J Clin Endocrinol Metab. 2004;89:49–60.

[8] Breder ISS, Garmes HM, Mazzola TN, et al. Three new Brazilian cases of 17α-hydroxylase deficiency: clinical, molecular, hormonal, and treatment features. J Pediatr Endocrinol Metab. 2018;31:937–942.

[9] Santangelo R, González-Andrade F, Børsting C, et al. Analysis of ancestry informative markers in three main ethnic groups from Ecuador supports a trihybrid origin of Ecuadorians. Forensic Sci Int Genet. 2017;31:29–33.

[10] Homburger JR, Moreno-Estrada A, Gignoux CR, et al. Genomic insights into the ancestry and demographic history of South America. PLoS Genet. 2015;11:e1005602–e1005626.

[11] Paz-y-Miño C, Beaty D, López-Cortés A, et al. Frequency of GJB2 and del(GJB6-D13S1830) mutations among an Ecuadorian mestizo population. Int J Pediatr Otorhinolaryngol. 2014;78:1648–1654.

[12] Fernández-Cancio M, García-García E, González-Cejudo C, et al. Discordant genotypic sex and phenotype variations in two Spanish siblings with 17α-hydroxylase/17,20-lyase deficiency carrying the most prevalent mutated CYP17A1 alleles of Brazilian patients. Sex Dev. 2017;11:70–77.

[13] Moreira AC, Leal AMO, Castro M. Characterization of adrenocorticotropin secretion in a patient with 17α-hydroxylase deficiency. J Clin Endocrinol Metab. 1990;71:86–91.

[14] Teeuw ME, Hagelaar A, Ten Kate LP, et al. Challenges in the care for consanguineous couples: an exploratory interview study among general practitioners and midwives. BMC Fam Pract. 2012;13:1.

[15] Werck-Reichhart D, Feyereisen R. Protein family review Cytochromes P450 : a success story. Genome Biol. 2000;1:1–9.

[16] Kardelen AD, Toksoy G, Baş F, et al. A rare cause of congenital adrenal hyperplasia: clinical and genetic findings and follow-up characteristics of six patients with 17-hydroxylase deficiency including two novel mutations. JCRPE J Clin Res Pediatr Endocrinol. 2018;10:206–215.

[17] Yanase T, Imai T, Simpson ER, et al. Molecular basis of 17α-hydroxylase/17,20-lyase deficiency. J Steroid Biochem Mol Biol. 1992;43:973–979.

[18] Bee YM, Manju C, Papari-Zareei M, et al. Phenotypic variation in a Chinese family with 46,XY and 46,XX 17α-hydroxylase deficiency. Gynecol Endocrinol. 2012;28:322–325.

[19] Wang YP, Zhao YJ, Zhou GY, et al. CYP17A1 gene mutations and hypertension variations found in 46, XY females with combined 17α-hydroxylase/17, 20-lyase deficiency. Gynecol Endocrinol. 2014;30:456–460.

[20] Martinez-Arguelles DB, Papadopoulos V. Epigenetic regulation of the expression of genes involved in steroid hormone biosynthesis and action. Steroids. 2010;75:467–476.

[21] Benetti-Pinto CL, Vale D, Garmes H, et al. 17-Hydroxyprogesterone deficiency as a cause of sexual infantilism and arterial hypertension: laboratory and molecular diagnosis – a case report. Gynecol Endocrinol. 2007;23:94–98.

[22] Auchus RJ. Steroid 17-hydroxylase and 17,20-lyase deficiencies, genetic and pharmacologic. J Steroid Biochem Mol Biol. 2017;165:71–78.

[23] Richard-Eaglin A. Male and female hypogonadism. Nurs Clin North Am. 2018;53:395–405.