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Case Report

Wolf-Hirschhorn (4p-) syndrome: Prenatal diagnosis, molecular cytogenetic characterization and association with a 1.2-Mb microduplication at 8p22-p21.3 and a 1.1-Mb microduplication at 10p15.3 in a fetus with an apparently pure 4p deletion

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Abstract

Objective: To present prenatal diagnosis and molecular cytogenetic characterization of Wolf-Hirschhorn syndrome (WHS) associated with microduplications at 8p and 10p in a fetus with an apparently pure 4p deletion.
Case Report: A 35-year-old gravida 2, para 1 woman underwent amniocentesis at 18 weeks of gestation because of advanced maternal age. Her husband was 38 years of age. There was no family history of congenital malformations. Amniocentesis revealed a karyotype of 46,XY,del(4p16.1). The parental karyotypes were normal. Array comparative genomic hybridization (aCGH) analysis revealed a 6.5-Mb deletion at 4p16.3-p16.1, a 1.2-Mb microduplication at 8p22-p21.3, and a 1.1-Mb microduplication at 10p15.3, or arr cgh 4p16.3p16.1 (0–6,531,998 bp)×1, 8p22p21.3 (18,705,388–19,940,445 bp)×3, 10p15.3 (0–1,105,065 bp)×3. Polymorphic DNA marker analysis confirmed a paternal origin of 4p deletion. Prenatal ultrasound revealed facial dysmorphism and hypospadias. The aCGH analysis of the parents revealed no genomic imbalance. Fluorescence *in situ* hybridization study showed an unbalanced reciprocal translocation between chromosomes 4 and 10 at bands 4p16.1 and 10p15.3. The cytogenetic result, thus, was 46,XY,der(4)t(4;10)(p16.1;p15.3),dup(8)(p21.3p22). The parents elected to terminate the pregnancy, and a 470-g malformed fetus was delivered.
Conclusion: The present case provides evidence that an apparently pure 4p deletion can be associated with subtle chromosome imbalances in other chromosomes.

Keywords: 4p deletion; 8p22-p21.3 duplication; 10p15.3 duplication; Prenatal diagnosis; Wolf-Hirschhorn syndrome

Introduction

Wolf-Hirschhorn (4p-) syndrome (WHS) (OMIM 194190) is a contiguous gene deletion syndrome that was first described independently by Wolf et al [1] and Hirschhorn et al

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[2] as multiple congenital anomalies and mental retardation caused by partial deletion of 4p. WHS is associated with a hemizygous deletion of chromosome 4p16.3 and is characterized by the "Greek warrior helmet" appearance of the nose, high forehead, prominent glabella, hypertelorism, high arched eyebrows, protruding lips, epicanthic folds, pre- and postnatal growth deficiency, mental retardation, seizures, hypotonia, and closure defects, such as cleft palate, hypospadias, ocular colobomas and cardiac septal defects [3,4]. The frequency of WHS is estimated as 1/20,000–1/50,000 births, and there is a female: male ratio of 2:1 [3]. Here, we report prenatal diagnosis and molecular cytogenetic characterization of a fetus with WHS and microduplications of 8p and 10p. To our knowledge, such a case has not previously been described.

Case report

A 35-year-old gravida 2, para 1 woman was referred for amniocentesis at 18 weeks of gestation because of advanced maternal age. Her husband was 38 years of age. There was no family history of congenital malformations. Amniocentesis revealed a karyotype of 46,XY,del(4p16.1) (Fig. 1). The parental karyotypes were normal. The parents requested repeated amniocentesis at 21 weeks of gestation. Array comparative genomic hybridization (aCGH) analysis of uncultured amniocytes using oligonucleotide-based Oligo HD Scan (CMDX, Irvine, CA, USA) revealed a 6.5-Mb deletion at 4p16.3-p16.1, a 1.2-Mb microduplication at 8p22-p21.3, and a 1.1-Mb microduplication at 10p15.3 or arr cgh 4p16.3p16.1 (0–6,531,998 bp)×1, 8p22p21.3 (18,705,388–19,940,445 bp)×3, 10p15.3 (0–1,105,065 bp)×3 (Fig. 2) [University of California Santa Cruz genome browser on human, March 2006 (National Center for Biotechnology Information 36/human genome 18) assembly]. Metaphase fluorescence *in situ* hybridization (FISH) analysis of cultured amniocytes using a 10p15.3-specific bacterial artificial chromosome (BAC) clone probe RP11-145I2 (214,406–216,399; spectrum red) and a 4p16.1-specific BAC clone probe RP11-89K12 (6,207,318–6,377,316; spectrum green) showed absence of a green signal and presence of a red signal on the short arm of the derivative chromosome 4, presence of a red signal on each of the two chromosomes 10, and presence of a green signal on one chromosome 4 (Fig. 3). The result was consistent with an unbalanced reciprocal translocation between chromosomes 4 and 10 at bands 4p16.1 and 10p15.3. The cytogenetic result, thus, was 46,XY,der(4)t(4;10)(p16.1;p15.3),dup(8)(p21.3p22). Polymorphic DNA marker analysis confirmed a paternal origin of the deletion (Fig. 4, Table 1). Prenatal ultrasound revealed midface hypoplasia, micrognathia, hypertelorism, and hypospadias (Fig. 5). The aCGH analysis of the parents revealed no genomic imbalance. The parents elected to terminate the pregnancy, and a 470-g malformed fetus was delivered with facial dysmorphism and ear tags (Fig. 6). Postnatal cytogenetic analysis of the cord blood confirmed the prenatal diagnosis.

Discussion

The WHS critical region at 4p16.3 includes WHS candidate genes of *WHSC1* (WHS candidate gene 1) (OMIM 602952) [5], *WHSC2* (WHS candidate gene 2) (OMIM 606026) [6,7,8], and *LETM1* (leucine zipper/EF-hand-containing transmembrane protein 1) (OMIM 604407) [9]. WHSC1 functions in transcriptional regulation together with developmental transcription factors to prevent the inappropriate transcription and the consequent pathophysiologies. WHSC2 is a subunit of negative elongation factor (NELF) complex which functions in histone mRNA maturation. LETM1 is a regulator of

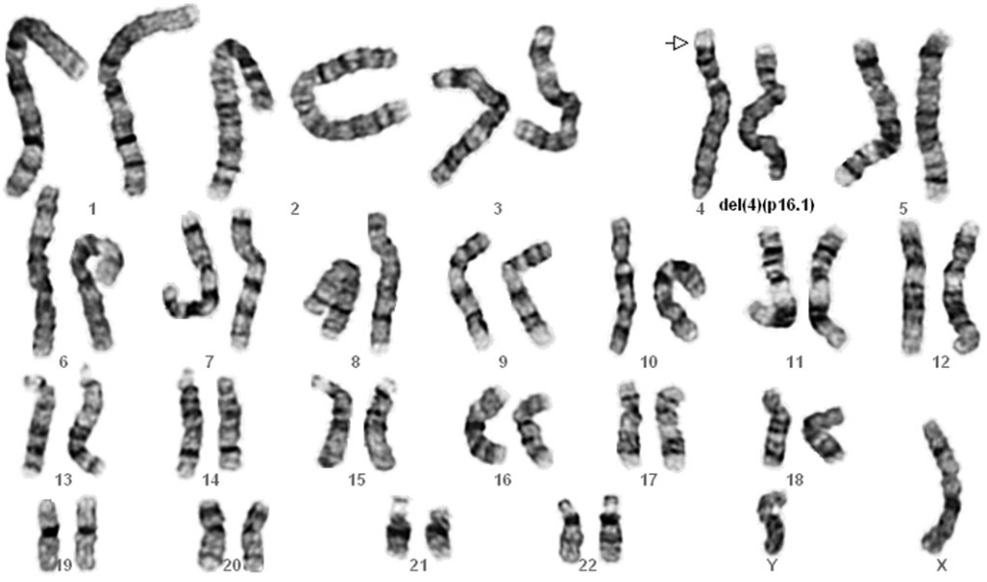
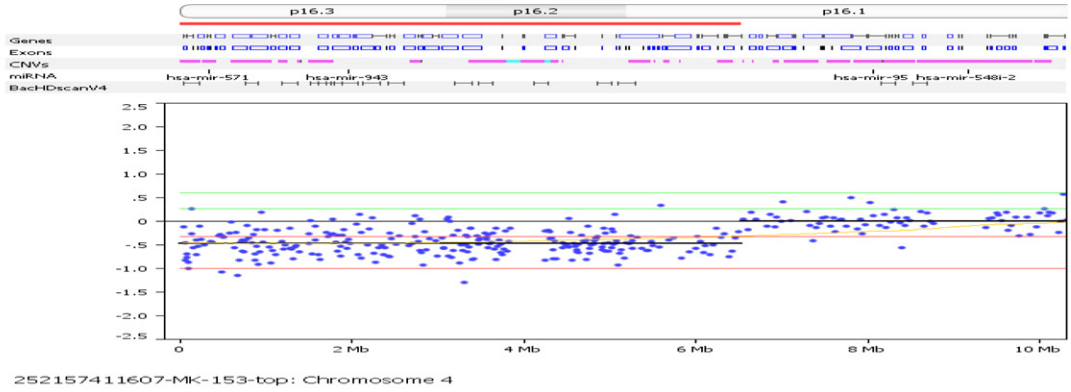


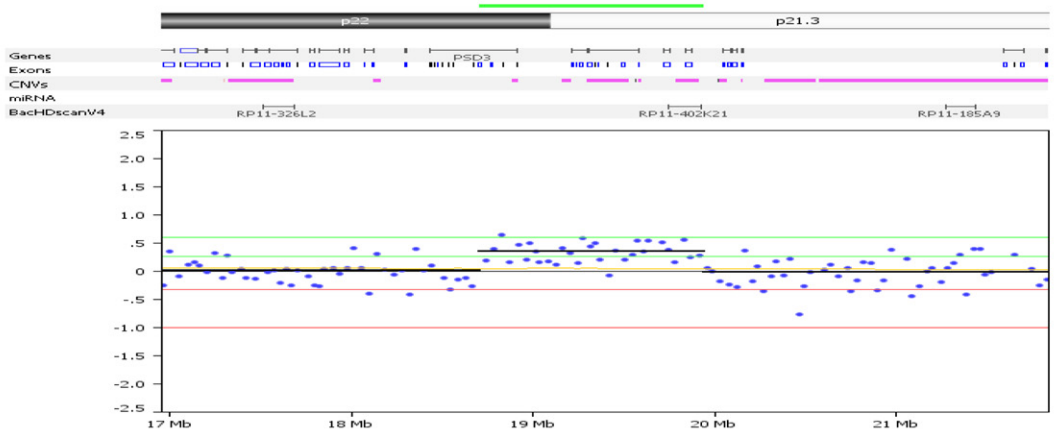
Fig. 1. A karyotype 46,XY,del(4)(p16.1). The arrow indicates the breakpoint. del = deletion.

arr 4p16.3p16.1 (0-6,531,998)x1 6.5Mb deletion



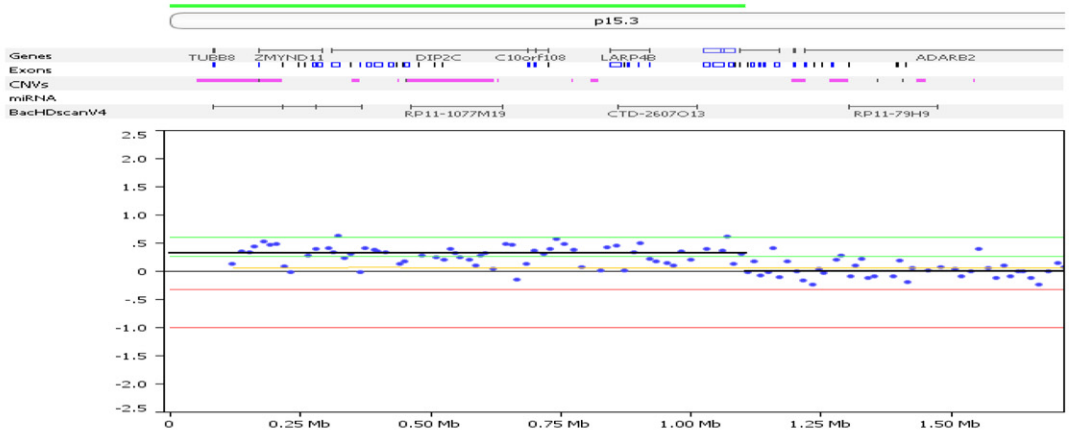
252157411607-MK-153-top: Chromosome 4

arr 8p22p21.3(18,705,388-19,940,445)x3 1.2Mb microduplication



252157411607-MK-153-top: Chromosome 8

arr 10p15.3(0-1,105,065)x3 1.1Mb microduplication



252157411607-MK-153-top: Chromosome 10

Fig. 2. Array comparative genomic hybridization (aCGH) analysis using oligonucleotide-based Oligo HD Scan (CMDX, Irvine, CA, USA) reveals a 6.5-Mb deletion (0–6,531,998 bp) at 4p16.3-p16.1, a 1.2-Mb microduplication (18,705,388–19,940,445 bp) at 8p22-p21.3, and a 1.1-Mb microduplication (0–1,105,065 bp) at 10p15.3.

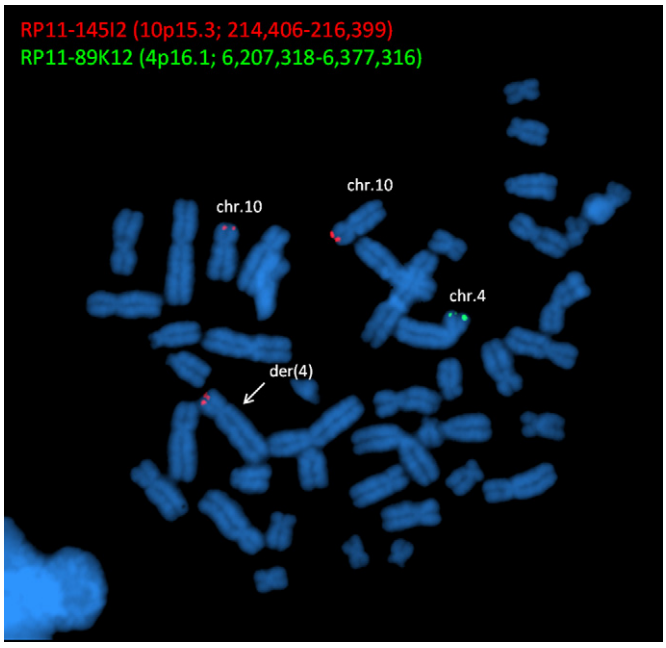


Fig. 3. Metaphase fluorescence *in situ* hybridization analysis of cultured amniocytes using a 10p15.3-specific bacterial artificial chromosome (BAC) clone probe RP11-145I2 (214,406–216,399; spectrum red) and a 4p16.1-specific BAC clone probe RP11-89K12 (6,207,318–6,377,316; spectrum green) shows absence of a green signal and presence of a red signal on the short arm of the derivative chromosome 4 [der(4)], presence of a red signal on each of the two chromosomes 10 (chr. 10), and presence of a green signal on one chromosome 4 (chr. 4).

mitochondrial Ca^{2+} and H^{+} concentrations and functions as a mitochondrial Ca^{2+}/H^{+} antiporter. It has been suggested that characteristic WHS facial dysmorphism is associated with haploinsufficiency of *WHSC1*, and seizure is associated with

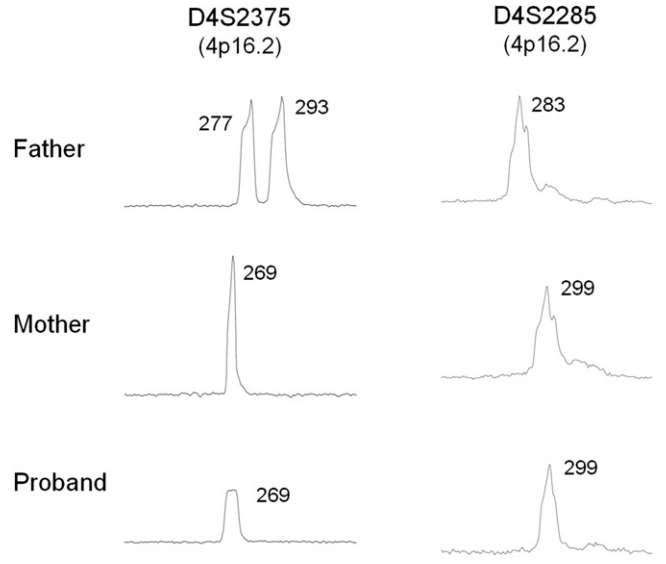


Fig. 4. Representative electrophoretograms of quantitative fluorescent polymerase chain reaction assays at short tandem repeat markers specific for chromosome 4p16.2 using fetal and parental DNAs. With the markers D4S2375 (4p16.2) and D4S2285 (4p16.2), only the allele of 269 bp (maternal) and 299 bp (maternal), respectively, is present in the fetus, indicating maternal inheritance in 4p16.2 and a paternal origin of the deletion.

Table 1
Molecular results using polymorphic DNA markers specific for chromosome 4p*.

Markers	Location	Father	Mother	Fetus
D4S2375	4p16.2	277, 293	269, 269	269
D4S2285	4p16.2	283, 283	299, 299	299
D4S2366	4p16.1	113, 121	113, 113	113, 121

* Alleles (basepair sizes) are listed below each individual.

haploinsufficiency of *LETM1* [4]. The present case had a 6.5-Mb deletion of 4p16.3-p16.1 of paternal origin and haploinsufficiency of the genes of *WHSC1*, *WHSC2*, and *LETM1*.

Prenatal diagnosis of WHS has been well described [10–16]. The prenatal ultrasound findings of WHS include intrauterine growth restriction (IUGR), facial dysmorphism of midface hypoplasia and "Greek warrior helmet" appearance of the nose, midline fusion defects such as facial cleft, hypospadias, agenesis of the corpus callosum and cardiac septal defects, congenital diaphragmatic hernia, renal hypoplasia,

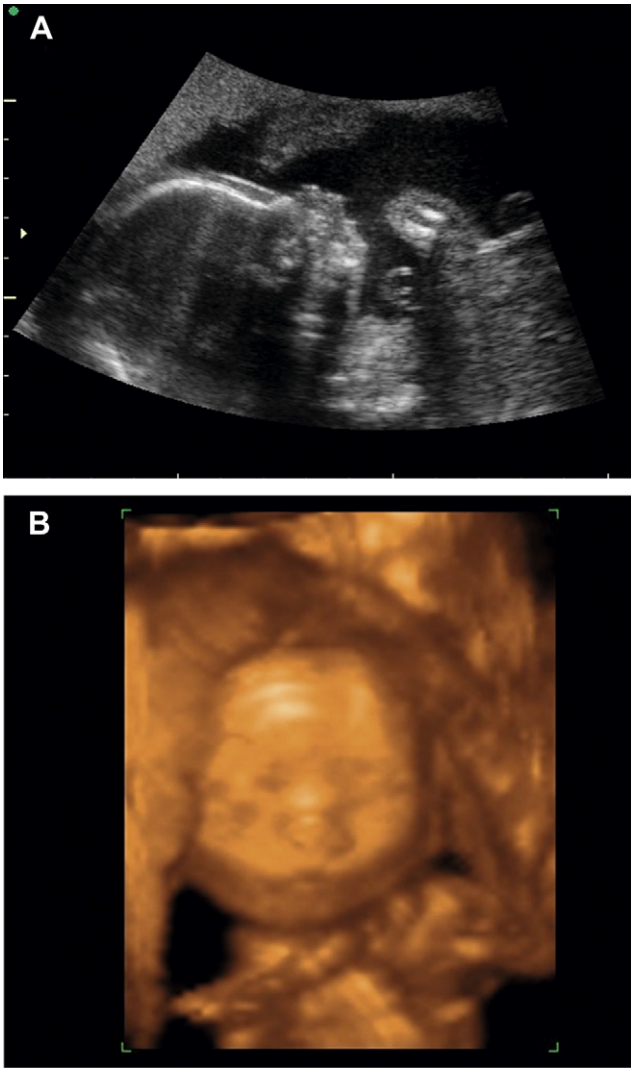


Fig. 5. (A) Two-dimensional and (B) three-dimensional ultrasound demonstration of midface hypoplasia and micrognathia.

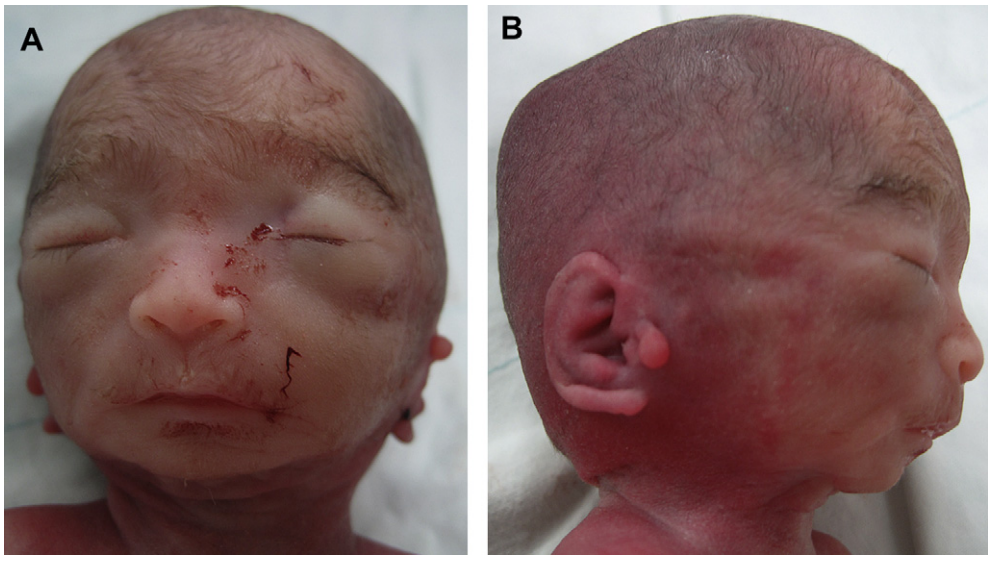


Fig. 6. (A) and (B) The craniofacial appearance with low-set ears and ear tags of the fetus at birth.

foot deformity, increased nuchal translucency and cystic hygromas. The present case was associated with facial dysmorphism and hypospadias on prenatal ultrasound.

The peculiar aspect of the present case is the association with a 1.2-Mb 8p microduplication at 8p22-p21.3, a 1.1-Mb 10p microduplication at 10p15.3 and an unbalanced reciprocal translocation between chromosomes 4 and 10 at bands 4p16.1 and 10p15.3. Unbalanced reciprocal translocation between 4p16 and 10p15 associated with WHS has been previously reported. Zollino et al [4] reported a 3.5-Mb 4p deletion and a 4-Mb 10p duplication in a WHS patient with an unbalanced translocation of t(4;10)(p16.3;p15)mat. The present case had gene dosage increase of isopentenyl-diphosphate delta-isomerase (*IDI*) [IDI1 (OMIM 604055) and IDI2] at 10p15.3. IDI plays an essential role in the biosynthesis of cholesterol. Recently, Kato et al [17] found a segmental copy-number gain in many patients with sporadic amyotrophic lateral sclerosis in the *IDI1*/*IDI2* gene region at 10p15.3 subtelomere. The *IDI1*/*IDI2* gene region contains multiple low-copy repeats and instability which may trigger a segmental gain by an unequal crossing-over or end-joining event [18,19]. Unbalanced translocations account for 22% of the cases with WHS [4]. In cases of unbalanced translocations associated with WHS, the aberrations usually originate in the paternal meiosis, and partial trisomy 8p caused by unbalanced t(4;8)(p16;p23) is the most frequent concomitant aneuploidy [4]. The frequent occurrence of t(4;8)(p16;p23) has been hypothesized to be due to the locations of olfactory receptor (OR)-gene clusters on both 4p16 and 8p23 [20], non-allelic homologous recombination between the OR-gene clusters [21], and extensive normal copy number variation of a β-defensin antimicrobial gene cluster of *DEFB4*, *DEFB103*, and *DEFB104* at 8p23.1 [22].

In summary, we have presented prenatal diagnosis and molecular cytogenetic characterization of WHS associated with microduplications at 8p and 10p. The present case provides evidence that a seemly pure 4p deletion of paternal origin can be associated with subtle chromosome imbalances in other chromosomes. Our presentation highlights the usefulness of aCGH and FISH in genetic analysis of prenatally detected chromosome aberration.

Acknowledgments

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