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CLINICAL REPORT

De novo c.2455C>T mutation of *NPR2* gene in a fetus with shortened long bones and a ventricular septal defect conceived by a mother with a fragile site at 16q22.1 and a father with a rare heterochromatic variant of chromosome 4 from Vietnam

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Abstract

Background: A heterozygous natriuretic peptide receptor 2 (*NPR2*) gene c.2455C>T mutation was identified as a cause of familial idiopathic short stature (ISS). Only two cases with this mutation were reported previously, and the probands with ISS had no organ system defects.

Methods: Next-generation sequencing (NGS) was performed on an amniotic fluid DNA sample of a fetus with shortened long bones and a small ventricular septal defect detected by an obstetric ultrasound examination. The pathogenic variant of the fetus was confirmed by Sanger sequencing. Sanger sequencing, G-banded, and C-banded karyotyping of the fetus's parents were subsequently performed.

Results: A de novo *NPR2* gene c.2455C>T, p.(Arg819Cys) mutation was identified in the fetus. No microdeletion or microduplication was identified in the fetus by copy number variation sequencing with a maximum resolution of 400 kb. The two previous miscarriages experienced by the fetus's parents were interpreted as a result of chromosomal aberrations, including a maternal fragile site at 16q22.1 and a rare paternal variant involving in a large G-band-positive and C-band-positive block of paracentric heterochromatin of chromosome 4p.

Conclusion: This report provides clinical signs of a de novo heterozygous *NPR2* gene c.2455C>T mutation in the fetus and shows paternal chromosomal aberrations causing repeated pregnancy loss.

KEYWORDS

fragile site at 16q22.1, *NPR2* gene c.2455C>T, repeated pregnancy loss, shortened long bones, variant of paracentric heterochromatin of chromosome 4p

1 | INTRODUCTION

The natriuretic peptide receptor 2 (*NPR2)* gene (OMIM accession number: 108961) is located on chromosome 9p13.3 and encodes natriuretic peptide receptor B (NPR-B). The interaction between NPR-B and its ligand, C-type natriuretic peptide (CNP), plays an important role in endochondral bone growth. In 2004, Bartels et al., (2004) identified biallelic *NPR2* mutations responsible for causing acromesomelic dysplasia, type Maroteaux (AMDM) and found that obligate carriers were shorter than expected when compared to matched controls. Subsequently, several studies showed that homozygous or compound heterozygous mutations of the *NPR2* gene caused a severe and disproportionate short stature, whereas heterozygous mutations of this gene seemed to be associated with mild and variable growth impairment without a distinct skeletal phenotype (Wang et al., 2015). Dozens of homozygous *NPR2* mutations causing AMDM, as well as heterozygous *NPR2* mutations causing idiopathic short stature (ISS), have since been reported (Hwang et al., 2020; Irfanullah et al., 2018; Tran et al., 2019; Wang et al., 2015). However, only two families have been reported as having a heterozygous *NPR2* gene c.2455C>T mutation (NM_003995.3(*NPR2*):c.2455C>T p.(Arg819Cys)) causing ISS. Both probands had inherited the c.2455C>T mutation from their mothers and had no organ system defects (Hisado-Oliva et al., 2015; Vasques et al., 2013). Here, we report a de novo c.2455C>T p.(Arg819Cys; +++RNA not analyzed) mutation of the *NPR2* gene of a fetus with shortened long bones and a heart defect. The fetus was conceived by a couple with a bad obstetric history and with chromosomal aberrations including a maternal fragile site at 16q22.1 and a paternal rare heterochromatic variant of chromosome 4.

2 | METHODS

2.1 | Ethical compliance

All procedures of this study were approved by the Ethics Committee of the Hospital of Hue University of Medicine and Pharmacy, Vietnam (#660, approval date: 30 May 2019; #1402, approval date: 29 October 2019). Written informed consent was obtained from the fetus's parents.

2.2 | Patient

A 31-year-old G3P0020 (two previous miscarriages) pregnant woman was referred to the Hospital of Hue University of Medicine and Pharmacy, Vietnam for a prenatal examination because of a small-for-gestational-age (SGA) fetus detected at 20^{+3} weeks of gestational age (GA).

2.3 | Obstetric ultrasound examination

Obstetric ultrasound examinations (WS80A, Samsung Medison, Korea) were performed in order to monitor fetal growth every 2 weeks. Doppler ultrasound of the umbilical artery was used to assess fetal hypoxia.

2.4 | Genomic analysis

Genomic DNA was extracted from the fetal amniotic fluid at 21^{+2} weeks of GA using ReliaPrep^{TM} gDNA Tissue Miniprep System (Promega Corp., Madison, Wisconsin, USA), and was subjected to copy number variation sequencing (CNV-seq) by a next-generation sequencing (NGS) method that can identify chromosomal abnormalities with a maximum resolution of 400 kilobases (NextSeq, Illumina, USA). Another subsequent NGS assay was performed in order to detect variants for a panel of 4503 genes. The pathogenic variant of the fetus was confirmed by Sanger-directed sequencing (Applied Biosystems 3730 Genetic Analyzer, Thermo Fisher Scientific, USA). Primers for directed sequencing were designed according to the published DNA sequence of *NPR2* gene (GenBank accession number: NC_000009.12). This Sanger sequencing method was also performed to determine corresponding alleles of the fetus's parents.

2.5 | Karyotyping

Peripheral lymphocytes of the fetus's parents were subjected to G-banded karyotyping at a resolution of 400–550 bands and followed by C-banded karyotyping. At least 30 metaphases were analyzed for each case. Nomenclatures were assigned for karyotypes according to the international system for human cytogenomic nomenclature (ISCN) 2016 (McGowan-Jordan et al., 2016).

3 | RESULTS

An obstetric ultrasound examination at 21^{+2} weeks of GA revealed decreased fetal measurements below the third percentile of INTERGROWTH-21st, including an estimated fetal weight (EFW) of 189 gr, biparietal diameter (BPD) of 45 mm, head circumference (HC) of 157 mm, and abdominal circumference (AC) of 119 mm, combined with shortened long bone measurements, including a femur length (FL) of 22 mm, humerus length (HL) of 20 mm, left radius length of 18 mm, right radius length of 17 mm, left tibia length of 17 mm, and right tibia length of 18 mm. A hypoplastic nasal bone (2.6 mm) was also observed.

At 26 weeks of GA, the fetus still showed lower than expected ultrasound measurements for the GA (below the third percentile), including an EFW of 401 gr, BPD of 59 mm, HC of 209 mm, AC of 160 mm, FL of 33.3 mm, left humerus length of 29 mm, right humerus length of 27 mm, left radius length of 25 mm, right radius length of 24 mm, left ulna length of 25 mm, right ulna length of 24 mm, left tibia length of 28 mm, right tibia length of 27 mm, left fibula length of 27 mm, and right fibula length of 27 mm. The fetal ultrasound measurements were not improved in the next examinations (Figure 1a–d). In addition, a ventricular septal defect, 1.7 mm in size, was detected from 23^{+2} weeks of GA; this defect had reached 2.17 mm in size at 27 weeks of GA (Figure 1e). Doppler ultrasound was used to assess fetal hypoxia and revealed a reduced umbilical artery diastolic flow at 26 weeks of GA and, subsequently, an absent umbilical artery diastolic flow at 27 weeks of GA (Figure 1f). Unfortunately, this pregnancy was ended by preeclampsia occurring at 37 weeks of GA.

CNV-seq analysis did not show microdeletion or microduplication in the fetus (Figure 2a), whereas NGS assay for a panel of 4503 genes revealed a heterozygous c.2455C>T, p.(Arg819Cys) mutation of the *NPR2* gene. This mutation was confirmed by Sanger sequencing (Figure 2b).

The fetus's parents were identified by Sanger sequencing as wild-type homozygotes at the c.2455 position of the *NPR2* gene (Figure 2c,d). In addition, karyotyping revealed that the wife had a fragile site at 16q22.1 (Figure 2e), whereas the husband had a rare variant of chromosome 4 involving in a large G-band-positive and C-band-positive block of paracentric heterochromatin of chromosome 4p (Figure 2f,g). Both parents were of normal height; the wife was 154 cm in height

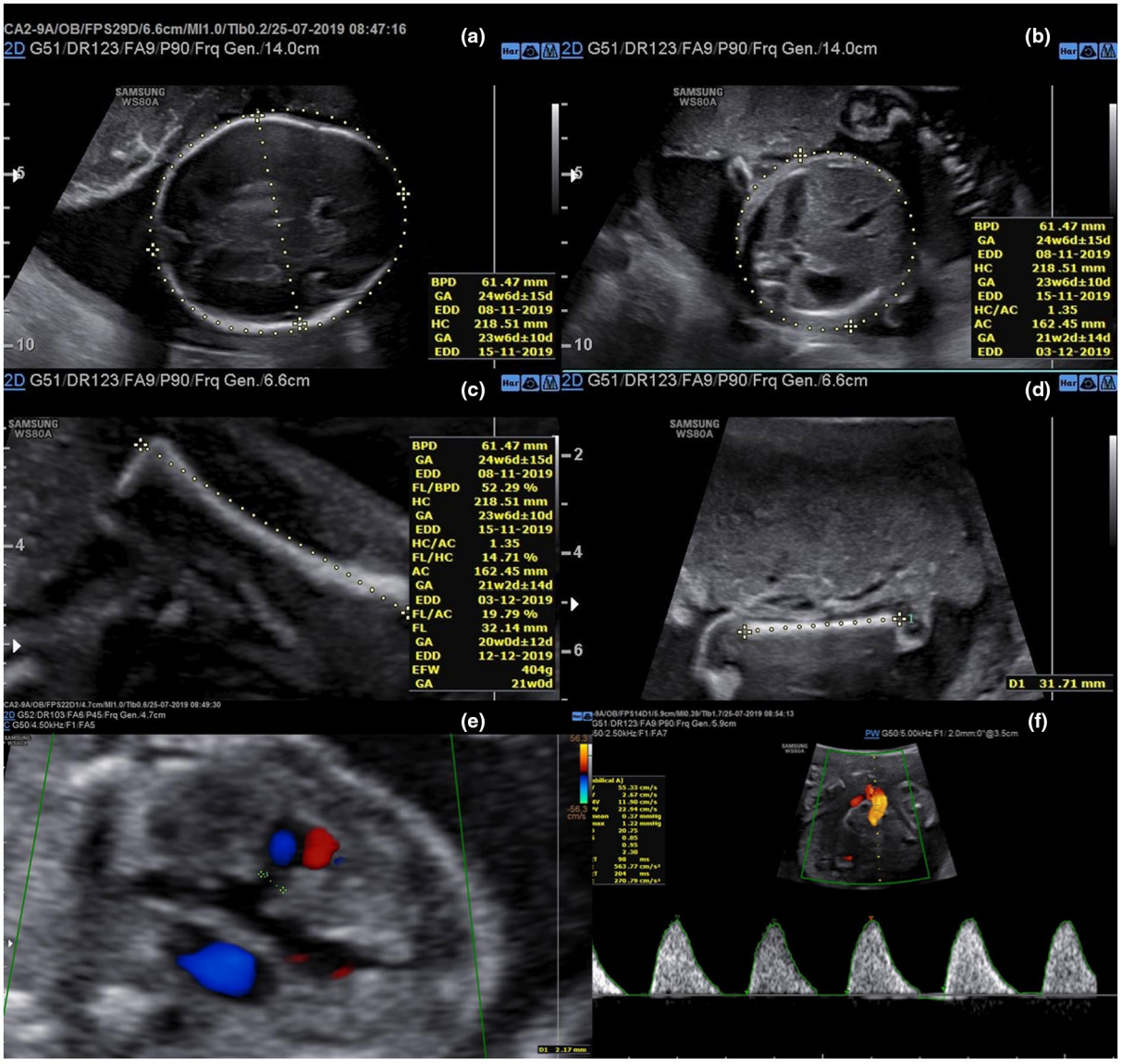

FIGURE 1 Ultrasound features of the fetus at 27 weeks of GA. (a) BPD of 61.47 mm and HC of 218.51 mm; (b) AC of 162.45 mm; (c) FL of 32.14 mm; (d) HL of 31.71 mm; (e) ventricular septal defect, 2.17 mm in size; (f) absent umbilical artery diastolic flow

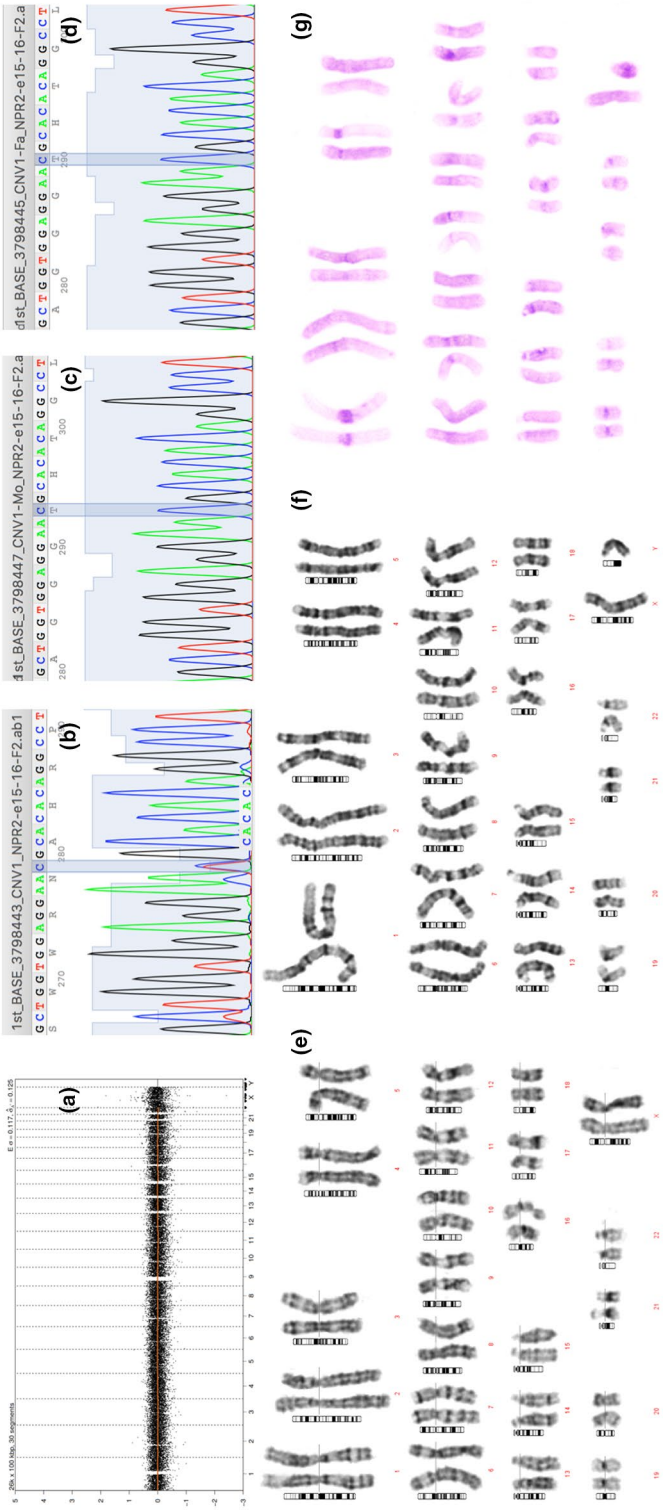

FIGURE 2 Analysis of *NPR2* gene mutation and chromosomal disorders. (a) CNV-seq result of the fetus; (b) Sequencing trace of the fetus: *NPR2* c.2455C>T mutation; (c) Sequencing trace of the mother; (d) Sequencing trace of the father; (e) G-banding karyotype of the mother, showing a fragile site at 16q22.1; (f)) G-banding karyotype of the father, showing a heterochromatic variant of the 4p paracentric region; (g) C-banding karyotype of the father showing a heterochromatic variant of the 4p paracentric region

(the average height of Vietnamese women is 153.4 cm) and the husband was 170 cm in height (the average height of Vietnamese men is 164.4 cm).

4 | DISCUSSION

NPR2 mutations were detected in approximately 2–6% of patients with ISS (Hisado-Oliva et al., 2015; Vasques et al., 2013), but the c.2455C>T (p.(Arg819Cys)) mutation was quite rare in these patients. The p.(Arg819Cys) mutation is located between the kinase homology and guanylyl cyclase domains of NPR-B protein, a receptor for CNP. Vasques *et al*. postulated that the c.2455C>T mutation caused a dominant negative effect on signal transduction by the NPR-B receptor, although some normal trafficking to the plasma membrane still continued (Vasques et al., 2013). This suggested that the c.2455C>T mutation might cause somewhat less damage to longitudinal bone growth when compared to other mutations.

In 2013, Vasques *et al*. reported the first case of this mutation: a Brazilian boy with a heterozygous c.2455C>T (p.(Arg819Cys)) mutation of the *NPR2* gene. This patient had a proportionally short stature (−2.8 SD score) at his first examination at the age of 11.1 years. His mother carried the heterozygous c.2455C>T mutation and had a normal height (−1.7 SD score; Vasques et al., 2013). The second case, reported by Hisado-Oliva et al., (2015), was a 10.2-year-old Spanish boy with a height of 123 cm (−2.3 SD score). He was diagnosed with Leri–Weill dyschondrosteosis with muscular hypertrophy. His mother had a short stature, at 145.6 cm (−2.7 SD score). He and his mother both carried the c.2455C>T mutation. Hisado-Oliva *et al* compared these two cases and postulated that the phenotypic expression of the c.2455C>T (p.(Arg819Cys)) mutation was highly variable in two examined families (Hisado-Oliva et al., 2015).

Our current case was different. This was a female fetus with shortened long bones, and the heterozygous c.2455C>T mutation was found in amniotic fluid DNA by NGS analysis and confirmed by Sanger sequencing. This mutation was identified as de novo because the parents were not carriers. To the best of our knowledge, this is the first case of a de novo c2455C>T mutation in a fetus with shortened long bones.

This fetus also had a small-sized ventricular septal defect (2.17 mm at 27 weeks of GA). CNV-seq analysis did not reveal any microdeletion or microduplication larger than 400 kb in size. It was very difficult to assume a cause of this heart defect. The ventricular septal defect is not a common finding of the *NPR2* gene mutation. Blaser's study reported cardiac hypertrophy, left ventricular dysfunction, and interstitial cardiac fibrosis with age in NPR2-deficient mice (Blaser et al., 2018). Langenickel's study on transgenic rats provided evidence linking reduced NPR-B signaling to cardiac hypertrophy (Langenickel et al., 2006). However, no ventricular septal defect was observed in these in vitro studies. The combination of a ventricular septal defect and shortened long bones in the fetus with de novo c.2455C>T mutation has not been previously reported. Further studies will be necessary to assess the relationship between ventricular septal defect and this mutation.

The parents of this fetus had a bad obstetric history, including two previous miscarriages. Analysis of their karyotypes revealed a fragile site at 16q22.1 in the wife and a rare variant of chromosome 4 involving in a large G-band-positive and C-band-positive block of paracentric heterochromatin of chromosome 4p of the husband. The chromosomal variants in the fetus's parents might be associated with the two previous spontaneous miscarriages. The husband's variant of chromosome 4, which was very rare, was previously reported as a rather extreme polymorphism that was transmitted from generation to generation (Docherty & Bowser-Riley, 1984; Hansmann et al., 1982). The bearers of this variant of chromosome 4 did not show any clinical signs of their chromosomal aberrations. Consequently, this variant could not be easily linked to recurrent pregnancy loss.

Chromosome 16q22.1 contains two fragile sites, FRA16B and FRA16C (Martorell et al., 2014). Previously, these fragile sites were not associated with either clinical problems or phenotypic effects (Schmid et al., 1986). However, Garcia-Sagredo reported a man with a fragile site at 16q22.1 who fathered a son with a de novo balanced translocation (1;16) at the same breakpoint (García-Sagredo et al., 1983). Aswini reported a non-consanguineous couple with repeated pregnancy loss who presented with FRA16B expression (Aswini et al., 2012). Recently, Martorell reported a couple with difficulty achieving pregnancy, where the wife was a carrier of t(11;22)(q23;q11.2) and the husband had a high expression of fragile site at 16q22.1 (FRA16B/C) in his peripheral blood lymphocytes (Martorell et al., 2014). The husband's sperm and the embryos both showed chromosome 16 abnormalities, indicating that the fragile site at 16q22.1 might cause chromosomal disorders involving the same breakpoint and non-disjunction during meiosis, thereby leading to pregnancy losses.

In conclusion, to our knowledge, this is the first report of a de novo c.2455C>T mutation of the *NPR2* gene in a fetus with shortened long bones and a small ventricular septal defect. The maternal fragile site at 16q22.1 and the paternal heterochromatic variant of the 4p paracentric region might be involved in the couple's repeated pregnancy losses.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

Thi Minh Thi Ha performed clinical evaluation, collected medical history details, evaluated the Sanger sequencing data and karyotypes, wrote the draft, and supervised the study. Tran Thao Nguyen Nguyen performed obstetric ultrasound examinations. Thi Mai Ngan Nguyen performed karyotyping. Huu Nguyen Nguyen performed the next-generation sequencing. All authors revised the draft, read, and approved the final version of the article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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