

CASE REPORT

Interstitial 20p13 microdeletion including *PRNP* and adjacent genes in a fetus with congenital abnormalities—First case report

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Abstract

We present a prenatal case with congenital anomalies that revealed a 759 kb microdeletion at 20p13 possibly implicating *PRNP* and adjacent genes.

KEYWORDS

abnormal ultrasound, CMA, prenatal, *PRNP* deletion

1 | INTRODUCTION

PRNP is mainly expressed in the central and peripheral nervous system. We report a fetus with congenital anomalies with 20p13 deletion encompassing *PRNP* and adjacent genes, and 116 Kb gain was found at 22q.11.21. *PRNP* and adjacent genes have a possible role in congenital abnormalities.

Prion proteins are encoded by *PRNP* which is located at 20p13. About 15% of prion diseases are considered to be related to genetic mutations of *PRNP*. The *PRNP* gene has two exons and the open reading frame is located in exon two. It is expressed broadly, almost ubiquitously, though enriched in the CNS. Coding mutations in *PRNP* are associated with prion disease by resulting in misfolded prion proteins. While the normal function of the nonpathogenic (cellular) form of prion protein (PrPC) is unknown, the pathogenic form (PrPSc) has a propensity to accumulate¹. Mutations identified in *PRNP* are missense, nonsense, and insertions or a deletion. More than 30 mutations in the *PRNP* have been identified in people with familial forms of prion disease^{2,3}. It is reported that the loss of *PRNP* function does not play a major role in the etiology of prion disease.^{4,5} In other organisms like mice and zebrafish,⁶ *PRNP* is not required for normal developmental process.

2 | CLINICAL PRESENTATION

We present a fetus with congenital anomalies including bilateral clubbed feet, rocker-bottom feet, and absent cavum septum pellucidum as well as intrauterine growth retardation (IUGR) identified at 29-week in a 21-year-old pregnant woman (G4P2A1). The parturient had a history of ADHD, bipolar disorder, chronic depression, smoking, and use of THC (tetrahydrocannabinol) during pregnancy. It was also reported that she was taking psychiatric medication which she stopped after finding out that she was pregnant. She had an uncomplicated C-section at 37w0d. APGARS scores were 9 at 1 minute, 9 at 5 minutes. The baby was born active, pink, and vigorous. She did not require any interventions in the delivery room. Neonatal evaluation revealed that the baby was small for gestational age. Her birth weight was 1.810 kg (0.3 percentile), length was 41.0 cm (0.1 percentile), and head circumference was 33.0 cm (39.7 percentile). Her left foot had a rocker-bottom appearance with dorsal deviation. Additionally, her right foot was dorsiflexed with resistance to extension of the ankle. No cardiac or respiratory abnormalities were seen and neurologic reflexes including Moro, pupillary, fair suck were normal. Cranial ultrasound findings revealed enlargement of the lateral ventricles (ventriculomegaly) with absence of septum pellucidum. Cranial/orbit

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MRI revealed septo-optic dysplasia with partial agenesis of the corpus callosum.

3 | METHODS

3.1 | Chromosome microarray analysis (CMA) and fluorescence in situ hybridization (FISH)

For CMA analysis, DNA was extracted from amniotic fluid using QIAamp[®] DNA Blood Mini Kit (Cat #51106). The DNA concentration was determined by using Nanodrop ND-2000 spectrometer (Thermo Scientific). CMA experiments were performed on SurePrint G3 ISCA CGH + SNP Microarray Kit, 4x180K v2.0 platform (Agilent Technologies), featuring approximately 110 715 custom oligonucleotides + 59 647 SNPs (60 mers) and covering 1282 ISCA regions, resulting in a 25.3 Kb resolution. As per the manufacturer's recommendations, DNA of the patient was referenced against Agilent Human Reference DNA female (5190-4370/4371) using Agilent's SureTag Complete DNA labeling Kit (Cat # 5190-4240). Patient's data were scanned (Agilent Model #G2505C) at 3 μ m resolution and visualized (Cytogenomics software) with log₂ threshold ratios of -0.25 for losses and 0.25 for gains.

The BAC probe (RP11-960N2, spectrum orange) and control probe (TelVysion 20p, spectrum green) were labeled according to the manufacturer's instructions (Cat #05J03-030, Abbott Laboratories). The manufacturer's instructions and standard protocols were followed for hybridization. Slides were analyzed using a Nikon (Eclipse 80i) fluorescence microscope attached with a CCD camera. For image acquisition and analyses, Applied Spectral Imaging (ASI) software was used. Ten metaphase and 100 interphase cells were analyzed for confirmation of CMA findings.

4 | RESULTS

Amniotic fluid cytogenetic analysis revealed a normal 46,XX karyotype by G-banding technique. Follow-up CMA on cultured cells showed a 759 Kb heterozygous loss at 20p13 and a 116 Kb gain at 22q.11.21. The array and FISH result: ish del(20)(p13p13)(RP11-960N2)[10/10],dup(22)(q11.21q11.21)(PRODH+)[10/10].arr[GRCh37/hg19]20p13(4,018,739x2,4,053,819-4,813,038x1,4,834,291x2),22q11.21(18,877,583x2,18,894,835-19,010,508x3,19,036,997x2). The loss at 20p13 encompassed *PRNP*, *SMOX* (Spermine Oxidase) *ADRA1D* (Alpha-1-adrenergic receptors); *PRND* (Prion Like Protein Doppel); *RASSF2* (Ras Association Domain Family Member 2); *LINC01433* (Long Intergenic Non-Protein Coding RNA 1433); and *PRNT* (Prion

Locus LncRNA, Testis Expressed). The gain of 116 Kb at 22q.11.21 included *DGCR6*, *PRODH*, and *DGCR5*. FISH analysis on 10 metaphase and 100 interphase cells confirmed the 20p13 deletion (Figure 1A,B,C). Parental samples were not available to determine the nature and origin of 20p13 deletion and 22q11.21 duplication. No follow-up information on child's development is available.

5 | DISCUSSION

We performed a database review in DECIPHER (Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources)⁷ and ClinGen. The criteria used for the database search was to match the exact deletion of our case - 20p13 (4,018,739x2,4,053,819-4,813,038x1,4,834,291x2). Complete *PRNP* deletions have rarely been reported in these databases. In ClinGen (<https://clinicalgenome.org/>) one partial deletion was found (nssv1495051). In DECIPHER (<https://decipher.sanger.ac.uk/>), two deletions were found (ID numbers: 332440, 366633). We also did a literature review in PubMed for “prenatal prion” or “*PRNP* deletion” as search terms, however, we could not find cases similar to the one in this report. The DECIPHER cases showed 20p13 deletion overlapping approximately 21% with our case encompassing *PRNP*, *PRND*, and *PRNT* (Size 159.37 Kb; Coordinates: GRCh38: 4,638,741-4,798,107x1). Both these cases are siblings from a single family with autistic behavior and global developmental delay, inheritance unknown (personal communication from DECIPHER).

⁸Demonstrated in PrP-null mice hippocampal slices a weakened GABAA (gamma-aminobutyric acid type A) receptor-mediated fast inhibition and impaired long-term potentiation. Based on this, the authors argued that a loss of function of PrPC may contribute to the early synaptic loss and neuronal degeneration typically found in Creutzfeldt-Jakob disease.⁹ Miranda et al studied mouse embryonic stem cells (ESC) in order to identify the role of *PRNP* in stem cell differentiation. The functionality of *PRNP* in mouse Prnp-null (KO) and WT embryonic stem cell (ESC) lines was examined during the embryonic body differentiation. They revealed a relationship between *PRNP* and several key pluripotency genes such as *Nanog* and *Oct3/4*. Their results provided the evidence that PRNP participates in the self-renewal/differentiation status of stem cells during early embryogenesis.^{9,10} Young et al provided strong evidence for the role of prion family proteins in early mouse embryogenesis. They studied a prion-related protein named Shadoo which has partially overlapping biological properties and brain distribution with the prion protein. They reported that the Shadoo-encoding gene knockdown in PrnP-knockout mouse embryos resulted in a lethal phenotype. Their study also explains the important

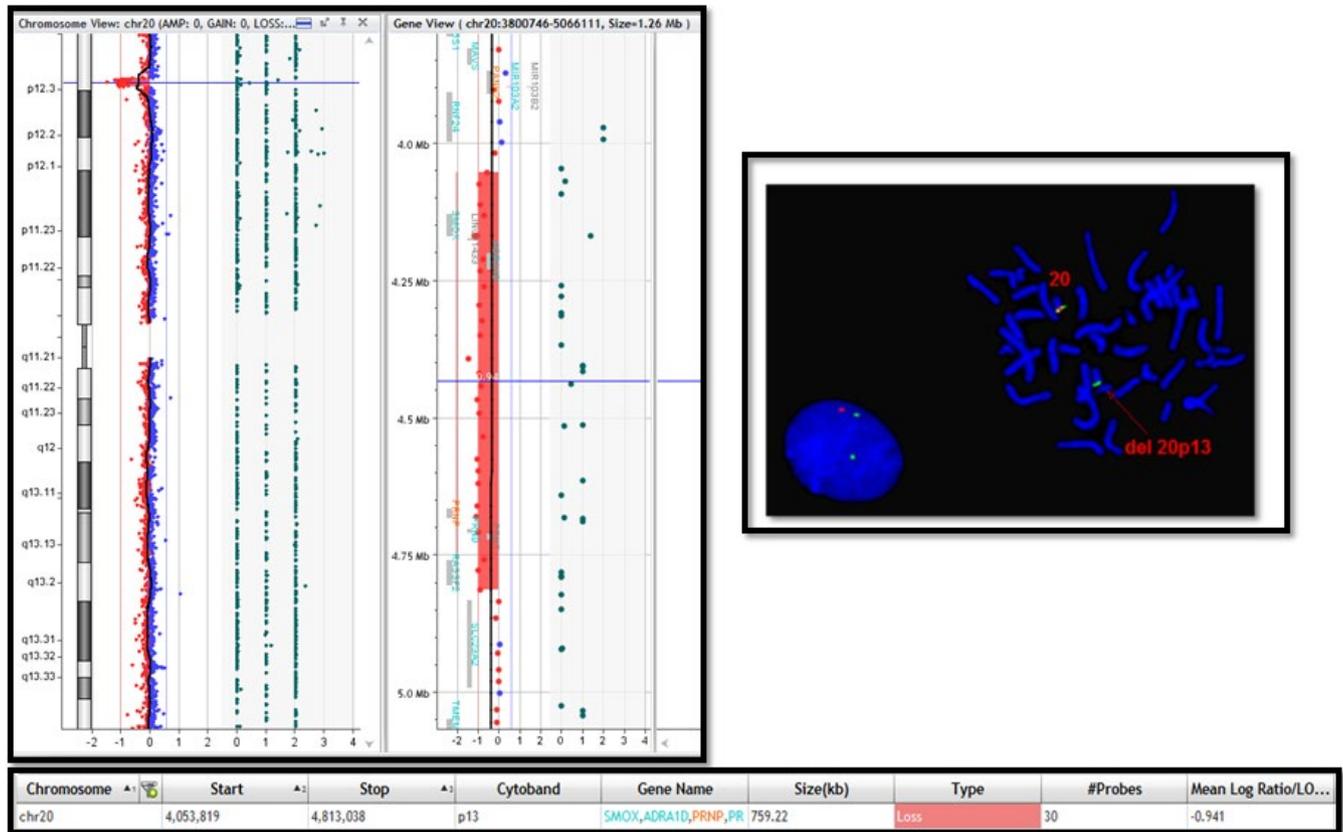


FIGURE 1 A, Chromosome view of chromosome 20 showing the deletion in the 20p13 region. B, Fluorescence in situ hybridization (FISH) confirmation of CMA finding, (RP11-960N2 [20p13] BAC probe, Spectrum Orange; TelVysion 20p [control], Spectrum Green). C, Log2 ratio of deletion in 20p13

function of these two proteins together in mammalian embryogenesis.¹⁰ Studies with zebrafish have also showed strong findings highlighting the importance of prion protein function in early embryonic development. The amenability of zebrafish to global technologies has generated data indicating the existence of “anchorless” splice variants of PRNP in the early embryo.¹¹ Nourizadeh-Lillabadi studied the PRNP2 gene in the zebrafish genome and showed impaired brain and neuronal development in PRNP2 deleted embryos. They also reported apoptosis and high mortality at 24 hours postfertilization in the same embryos. The study suggests that PRNP2 knockdown has an important biological function for the zebrafish in affecting gene networks controlling neurogenesis and embryo development.¹¹ Interestingly, *PRND* is also deleted in our case and may have played a role in the congenital abnormalities observed. All of the above studies suggest an important role of *PRNP* and its close family of genes during embryogenesis. It is also well known that whole-gene deletions or duplications can be associated with diverse phenotypic abnormalities. It remains to be seen if heterozygous deletion of *PRNP* and adjacent genes also has an impact in human embryogenesis. Additional similar cases will be helpful in this regard.

Our case also revealed a 116 Kb gain at 22q.11.21 encompassing *DGCR6*, *PRODH*, and *DGCR5*. A causal effect of duplications in this region on stand-alone conotruncal heart defects has been suggested by Gao et al.¹² Our case has a much smaller duplication and is most likely a benign finding. Marijuana exposure during pregnancy has been reported to be associated with low birth weight and preterm delivery. This is also confounded by cigarette smoking, alcohol consumption, and substance abuse.¹³ We have limited information on the type and duration of psychiatric medication exposure to the fetus. It is also possible that several genes included in 20p13 deletion segment may be responsible for the abnormal phenotype in our case.

6 | CONCLUSION

We have reported the first prenatal case with a 759 Kb heterozygous deletion at 20p13 with congenital abnormalities. Two overlapping cases in DECIPHER with a 159 Kb deletion encompassing *PRNP*, *PRND*, and *PRNT* with autistic behavior and global development delay supports a possible pathogenic role of *PRNP* and adjacent genes. Future similar

cases may support haploinsufficiency of these genes in early embryogenesis.

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CONFLICT OF INTEREST

All the authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

PO: wrote the manuscript, MAI: supervised the DNA microarray analysis, interpreted the data, and reviewed the final draft of manuscript. All authors read and approved the final manuscript.

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