ELECTRONIC LETTER

Horizontal gaze palsy with progressive scoliosis can result from compound heterozygous mutations in *ROBO3*

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Background: Horizontal gaze palsy with progressive scoliosis (HGPPS) is an autosomal recessive disorder characterised by congenital absence of horizontal gaze, progressive scoliosis, and failure of the corticospinal and somatosensory axon tracts to decussate in the medulla. We previously reported that HGPPS patients from consanguineous pedigrees harbour homozygous mutations in the axon guidance molecule *ROBO3*.

Methods: We now report two sporadic HGPPS children of non-consanguineous parents who harbour compound heterozygous mutations in *ROBO3*. The mother of one of the children also had scoliosis DNA was extracted from a blood sample from each participant using a standard protocol, and the coding exons of *ROBO3* were amplified and sequenced as previously described.

Results: Each patient harboured two unique heterozygous mutations in *ROBO3*, having inherited one mutation from each parent.

Conclusions: HGPPS can result from compound heterozygous mutations. More comprehensive examinations of parents and siblings of HGPPS patients are required to determine if the incidence of scoliosis in individuals harbouring heterozygous *ROBO3* mutations is greater than in the general population.

Horizontal gaze palsy with progressive scoliosis (HGPPS) is a rare disorder characterised by congenital absence of horizontal conjugate eye movements with progressive scoliosis developing in childhood and adolescence.[1] We previously studied ten consanguineous pedigrees that segregated HGPPS as an autosomal recessive trait and reported that the affected individuals harbour ten different homozygous mutations in the axon guidance molecule *ROBO3*, including missense, nonsense, frameshift, and splice site mutations.[2] The descending corticospinal and ascending sensory tracts in the affected individuals failed to cross the midline of the medulla early in development.[2] Similarly, removal of *Rig-1* (*Robo3*) in mice results in failure of the hindbrain precerebellar axons and neurons,[3] and spinal cord commissural axons[4] to cross the midline.

METHODS

We have now studied the genetic basis of sporadic HGPPS in two children from non-consanguineous pedigrees. The affected children and their parents (fig 1; pedigrees PB and PC) were enrolled in our ongoing research study of congenital eye movement disorders approved by the Children's Hospital Boston Institutional Review Board. Informed consent was obtained, each participant was examined and donated a blood sample from which genomic DNA was extracted using a standard protocol, and the coding exons of *ROBO3* were amplified and sequenced as previously described.[2] Parental and control DNA were examined for the presence or absence of each sequence variant identified by either denaturing high performance liquid chromatography (DHPLC; Transgenomic, Omaha, NE) or by the ABI 7900HT Fast Real-Time PCR system (Applied Biosystems, Foster City, CA).

RESULTS

Case 1

The affected son in pedigree PB (fig 1A; PBII:1) is the offspring of non-consanguineous parents of Irish and German descent. Pregnancy was normal. He was born at term to a 35 year old primigravida mother by caesarean section for failure of labour to progress with a birth weight of 3800 g and Apgar scores of 9 and 9 at 1 and 5 min. His mother has mild scoliosis that has not required medical attention, and family history was otherwise negative for ocular and musculo-skeletal disease.

At his 2 month well child visit, the baby was noted to have a large head and to be looking upwards to the right. By 4 months he had an obvious torticollis and was referred for neurological evaluation. At 5.5 months, he could roll from front to back only from his left side. His head circumference was at the 90th percentile and he had a right torticollis and plagiocephaly. He had absent bilateral horizontal gaze, marked lower thoracic scoliosis convex to the left, truncal hypotonia, and brisk symmetric reflexes. When last seen for neurological follow up at 11 months of age, he was not yet able to sit independently; the rest of his examination remained unchanged.

Ophthalmological examination at 13 months of age showed a left head tilt of about 20°. He fixated on targets but could only follow them in up and downgaze. Apart from intermittent bouts of pendular nystagmus of mild amplitude, horizontal eye movements were absent. There was no significant refractive error and the anterior ocular segment and retinal examinations were normal.

Spine x rays at 5.5 months of age showed a moderate scoliosis (40°) convex to the left at the T11 and T12 level, as well as some kyphosis (fig 2A). There were no rib or vertebral anomalies. Brain MRI scan showed a midline cleft in a portion of the anterior surface of the pons with a smaller anterior-posterior diameter of the pons itself, and a more prominent cleft in the medulla resulting in the medulla having a bifid appearance (fig 2B). In addition, there were prominent CSF spaces anterior to the frontal lobes and widened sylvian fissures suggesting that the macrocephaly resulted from benign external hydrocephalus.

Case 2

The affected daughter of pedigree PC (fig 1A; PCII:1) is the offspring of non-consanguineous parents of English/Irish and Acadian descent. Pregnancy was normal and she was born at 42 weeks gestation to a 28 year old G2P1 mother by induced

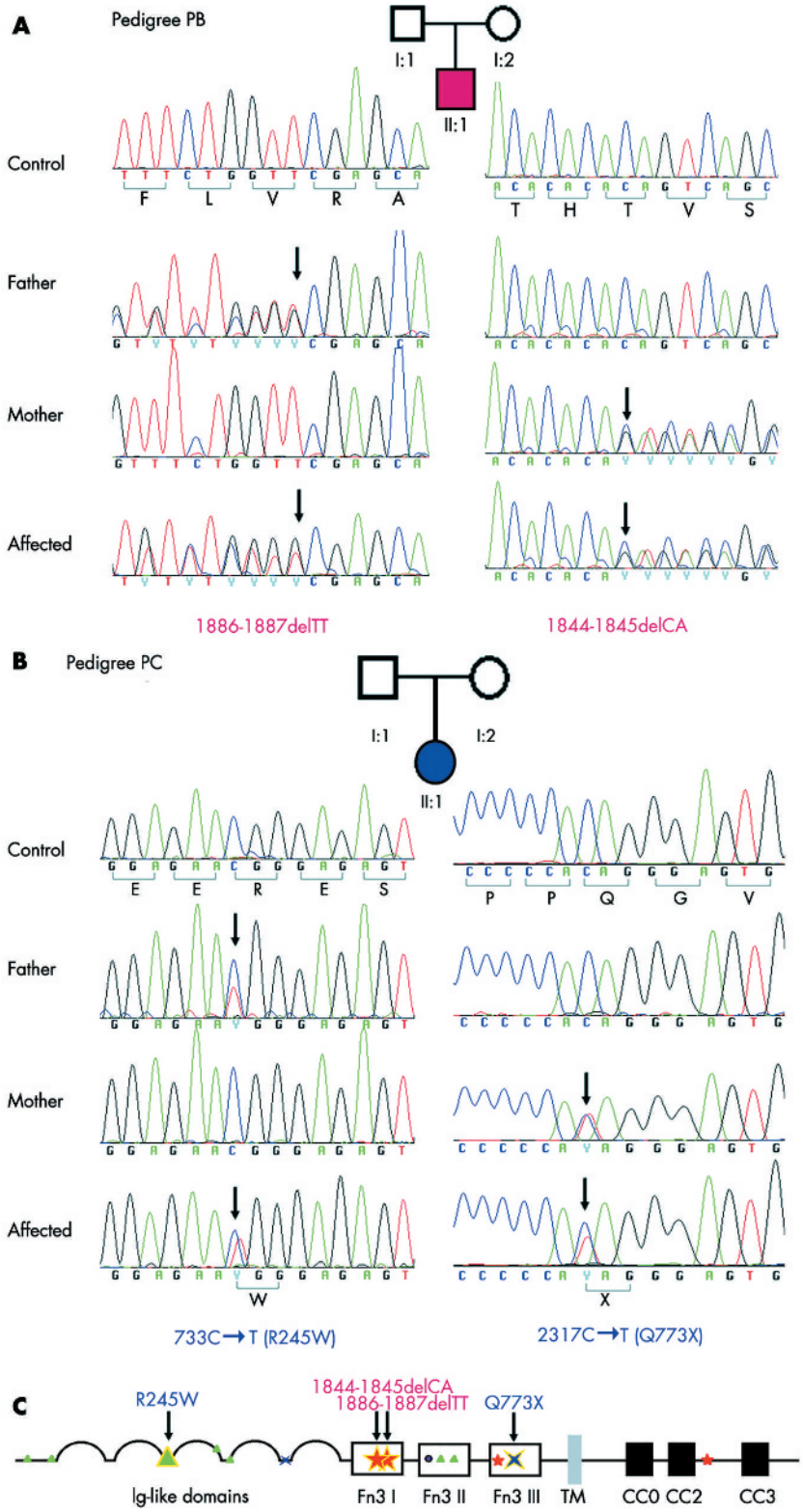

Figure 1 HGPPS pedigrees, chromatographs, and ROBO3 protein structure. (A, B) Pedigrees PB (A) and PC (B) and the four heterozygous *ROBO3* mutations. Squares: males; circles: females; filled symbol: affected status. Below each pedigree, the first row shows the paternal and the second the maternal mutation. Each column contains sequence from an unaffected control (row 1 with the corresponding normal amino acid residue depicted under each control triplet codon). Note that each parent harbours one normal and one mutated allele, and each child has inherited both of the parents' mutated alleles, as indicated by the arrows. In column 1, PBI:1 and PBII:1 sequence is from the reverse strand in order to present sequence that does not overlap with the maternal deletion located 42 bases upstream. (C) Predicted ROBO3 topology with the locations of the four heterozygous mutations indicated by arrows above the protein. The four new and 10 previously reported homozygous mutations[2] are superimposed on the protein structure as large yellow-outlined and small symbols, respectively. Green triangles: missense; blue crosses: nonsense; purple circle: splice site; red stars: frameshift mutations. All four new mutations fall in the extracellular domain of the ROBO3 protein, similar to nine of the 10 mutations previously reported.

vacuum delivery. Her birth weight was 3065 g and Apgar scores were 9 and 9 at 1 and 5 min. There was no family history of ocular or musculo-skeletal disease. At birth she was noted to have a left head tilt, drooping of the right side of her mouth with decreased blink on the right, and wasting of the muscles of the left side of her neck. An ultrasound showed ''muscular asymmetry'' in the neck.

The patient was developmentally delayed, rolling at 8–9 months, sitting at 12 months, and taking her first steps at 14 months. Physical therapy started at age 9 months and improved her left head tilt. Examination by a neurologist at 14 months revealed moderate convex right thoraco-lumbar scoliosis, poor horizontal ocular motility, and generalised hypotonia. Examination by a paediatric ophthalmologist at 18 months of age revealed complete lack of abduction of either eye and diminished adduction of both eyes. Independent walking occurred at 21 months despite progression of her scoliosis.

The authors first examined the patient when she was 5 years of age. Visual acuity measured OD 20/40, OS 20/50 wearing glasses with a mild hyperopic astigmatism correction. Pupils were normal and there was full vertical motility. Horizontal saccades and pursuit movements were absent, and vestibulo-ocular reflex testing produced no horizontal eye movements. She substituted convergence to produce adduction of the fixating eye. Low amplitude, moderate frequency horizontal/torsional nystagmus was present. There was no globe retraction. A ~15 prism diopter esotropia was present at distance and near in both primary position and with her abnormal left head tilt posture. There was a slightly slower blink on the left side. The remainder of the ocular exam was normal.

Spine x rays showed profound scoliosis (fig 2C). Cardiac echography and brainstem auditory evoked potentials were normal. Review of previous brain MK imaging done at 4 years of age revealed a scalloped appearance of the dorsal pons and flattening of the medullary olives (fig 2D), and somatosensory potentials showed a ''brainstem disturbance''.

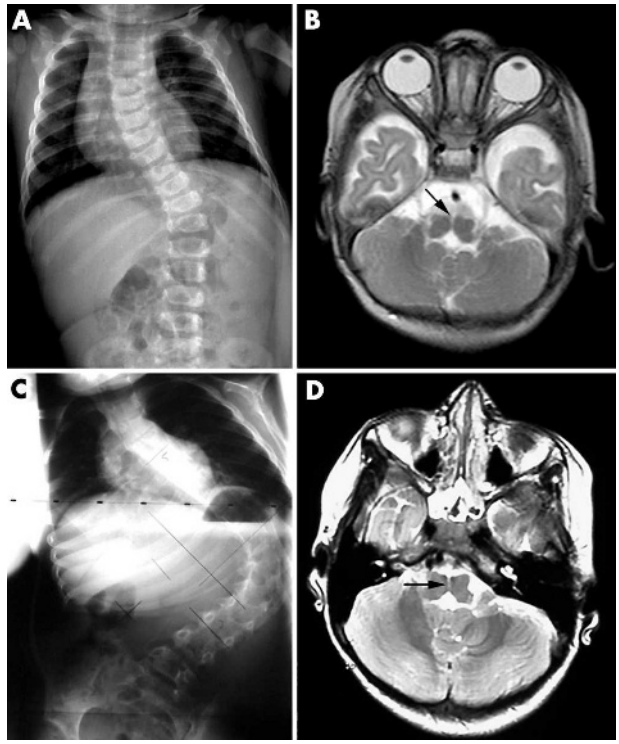

Figure 2 HGPPS imaging of case 1 (PB II:1; A, B) and case 2 (PC II:1; C, D). PA spine x rays demonstrated mild scoliosis in case 1 (A) and profound scoliosis in case 2 (C; linear pen marks are artifacts of scoliosis angle measurements). Axial MR brain images through the medulla of case 1 (B) and case 2 (D) demonstrating a flattened and butterfly-like appearance of each patient's medulla, accompanied by a midline cleft (arrows), and consistent with the absence of decussation of the corticospinal tract fibres. (These images are reproduced with permission.)

ROBO3 analysis

Sequence analysis of *ROBO3* in case 1 (PB II:1) revealed two unique heterozygous 2 bp deletions located 42 bp apart from one another in exon 12 (fig 1A; 1844–1845delCA, 1886–1887delTT). These deletions shift the reading frame and create premature stop codons after 23 and nine altered amino acids, respectively (fig 1C). The father (PB I:1) is heterozygous for the 1886–1887delTT allele, while the mother (PB I:2) is heterozygous for the 1844–1845delCA allele (fig 1A).

Sequence analysis of *ROBO3* in case 2 (PC II:1) revealed two heterozygous point mutations (fig 1B). A heterozygous 733C→T missense mutation was found in exon 4, and is predicted to result in the non-conservative substitution of a positively charged arginine by an aromatic tryptophan at residue 245 (R245W), located between the second and the third immunoglobulin (Ig)-like motifs (fig 1C). A heterozygous 2317C→T nonsense mutation was identified in exon 15, and is predicted to result in the conversion of a glutamine to a stop codon at residue 773 (Q773X) in the third fibronectin-like motif (fig 1C). The father (PC I:1) harbours a heterozygous 733C→T mutation, while the mother (PC I:2) harbours a heterozygous 2317C→T mutation (fig 1B).

None of the four mutations have been reported previously, and none were present on 174 chromosomes of mixed ethnicity. The two heterozygous out-of-frame deletions in PB II-1 and the nonsense mutation in PC II-1 are each predicted to result in premature termination of the ROBO3 protein. The arginine at amino acid residue 245 is conserved in mouse and *Drosophila*, supporting our hypothesis that its substitution by a tryptophan may result in loss of function of this mutated *ROBO3* allele as well. Three previously unreported *ROBO3* polymorphisms were also detected in this study: 22/96 controls (23%) harboured 1247G→A (R416H) in exon 8; 2991G→A (A997A) in exon 21; and 19/85 controls (22%) harboured a 6 bp deletion (4094–4099delGGAGTC) in exon 27.

DISCUSSION

We have identified two children with sporadic HGPPS resulting from compound heterozygous *ROBO3* mutations, inherited from parents who are each heterozygous for one of their offspring's mutations. In both patients, torticollis and plagiocephaly were detected prior to the diagnosis of scoliosis. Torticollis has been reported previously in several infants with HGPPS.[5–8] Given the clinical and radiological similarities between these HGPPS patients and those previously reported, it seems likely that their *ROBO3* mutations also lead to complete loss of ROBO3 function.[2 9] This report of *ROBO3* mutations in the offspring of non-consanguineous relationships reinforces the importance of considering this diagnosis in infants and children who present with torticollis, scoliosis, or abnormalities of horizontal gaze such as Duane anomaly.

It is notable that PB I:2, the mother of PB II:1, harbours a heterozygous *ROBO3* mutation and has mild scoliosis. Similarly, there are previous reports of scoliosis in relatives of HGPPS patients who harbour, or are at risk of harbouring, heterozygous *ROBO3* mutations. The father of an affected HGPPS patient from Crete had mild scoliosis with onset in adolescence[10] and carries a heterozygous E319K *ROBO3* mutation.[2] Two consanguineous siblings with HGPPS who

harbour the homozygous L5P *ROBO3* mutations[2] are reported to have a sister and cousin with scoliosis.[11] Lastly, scoliosis was reported in the mother of an affected individual from a consanguineous family which has not undergone genetic analysis.[12] More comprehensive examinations of parents and siblings of HGPPS patients are required to determine if the incidence of scoliosis in individuals harbouring heterozygous *ROBO3* mutations is greater than in the general population.

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