

An Unusual Diagnostic Journey Through MLPA: From Spinal Muscular Atrophy to a Severe Case of Prader-Willi Syndrome

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ABSTRACT

Background: Prader-Willi Syndrome (PWS) is a multisystemic disorder characterized by dysmorphic facies, hypotonia, developmental delay, cognitive impairment, hypogonadism, and obesity. It is caused by the absence of expression of paternally derived genes on chromosome 15. Here, we report the diagnostic journey of a case with severe neonatal hypotonia.

Case Report: A neonatal patient was referred for the prediagnosis of spinal muscular atrophy (SMA). During the SMA Multiplex Ligation-dependent Probe Amplification (MLPA) analysis, a diminished signal of a reference probe on the 15q11.2 was revealed. Later, it was confirmed that she had a deletion confined to 15q11.2-q13.1, with a methylation pattern compatible with PWS.

Conclusion: Since hypotonia might be the only finding in newborns with PWS, this case was presented to emphasize the importance of a comprehensive approach to such patients.

Keywords: Prader-Willi Syndrome, Spinal muscular atrophy, MLPA, PWS, SMA, hypotonia.



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INTRODUCTION

Prader-Willi Syndrome (PWS) is a multisystemic disorder caused by a lack of expression of the paternally-inherited imprinted genes in the PWS region of chromosome 15. Individuals with PWS exhibit variable phenotypical features depending on the systems affected and the age of the proband. Its clinical manifestations include severe hypotonia, feeding difficulty in infancy followed by global developmental delay, and food-seeking behavior leading to morbid obesity in childhood. The patients commonly have characteristic facial features, including a narrow bitemporal diameter, upslanting and almond-shaped palpebral fissures, thin vermilion of the upper lip, and downturned corners of the mouth. As the individual ages, short stature, scoliosis, hypogonadism, cognitive impairment, and behavioral disturbances also become evident.¹ The main genetic mechanisms resulting in PWS are paternal deletion, which is attributed to 60% of the cases, maternal disomy 15, which is attributed to 35%, and rarely imprinting defects.² DNA methylation analysis is the sole molecular technique capable of diagnosing PWS caused by all three mechanisms. Spinal muscular atrophy (SMA), on the other hand, is a relative-

ly common autosomal recessive neurodegenerative disease affecting the anterior horn cells of the spinal cord, leading to a deterioration of motor functions. Approximately 95% of the patients harbor a homozygous exon 7 deletion of the Survival Motor Neuron 1 (SMN1) gene located in the 5q13 region.³ The method of choice for diagnosis is the multiplex ligation-dependent probe amplification (MLPA) technique.⁴ Since neonatal floppiness is a shared symptom of PWS and SMA, a differential diagnosis is necessary. This case report aims to emphasize the importance of a comprehensive approach to patients by reporting a PWS diagnosis in a case initially prediagnosed with SMA.

CASE REPORT

A three-month-old female infant was referred to the Medical Genetics department with a prediagnosis of SMA based on the findings of hypotonia, poor suck, myoclonus, developmental hip dysplasia, and absence of deep tendon reflexes (on 21/09/2021). Antenatally, microcephaly and intrauterine growth retardation were observed, without any history of toxin or drug exposure. She was delivered at 40 weeks via C-section. Due to poor suck and hypotonia, she was hospitalized in the intensive care unit with oxygen and feeding support. Her non-related parents had no health complaints, and she had a phenotypically healthy 3-year-old sister. The patient's karyotype analysis resulted in 46, XX. Then, she underwent SMA-MLPA analysis using the SALSA MLPA Probemix P060 kit (MRC Holland, Amsterdam, Holland). The copy number of SMN1 gene exon 7 was found to be normal. However, the signal of the 245-nucleotide-size reference probe localized on the 15q11.2 region was consistently diminished in repetitive studies and drew particular attention (Fig. 1).

Considering that neonatal hypotonia is a common finding in Prader-Willi Syndrome, a comprehensive physical examination was conducted. Her height and weight were measured as 57 cms [-1.27 Standard Deviation (SD)] and 4600 grams (-1.75 SD), respectively. The measured head circumference was 37 cms (-2.49 SD). The patient was found to have dysmorphic features, including bitemporal narrowing, almond-shaped eyes, blue irides, microretrognathia, thin vermilion of the lips, high palate, sparse hair, and orange eyebrows (Fig. 2a–d). She experienced myoclonic seizures and exhibited generalized retinal pigment atrophy. Central hypothyroidism was also identified with a Thyroid-Stimulating Hormone (TSH) level of 0.0683 mU/L and a free T4 level of 0.711 ng/dl. Brain magnetic resonance imaging and the initial echocardiography yielded normal results. PWS Methylation-specific (MS)-MLPA revealed a deletion of the 15q11.2 to 15q13.1 region and a methylation pattern compatible with PWS (Fig. 1). The deletion was also

detected using fluorescence in-situ hybridization (FISH) with the Diagen kit. To determine the exact boundaries of the deletion, array-comparative genomic hybridization (CGH) analysis was performed on the SurePrint G3 Unrestricted CGH ISCA v2 8x60K microarray platform (Agilent Technologies, California, United States). It was found that she had a heterozygous 5755 kbp deletion confined to the 15q11.2-q13.1 banding regions including the Makorin RING finger protein 3 (*MKRN3*), MAGE family member L2 (*MAGE L2*), Necdin (*NDN*), Chromosome 15 open reading frame 2 (*C15orf2*), Small nuclear ribonucleoprotein polypeptide N (*SNRPN*), Paternally expressed 5 (*PAR5*), Imprinted in Prader-Willi syndrome (*IPW*), Paternally expressed 1 (*PAR1*), Paternally expressed 4 (*PAR4*), Ubiquitin protein ligase E3A (*UBE3A*), ATPase phospholipid transporting 10A (*ATP10A*), Gamma-aminobutyric acid type A receptor beta3 subunit (*GABRB3*), Gamma-aminobutyric acid type A receptor alpha5 subunit (*GABRA5*), Gamma-aminobutyric acid type A receptor gamma3 subunit (*GABRG3*), Oculocutaneous albinism II (*OCA2*) and HECT and RLD domain-containing E3 ubiquitin protein ligase 2 (*HERC2*) genes. This region is between the BP2-BP3 breakpoints, indicating a PWS type 2 deletion. At the age of 7 months, she was hospitalized with an infection, anemia, and feeding and respiratory issues. Subsequent echocardiography revealed non-compaction cardiomyopathy, myocarditis, and sinus tachycardia. The patient passed away due to heart failure. The genetic testing of the parents was normal. Detailed genetic counseling was provided to the family, including an explanation of the preimplantation genetic testing (PGT) and prenatal diagnosis options. Informed consent for the publication of the patient's and their data was obtained from the parents.

DISCUSSION

Prader-Willi Syndrome is the first identified genomic imprinting disorder caused by a lack of paternally expressed genes on the 15q11-q13 region, mostly due to deletion.⁵ In this study, the proband had severe neonatal hypotonia and feeding difficulty that was initially confused with the clinical presentation of SMA. The proband exhibited a distinct craniofacial appearance consistent with PWS. This syndrome affects the hypothalamic-pituitary axis, leading to various endocrinopathies, including growth hormone deficiency, hypogonadism, hypothyroidism, and adrenal insufficiency.⁶ Our patient was being treated for central hypothyroidism, and no central adrenal insufficiency or other endocrinopathies were detected during the follow-up. In the literature, Bohonowych et al.⁷ reported a high prevalence of vision and eye problems in PWS patients, including strabismus, myopia, hyperopia, astigmatism, and amblyopia. Our patient exhibited retinal pigment epithelium (RPE) atrophy,

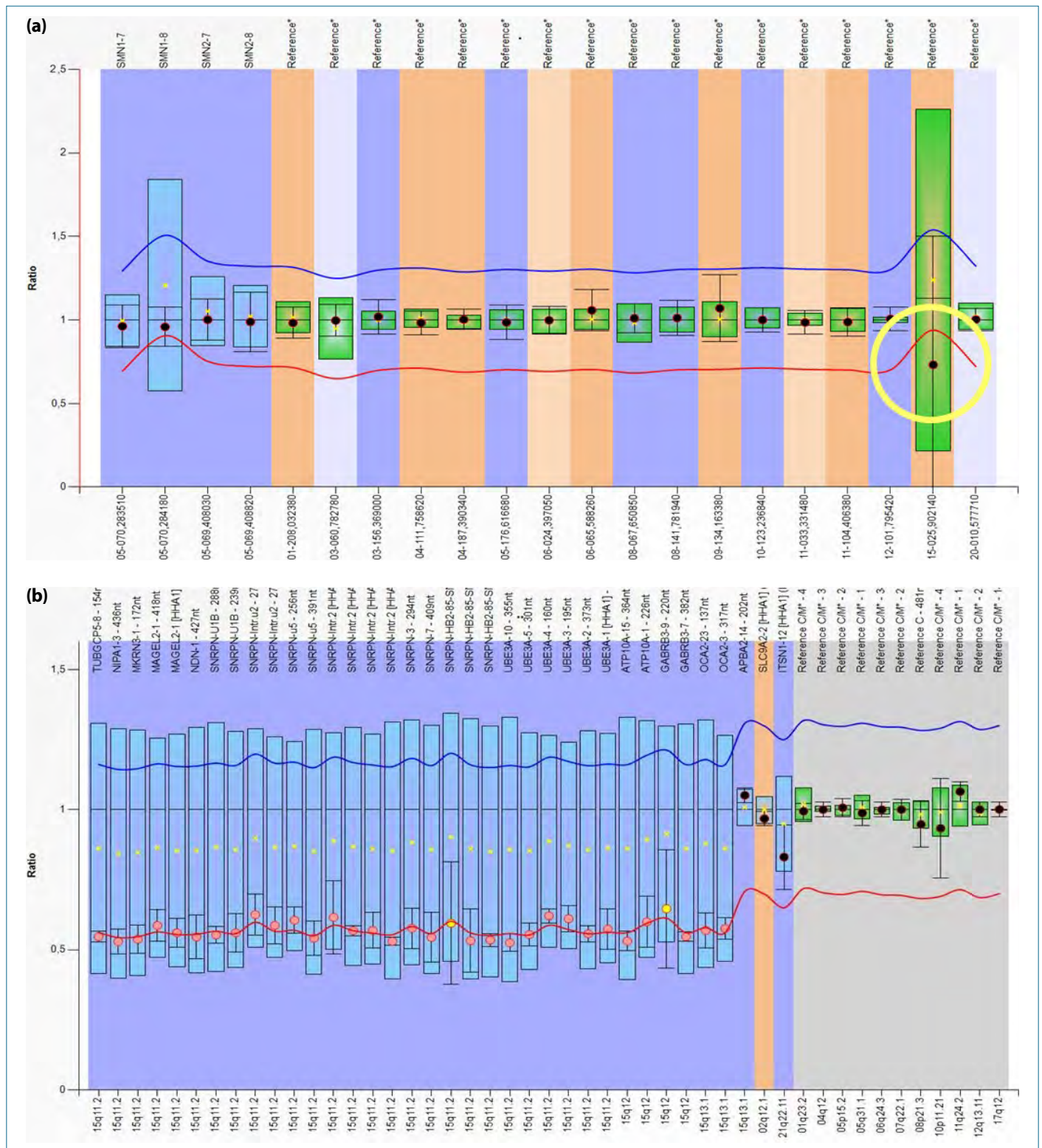


Figure 1. MLPA analysis results using the Coffalyser program. **(a)** MLPA analysis results for SMN1 and SMN2 genes. The signal of one of the reference probes located on chromosome 15 is shown to be diminished (indicated by the circle). **(b)** MLPA analysis depicting a type II heterozygous deletion within the regions of 15q11.2-q13 in the patient (~50% reduction in the peak area of the amplification product).

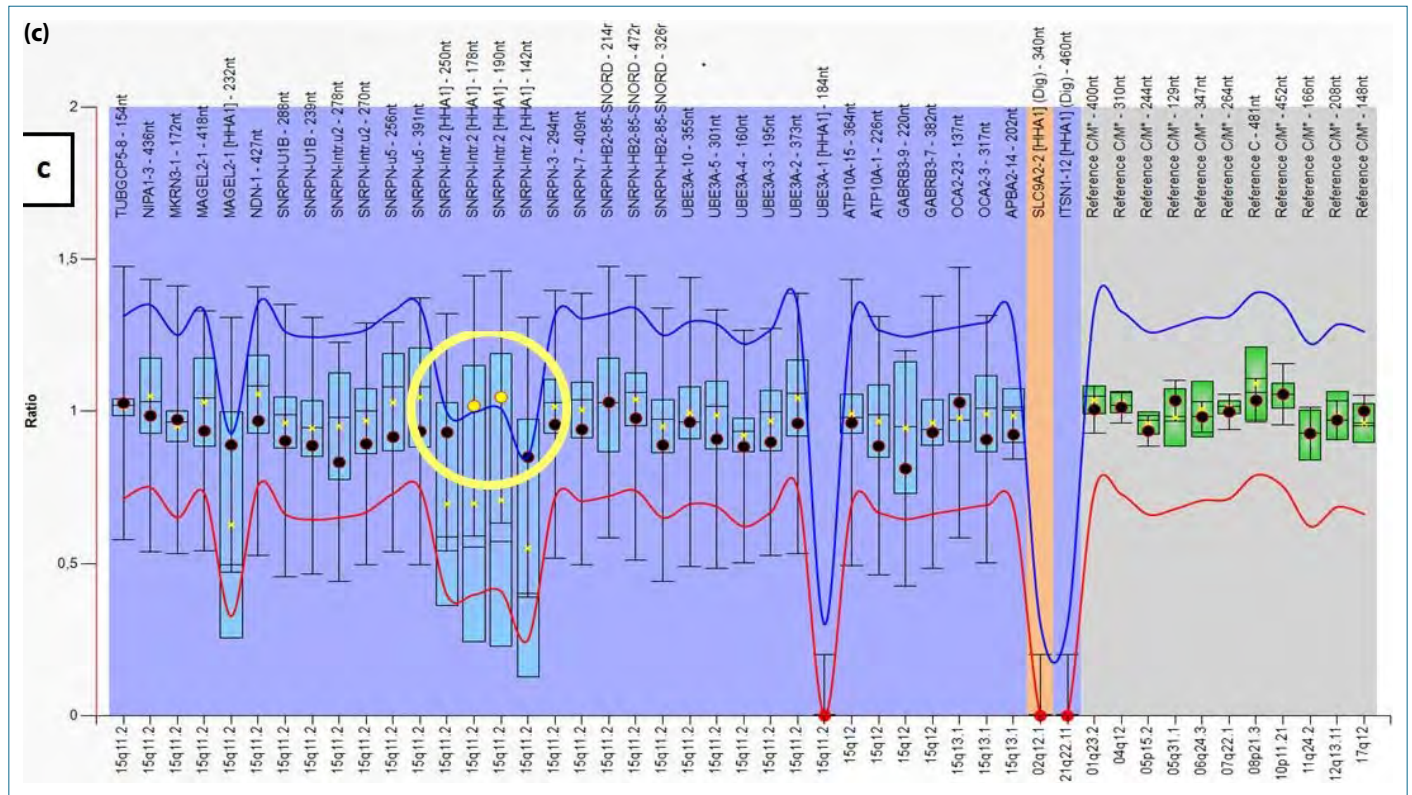


Figure 1 (cont.). (c) Detection of hypermethylation of two probes in the *SNRPN* CpG island after digestion with the methylation-sensitive *HhaI* restriction endonuclease (indicated by the circle).

making her the third reported case of PWS with this rare finding in the literature. Additionally, the case presented developmental hip dysplasia, which is known to be one of the most common complications of the syndrome, occurring in 10–30% of patients.⁸ Elia et al.⁹ mentioned that only a minor proportion of PWS patients experience epileptic seizures. Although the seizure types vary among cases, the most common seizure type reported was focal seizures. Our proband had myoclonic epilepsy. In PWS patients, respiratory failure and cardiac problems combined are the leading causes of death. In our index case, heart failure resulting from cardiomyopathy and myocarditis led to her demise.

Prader-Willi Syndrome can result from several genetic mechanisms, including paternal deletion, maternal uniparental disomy 15 (UPD15), and imprinting defects. The typical deletion in the 15q11.2-q13 region extends from one of the two proximally located breakpoints (BP1, BP2) to the distal breakpoint (BP3). Type 1 deletion, from BP1 to BP3, is slightly larger and has been associated with poorer intellectual abilities and adaptive behavior compared to type 2 deletion (BP2 to BP3).¹⁰ It has been shown that cases caused by deletion are more severely affected in terms of facial dysmorphism and pigmen-

tion due to loss of the *OCA2* gene, and they more commonly experience speech disorders and feeding difficulties. Type 2 deletion has also been associated with milder cognitive and behavioral issues in the literature. In our case, with a heterozygous type 2 deletion, the clinical condition was aggressive. She exhibited generalized hypopigmentation, required nasogastric throughout her entire life, and could not survive the neonatal period.

During infancy, PWS can resemble spinal muscular atrophy, another neonatal neuromuscular disorder caused by degeneration of motor neurons. These diseases need to be differentiated clinically or through genetic testing.

CONCLUSION

In this study, although the patient had a typical deletion, her condition was more severe compared to the other cases reported in the literature. She passed away due to heart failure at the age of 7 months. By reporting this case, we emphasize the consideration of Prader-Willi Syndrome when evaluating a case with neonatal hypotonia and stress the importance of comprehensive assessments for early and accurate diagnosis.

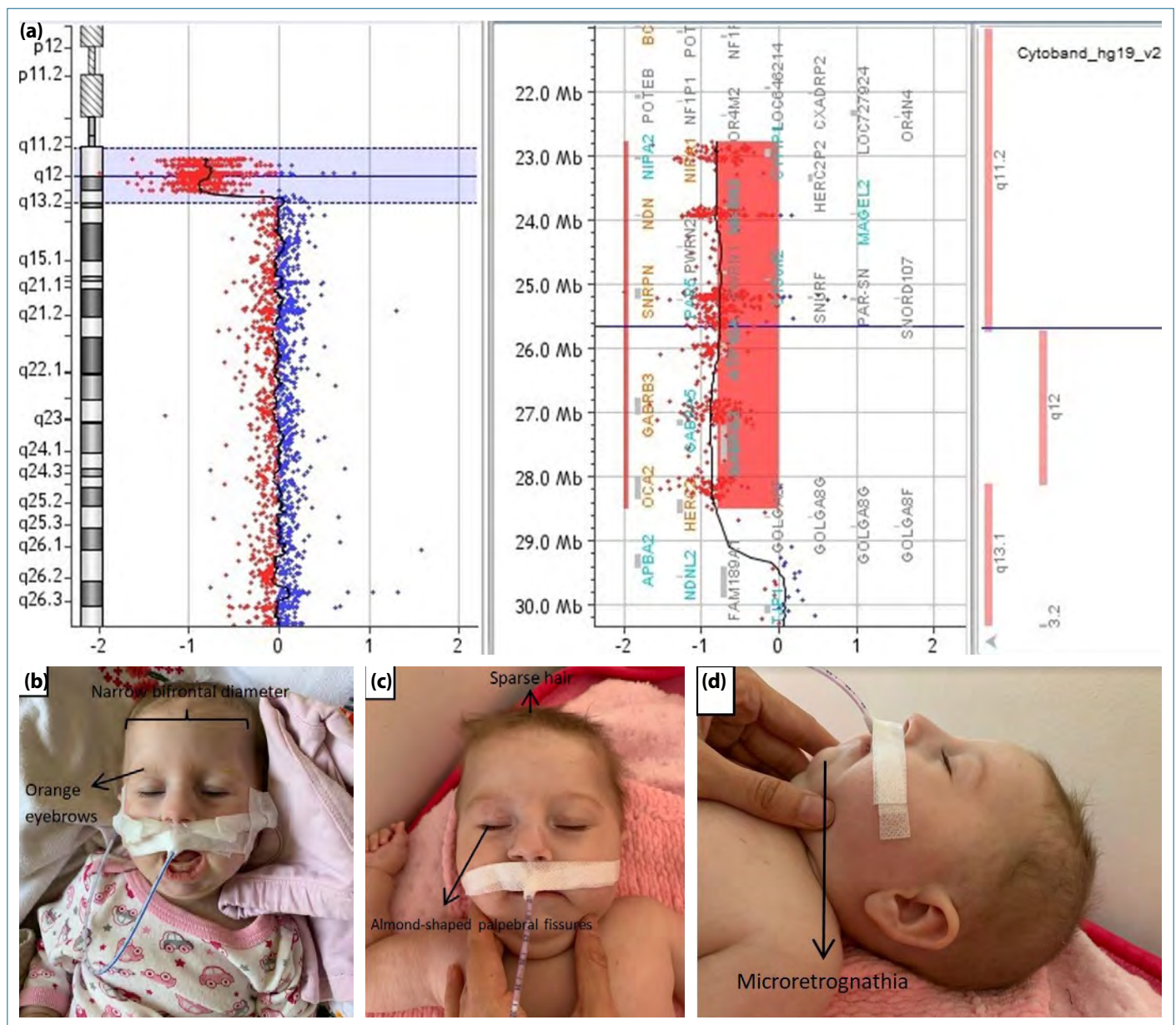


Figure 2. (a) Cytogenomic analysis. **(a)** CGH plot showing a de novo 5755 Kbp deletion confined to the 15q11.2-q13.1 banding regions in our patient. This deletion includes the genes *MKRN3*, *MAGEL2*, *NDN*, *C15orf2*, *SNRPN*, *PAR5*, *IPW*, *PAR1*, *PAR4*, *UBE3A*, *ATP10A*, *GABRB3*, *GABRA5*, *GABRG3*, *OCA2* and *HERC2*. **(b–d)** Photographs of the proband showing narrow bifrontal diameter, almond-shaped palpebral fissures, thin vermilion of the upper lip, orange eyebrows, sparse hair, and microretrognathia. **(b)** Photograph taken at the age of 3 months. **(c, d)** Photographs taken at the age of 5 months.

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Informed Consent: Written, informed consent was obtained from the patient's family for the publication of this case report and the accompanying images.

Author Contributions: Concept – EG, BOA, HT; Design – EG, GK; Supervision – HT, BOA; Resource – AGZ, MSY; Materials – BOA, HT; Data

Collection and/or Processing – EG, HT, BOA, GK; Analysis and/or Interpretation – EG, BOA, GK, HT; Literature Search – EG, BOA, HT; Writing – BOA, EG, HT; Critical Reviews – EG, AGZ, MSY.

Conflict of Interest: The authors have no conflict of interest to declare.

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