

CASE REPORT

A 9-YEAR-OLD-GIRL WITH PHELAN McDERMID SYNDROME, WHO HAD BEEN DIAGNOSED WITH AN AUTISM SPECTRUM DISORDERGörker I^{1,*}, Gürkan H², Demir Ulusal S², Atlı E², İkbal Atlı E²

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ABSTRACT

Phelan McDermid Syndrome (PHMDS) (OMIM #606232), is a contiguous gene disorder resulting from deletion of the distal long arm of chromosome 22. The 22q13.3 deletions and mutations that lead to a loss of a functional copy of *SHANK3* (OMIM *606230) cause the syndrome, characterized by moderate to profound intellectual disability, severely delayed or absent speech, hypotonia, and autism spectrum disorder (ASD) or ASD traits. In this study, we present the case of a 9-year-old girl who had earlier been diagnosed with an ASD. Our findings were a clinically mild intellectual disability, rounded face, pointed chin but no autistic findings. We learned that her neuromotor development was delayed and she had neonatal hypotonia in her history. A heterozygous deletion of *MLC1*, *SBF1*, *MAPK8IP2*, *ARSA*, *SHANK3* and *ACR* genes, located on 22q13.33, was defined by multiplex ligation-dependent probe amplification (MLPA). Deletion of 22q13.3 (*ARSA*) region was confirmed by a fluorescent *in situ* hybridization (FISH) technique. The 22q13.3 deletion was found to be *de novo* in our patient, and she was diagnosed with PHMDS. We confirmed the 22q13.3 deletion and also determined a gain of 8p23.3-23.2 by array comparative genomic hybridization (aCGH). Fluorescent *in situ* hybridization was performed to determine

whether the deletion was of parental origin and to identify regions of chromosomes where the extra 8p may have been located. The parents were found to be normal. The extra copy of 8p was observed on 22q in the patient. She is the first case reported in association with the 22q deletion of 8p duplications in the literature.

Keywords: Autism spectrum disorder (ASD); Phelan McDermid Syndrome (PHMDS); 22q13.3 deletion.

INTRODUCTION

The 22q13.3 deletion syndrome, also known as Phelan McDermid Syndrome (PHMDS) (OMIM #606232), is a contiguous gene disorder resulting from deletion of the distal long arm of chromosome 22 [1]. 22q13.3 deletions and mutations that lead to a loss of a functional copy of *SHANK3* (OMIM *606230) cause the syndrome, characterized by moderate-to-profound intellectual disability, severely delayed or absent speech, hypotonia, and autism spectrum disorder (ASD) or ASD traits [2]. This syndrome results from a *de novo* deletion of chromosome 22 in 80.0-85.0% of individuals, and approximately 70.0% of the deletions are paternal in origin; it also results from an unbalanced chromosome rearrangement involving chromosome 22 in 15.0-20.0% of cases, of which approximately 50.0% are inherited from a balanced carrier parent. The rearrangement is equally likely to be inherited from the mother or the father [1]. In this study, we present the case of a 9-year-old girl who had been diagnosed with ASD, assessed and diagnosed with PHMDS using genetic analyses and the results are discussed.

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CASE REPORT

Our case, a 9-year-old girl and the only child of her family who have taken individual training for her social development, was examined at the Child and Adolescent Psychiatry Department, Trakya University, Edirne, Turkey. It was learned that she had been diagnosed with ASD some years ago and had both pharmacotherapy and individual training for her treatment. Our findings were a clinically mild intellectual disability, rounded face, pointed chin and no autistic findings. We learned that her neuromotor development was delayed and she had neonatal hypotonia in her history. The parents had no history of mental disorder or genetic illness. She was born by spontaneous delivery. She weighed 2850 g and was 50 cm long at birth and she was breast-fed by her mother for 18 months. She sat up in her 10th month and walked in her 16th month. Her language development was delayed. Her mother said that she showed head banging behavior between 7 months and 1 year. At 4 years old, she exhibited irritability and aggressive behavior towards her peers and teachers at the crèche she was attending, and risperidone treatment was begun. The intelligence test could not be applied because of her speech problems. She had no history of febrile convulsions. The results of neurological examination and the routine biochemical blood tests were normal. We discontinued her risperidone treatment in order to assess her mental state. Consequently, there was a remanifestation of her aggressive behavior and irritability. Subsequently, the risperidone treatment was restarted and she became stabilized. Then her genetic analyses were examined at the Department of Medical Genetics, Trakya University, Edirne, Turkey. Cytogenetic analysis was performed with GTG-banded chromosomes from cultured lymphocytes. Genomic DNA of the patient was isolated according to the instructions of the manufacturer (EZ1 Advanced Instruments; Qiagen GmbH, Hilden, Germany) from peripheral blood lymphocytes. Genomic regions previously related to mental retardation and possible deletion/duplication regions were analyzed by the multiplex ligation-dependent

probe amplification (MLPA) method (MRC-Holland, Amsterdam, The Netherlands), using P064-C1 mental retardation and P373-B1 microdeletion probe mixes, respectively. The DNA concentration of samples was optimized as 50 ng/μL for the array comparative genomic hybridization (aCGH) study. For the aCGH method, SurePrint G3 Human microarrays 8 × 60 K (Agilent Technologies, Palo Alto, CA, USA) were used, according to the manufacturer’s instructions. Microarray slides were scanned with 3 mm resolution in the microarray scanner system (Agilent Technologies). Feature Extraction 12.0.1.1 and Cytogenomics 2.9.2.4 (Agilent Technologies) software was used for the analysis of the samples. The subtelomeric FISH technique was performed using a commercially available set of probes Aquarius® Subtelomere Specific Probe (Cytocell Ltd., Cambridge, Cambridgeshire, UK), according to the manufacturer’s instructions. The probes are targeted to the sub-telomeric regions mostly at a 100-300 kb distance from the end of each of the chromosome arms, excluding the short arms of acrocentric chromosomes.

RESULTS

Cytogenetic analysis of GTG-banded chromosomes from cultured lymphocytes revealed a normal female karyotype. A heterozygous deletion of *MLC1*, *SBF1*, *MAPK8IP2*, *ARSA*, *SHANK3* and *ACR* genes, located on 22q13.33, was defined in the analysis of the P373-B1 MLPA (MRC-Holland) probe mix. Deletion of the 22q13.3 (ARSA) region was confirmed by the FISH technique using DiGeorge Region Probe (Vysis DiGeorge Region Probe-LSI TUPLE1 (HIRA) SpectrumOrange/TelVysion; Abbott Molecular, Abbott Park, IL, USA) (Figure 1). We applied chromosomal microarray analysis to specify the breakpoints of the deletion in the 22q13.3 region. As a result of the aCGH study, we confirmed the 22q13.3 deletion, in addition, we determined a gain of 8p23.3-23.2 (Figure 2). The copy number variation (CNV) regions, CNV size, breakpoints of the CNV and aberration type of the patient are shown in Table 1. The FISH analysis

Table 1. Copy number variants detected in the patient.

Chromosome	Cytoband	Start	Stop	Array Aberration	Aberration Size (bp)	Genes
8	p23.3-p23.2	191,531	5,248,586	duplication	5,057,056	12 genes ^a
22	q13.31-q13.33	46,863,086	51,178,264	deletion	4,315,178	45 genes ^b

^a *ZNF596, FBXO25, C8orf42, ERICH1, LOC286083, DLGAP2, CLN8, MIR596, ARHGEF10, KBTBD11, MYOM2, CSMD1.*

^b *CELSR1, GRAMD4, CERK, TBC1D22A, FLJ46257, MIR3201, FAMI9A5, C22orf34, BRD1, LOC90834, ZBED4, ALG12, CRELD2, PIM3, IL17REL, MLC1, MOV10L1, PANX2, TRABD, SELO, TUBGCP6, HDAC10, MAPK12, MAPK11, PLXNB2, FAM116B, PPP6R2, SBF1, ADM2, MIOX, LMF2, NCAPH2, SCO2, TYMP, ODF3B, KLHDC7B, C22orf41, CPT1B, CHKB-CPT1B, CHKB, LOC100144603, MAPK8IP2, ARSA, SHANK3, ACR.*

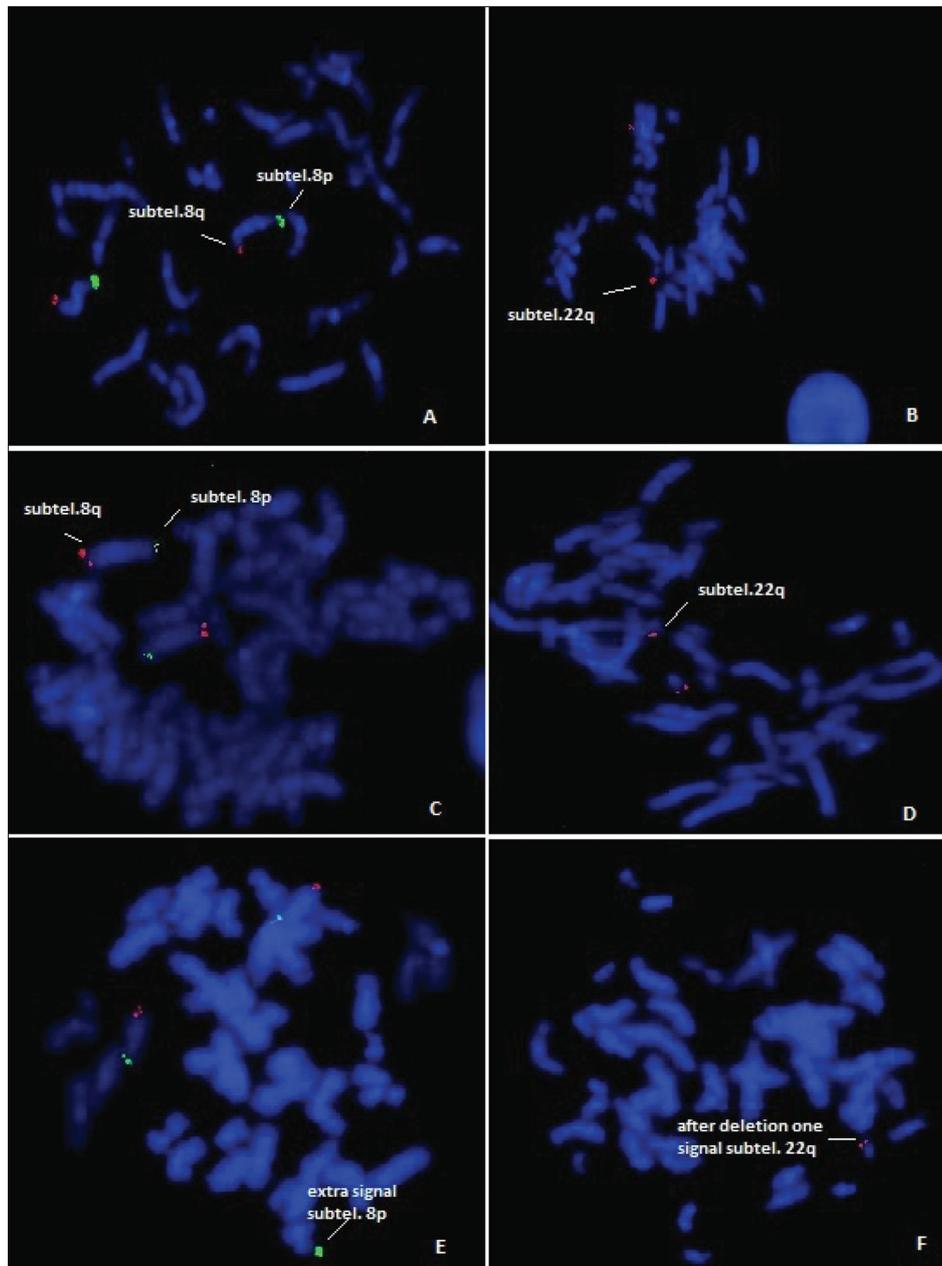


Figure 1. Subtelomeric FISH image of chromosome 8 and 22 (A-B: father; C-D: mother; E-F: patient, duplication 8p and deletion 22q signals).

performed with Aquarius® Subtelomere Specific Probe Set (Cytocell Ltd.) to determine the location of duplicated segment of 8p revealed that it was located on the deleted part of 22q. The parents were found to have a normal karyotype in cytogenetic analyses. Both the 8p23.3-23.2 gain and 22q13.3 deletion were not present in the parents according to the analyses by MLPA and FISH probes. Therefore, the 22q13.3 deletion and 8p23.3-23.2 gain were the result of a *de novo* event in the patient.

DISCUSSION

The genetic analyses of our patient showed that the 22q13.3 deletions leading to the loss of a functional copy of *SHANK3* caused PHMDS. She has neurodevelopmental delay, mild intellectual disability assessed clinically, speech problems, behavior problems and minor dysmorphic features, as defined in references of PHMDS. *SHANK3* remained a candidate gene as its expression

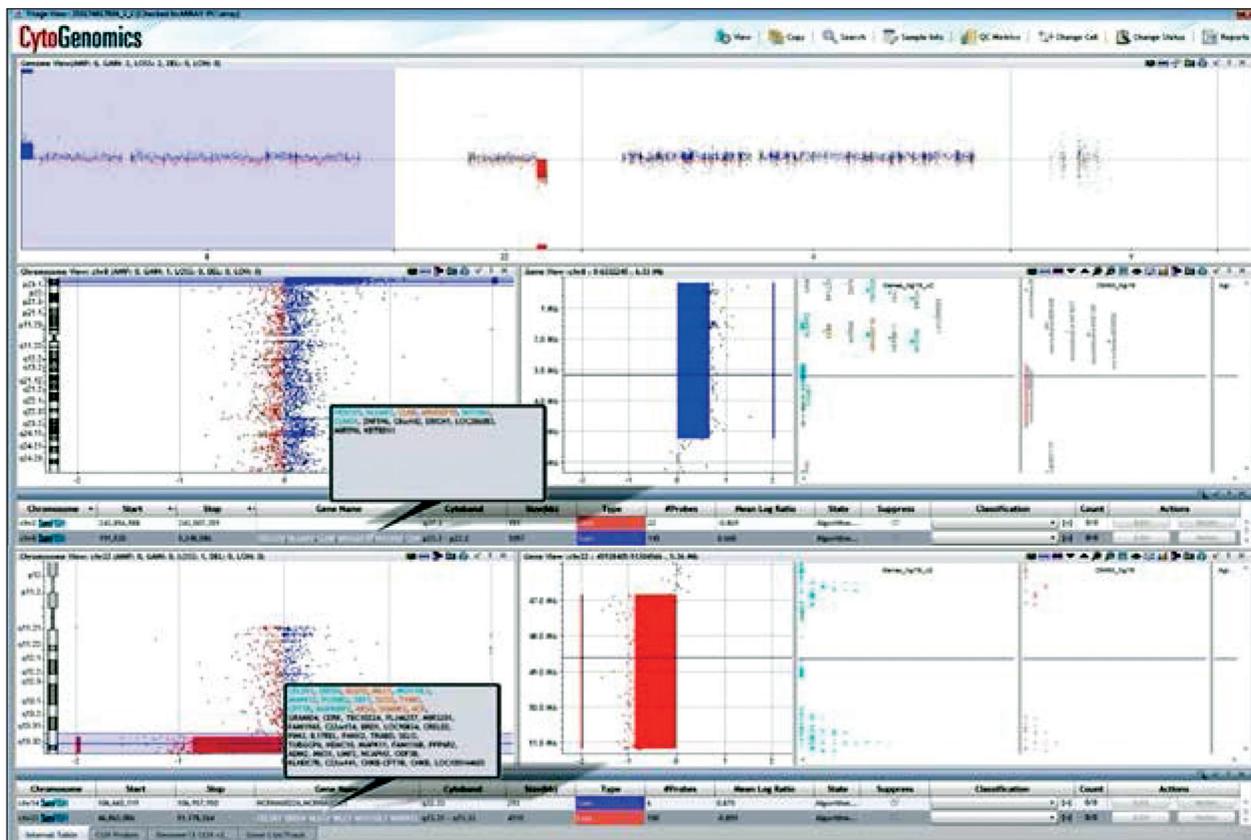


Figure 2. The 8p23.3-23.2 gain and 22q13.33 deletion in the patient.

is found in many regions of the brain, and the protein encoded is localized to the postsynaptic density, where it binds with other proteins that help maintain structural integrity. It is expressed at low levels in all brain regions during early postnatal development; peak expression correlates with a significant increase in synaptogenesis and synaptic maturation [3]. *SHANK3*, located on chromosome 22q13.3, is predominantly expressed in the cerebral cortex and cerebellum, and it is localized at excitatory synapses where it binds to neuroligins in post-synaptic boutons. It contains multiple protein-protein interaction domains and functions. As for neuroligins, several studies reported rare mutations or genomic deletion encompassing the *SHANK3* locus in as many as 0.85% of all ASD individuals [4]. SHANK proteins are master scaffolding proteins of the synaptic density of glutamatergic synapses and are critical determinants of glutamate transmission and spine dynamics. Loss of a functional copy of *SHANK3* accounts for about 0.5% of the cases of ASD and/or developmental delay, and there is likely wider role for *SHANK3* and glutamate signalling abnormalities in ASD and related neuro-developmental disorders [5]. Soorya *et al.* [6] evaluated ASD in a sample of 32 patients with *SHANK3*

haploinsufficiency and showed that 84.0% met the criteria for ASD, including 75.0% meeting autism criteria. These findings indicate that PHMDS is one of the more highly penetrant causes of autism [2]. The high prevalence of ASD in PHMDS has led to further investigation of the role of *SHANK3* in ASD and its potential overlap with other known genetic causes of ASD. Approximately 20.0% of ASD has been associated with specific chromosomal rearrangements, and more than 100 genes have been implicated. There is significant overlap in cellular dysfunction underlying many genetic subtypes of ASD, including deficits in synaptic function, synaptic plasticity, and excitatory glutamatergic signal transmission. Therefore, ASD has been hypothesized to occur as a result of synaptic dysregulation due to hypo- or hyper-connectivity, depending on the genetic insult and the role of affected proteins. As technology has progressed, increasingly sophisticated and higher resolution genetic analyses have allowed for greater detection of 22q13 deletions and *SHANK3* mutations. Improved access to, and greater appreciation of the need for genetic testing, will inevitably lead to increased diagnosis of PHMDS in cases of ASD, intellectual disability, and developmental delay [3].

Duplication of 8p is a rare euchromatic variant, and some overlapping variations are associated with different phenotypes (severe, mild and unaffected) [7]. It has been reported in the literature that duplications of the 8p23.1 region have not a distinct effect on the phenotype [8,9]. However, Kennedy *et al.* [10] reported a 16-year-old girl with an 8p23.1 duplication who has a normal development but has congenital heart disease. Glancy *et al.* [7] suggested that an increased copy number of the *MCPHI* gene, located in a duplicated locus between 8p23.1-8p23.2 [6.47 Mb between 3,848,594 and 10,323,426 bp] was associated with speech delay, ASD and learning deficits [7]. Although we found a gain of 1,399,992 bp on the 8p23.2 locus in our patient, the duplicated segment was not included in the *MCPHI* gene. Thus, we conclude that the mild intellectual disability finding in our patient was because of the deleted 22q region. To the best of our knowledge, this is the first case reported to have a 22q deletion and 8p23.2 duplication.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

REFERENCES

1. Phelan K, McDermid HE. The 22q13.3 deletion syndrome (Phelan-McDermid Syndrome). *Mol Syndromol.* 2012; 2(3-5): 186-201.
2. Betancur C, Buxbaum JD. SHANK3 haploinsufficiency: A “common” but underdiagnosed highly penetrant monogenic cause of autism spectrum disorders. *Mol Autism.* 2013; 4(1): 17. doi: 10.1186/2040-2392-4-18.
3. Costales JL, Kolevzon A. Phelan-McDermid Syndrome and SHANK3: Implications for treatment. *Neuro-therapeutics.* 2015; 12(3): 620-630.
4. Persico AM, Napolioni V. Autism genetics. *Behav Brain Res.* 2013; 15; 95-112. doi: 10.1016/j.bbr.2013.06.012.
5. Bozdagi O, Tavassoli T, Buxbaum JD. Insulin-like growth factor-1 rescues synaptic and motor deficits in a Mouse model of autism and developmental delay. *Mol Autism.* 2013; 4(1): 9. doi: 10.1186/2040-2392-4-9.
6. Soorya L, Kolevzon A, Zweifach J, Lim T, Dobry Y, Schwartz L, *et al.* Prospective investigation of autism and genotype-phenotype correlations in 22q13 deletion syndrome and SHANK3 deficiency. *Mol Autism.* 2013; 4(1): 18. doi: 10.1186/2040-2392-4-18.
7. Glancy M, Barnicoat A, Vijeratnam R, de Souza S, Gilmore J, Huang S, *et al.* Transmitted duplication of 8p23.1-8p23.2 associated with speech delay, autism and learning difficulties. *Eur J Hum Genet.* 2009; 17(1): 37-43.
8. Harada N, Takano J, Kondoh T, Ohashi H, Hasegawa T, Sugawara H, *et al.* Duplication of 8p23.2: A benign cytogenetic variant? *Am J Med Genet.* 2002; 111(3): 285-288.
9. Barber JC, Maloney V, Hollox EJ, Stuke-Sontheimer A, du Bois G, Daumiller E, *et al.* Duplications and copy number variants of 8p23.1 are cytogenetically indistinguishable but distinct at the molecular level. *Eur J Hum Genet.* 2005; 13(10): 1131-1136.
10. Kennedy SJ, Teebi AS, Adatia I, Teshima I. Inherited duplication, dup(8)(p23.1p23.1) pat, in a father and daughter with congenital heart defects. *Am J Med Genet.* 2001; 104(1): 79-80.

