

*Clinical Report***Donnai–Barrow Syndrome (DBS/FOAR) in a Child With a Homozygous *LRP2* Mutation Due to Complete Chromosome 2 Paternal Isodisomy**

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Donnai–Barrow syndrome [Faciooculoacousticorenal (FOAR) syndrome; DBS/FOAR] is a rare autosomal recessive disorder resulting from mutations in the *LRP2* gene located on chromosome 2q31.1. We report a unique DBS/FOAR patient homozygous for a 4-bp *LRP2* deletion secondary to paternal uniparental isodisomy for chromosome 2. The proband inherited the mutation from his heterozygous carrier father, whereas the mother carried only wild-type *LRP2* alleles. This is the first case of DBS/FOAR resulting from uniparental disomy (UPD) and the fourth published case of any paternal UPD 2 ascertained through unmasking of an autosomal recessive disorder. The absence of clinical

symptoms above and beyond the classical phenotype in this and the other disorders suggests that paternal chromosome 2 is unlikely to contain imprinted genes notably affecting either growth or development. This report highlights the importance of parental genotyping in order to give accurate genetic counseling for autosomal recessive disorders.

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**Key words:** Donnai–Barrow (DBS/FOAR) syndrome; uniparental isodisomy (UPD); paternal chromosome 2; reduction to homoallelism

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**INTRODUCTION**

Donnai–Barrow syndrome [Faciooculoacousticorenal (FOAR) syndrome; DBS/FOAR] [OMIM 222448] is a rare autosomal recessive disorder characterized by agenesis of the corpus callosum, enlarged anterior fontanelle, hypertelorism, high myopia, severe sensorineural deafness, congenital diaphragmatic hernia, and low molecular weight proteinuria with notable excretion of retinol-binding (RBP) and vitamin D-binding (DBP) proteins [Holmes and Schepens, 1972; Donnai and Barrow, 1993; Kantarci et al., 2007]. No single feature is

Abbreviations used: DBS/FOAR, Donnai–Barrow syndrome; UPD, uniparental disomy; RBP, retinol-binding protein; DBP, vitamin D-binding protein; *LRP2*, low-density lipoprotein receptor-related protein 2; STR, short tandem repeats.

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pathognomic for DBS/FOAR; rather the diagnosis should be entertained when several of the above listed features are present in combination. Small deletions or insertions causing frameshifts, as well as conserved splice site, nonsense and missense mutations of low-density lipoprotein receptor-related protein 2 (*LRP2*) gene in seven DBS/FOAR families were recently reported [Kantarci et al., 2007]. The 79 exon *LRP2* gene mapping to human chromosome 2q31.1 encodes megalin, an endocytic transmembrane glycoprotein [Fisher and Howie, 2006].

Engel [1980] first described the phenomenon of UPD in humans, representing the unusual situation in which both members of a chromosome pair are inherited from only one parent with no contribution from the other parent. The presence of both homologous chromosomes from only one parent is known as “uniparental heterodisomy,” while the presence of two copies of the same chromosome from the same parent is known as “uniparental isodisomy.” UPD arises from different mechanisms including trisomy rescue, monosomy rescue, gametic complementation, postfertilization error via somatic recombination or gene conversion, and somatic replacement of a derivative chromosome [Kotzot, 1999; Kotzot and Utermann, 2005]. According to a survey of all reported cases, maternal UPD is more common than paternal UPD (approximately 3:1) [Kotzot, 1999]. Since nondisjunction events occur mostly in maternal meiosis I, heterodisomy is more frequent in maternal UPD, while isodisomy preferentially arises in paternal UPD. Prader–Willi syndrome is the prototypical example of maternal heterodisomy for chromosome 15, while Angelman syndrome typifies paternal isodisomy for chromosome 15 [Kotzot, 1999; Kotzot and Utermann, 2005].

We report the first case of paternal isodisomy for chromosome 2 ascertained through homozygosity for a mutant *LRP2* allele in a patient clinically diagnosed with DBS/FOAR.

### CLINICAL REPORT

The proband, a 9-year-old boy, was born to healthy unrelated Caucasian parents aged 34 years (mother) and 40 years (father) (Fig. 1). He has one healthy sister and two healthy maternal half siblings. During pregnancy a small exomphalos was detected by ultrasound. He was delivered at term by normal vaginal delivery. Based on postnatal clinical examination and imaging studies, he was noted to have marked hypertelorism, bilateral coloboma, absence of the corpus callosum, malrotation of the gut, bilateral inguinal hernias, but not congenital diaphragmatic hernia. He had his omphalocele reduced on day 1, his inguinal hernias repaired at 1 year of age, and definitive surgery for his malrotation at 18 months of age.

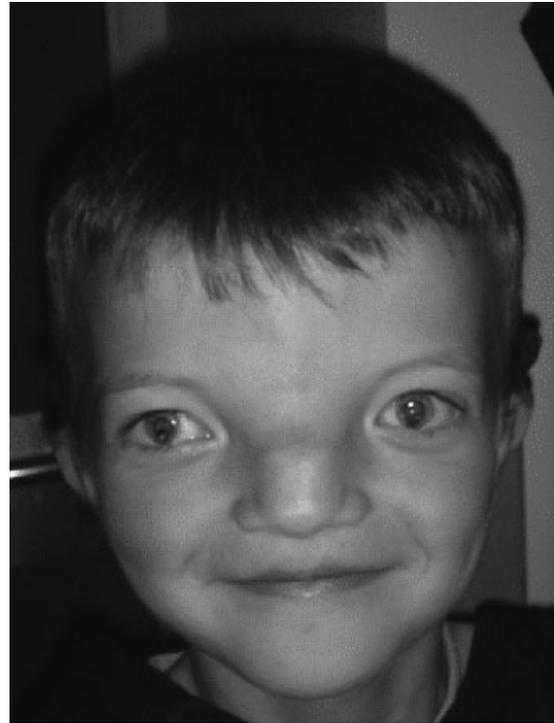


Fig. 1. Proband at 8 years of age with characteristic craniofacial features of DBS/FOAR syndrome. Note hypertelorism, downslanting palpebral fissures, and iris colobomas.

At 4 months of age his head circumference was 44.5 cm (95th centile), height 60.6 cm (90th centile), and weight 5.78 kg (90th centile). MRI scan of the brain confirmed the corpus callosum agenesis, and also revealed a frontal encephalocele with a widened anterior fossa, and a Chiari 1 malformation with cerebellar tonsils extending to C1.

His ocular manifestations include bilateral iris and chorioretinal colobomas, high myopia, right inferior cataract, and left posterior lenticonus, diagnosed at 3 months of age. His myopia is associated with enlarged globes (axial length measuring 30 mm at age 7 years), bilateral posterior staphylomata, and he received prophylactic 360 degree laser retinopexy to prevent retinal detachment. His glasses prescription is OD  $-15.00$  D, OS  $-19.25/-2.00$  axis  $92^\circ$ , although he usually wears contact lenses, and achieves corrected visual acuities of OD 20/200, OS 20/100. Although he had normal electrodiagnostic results at the age of 7 years, over the past 2 years the patient reported visual deterioration, especially at night. Repeat electroretinography age 9 years revealed generalized retinal dysfunction involving both the rod and cone systems mainly at the photoreceptor/retinal pigment epithelial interface. Ocular measurements at 6 years of age were 45, 70, and 110 mm for inner canthal, interpupillary, and outer canthal distances, respectively.

Cochlear implants were inserted at age 4 due to severe bilateral deafness, revised at age 6, and again at age 8. He attends a mainstream school and has specialist support for his visual and hearing deficits. He demonstrates some developmental delay, attending a class two years behind his peers. However, he is making good progress in this class, and much of his developmental delay is thought to be explained by his bisensory impairment, and gaps in his schooling due to frequent hospitalizations.

Chromosomal analysis revealed a normal male karyotype (46,XY) (data not shown).

**MATERIALS AND METHODS**

**DNA Extraction and Urine Studies**

Biological samples were obtained after informed and written consent was received. Peripheral blood and saliva derived genomic DNA samples from each family member were isolated using the Nucleon

genomic DNA extraction kit (Tepnel Life Sciences, Manchester, UK) according to the manufacturer's recommendations. A urine sample from the patient was collected for biochemical urinalyses, protein electrophoresis, and western blotting as described previously [Kantarci et al., 2007].

**Molecular Studies**

Seventy-nine coding exons and flanking intronic base pairs of the *LRP2* gene were sequenced using the ABI 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA) on peripheral blood derived genomic DNA from the proband, and analyzed using Sequencher DNA sequence analysis software (Ann Arbor, MI). Additional genotyping analyses were performed in the proband, his parents, and healthy sister on both peripheral blood and saliva derived genomic DNA (Fig. 2). The primer sequence list is available [Kantarci et al., 2007].

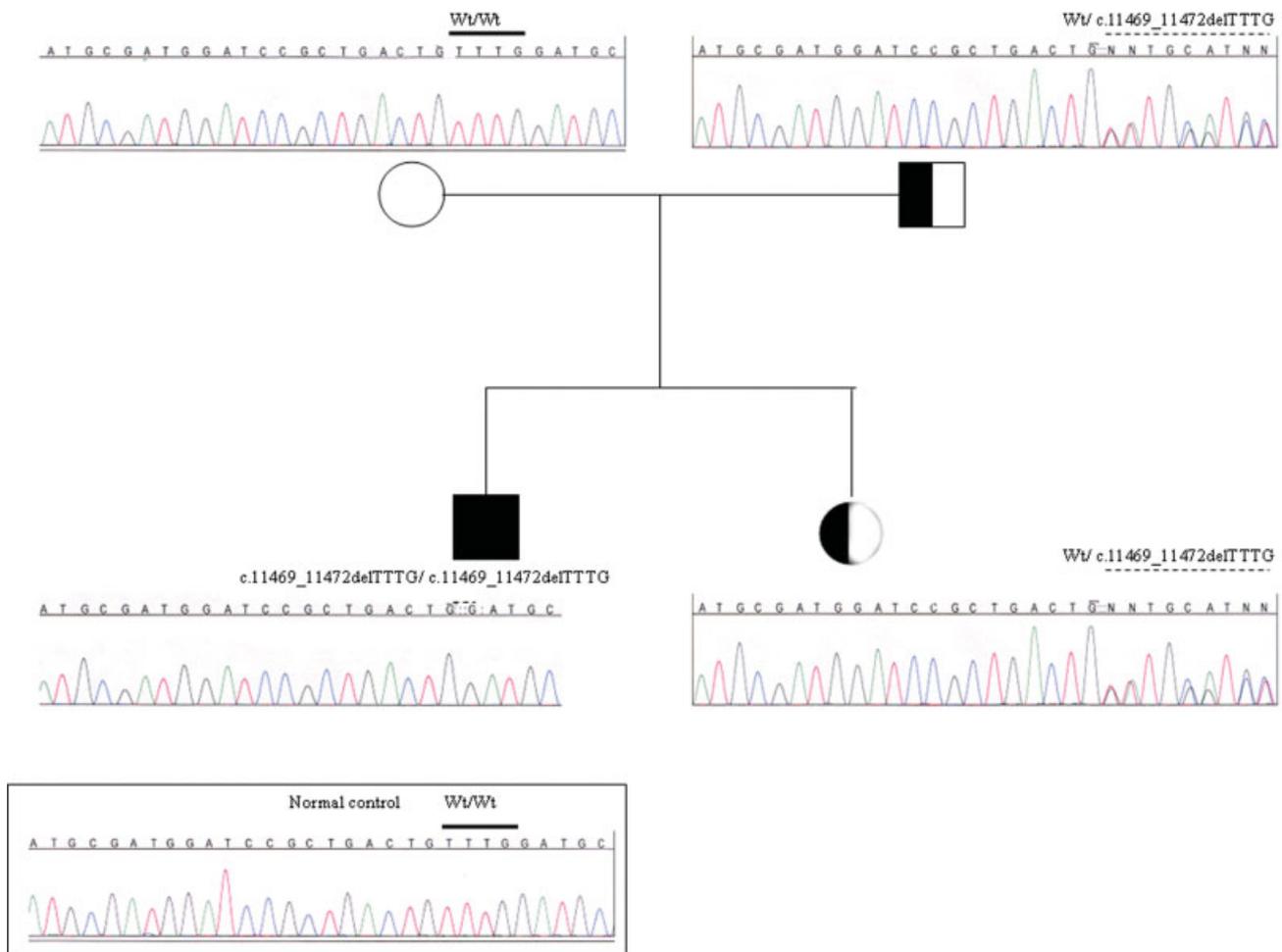


FIG. 2. The family pedigree and sequencing chromatograms of the *LRP2* gene showing a homozygous 4-bp deletion (c.11469\_11472delTTTG, exon 60) in the proband (indicated by the small bar), a heterozygous deletion in the father and the sister (indicated by the long dashed bar). The mother does not carry the mutation (indicated by the solid bar); appearance of her chromatogram is comparable to that of a normal control. Wt, wild-type.

Biological parentage was tested using the PowerPlex<sup>®</sup> 16 System (Promega Corp., Madison, WI). A total of 19 STR markers spanning both arms of chromosome 2 [D2S2584 (2p25.3), D2S2166 (2p25.2), D2S272 (2p24.3), D2S165 (2p23.2), D2S177 (2p22.2), D2S134 (2p14), D2S139 (2p12), D2S113 (2q11.2), D2S160 (2q13), D2S114 (2q21.2), D2S142 (2q24.1), D2S306 (2q24.2), D2S2330 (2q24.3), D2S326 (2q31.1), D2S152 (2q32.1), D2S72 (2q33.2), D2S126 (2q36.1), D2S206 (2q37.1), and D2S125 (2q37.3)] were used to investigate for possible uniparental disomy (Fig. 3).

## RESULTS

The patient's clinical features were consistent with Donnai-Barrow syndrome (DBS/FOAR) (Fig. 1). Biochemical analysis of his urine sample demonstrated massive proteinuria; protein electrophoresis and Western blotting confirmed the presence of characteristic low molecular moieties with increased spillage of RBP and DBP as previously published [Kantarci et al., 2007].

The *LRP2* gene demonstrated a homozygous 4-bp deletion (c.11469\_11472delTTTG) in exon 60 by direct sequencing (Fig. 2). The mutation is predicted to create an early stop codon (p.Cys3823TrpfsX159) corresponding to the low density lipoprotein (LDL)-receptor class A 33 domain, which would prematurely truncate the megalin protein.

Parental genomic DNA was then genotyped to investigate carrier status. We confirmed that the father was a heterozygous carrier for the 4-bp deletion, whereas the mother was found to be homozygous wild-type (Fig. 2). Biological parentage was confirmed (data not shown).

Given the proband's apparent homozygous 4-bp deletion, which could only be identified in the carrier father, we tested for UPD using 19 STR markers spanning the entire length chromosome 2. The proband was homozygous with a single paternal allele for all markers tested suggesting that reduction to homoallelism for the mutant *LRP2* allele was due to paternal uniparental isodisomy (Fig. 3), rather than a submicroscopic deletion of the chromosome 2q31.1 region on the maternally inherited chromosome 2 homologue. The proband's healthy sister, a

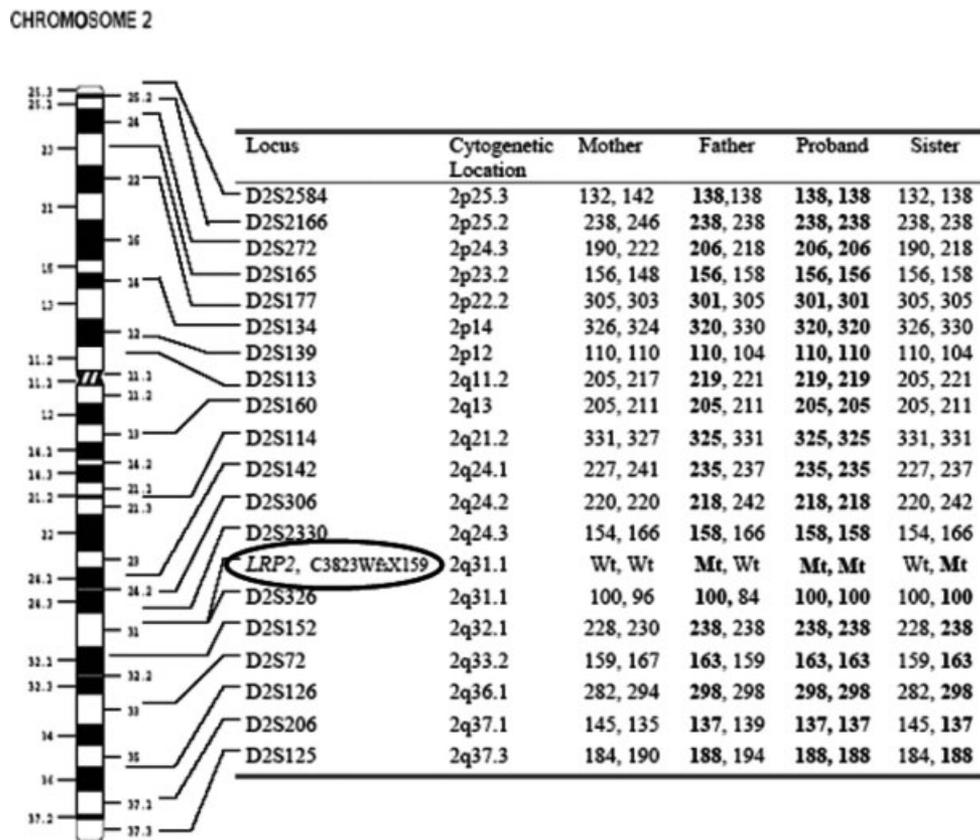


FIG. 3. Idiogram of chromosome 2 showing genotype analyses of family members using 19 short tandem repeat (STR) markers from chromosome 2. Proband was homozygous for all markers as well as the *LRP2* mutation (encircled in bold) which originated only from the carrier father. A total of 14 informative markers indicated paternal isodisomy for chromosome 2. The healthy sister showed normal bi-parental inheritance on chromosome 2; note possible recombination between paternal markers D2S2330 and *LRP2*.

heterozygous carrier of the mutation, showed biparental inheritance for chromosome 2 (Figs. 2 and 3).

The results of genotyping and STR marker analyses were concordant between peripheral blood and saliva derived DNA on all family members.

## DISCUSSION

Mutations in the *LRP2* gene encoding megalin were recently shown to cause DBS/FOAR syndrome [Kantarci et al., 2007]. The specific frameshift mutation in this patient, expected to produce a truncation close to the transmembrane domain, raises the question as to whether a residual molecule could be nonfunctional possibly through nonsense-mediated decay.

Here we describe a unique etiology for DBS/FOAR in a patient born to nonconsanguineous parents. The patient was homozygous for a novel 4-bp *LRP2* deletion which was carried as a heterozygous mutation in the father, but was absent in the mother. Analysis of STR markers spanning both arms of chromosome 2 revealed that the proband inherited a paternal haplotype but no maternal haplotype, consistent with paternal uniparental isodisomy for chromosome 2 (Fig. 3). However, heterozygosity of small regions due to double crossovers between the loci tested cannot be excluded.

In humans, UPD can give rise to abnormal phenotypes by several mechanisms including: pre-natal or postnatal trisomy mosaicism, genomic imprinting, or unmasking of recessive gene disorders due to reduction to homoallelism [Kotzot, 1999; Kotzot and Utermann, 2005]. As comprehensively reviewed by Kotzot and Utermann [2005], 23% of paternal and 10% of maternal whole chromosome UPD cases, after excluding chromosome 15, were ascertained due to the presence of an autosomal recessive disorder. In such cases, only molecular analyses can demonstrate that reduction to homoallelism occurs due to UPD, otherwise UPD is likely to go undiscovered [Kotzot, 1999; Kotzot and Utermann, 2005; Engel, 2006].

Careful phenotypic analysis of patients whose recessive disorders are caused by UPD-associated homoallelism can be instructive for identification of parent-of-origin differences in gene expression. If the phenotypes of these cases are expanded or more severe than expected, it is possible that these additional features are caused by UPD and a failure to inherit imprinted genes, though in some cases mosaicism for a residual trisomic cell line cannot be excluded. Distinct phenotypic features have been reported due to imprinted regions in association with uniparental inheritance of maternally derived chromosomes 7, 14, and 15 and paternally derived chromosomes 6, 11, 14, and 15.

The presence of imprinting effects for maternally derived chromosomes 2, 16, and 20 and for paternally derived chromosome 20 remain unknown due to the limited number of case reports or data that cannot be interpreted in support of this interpretation. And finally, there are no reported cases of whole chromosome maternal UPD 5, 11, 18, and 19 or paternal UPD 3, 4, 12, 17, 18, and 19, suggesting they either do not occur, are not compatible with life, or produce no phenotypic manifestations [Kotzot, 1999; Kotzot and Utermann, 2005].

Cases of UPD of chromosome 2 (UPD 2) have been infrequently reported. Besides a handful of maternal UPD 2 cases demonstrating confined placental trisomy 2 mosaicism and intrauterine growth retardation [Harrison et al., 1995; Webb et al., 1996; Hansen et al., 1997; Shaffer et al., 1997; Wolstenholme et al., 2001], only three maternal UPD 2 cases associated with an autosomal recessive disorder have been detected, including one case with a thyroid peroxidase (*TPO*) (2p25.3) mutation causing severe congenital hypothyroidism [Bakker et al., 2001] and two cases with mitochondrial trifunctional protein  $\alpha$ -subunit (*TFP $\alpha$* ) (2p23.3) mutations causing lethal trifunctional protein deficiency [Spiekerkoetter et al., 2002].

Evidence as to the presence or absence of imprinted genes on paternal chromosome 2 is very limited [Chavez et al., 2000; Thompson et al., 2002; Petit et al., 2005]. To our knowledge, our patient is only the fourth case of paternal UPD 2 ascertained through an autosomal recessive disorder. The first report described reduction to homoallelism for a paternal 5 $\alpha$ -reductase type 2 (*SRD5A2*) (2p23.1) mutation in a child with 5 $\alpha$ -reductase 2 deficiency [Chavez et al., 2000]. The subsequent reports identified homoallelism for paternal mutations of c-met proto-oncogene tyrosine kinase (*MERTK*) (2q13) in a case with retinal dystrophy [Thompson et al., 2002] and of uridine diphosphate glucuronosyltransferase (*UGT1A1*) (2q37.1) in a case with Crigler-Najjar type 1 syndrome [Petit et al., 2005]. Given the "typical" phenotypes found among the four paternal UPD 2 cases, including the present case, combined with the absence of paternally expressed imprinted mouse genes in regions syntenic to human chromosome 2 (<http://www.mgu.har.mrc.ac.uk/research/imprinting/imprin-viewdatagenes.html>) it is reasonable to surmise there are few or no paternally imprinted chromosome 2 genes that notably affect growth and development.

Based on the findings in our case, the presence of homozygous *LRP2* mutations in DBS/FOAR patients from nonconsanguineous families should lead to consideration of UPD as a possible causal mechanism. If UPD is proven, this dramatically alters genetic counseling for parents of children with "autosomal recessive" disorders such as DBS/FOAR, thereby reducing their recurrence risk from 1 in 4 to negligible.

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