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Two Novel *STRA6* Mutations in a Patient with Anophthalmia and Diaphragmatic Eventration

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To the Editor

Anophthalmia/microphthalmia and diaphragmatic defects are a rare but recognized phenotypic combination [Steiner et al., 2002]. These two malformations have been described together with pulmonary agenesis in the Matthew-Wood syndrome [MWS; OMIM 601186; Berkenstadt et al., 1999]. Pulmonary hypoplasia/agenesis, Diaphragmatic hernia/eventration, Anophthalmia/microphthalmia, and Cardiac defects, or PDAC syndrome, overlaps with MWS [for selected recent reports, see Ceylaner et al., 2006; Li et al., 2006; Chitayat et al., 2007; Martinovic-Bouriel et al., 2007]. Recently mutations in the *STRA6* gene were identified in a phenotype that overlaps with both MWS and PDAC [Golzio et al., 2007; Pasutto et al., 2007], with clinical features of affected families comprising pulmonary agenesis, diaphragmatic defects, anophthalmia or severe microphthalmia, cardiac defects, pancreatic malformations and intrauterine growth retardation. The *STRA6* gene mutations were first identified in two large consanguineous families using a positional cloning approach, and absence of the Stra6 protein on a Western blot from one child who had a homozygous frameshift mutation, was demonstrated [Pasutto et al., 2007]. In a further 13 unrelated individuals with eye, diaphragm, heart or lung malformations, four different missense mutations in *STRA6* were identified that were predicted either to affect the structure of the loops connecting the transmembrane domains, alter the SH2 domain binding motif, or occur at a site that could compromise downstream signal transduction [Pasutto et al., 2007]. Following a second paper reporting two homozygous frameshift mutations [Golzio et al., 2007], a total of eight different frameshift or missense mutations in *STRA6* have now been reported in eight individuals. However there is evidence for genetic heterogeneity, as some patients with similar phenotypes have been negative for *STRA6* mutations [Table I; Golzio et al., 2007; Pasutto et al., 2007], and mutational analysis of the original patients reported as having MWS has not been published. Interestingly, missense mutations have been associated with a more severe phenotype than the two truncating frameshift mutations [Pasutto et al., 2007]. We report on the detection of two novel *STRA6* mutations in a male fetus with anophthalmia and diaphragmatic eventration to add to the phenotype-genotype data on these intriguing conditions.

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The baby has been described in Steiner et al. [2002] as patient 2 or twin A. The baby was a twin born to a 25-year-old female following in-vitro fertilization. An assisted forceps delivery was performed at 38 weeks of gestation because of reduced growth and a reduced amniotic fluid volume. Birth weight was 2400 g (<10th centile). Ventilator support was provided for respiratory distress. The baby was hypotonic and had multiple congenital anomalies comprising anophthalmia with short, fused palpebral fissures, bilateral diaphragmatic eventration, cryptorchidism and bilateral inguinal hernias. The face was flat with bitemporal narrowing, a mildly prominent nose and anteverted nares, full cheeks, a long philtrum and a small jaw. After staged surgeries, the respiratory distress intensified and support was withdrawn. Autopsy confirmed the anophthalmia and the orbits lacked globes but contained fibroadipose tissue, skeletal muscle and retinal rudiments and the optic nerves were small. Other findings were a thin corpus callosum, ostium primum and fossa ovalis atrial septal defects, severe right ventricular hypertrophy, incomplete lobation of the lungs and a high and arched palate. The diaphragm showed no evidence of primary neuromuscular disease. Histological examination of the lung tissue showed bilateral, diffuse alveolar damage resembling bronchopulmonary dysplasia, parenchymal hemorrhage and focal bronchopneumonia. The karyotype was reported as 46,XY.

DNA was extracted from a section from a paraffin block after treatment with xylene, digestion with proteinase K and salting-out according to standard procedures (QIAamp® DNA Mini kit, Qiagen, Valencia, CA). The coding exons and the exon-intron boundaries of the *STRA6* gene were sequenced in both directions using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) on an ABI 3730 machine. All sequences were analyzed manually with the Sequencher software program (Gene Codes Corporation, Ann Arbor, MI). After the detection of two sequence variants in exon 2 of the gene, the polymerase chain reaction (PCR) product for exon 2 was cloned in to a TOPO-TA vector (Invitrogen, Carlsbad, CA) and sequenced to determine if the sequence alterations were present on the same (cis) or separate (trans) chromosomes.

We identified two novel sequence alterations in exon 2 of *STRA6* - an insertion of two C nucleotides, c.277insCC, predicting a frameshift with a premature stop codon, p.G13PfsX72, and c.310G>A, predicting p.W23X, a nonsense mutation (Fig. 1). Cloning of these two sequence alterations showed that they were in trans, or present on different chromosomes (data not shown). Parental samples were unavailable for testing. These two sequence alterations are both predicted to cause premature protein truncation early in the protein, similar to several other disease-causing mutations (Table I). Our findings are thus consistent with the autosomal recessive inheritance in this child. We also attempted to extract DNA from the paraffin blocks pertaining to the first patient reported by Steiner et al. [2002], but were unable to obtain DNA of sufficient quality for sequencing.

Relatively few *STRA6* mutations have been described (Table I). An analysis of the phenotypic features confined to patients with known mutations in *STRA6* or the affected siblings of these individuals shows that all patients who received an eye examination had bilateral anophthalmia (12/12; Table I). Interestingly, a brother who was heterozygous for a frameshift mutation resulting in premature protein truncation had isolated bilateral coloboma of the retina and iris. Cardiac defects were the second commonest feature and were present in 10/13 (77%). The heart malformations were varied, including tetralogy of Fallot, pulmonary stenosis, a single ventricle, ventricular and atrial septal defects, truncus arteriosus, absent pulmonary artery and patent ductus arteriosus (Table I). Pulmonary agenesis or hypoplasia was noted in 8/13 (62%) and CDH or diaphragmatic eventration in 8/13 (62%). Intra-familial variation was common for the cardiac, pulmonary and diaphragmatic malformations.

In addition to these four cardinal features, the phenotype of patients with *STRA6* mutations is wide-ranging and pleiotropic. Dysmorphic features were found in 6/13, with distinctive, broad, flaring, and only upward-growing eyebrows, wide nasal bridge, large and low-set ears and micrognathia in addition to the features described in this twin. Four out of 13 had renal anomalies that were usually relatively mild, such as a right pelvic kidney, horseshoe kidney, bilateral hydronephrosis and renal hypoplasia. Genital anomalies comprised bicornuate uterus, uterine dysplasia and cryptorchidism, and unilateral or bilateral inguinal hernias were present in two cases. Two patients had duodenal stenosis and two had pancreatic malformations, including an absent pancreas and an annular pancreas. Cerebral malformations were rare, with one occurrence of a thin corpus callosum and two instances of small or absent optic nerves. Growth was reported to be normal in three individuals, but three had intra-uterine growth retardation and three developed postnatal growth delay or failure to thrive. Survival was poor, and at the time of reporting, only two of the 13 patients were alive at the ages of three months and 14 years. In those that survived the neonatal period, developmental delay was usual, although one patient was reported to be cognitively normal until 22 months of age. All patients were consanguineous except for the three individuals from the family designated MWS4 [Pasutto et al., 2007] and this twin [Steiner et al., 2002].

The phenotypic criteria for testing for *STRA6* mutations have not yet been fully defined, but it has been suggested that testing should be offered in patients with severe microphthalmia or anophthalmia who also have one or more additional feature comprising pulmonary defects (pulmonary agenesis, pulmonary hypoplasia or unilobar lung), diaphragmatic defects (eventration or hernia), and cardiovascular malformations involving the common aorticopulmonary trunk or pulmonary arteries [Golzio et al., 2007].

Vitamin A is transported as a complex bound to retinol binding protein (RBP), but until recently, the mechanism responsible for absorption of the vitamin A-RBP complex into cells was poorly understood. Stra6 was established as a specific membrane receptor for RBP, binding RBP with high affinity and enabling vitamin A uptake. The intake of vitamin A is vital to adequate retinoic acid signaling and downstream gene transcription. Stra6 confers RBP binding to transfected cells and is found in the cellular localizations expected of the RBP receptor in native tissues [Kawaguchi et al., 2007]. Consistent with the diverse phenotype of these patients, *STRA6* is widely expressed in embryonic development and in adult organ systems [Kawaguchi et al., 2007; Pasutto et al., 2007]. The intra-familial variability seen with *STRA6* mutations may relate to environmental (vitamin A levels) factors and to genetic modifiers. The severity and poor survival in patients with *STRA6* mutations are testimony to the importance of vitamin A and similar micronutrients in human development.

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Figure 1.

Fig. 1a Chromatogram showing an insertion of two CC nucleotides, c.277insCC, predicting a frameshift with a premature stop codon, p.G13PfsX72. Nucleotide numbering is according to nucleotide position for transcript ENSG00000137868 (<http://www.ensembl.org>).

Fig. 1b Chromatogram showing c.310G>A, predicting p.W23X. Nucleotide numbering is according to nucleotide position for transcript ENSG00000137868 (<http://www.ensembl.org>).

Table 1

Clinical Features of Patients with Mutations in the *STRA6* Gene

Patient, Sex	Mutation	Eyes	Lungs	Diaphragm	Cardiovascular	Survival	Other	Reference
Fam. 1-IV:2; F	p.P293L	Bilat. An.	Alveolar capillary dysplasia	-	Pulmonary stenosis*; PDA	Died 6 m	Right pelvic kidney; Developmental delay; Normal growth; Dysmorphism	Pasutto et al., 2007
Fam. 1-IV:4; F	Presumed p.P293L	Bilat. An.	-	-	Pulmonary atresia; Single ventricle	Died 2 d	Normal growth	Pasutto et al., 2007
Fam. 2-IV:1; M	p.G50A fs X22 Homozygous	Bilat. An.	-	CDH	ASD; VSD	Alive 14 y	Mental retardation; Short stature; Absent optic nerves; Dysmorphism	Pasutto et al., 2007
Fam. 2-IV:3; Not stated	p.G50A fs X22 Homozygous	Bilat. An.	-	CDH	-	TOP 23 w	Dysmorphism	Pasutto et al., 2007
MWS1-EE; M	p.R655C	Bilat. An.	Hypoplasia	Left-sided eventration	-	Died 3 m	Polyhydramnios; Hypotonia; Poor feeding; Failure to thrive; Right inguinal hernia	Pasutto et al., 2007
MWS1-EE Brother; M	Presumed p.R655C	Bilat. An.	-	-	Truncus arteriosus*; PDA; Pulmonary atresia	Died 22 m	Postnatal short stature; Not delayed	Pasutto et al., 2007
MWS4-BE; F	p.T644M	Bilat. An.	Hypoplasia	R CDH	-	Alive 3 m	Bilateral hydronephrosis; Normal growth	Pasutto et al., 2007
MWS4-BE Brother; M	Presumed p.T644M	Unknown	Hypoplasia, unilobar left lung	-	TOF, PDA	Died 1 d	Horseshoe kidney; Cryptorchidism; Hypoplastic renal arteries	Pasutto et al., 2007
MWS4-BE Sister; F	Presumed p.T644M	Bilat. An.	Hypoplasia, unilobar lungs	-	PDA, Coarct. of aorta	Died 1 d	Uterine dysplasia	Pasutto et al., 2007
MWS6-BK; F	p.P90L; p.T321P	Bilat. An.	Bilateral hypoplasia	L CDH; R eventration	PDA	Died 1 d	Renal hypoplasia; Bicornuate uterus; Prominent labia minora; Meckel diverticulum; Normal growth	Pasutto et al., 2007
Fetus 1; M	p.D17A fsX55 Homozygous	Bilat. An.	Bilateral Agenesis	Bilateral eventration	Absent PA branches	Died 31 wg	Annular pancreas; Duodenal stenosis; IUGR; Dysmorphism	Golzio et al., 2007

Patient, Sex	Mutation	Eyes	Lungs	Diaphragm	Cardiovascular	Survival	Other	Reference
Fetus 2:F	p.G176G fsX59 Homozygous	Bilat. An.	Bilateral Agenesis	Bilateral eventration	Absent PA, trunk; VSD	Died 28 wg	Absent pancreas; Duodenal stenosis; IUGR; Dysmorphisms	Golzio et al., 2007
Patient 2; M	p.G13PfsX72; p.W23X	Bilat. An.	Incomplete lung lobation; Diffuse bilateral parenchymal lung disease	Bilateral eventration;	PDA; ASD; Right ventricular hypertrophy	Died Neonatal period	IUGR (twin pregnancy); Hypotonia; Dysmorphisms; Bilateral inguinal hernias; Cryptorchidism; Thin corpus callosum; Small optic nerves	Steiner et al., 2002