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Primary Ovarian Failure in Addition to Classical Clinical Features of Coats Plus Syndrome in a Female Carrying 2 Truncating Variants of CTC1

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Established Facts

- Coats plus syndrome is an autosomal recessive multisystemic and pleiotropic disorder affecting body growth, eyes, brain, bone, and gastrointestinal tract.
- The phenotype is believed to result from telomere dysfunction, with accumulation of DNA damage, cellular senescence, and stem cell depletion.
- Most reported patients are compound heterozygous for a truncating mutation and a missense variant, suggesting that the presence of 2 truncating variants would be associated with a severe phenotype, lethal in utero.

Novel Insights

- This is the first report of a patient carrying 2 truncating conserved telomere maintenance component 1 variants. Low levels of wild-type transcripts found in this patient may have prevented embryonic lethality.
- In addition, we are the first to report that Coats plus syndrome is associated with ovarian failure. We speculate that gonadal failure is caused by telomere shortening in oocytes and granulosa cells and/or loss of chromosome cohesion, resulting in accumulation of DNA damage with stem cell depletion, cell arrest, and early decrease in follicular reserve.

Keywords
Coats plus syndrome · CTC1 gene · Ovarian failure · Brain calcifications · Short stature

Abstract
Coats plus syndrome is an autosomal recessive multisystemic and pleiotropic disorder affecting the eyes, brain, bone, and gastrointestinal tract, usually caused by com-

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pound heterozygous variants of the conserved telomere maintenance component 1 gene (*CTC1*), involved in telomere homeostasis and replication. So far, most reported patients are compound heterozygous for a truncating mutation and a missense variant. The phenotype is believed to result from telomere dysfunction, with accumulation of DNA damage, cellular senescence, and stem cell depletion. Here, we report a 23-year-old female with prenatal and postnatal growth retardation, microcephaly, osteopenia, recurrent fractures, intracranial calcification, leukodystrophy, parenchymal brain cysts, bicuspid aortic valve, and primary ovarian failure. She carries a previously reported maternally inherited pathogenic variant in exon 5 (c.724_727del, p.(Lys242Leufs*41)) and a novel, paternally inherited splice site variant (c.1617+5G>T; p.(Lys480Asnfs*17)) in intron 9. *CTC1* transcript analysis showed that the latter resulted in skipping of exon 9. A trace of transcripts was normally spliced resulting in the presence of a low level of wild-type *CTC1* transcripts. We speculate that ovarian failure is caused by telomere shortening or chromosome cohesion failure in oocytes and granulosa cells, with early decrease in follicular reserve. This is the first patient carrying 2 truncating *CTC1* variants and the first presenting primary ovarian failure.

Introduction

Coats plus syndrome or cerebroretinal microangiopathy with calcifications and cysts (CRMCC, MIM #612199) is a rare pleiotropic and multisystem autosomal recessive disorder compromising the eyes, brain, bone, and gastrointestinal tract [1]. The clinical phenotype of CRMCC is wide, variable, and of progressive nature including pre- and postnatal growth retardation, retinal exudates and bilateral retinal telangiectasia, intracranial calcifications, leukodystrophy, occasionally parenchymal brain cysts, and in some cases, a tendency to life-threatening gastrointestinal bleeding, infections and/or liver failure that can cause death before the age of 30 years [1–4].

The molecular basis of most patients affected by this disorder is a biallelic pathogenic variant in the conserved telomere maintenance component 1 (*CTC1*) gene (MIM #613129), located on chromosome 17p13.1 [5, 6]. Most patients carry a truncating variant in combination with a missense variant. Since no patient carrying 2 truncating mutations had been discovered, it was assumed that such combination would be lethal in utero [6]. Besides *CTC1* variants, also biallelic variants in *STN1* or the shelterin component *POT1* can cause Coats plus syndrome [7].

The *CTC1* gene contains 23 exons and encodes a 1,217 amino acid protein. This protein interacts with STN1 (oligonucleotide/oligosaccharide-binding fold-containing protein 1) and TEN1 (telomerase capping complex subunit homolog) to form a trimeric CST complex, which is essential in the regulation of telomere extension by telomerase and C-strand fill-in synthesis by DNA polymerase alpha-primase [7, 8]. The CST complex promotes telomere duplex replication and the rescue of stalled DNA replication at nontelomeric sites, suggesting genome-wide roles during replication [9–11]. This dual role of CST complex is thought to explain the wide spectrum of symptoms and clinical features associated with diseases caused by CST mutations.

Here, we describe the phenotype and genotype of a 23-year-old woman with several classical features of Coats plus syndrome, and so far unreported primary ovarian failure. Exome sequencing revealed compound heterozygosity for a previously reported pathogenic variant and a novel splice site variant in *CTC1*.

Case Report/Case Presentation

A 23-year-old woman known with severe microcephalic short stature and bilateral peripheral deafness was referred to the pediatric endocrinology clinic for primary amenorrhea and recurrent fractures of long bones. She was born after a third pregnancy of healthy nonconsanguineous parents originating from a rural area around Santiago de Chile. The oldest child is healthy and the second pregnancy resulted in a stillbirth female with multiple malformations, including hypoplasia of both thumbs. Heights and comorbidities of the family members are shown in the pedigree (Fig. 1a).

The proband was born full term and small for gestational age with congenital limb malformations and bilateral narrowing of external auditory canals. At follow-up, she showed delayed psychomotor development, severe microcephalic growth failure, deafness due to severe narrowing of both external auditory canals, and impaired cognitive function (IQ 65). Cytogenetical analysis showed a 46,XX karyotype. The brain CT showed basal ganglia calcification and severe narrowing of both external auditory canals. A detailed list of clinical features compared with 3 previous reports is shown in online supplementary Table 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000520410). She was lost to follow-up after 7 years of age.

At 23 years of age, the medical history revealed 2 orthopedic surgeries (at 15 and 21 years) due to fractures of the right elbow and tibia, secondary to trauma of varied intensity. Menarche had not taken place. The growth curve, progeroid appearance, and sparse hair of normal color, and hand and foot malformations are shown in Figure 1b–d, respectively. A radiological survey showed

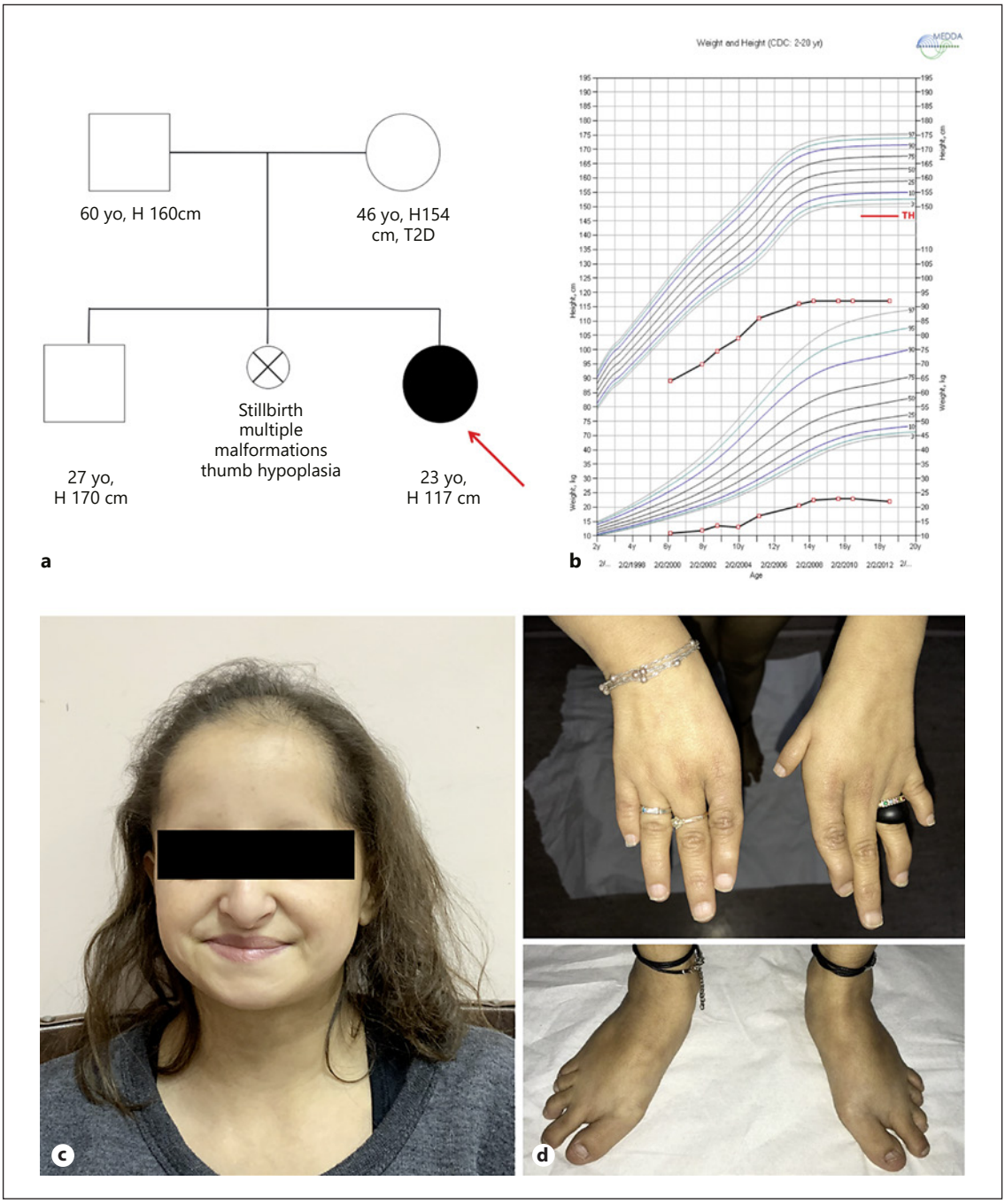

Fig. 1. a Pedigree. **b** Growth curve. **c** Facial characteristics (triangular face, micrognathia, sparse hair, and hypotelorism). **d** Hand and foot anomalies (right thumb agenesis, left thumb hypoplasia, and feet malformations).

thick intracranial calcifications, generalized osteopenia**,** bilateral radio-humeral fractures, agenesis of right thumb and hypoplasia of left thumb, and feet malformations with bilateral hypoplasia of the fourth and fifth phalanges (Fig. 2a, b). Skull X-ray and a brain CT confirmed thick calcifications in the bilateral basal ganglia (Fig. 2c, d). A brain MRI showed bilateral parietal periventricular white matter signal alterations on T2-FLAIR sequences, associated with small cysts at the same location and vermis hypoplasia (Fig. 2e, f). Echocardiographical evaluation showed a bicuspid aortic valve. Clinically, she has remained without specific neurological, ophthalmological, or gastrointestinal symptoms, but the alopecia has been progressive (Fig. 1c). Laboratory tests revealed hy-

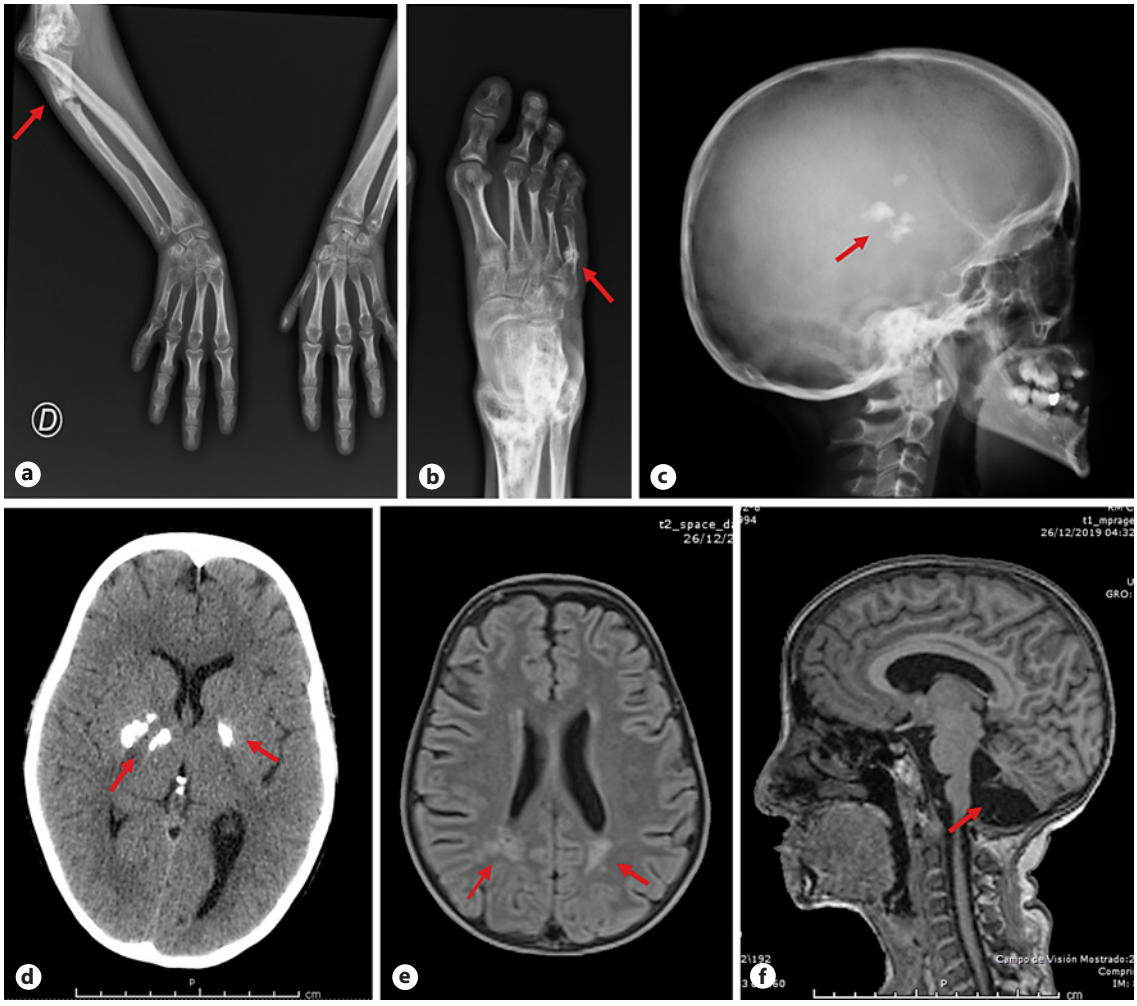

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Fig. 2. Imaging studies. **a** Hands X-ray: right radio-humeral fracture, agenesis of right thumb-metacarpal-scaphoid, hypoplasia of left thumb-metacarpal-scaphoid. **b** Right foot X-ray: fracture of the fifth left metatarsal partially consolidated, tibia and talus with osseous sclerosis and foci of osteopenia and articular narrowing. **c** Skull X-ray showing the presence of intracranial calcifications. **d** Brain CT scan showing bilateral calcifications in the thalamus. **e** Brain MRI (T2-FLAIR) showing alteration of bilateral posterior parietal periventricular white matter signal. **f** Brain MRI showing vermis hypoplasia.

pergonadotropic hypogonadism and a pelvic ultrasound confirmed a prepubertal aspect of ovaries and uterus. Further details are presented in online supplementary Tables 1 and 2.

The patient and her parents provided written informed consent for the clinical and genetic analysis and presentation of clinical data, identifiable patient images, and radiographic and genetic information. The publication of the clinical case was approved by the Ethics Committee of the San Juan de Dios Hospital, Santiago de Chile.

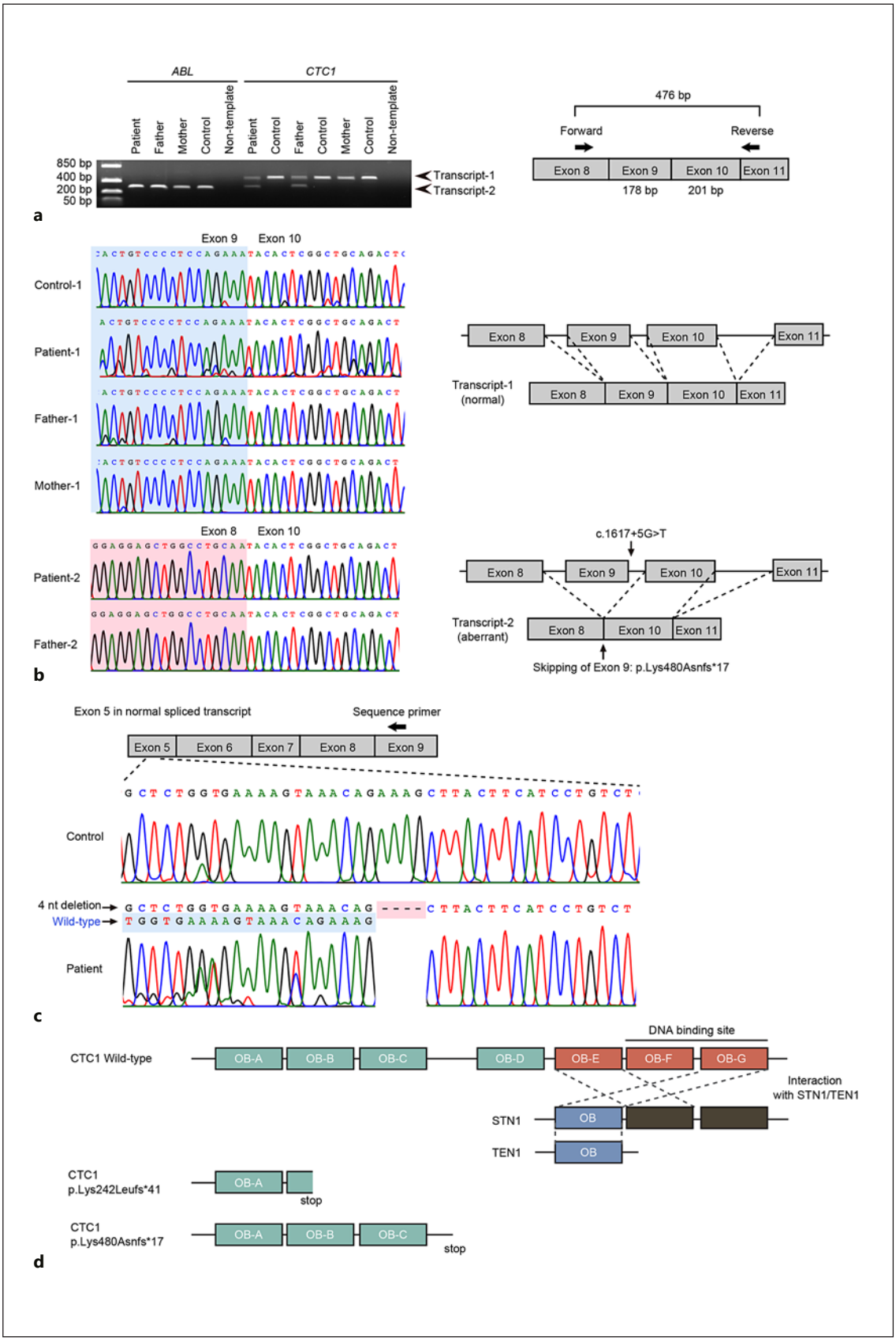
Genetic Analysis

Exome sequencing analysis was performed at the Laboratory for Diagnostic Genome Analysis and transcript analysis at the Laboratory for Pediatric Immunology (Leiden University Medical Center, Leiden, the Netherlands). Further details are presented in the online supplementary information.

Results

The recessive inheritance filter revealed compound heterozygous variants in *CTC1*. The maternally inherited variant (exon 5: c.724_727del p.(Lys242Leufs*41)) has been reported previously as a pathogenic variant [5]. The paternally inherited variant in intron 9 (c.1617+5G>T) is novel and predicted to lead to loss of the splice donor site and skipping of exon 9, resulting in a truncated protein (p.(Lys480Asnfs*17)) (online suppl. information).

To assess the effect of the splice site variant, molecular studies on the patient's RNA were performed. Reverse transcription PCR encompassing exon 9 and 10 followed



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by sequencing analysis demonstrated that the patient and the father had transcripts that differed from the mother and controls (Fig. 3a). Sequencing of the aberrant transcript showed that exon 9 is skipped, which is predicted to result in a frameshift and premature termination of the protein (p.(Lys480Asnfs*17), Fig. 3b). Sequencing of reverse transcription PCR products of normally spliced transcripts (encompassing exon 5–9) demonstrated mainly the 4 nucleotide deletions in exon 5. However, also a small trace of wild-type sequence was detected, indicating that the patient had a low level of wild-type transcript (Fig. 3c). Both truncating variants are predicted to result in the absence of interaction sites with STN1/TEN1 and DNA-binding sites [12] (Fig. 3d).

Discussion/Conclusion

We describe the phenotype and genotype of a 23-year-old woman with various typical features consistent with CRMCC, a condition with a broad (multisystemic) and variable phenotype, which makes the diagnosis challenging. Clinical features in our patient include severe pre- and postnatal microcephalic growth failure, osteopenia with recurrent fractures, intracranial calcifications, leukodystrophy, and parenchymal brain cysts, in addition to less common features such as congenital limb malformations, bilateral peripheral deafness caused by narrowing of the auditory canal, microcephaly, oligodontia with root teeth dwarfism, and bicuspid aorta valve. A novel observation is primary amenorrhea due to ovarian failure.

The dual role of CST at telomeric and nontelomeric sites may be related to the wide spectrum of symptoms and clinical features associated with diseases caused by *CTC1* variants. *CTC1* has an essential role in promoting efficient homeostasis of telomeres, and in fact, several patients presented shortening of telomeres [5, 6]. However, *CTC1* defects also lead to nontelomeric DNA damage, with activation of DNA damage response elements, global genome instability, cellular apoptosis/senescence, and finally stem cells depletion in different tissues. This suggests that the pathogenesis of the syndrome may consist of a cellular proliferation failure with stem cell depletion [9–11].

In previous reports, most cases of early-onset CRMCC are compound heterozygotes with a combination of a truncating variant (frameshift or nonsense) and a missense variant [5, 13]. Our patient is the first who carries a truncating variant (a previously reported pathogenic variant in exon 5 inherited maternally, c.724_727del, p.(Lys242Leufs*41)) and a novel paternally inherited heterozygous splice site variant (c.1617+5G>T, p.(Lys480Asnfs*17)) which is predicted to result in a truncated protein. The first variant was shown to generate a truncated protein or low levels of CTC1 protein, resulting in telomere dysfunction, with massive telomere loss, chromosome fusions, and global ATR-dependent DDR activation in the mouse [14].

Truncating variants are able to function in a dominant-negative fashion to repress the expression of their missense counterparts [13], resulting in a more severe disease phenotype [5, 15], and it was assumed that carrying 2 truncating mutants would be lethal in utero [6]. *CTC1* transcript analysis revealed that the patient had a low level of wild-type transcript, as well as the 2 aberrant transcripts. We speculate that the presence of 2 truncated proteins caused the severe phenotype of the patient and probably the intrauterine death in an earlier pregnancy; the expression of wild-type transcripts (though at a low level) in the patient may have prevented embryonic lethality.

The novel clinical feature of CRMCC presented by our patient is primary ovarian failure. So far, hypergo-

Fig. 3. Effect of the splicing site mutation and analysis of *CTC1* transcripts in the patient. **a** RT-PCR analysis of splicing site variant. Left: RT-PCR for *CTC1* using primers in exon 8 and 11, and *ABL* (endogenous control). The patient and the father demonstrated 2 bands (transcript-1 and -2), while the mother and controls showed 1 band (transcript-1). Right: Scheme of *CTC1* transcript (exon 8–11) and primers. **b** Sanger sequencing analysis of transcript-1 and -2. Left: Sanger sequencing of transcript-1 (control-1, patient-1, father-1, and mother-1) and transcript-2 (patient-2 and father-2). Transcript-1 demonstrated normal splicing and transcript-2 demonstrated aberrant splicing, skipping exon 9. Right: Scheme of splicing pattern of transcript-1 (upper) and transcript-2 (lower). **c** Sanger sequencing of normal spliced transcript. Normal spliced transcript was amplified by RT-PCR using primers in exon 5 and 9 and sequenced using a primer in exon 9. The patient demonstrated mainly the 4 nt deletion with a small trace of wild-type sequence. **d** A cartoon of CTC1 domains, their interaction with STN1 and TEN1 and the consequences for the 2 truncating gene variants carried by our patient. The upper part shows the 7 oligonucleotide-oligosaccharide-binding fold domains (OB domains, OB-A to G). OB-F and OB-G contain DNA-binding sites, and OB-E and OB-G contain interaction sites with STN1/TEN1 (adapted from [12]). The middle and bottom parts show the truncations encountered in the patient (p.(Lys242Leufs*41) and p.(Lys480Asnfs*17), respectively), demonstrating that these truncations result in absence of interaction sites with STN1/TEN1 and DNA-binding sites. CTC1, conserved telomere maintenance component 1 gene; RT-PCR, reverse transcription PCR.

nadotropic hypogonadism has only been reported in a 39-year-old Brazilian male [16]. We consider 2 possible causes of ovarian failure in this syndrome. First, ovarian failure may be caused by telomere shortening of oocytes and granulosa cells, with accumulation of DNA damage, which could produce cell arrest and early decrease in follicular reserve. In support of this assumption, experimental genetic or pharmacological shortening of telomeres in mice oocytes produced a phenotype remarkably similar to age-related oocyte dysfunction in women, with meiotic dysfunction, apoptosis, delayed, and arrested embryonic cell cycles as well as embryo fragmentation and general genomic instability [17, 18]. Second, a recent report [19] showed a sister chromatid cohesion loss in CST-depleted cells, while premature chromosome cohesion failure has previously been linked to premature ovarian failure [20]. Since both mechanisms would result in DNA damage, the occurrence of ovarian failure could be expected because gonadal failure has been part of several syndromes associated with severe short stature (primordial dwarfism) due to several DNA repair syndromes [21, 22]. These findings clearly indicate the sensitivity of gonadal tissues to the deleterious effects of abnormalities in DNA repair.

In conclusion, this is the first report on a patient with CRMCC carrying 2 truncating *CTC1* variants, in contrast to previous assumptions that such form of biallelic variants would cause embryonic lethality. It is also the first patient presenting with primary ovarian failure, besides multiple common and less common features of CRMCC. This finding in combination with documented gonadal failure in an affected male suggests that hypergonadotropic hypogonadism should be considered part of the phenotype of CRMCC.

Acknowledgments

The authors thank the patient and her family for their participation in this study.

Statement of Ethics

Written informed consent was obtained from the patient and her legal guardians for publication of this case report and accompanying images. On October 30th, 2019, in exempt resolution No. 024667, the Ethics Committee of Hospital San Juan de Dios approved publication of the clinical case.

Conflict of Interest Statement

J.M.W. is currently Editorial Board member of Hormone Research in Pediatrics Journal. The other authors declare no conflict of interest.

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Author Contributions

J.R. performed the clinical evaluation of the patient, made the literature review, and wrote the manuscript. J.R. and F.P. were involved in clinical data collection. S.T., T.D., M.B., H.D., I.P., M.B., and M.L. performed genomic analyses and evaluation of genomic database and contributed to comparative evaluation of the genetic and the clinical data. J.M.W., V.M., M.L., M.B., M.B., and H.D. participated in reviewing clinical data, and editing and writing the manuscript.

Data Availability Statement

The data supporting the findings of this clinical report are included in this article and its online supplementary material files. Further inquiries can be directed to the corresponding author upon reasonable request.

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