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Compound Heterozygosity for Mutations in *PAX6* in Patient with Complex Brain Anomaly, Neonatal Diabetes Mellitus, and Microophthalmia

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Abstract

We report on a patient with trisomy 21, microophthalmia, neonatal diabetes mellitus, hypopituitarism, and a complex structural brain anomaly who was a member of a large bilineal family with eye anomalies. The patient inherited a different mutation in *PAX6* from each parent and is the only known living and second reported patient with compound heterozygosity for mutations in *PAX6*. *PAX6* is a transcription factor involved in eye and brain development, and has roles in pancreatic and pituitary development. Clinical evaluation of the propositus and his parents demonstrated the effects of mutations of differing severity in multiple individuals.

Keywords

PAX6; Microophthalmia; Aniridia; Neonatal Diabetes Mellitus

Introduction

PAX6, located on chromosome 11p13, is a highly evolutionarily conserved transcription factor involved in ocular and neural development [Hanson et al., 1999; Simpson and Price, 2002]. Heterozygous deletions or loss-of-function mutations in *PAX6* can result in ocular anomalies [Jordan et al., 1992; Robinson et al., 2008]. Truncating mutations in the open reading frame are more commonly associated with aniridia; missense mutations occur with variable eye anomalies including keratopathy, congenital optic nerve defects, cataracts, isolated foveal hypoplasia, iris hypoplasia and (rarely) microophthalmia [Tzoulaki et al., 2005].

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PAX6 is expressed in the pancreas, and mutations in *PAX6* may result in abnormal glucose metabolism and defective processing of proinsulin [Wen et al., 2009]. In the human embryo, *PAX6* is expressed in the pituitary gland, and in mice, appears to play a role in pituitary development and function [Terzic and Saraga-Babic, 1999; Kioussi et al., 1999; Bentley et al., 1999]. Finally, heterozygous mutations in *PAX6* may also result in auditory processing deficits related to corpus callosum anomalies [Bamiou et al., 2007].

One individual has previously been described with compound heterozygous nonsense mutations in *PAX6* [Glaser et al., 1994]. That patient, who died at 1 week of age, had anophthalmia with fused eyelids, choanal atresia, microcephaly and a complex brain anomaly described as a large midline cavity with an absent corpus callosum, hypoplastic brainstem, near-absent olfactory bulbs, and polymicrogyria. Facial dysmorphisms included a small, malformed nose, a high-arched palate, and micrognathia. No other organ anomalies were noted on full autopsy, and endocrinologic abnormalities were not described.

We describe a 4-year-old male who additionally had trisomy 21 and who is the second reported patient (and the only to survive the neonatal period) with mutations in both *PAX6* alleles. We also depict a 4-generation pedigree in which there was clinical and molecular evidence that numerous relatives were *PAX6* mutation heterozygotes. This family provides evidence for the wide phenotypic spectrum associated with mutations in *PAX6*. The father's missense mutation resulted in much milder ophthalmologic anomalies than the mother's nonsense mutation, while the presence of both mutations in the propositus (and likely, his deceased brother) resulted in severe ophthalmologic, neurologic, and endocrinologic manifestations, the last of which has not previously been described in a human with two *PAX6* mutations.

Methods

The patient and his parents participated in our comprehensive clinical study on holoprosencephaly and related neurological disorders at the National Human Genome Research Institute, National Institutes of Health. Appropriate consent was obtained for all participants, including for photo publication. Sequence analysis for *SHH*, *ZIC2*, *SIX3*, and *TGIF*, the 4 most common holoprosencephaly-associated genes, was performed by methods previously described [Roessler et al., 1996; Brown et al., 1998; Wallis et al., 1999; Gripp et al., 2000]. Sequence analysis of *PAX6* was performed commercially (GeneDx, Gaithersburg, MD).

Clinical Report

When evaluated by us, the propositus was a 4-year-old Caucasian male with prenatally-diagnosed trisomy 21. He was initially diagnosed as having lobar holoprosencephaly. His complex medical history additionally included bilateral microophthalmia, choanal atresia, severe developmental delay, and renal dysplasia with recurrent urinary tract infections. Evidence for hypopituitarism included central hypothyroidism, secondary adrenal insufficiency, and a history of cryptorchidism and micropenis (now status-post testosterone treatment) making gonadotropin deficiency likely. He had neonatal-onset insulin-dependent diabetes mellitus which was very difficult to control, but abdominal MRI revealed no pancreatic anomalies.

Review of the propositus's brain MRI showed structural abnormalities not consistent with holoprosencephaly because of the lack of hemispheric fusion, but included multiple anomalies including agenesis of the corpus callosum, midline interhemispheric cyst, hypoplastic pons and vermis (absent inferiorly), possible Dandy-Walker malformation, dysplastic tectum, pituitary and hypothalamic hypoplasia, and a globular (though not fused)

basal ganglia. The thalamic nuclei were well-separated by a large third ventricle. Microcephaly and asymmetric microophthalmia were also evident (Fig 1).

On physical examination, the child displayed physical features consistent with his diagnosis of trisomy 21, as well as bilateral severe microophthalmia, extreme microcephaly (head circumference 50th centile for a 1-month-old with Down syndrome), and a smooth philtrum (Fig 1).

The propositus's mother had a history of aniridia, but reported no other medical problems. She had an extensive family history of autosomal dominant aniridia, though no previous genetic study had been initiated (Fig 2). On physical examination, no extraocular anomalies were appreciated. Ophthalmological examination showed bilateral aniridia, glaucoma, and corneal opacifications, as well as a dense cataract in the right eye (Fig 3). While she had not been previously diagnosed with diabetes, she had an elevated fasting glucose on our evaluation.

The propositus's father reported a history of cataracts in early childhood and eventual blindness, as well as hearing loss. He also had an extensive family history of similar visual problems and hearing loss (Fig 2). On examination, he had a high palate and dental crowding in addition to ocular anomalies. Ophthalmological examination showed bilateral microcornea, a right eye cataract, and left aphakia (absent lens) (Fig 3). He also had subtle iris hyoplasia and corectopia.

The propositus's brother, on whom DNA was not available for testing, was described as having very similar structural brain anomalies to the propositus. He additionally had neonatal diabetes mellitus and anophthalmia, and died in infancy.

Molecular Testing

Sequence analysis for mutations in the four most common holoprosencephaly-associated genes (*SHH, ZIC2, SIX3*, and *TGIF*) was negative.

The patient's father had a nonconservative missense mutation (c.112C>T, resulting in p.R38W) in the paired box domain of *PAX6*. This mutation has previously been reported in a patient with microphthalmia and aniridia [Henderson et al., 2007]. This is a highly evolutionarily conserved residue which mediates sequence-specific DNA binding [Xu et al., 1999; Hanson et al., 1999].

The patient's mother had a nonsense mutation (c.718C>T, resulting in p.R240X) in the homeobox domain of *PAX6*. This mutation has previously been reported in a patient with aniridia. The premature insertion of a stop codon in the homeobox domain is predicted to result in nonsense-mediated decay and a consequently functionally null allele [Wilson et al., 1995; Tzoulaki et al., 2007].

The propositus inherited both the maternal and paternal mutations in *PAX6*.

Discussion

This is the second reported and the only known surviving patient with mutations in both *PAX6* alleles. Analysis if the findings provides a unique example of the phenotypic effects of compound heterozygosity of mutations of *PAX6*. The propositus's hypopituitarism, diabetes mellitus, and brain and ophthalmologic anomalies can all be explained by the *PAX6* mutations. The mutations also explain the ophthalmologic phenotype in his parents, and additionally may explain impaired glucose tolerance in the propositus's mother.

The previously reported patient, who had a nonsense mutation in each *PAX6* allele, survived to the eighth day of life [Glaser et al., 1994]. The propositus's brother, who was described as having near-identical brain and ophthalmologic anomalies as the propositus, died in early infancy, and was not available for genetic testing. However, given the similar phenotypes and the 25% chance that the parents would give birth to a child with both mutations, it is likely that the deceased sibling inherited both mutant alleles. The morbidity associated with inheriting two mutations and the fact that the propositus additionally had trisomy 21 makes his survival and relative good health an especially rare event.

Finally, this case highlights the importance of a multidisciplinary approach which includes the evaluation of multiple family members in the diagnosis of unusual and complex patients.

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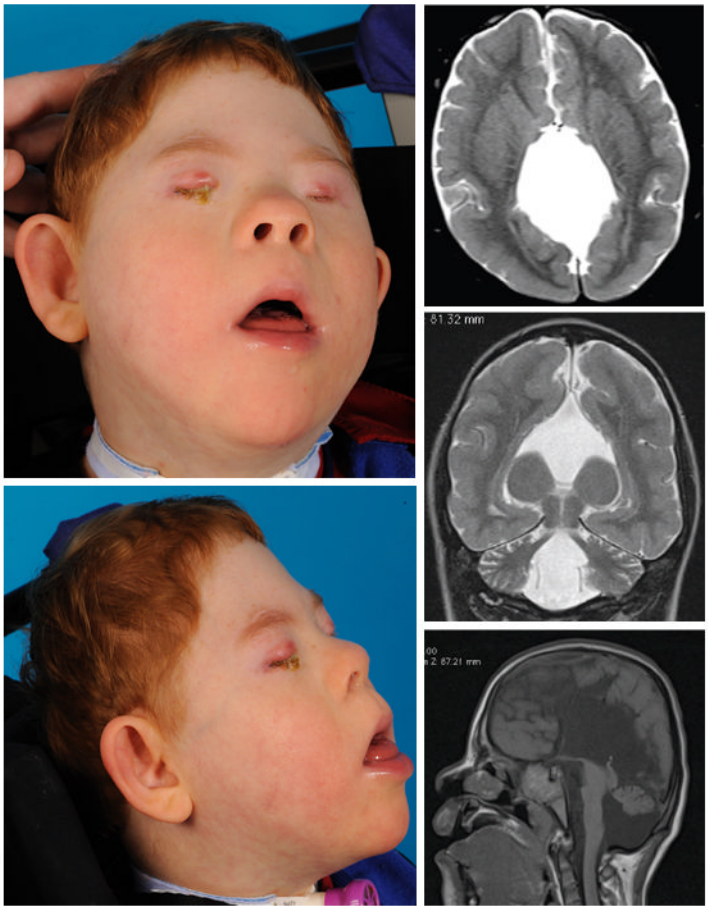

Figure 1.
Propositus with compound heterozygosity for mutations in *PAX6*. Facial features were consistent with trisomy 21, and were also notable for extreme microcephaly, microophthalmia, and a smooth philtrum. Axial, coronal, and sagittal (from top) brain MRI demonstrates complex structural brain anomaly in propositus.

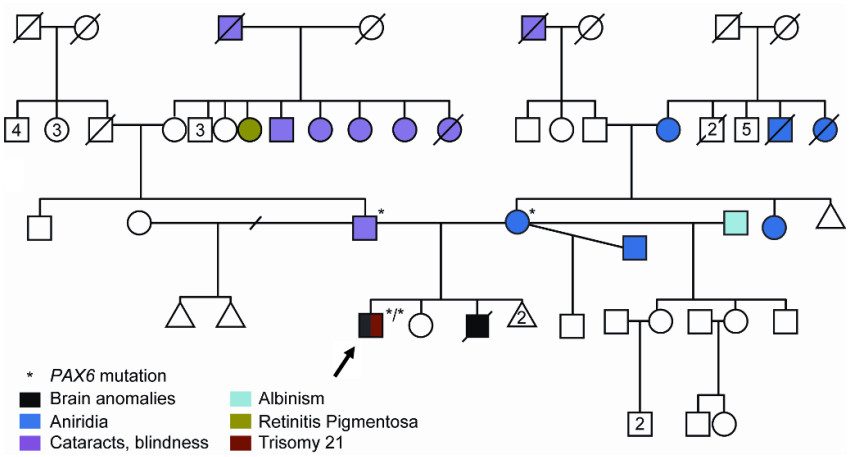

Figure 2.
Family pedigree.

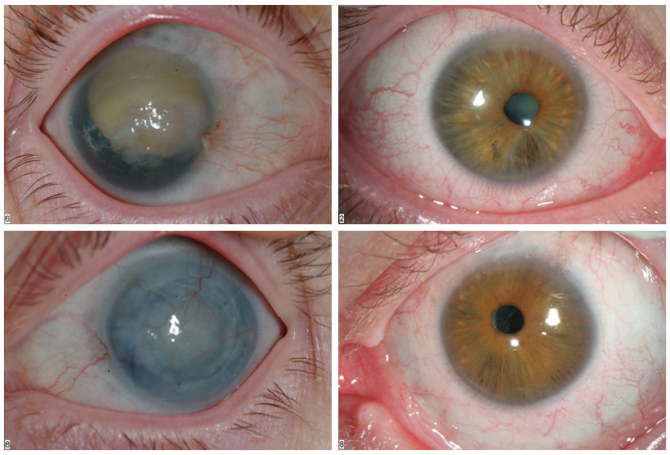

Figure 3.
Photos of parents' ocular findings (mother on left, father on right).