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Immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX): report of the first prenatal mutation testing

Immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX, MIM 304930) is a rare X-linked recessive disorder of immune regulation, characterized by enteropathy, eczema, anemia, thrombocytopenia and the involvement of the endocrine system, such as insulin-dependent diabetes mellitus (IDDM) and thyroiditis (Bennett and Ochs, 2001). IPEX is usually lethal in males during infancy or childhood. The carrier females are normal. *FOXP3*, the causative gene for IPEX, codes for scurfin, a 431-amino acid protein, member of the forkhead family of transcriptional regulators, which acts as a repressor of transcription, and is specifically expressed in CD25⁺ CD4⁺ T cells in the thymus and periphery (Khattari *et al.*, 2001, Ramsdell and Ziegler, 2003). *FOXP3* is made of 11 exons and is characterized by three functional motifs: a C2H2 zinc-finger domain, a leucine zipper (Zip) motif and a forkhead DNA-binding domain spanning exons 9, 10 and part of 11 (amino acids 337–420). This motif was originally identified in *Drosophila* and contains many of the sequence features important for mediating protein–DNA contacts (Bennett *et al.*, 2001).

Up to now, 14 mutations have been described that are associated with IPEX. (Bennett *et al.*, 2001; Kobayashi *et al.*, 2001; Wildin *et al.*, 2001, 2002; Gambineri *et al.*, 2003; Owen *et al.*, 2003).

In 2001, we collaborated on the first report of *FOXP3* mutations in IPEX (Wildin *et al.*, 2001), in the case of two male twins, who had died from a syndromic form resembling IPEX in their first months of life and whose family came to us for genetic counseling in 1999. They belonged to a large Italian family (Figure 1, family tree), in which, on the maternal side, three male babies were found to have shown severe dermatitis and died of 'diarrhea' in their very early infancy. After the death of the affected twins, the mother (IV2) and her sister (IV4) came to us for consultation, asking about their reproductive risk and the possibility for prenatal diagnosis. All the clinical symptoms were strongly suggestive of IPEX, but at that time, as the causative role of *FOXP3* was unknown, no direct genetic tests were available to confirm the diagnosis, to assess the carrier status in a female and to be used for prenatal

diagnosis. As autopsy specimens of the affected twins had been collected, DNA could be obtained, and linkage analysis using three microsatellite markers (DXS8083, DXS1003 and DXS1208) segregating with the syndrome was performed in many members of the family. The two sisters (V2 and V4) were found to be carriers of the disease-linked haplotype; they were informed about the reliability of prenatal diagnosis by linkage analysis and, as the death of the twins had been a devastating experience for them, they had decided not to become pregnant. As soon as the role of the *FOXP3* gene in IPEX etiology became clear, mutation analysis was performed in the DNA samples of the twins (Wildin *et al.*, 2001). In exon 11 of the gene, at position 1150, an A → G substitution was found that transforms the amino acid alanine at position 384 into threonine (A384T). This mutation falls within the forkhead-winged-helix domain of scurfin, disrupting the DNA-binding properties of the protein. It was therefore possible to confirm the carrier status of the mother and her sister and also to offer them a safe test for prenatal diagnosis. When V4 became pregnant, at the 12th week of gestation a chorionic villi sample was analyzed for sex by karyotype and the DNA was extracted by the phenol–chloroform method. Although the fetus turned out to be a female, the parents asked for the A384T mutation analysis.

We were aware that genetic testing on a female fetus was ethically questionable; nevertheless, we decided to perform the analysis, mainly for psychological reasons. The family members, after the devastating experience of the death of the twins, were very concerned with the scientific issues regarding IPEX and were also very cooperative, allowing the autopsy specimens of the twins to be used for research. *FOXP3* exon 11 was then amplified by specific primers, sequenced (forward and reverse) by the Big Dye Terminator 1.1 kit and the sequence products run and analyzed on an ABI Prism 377 sequencer (Figure 2). A panel of microsatellite markers was used to rule out any maternal contamination of the fetal sample. The female fetus was heterozygous for the A384T mutation. The pregnancy was continued and the baby is now 11 months and in good health.

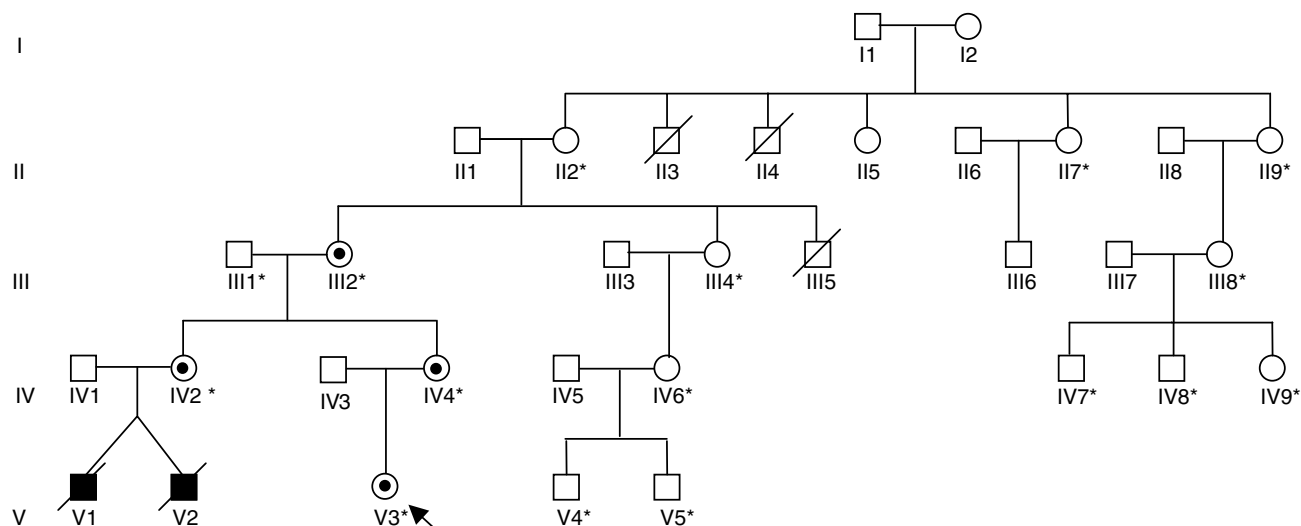


Figure 1—□, ○ Normal subjects, ■ affected male, ● carrier female, * subjects tested for the mutation

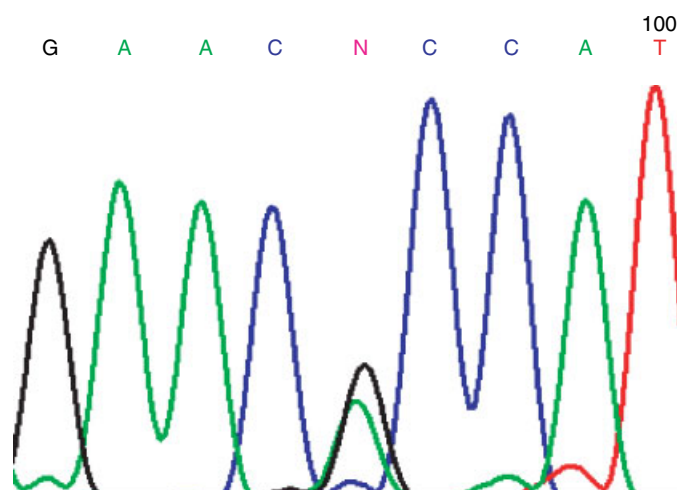


Figure 2—Electropherogram showing heterozygosity for the A384T mutation in the female fetus

Prenatal diagnosis of IPEX by linkage analysis has previously been reported (Bakke *et al.*, 2004). This is the first report of prenatal mutation testing. The possibility of detecting the mutations causative of this rare syndrome allows the correct diagnosis both in the prenatal and postnatal period, the detection of the carrier females and a proper genetic counseling for the families at risk.

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Prenatal sonography and magnetic resonance imaging of pulmonary sequestration associated with a gastric duplication cyst

A 34-year-old woman, gravida 1, para 0, was referred at 30 gestational weeks for evaluation of a solid lung lesion and an intra-abdominal cystic mass in the fetus. Level II ultrasound demonstrated a 17.1×28 mm well-circumscribed echogenic mass, lobar in shape, at the base of the left side of the fetal chest and an 8×4 mm noncommunicating subdiaphragmatic gastric duplication cyst near the cardia of the stomach (Figure 1). The color Doppler visualization of a systemic artery arising from the aorta feeding the lung lesion strongly favored the diagnosis of pulmonary sequestration. Ultrafast magnetic resonance imaging (MRI) scans at 32 gestational weeks depicted the detailed structure of the pulmonary sequestration and the noncommunicating gastric duplication cyst (Figure 2). At 39 gestational weeks, a 3530-g female baby was delivered uneventfully with Apgar scores of 9 and 10 at 1 min and 5 min, respectively. Her karyotype was 46,XX. Postnatal ultrasound and computed tomography scans with and without enhancement confirmed the prenatal diagnosis.

The present case represents the first report of prenatal diagnosis of concomitant pulmonary sequestration and gastric duplication by ultrasound and by MRI. Pulmonary sequestration has been found in as many as 23% of prenatally detected pulmonary lesions (Adzick *et al.*, 1998) and accounts for about 1.1–1.8% of all pulmonary resections (Carter, 1969). The gastric duplication cyst is the least common of all gastrointestinal duplications, representing only 4% of all enterogenous duplications (Pruksapong *et al.*, 1979). Prenatal sonographic diagnosis of either pulmonary sequestration (Slotnick *et al.*, 1990; Dolkart *et al.*, 1992; White *et al.*, 1994; Becmeur *et al.*, 1998) or gastric duplication (Bidwell and Nelson, 1986; Goyert *et al.*, 1991; Özmen *et al.*, 1997) has been well described. Currently, MRI has been increasingly used as a helpful supplement to sonography in prenatal imaging of fetuses with pulmonary sequestration (Hubbard *et al.*, 1999; Dhingra *et al.*, 2003) or gastric

duplication (Levine *et al.*, 1999; Granata *et al.*, 2003).

The concomitant occurrence of both pulmonary sequestration and gastric duplication is very unusual. To our knowledge, only six cases, all diagnosed postnatally, have been reported previously (Mahour *et al.*, 1971; McClelland *et al.*, 1977; Thornhill *et al.*, 1982; Richards *et al.*, 1996; Hadley and Egner, 2001; Saggese *et al.*, 2002). Approximately 50–60% of cases with extralobar pulmonary sequestration are associated with other developmental anomalies, whereas only 14% of cases with intralobar pulmonary sequestration have other anomalies (Savic *et al.*, 1979). The reported associated anomalies include connection with the foregut, foregut duplication or diverticulum, gastric duplication cyst, esophageal diverticulum, anomalous pulmonary venous return, pulmonary agenesis, eventration of the diaphragm, diaphragmatic hernia, ectopic pancreatic tissue, congenital absence of the pericardium, arteriovenous fistula, double ureter, cervical vertebral malformation, bronchogenic cleft cyst, absence of the mediastinal pleura, basilar artery aneurysm, horseshoe kidney, hydroureters, congenital megacolon, and esophageal bronchial fistula (McClelland *et al.*, 1977; Savic *et al.*, 1979). Among these, diaphragmatic hernia anomalies, other pulmonary anomalies, and cardiac malformations are common associated anomalies.

In conclusion, we have presented the prenatal sonography and MRI of pulmonary sequestration associated with a gastric duplication cyst. We suggest that prenatal identification of pulmonary sequestration should prompt a careful investigation of associated fetal structural anomalies.

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