

ARTICLE

Unmasking of a hemizygous *WFS1* gene mutation by a chromosome 4p deletion of 8.3 Mb in a patient with Wolf–Hirschhorn syndrome

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The Wolf–Hirschhorn syndrome (WHS (MIM 194190)), which is characterized by growth delay, mental retardation, epilepsy, facial dysmorphisms, and midline fusion defects, shows extensive phenotypic variability. Several of the proposed mutational and epigenetic mechanisms in this and other chromosomal deletion syndromes fail to explain the observed phenotypic variability. To explain the complex phenotype of a patient with WHS and features reminiscent of Wolfram syndrome (WFS (MIM 222300)), we performed extensive clinical evaluation and classical and molecular cytogenetic (GTG banding, FISH and array-CGH) and *WFS1* gene mutation analyses. We detected an 8.3 Mb terminal deletion and an adjacent 2.6 Mb inverted duplication in the short arm of chromosome 4, which encompasses a gene associated with WFS (*WFS1*). In addition, a nonsense mutation in exon 8 of the *WFS1* gene was found on the structurally normal chromosome 4. The combination of the 4p deletion with the *WFS1* point mutation explains the complex phenotype presented by our patient. This case further illustrates that unmasking of hemizygous recessive mutations by chromosomal deletions represents an additional explanation for the phenotypic variability observed in chromosomal deletion disorders.

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Introduction

Wolf–Hirschhorn syndrome (WHS (MIM 194190)) is characterized by growth delay, mental retardation, epilepsy, facial dysmorphisms, and midline fusion defects.¹ About 80% of patients carry a *de novo* terminal deletion of chromosome 4p. Identification of patients with small

interstitial deletions allowed the definition of the Wolf–Hirschhorn Syndrome Critical Regions 1 and 2 (WHSCR), that include a contiguous set of genes in chromosome band 4p16.3 located between ~1.6 and ~2.2 Mb from the telomere.^{1–3} About 20% of patients carry unbalanced translocations, most of which involve the short arms of chromosomes 4 and 8.⁴

The variability of the phenotype in patients with WHS has been related to the extent of monosomy 4p, and attempts have been made to attribute certain phenotypic features to specific genes.² Yet, patients with similar deletions showed considerable phenotypic differences. Several hypotheses have been proposed to account for

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this. Firstly, mutations in modifier genes located outside the deleted region may either enhance or suppress the severity of the disorder, as has been suggested in a mouse model of deletions in 17p11.2 associated with Smith–Magenis syndrome.⁵ Secondly, observations on monozygotic twins with identical 22q11.2 microdeletions but discordant clinical phenotypes⁶ suggest that post-zygotic mutational events, environmental influences, or stochastic factors affecting gene expression may cause phenotypic variation. Thirdly, position effects may affect clinical manifestations of chromosomal alterations, such that genes closely located to telomeres, for example in the WHSCR, may be particularly sensitive to gene silencing.¹ Fourthly, a functional polymorphism uncovered by the hemizygous deletion may add phenotypic variability to the effects of the deletion *per se*.⁷

As an extension of the previous observation, a fifth cause of phenotypic variability may be unmasking of autosomal recessive mutations by a hemizygous deletion. Here, we present a WHS patient with features reminiscent of the Wolfram syndrome (WFS (MIM 222300)), a progressive neurodegenerative syndrome characterized by diabetes insipidus, diabetes mellitus, optic atrophy, and deafness caused by recessive mutations in the *WFS1* gene located on chromosome 4p16.3.⁸ This patient was shown to carry an 8.3 Mb terminal deletion encompassing the WHSCR and the *WFS1* gene and a nonsense mutation in exon 8 in the *WFS1* allele on the structurally normal chromosome 4. To the best of our knowledge, this is the first WHS patient in whom unmasking of autosomal recessive mutations by a hemizygous deletion is demonstrated.

Materials and methods

Case history

The male patient was the first child of non-consanguineous, healthy parents with no family history for congenital malformations. Foetal ultrasound examination, performed at 33 weeks of gestation because of intrauterine growth retardation, revealed no other anomalies. Upon prenatal chromosome analysis, a normal male karyotype (46,XY) was found. A maternal epileptic seizure of unknown cause prompted a primary caesarean section at 38 weeks of gestation. At birth, the patient had a weight, length, and head circumference of <-2.5 SD. He was hypotonic and had glandular hypospadias and bilateral cryptorchidism.

During the first year of life, developmental delay became evident. At 2 months of age, the patient showed myoclonic seizures. Because of feeding difficulties and recurrent pulmonary aspiration, a percutaneous endoscopic gastrostomy tube was placed. Upon physical examination, features of WHS were noted (Table 1). Additional investigations revealed corpus callosum hypoplasia, sensorineural hearing loss, optic colobomas, and renal pelvis dilatation.

At the age of 1 year, the patient developed circulatory and respiratory insufficiency following viral infection, during which he became comatose. Laboratory investigations revealed hyperglycaemia with non-ketotic hypernatremia, and metabolic acidosis. After recovery, he remained insulin dependent, confirming diabetes mellitus. Anti-islet and anti-GAD antibodies were absent, ruling out an autoimmune origin. During a viral infection at the age of 3 years, he developed polyuria despite adequate management of his diabetes mellitus. Laboratory findings on plasma and urine samples indicated diabetes insipidus. The polyuria responded to desmopressin, and disappeared spontaneously after the infection had subsided. Notably, during subsequent viral infections, he developed metabolic acidosis with inappropriately high urinary pH. Distal renal tubular acidosis was demonstrated by laboratory investigations. An elevated lactate level prompted suspicion of a mitochondrial disorder. Upon a muscle biopsy, a moderately decreased ATP production was found (33 mU CS; 42–81 mU CS), as well as decreased oxidation rates. The respiratory chain complexes functioned normally.

At present, he is 5 years old and has profound motor and developmental retardation, seizures, feeding difficulties and insulin requiring diabetes. He is frequently hospitalized for intermittent viral illness leading to an elevated risk for metabolic crisis.

Classical and molecular cytogenetics

FISH was performed on metaphase chromosomes according to standard methods,⁹ except that after hybridization, slides were washed twice in $0.4 \times$ SSC/0.05% Tween-20 at 72°C for 5 min, followed by washes in $2 \times$ SSC/0.05% Tween-20 and $4 \times$ SSC/0.05% Tween-20 at room temperature for 5 min each. Commercially available probes were hybridized according to instructions provided by the manufacturer. DNA-probes included the GS-36-P21 4pter probe¹⁰ and the LSI-WHS/centromere 4 probe containing part of the *WHSC1* gene (Vysis Inc., Downers Grove, IL, USA). Probe RP11-34C20, located in band 4p15.33, was obtained from the Wellcome Trust Sanger Institute, Cambridge, UK. A FITC-labeled telomere-specific TTAGGG peptide nucleic acid (PNA)-probe was obtained from DAKO, Glostrup, Denmark.

For segmental aneuploidy profiling by array-CGH, 1 μ g of sonicated genomic DNA from our patient and 1 μ g of sonicated genomic DNA from a pool of 50 healthy male individuals were labeled using the BioPrime DNA Labeling System (Invitrogen, Carlsbad, CA, USA) with Cy3-dUTP and Cy5-dUTP (Amersham Biosciences, Little Chalfont, UK) and hybridized to array-slides containing DOP-PCR products of 3343 BAC DNA probes¹¹ using a GeneTAC Hybstation (Genomic Solutions, Ann Harbor, MI, USA) as described.¹² The fluorescence ratios of all autosomes excluding chromosome 4 covered a 95% confidence interval of 13.9%. Therefore, thresholds for copy number

Table 1 Clinical features of our patient and patients with Wolf–Hirschhorn syndrome, trisomy 4p,¹⁴ and Wolfram syndrome

Clinical features	Our patient	Wolf–Hirschhorn syndrome	Trisomy 4p16p15	Wolfram syndrome
<i>Wolf–Hirschhorn syndrome</i>				
Growth retardation	+	+	+	
Mental retardation	+	+	+	
Microcephaly	+	+	+	
Epilepsy	+	+	+	+
Hypotony	+	+	+	
Psychomotor retardation	+	+	+	
Hearing loss	+	+		+
Prominent glabella		+	+	
Broad nasal bridge	+	+	+	
Short philtrum		+		
Micrognathia		+		
Hypertelorism	+	+	+	
Epicanthal folds	+	+		
Coloboma of the eye	+	+	+	
Hypospadias	+	+	++	
Cryptorchidism	+	+	+	
Cleft lip/palate		+		
Cardiac defect		+	+	
<i>Wolfram syndrome</i>				
Diabetes mellitus	+			+
Diabetes insipidus	+			+
Optic atrophy				+
Hearing loss	+	+		+
<i>Trisomy 4p syndrome</i>				
Growth retardation	+	+	+	
Mental retardation	+	+	+	
Enlarged dysplastic ears			+	
Prominent glabella		+	+	
Bulbous nose			+	
Depressed nasal bridge			+	
Retrognathia			+	
Pointed chin			+	
Short neck			+	
Camptodactyly			+	
Arachnodactyly			+	
Rocker-bottom feet			+	

gain and loss were set at 2^{\log} Patient/Reference values of +0.139 and –0.139, respectively.

Molecular genetics

The *WFS1* gene consists of eight exons of which the first one is non-coding. Coding exons 2–8, together with the flanking intronic splice site sequences, were amplified from genomic DNA of the patient and his parents. Exons 2–7 were amplified as single fragments, whereas the much larger exon 8 was amplified in five overlapping fragments. Primer sequences and PCR conditions are available upon request. Genomic DNA was extracted from peripheral blood lymphocytes using standard methods. Sequencing was performed on a 3730 ABI automated sequencer using BigDye Terminator chemistry (Applied Biosystems). Genbank Accession Number NM_006005.2 is used as *WFS1* reference sequence in which the A of the ATG start codon is designated position 1. Mutations were numbered accordingly.

Results

Classical and molecular cytogenetics

By GTG-banding the patient was initially judged to have a normal 46,XY karyotype at the 550 band level. Using FISH with probe LSI-WHS, a hemizygous deletion of WHSC1 was demonstrated. Array-CGH showed an 8.3 Mb deletion of chromosome region 4pter→p16.1, including BAC probes RP1-36P21 and RP11-101J14, immediately adjacent to a 2.6 Mb duplication of region 4p15.33→p16.1, including BAC probes RP11-17I9 and RP11-34C20 (Figure 1). The deleted region included the WHSCR1 and WHSCR2 regions and the *WFS1* gene. FISH with probe RP11-34C20 produced uneven signal intensities on the two homologues of chromosome 4, with a single, stronger, and more terminally located signal on the chromosome 4 that harboured no LSI-WHS signal (Figure 2). This is consistent with duplicated RP11-34C20 signals in close proximity owing to an inverted duplication at the terminal part of the short arm

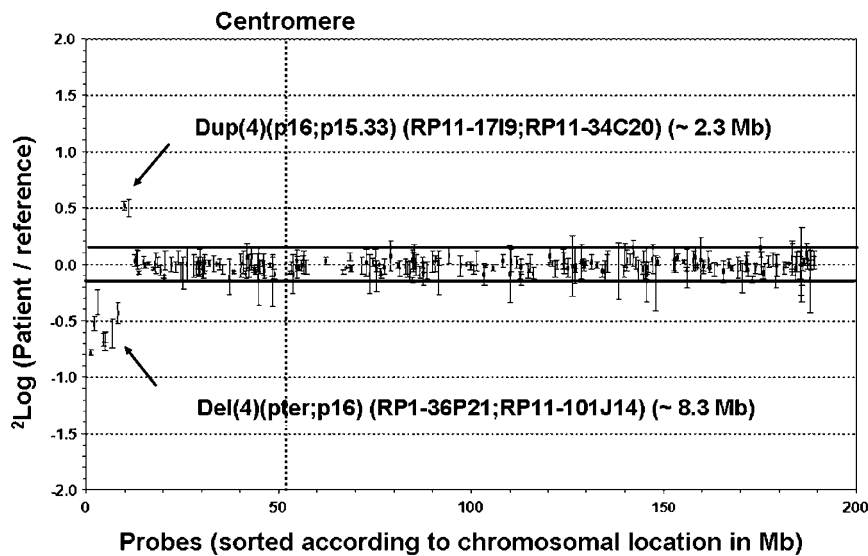


Figure 1 Segmental aneuploidy profile of chromosome 4 of the patient by array-CGH. Abscissa shows probes sorted according to chromosomal position, and the ordinate shows to ^2Log fluorescence ratios of patient vs reference DNA. The horizontal bars demarcate the 95% confidence interval; the vertical dotted line indicates the position of the centromere of chromosome 4.

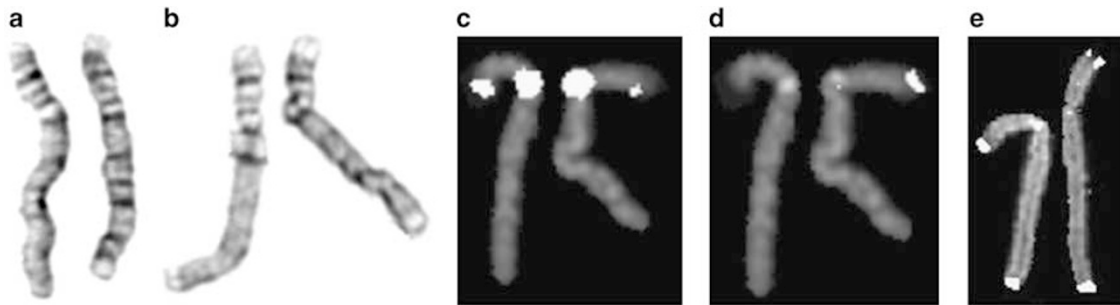


Figure 2 Partial GTG-banded karyotype of the patient (a and b), showing the normal chromosome 4 (right) and the inv dup del(4) (left). FISH using BAC-clone RP11-34C20 (c) results in a single, stronger signal on the inv dup del(4) chromosome (left). This chromosome does not carry an LSI-WHS signal (d). (e) A TTAGGG PNA-probe hybridizes to both termini of the inv dup del(4) chromosome and the normal chromosome 4.

of the chromosome 4 carrying the terminal deletion. Re-evaluation of the karyotype revealed subtle differences in the terminal part of the short arm of chromosome 4 (Figure 2). GTG-banding and FISH using the LSI-WHS probe did not reveal a deletion or duplication of chromosome 4 in the parents. In addition, array-CGH did not give evidence for segmental aneuploidies in chromosome 4 or in other chromosomes of the parents (results not shown). Therefore, the chromosomal rearrangement seen in the patient originated *de novo*. Whereas FISH using the GS-36-P21 probe indicated a subtelomeric deletion on the rearranged chromosome 4 (results not shown), a telomere-specific PNA probe hybridized to all chromosome termini, including the short arm of the inv dup del(4) chromosome were present (Figure 2). Based on these findings, we describe the karyotype as 46,XY,inv dup del(4):(p15.33→

p16.1::p16.1→qter)dn.ish(GS-36-P21-,WHSC1-,RP11-34C20++) (diagrammed in Figure 3).

Molecular genetics

Mutation analysis of the *WFS1* gene in the patient revealed a c.1096C>T transition in exon 8 of the *WFS1* gene (Figure 4). The mutation results in a premature stop codon at position 366 in the corresponding Wolfram protein (p.Gln366X), and has been previously reported as a causative mutation in two WFS families.¹³ Looking at the results of the sequence analysis, the mutation appears to be homozygous. However, as the patient has only one copy of the *WFS1* gene the mutation is in fact hemizygous. The mother of the patient was found to be a heterozygous carrier of this mutation. No mutations were detected in the *WFS1* gene of the father.

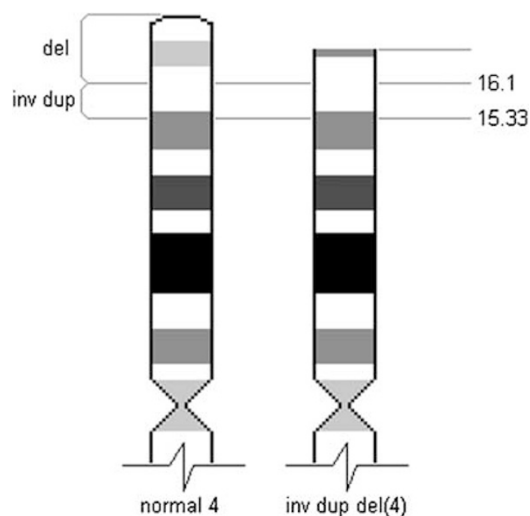


Figure 3 Partial ideogram of chromosome 4 showing the inv dup del(4).

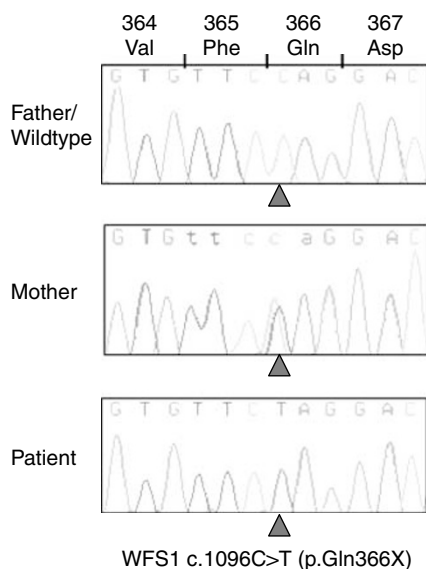


Figure 4 Standard sequence analysis showing a c.1096C>T (p.Gln366X) stop mutation in exon 8 of the *WFS1* gene (arrow). The mutation in the patient was inherited from the heterozygous mother. No *WFS1* mutation was found in the father of the patient.

Discussion

Our patient presented with key features of WHS. In contrast to the patients described by Patel *et al*,¹⁴ our patient has a relatively small duplication 4p. This difference explains the absence of many typical trisomy 4p features in our patient (Table 1). He also shared features with a case of a 46,XX,inv dup del(4)(:p14→p16.1::p16.1→qter)dn with both signs of WHS and trisomy 4p described by Beaujard *et al*.¹⁵ Comparison of the two is not justified, as this published case concerns a

prenatal diagnosis and the inverted duplication detected in this case is larger than the one in our patient. The combination of early-onset diabetes mellitus and diabetes insipidus is consistent with WFS, and corroborated by the molecular defect in the *WFS1* gene. No optic atrophy was found in our patient, which is expected, as it generally manifests itself in WFS patients at a later age. His hearing loss can be attributed to both WHS and WFS. Thus, the complex phenotype of our patient is consistent with both a deletion of the *WHSCR1* and a defective *WFS1* gene.

As both the *WHSCR* and the *WFS1* gene are located in chromosome region 4p16.3, a deletion of chromosome 4p16 may, in addition to causing WHS, unmask a hemizygous *WFS1* gene mutation, thus leading to the complex phenotype of our patient.

Molecular cytogenetic techniques contributed to resolve this puzzling combination of clinical phenotypes. By FISH, we detected a deletion of the *WHSCR1* locus on chromosome 4p16.3. This was corroborated by array-CGH, which demonstrated a terminal 8.3 Mb deletion of 4p covering both the *WHSCR1* and *WHSCR2* regions and including all the genes deemed to be involved in the development of the core features of WHS and additional midline defects.^{3,4} As our patient does not show some of the characteristic midline defects of WHS while being hemizygous for the *WHSCR1*, *WHSCR2*, *LETM1*, *FGFR3*, *TACC3* (also known as *AIN1*), *SLBP*, *HDNTNP*, and *HSPX153* loci, we conclude that mere hemizyosity for these genes does not suffice to explain the phenotype of our patient.^{1,3}

The presence of a 2.6 Mb duplication immediately adjacent to the 8.3 Mb deletion of chromosome 4pter→p16 prompted us to consider a potential contribution of a secondary chromosome imbalance, possibly associated with a cryptic chromosome translocation. FISH data obtained with probe RP11-34C20 contradicted a tandem duplication 4p16.1p16.3, a translocation between 4p16.2 and 11p15.5, or a translocation between 4p16.3 and 8p23.^{4,16} Moreover, our patient did not show the clinical features found in patients with a tandem duplication of 4p16.1–4p16.3.¹⁶ Thus, these data do not support a contribution of a possible position effect due to a putative translocation¹ to the observed clinical phenotype of our patient.

WFS1 mutation analysis in our patient demonstrated a hemizygous c.1096C>T nonsense mutation (p.Gln366X) in exon 8 of the gene, which was inherited from his carrier mother. This hemizygous *WFS1* mutation most likely explains the metabolic features of our patient, as the same mutation has been described by Strom *et al*¹³ in two other WFS families with patients showing diabetes mellitus at the age of 6–8 years and also progressive optic atrophy and deafness.¹³ This is the third family reported with the c.1096C>T mutation.

Various mechanisms have been proposed to explain the origin of interstitial inverted duplications adjacent to a

distal deletion. Such rearrangements have been documented for many chromosome arms, including 1q, 2q, 3p, 4p, 5p, 7q, 8p, 9p, and 11p.¹⁷ They may arise when, during the first meiotic division, refolding of a chromosome arm is mediated by repeated, inverted DNA-sequences, allowing intrachromatid synapsis and ectopic recombination. When the centromere splits during the second meiotic division, an asymmetric break in the recombinant chromosome produces an inv dup del chromosome.¹⁸ Addition or capture of telomere repeats (TTAGGG_n) results in telomere regeneration.¹⁹ The most frequently occurring inv dup del involves 8p, and is mediated by illegitimate recombination between two olfactory receptor gene clusters in 8p23.1 during meiosis in female carriers of a 4.7 Mb 8p inversion polymorphism.²⁰ Although olfactory receptor gene clusters and their corresponding inversion polymorphism are also located in 4p16.3, involvement of these gene clusters is unlikely in our case as the distal break occurred in 4p16.1. Kondoh *et al*¹⁷ also excluded involvement of the 4p16.3 olfactory receptor gene clusters in their patient.¹⁷ An alternative mechanism may consist of a two-chromatid break, followed by a *U*-type reunion of the broken ends producing a dicentric intermediate chromosome that is rescued by subsequent random breakage and telomere healing.²¹ Such an event, occurring during paternal meiosis,²² may be underlying our case, as the abnormal chromosome is of paternal origin.

Our patient highlights the complexity of clinical phenotypes in patients with chromosomal aberrations. To account for this, mutations in modifier genes located outside the deleted region, post-zygotic mutational events, and position effects affect expression of genes closely located to telomeres, and polymorphisms uncovered by the hemizygous deletion have been proposed.^{1,5–7} Findings in our patient prompt us to propose yet another mechanism: unmasking of autosomal recessive mutations by hemizygous deletions. Such a mechanism, as initially proposed by Hatchwell,²³ has been invoked by Riley *et al*²⁴ to explain features of both retinoblastoma and Wilson disease in a patient with a paternally derived interstitial deletion in chromosome 13q14.2–q22.2. In addition, Ikegawa *et al*²⁵ proposed that the pseudoachondroplasia seen in a patient with a *de novo* deletion of 11q21–q22.2 may be caused by ‘unmasking heterozygosity’ of a putative autosomal recessive mutation on the structurally normal chromosome 11. This mechanism was demonstrated in patients with Angelman/Prader Willi syndrome with a deletion in the 15q11–q13 region, in combination with oculocutaneous albinism related to hemizygous mutations in the *P* gene.^{26,27} In addition, unmasking a recessive deafness allele in the *MYO15A* is associated with sensorineural deafness in a patient with Smith–Magenis syndrome carrying the common 17p11.2 deletion.²⁸

Here, we demonstrate for the first time that a similar combination of a deletion and a recessive mutation may

explain complex phenotypes of certain patients with WHS or WFS. We propose that phenotypic variability among patients with similar deletion syndromes may in part be due to hemizygous expression of a recessive mutation on the non-deleted homologue. Given the high degree of segmental deletion polymorphisms in the genome of healthy individuals,^{29,30} exposure of hemizygous autosomal recessive mutations should be considered as a plausible mechanism of phenotypic variability among patients with chromosomal deletion syndromes.

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