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Congenital Diaphragmatic Hernia and Microtia in a Newborn With Mycophenolate Mofetil (MMF) Exposure: Phenocopy for Fryns Syndrome or Broad Spectrum of Teratogenic Effects?

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A newborn female infant born to a woman on immunosuppressive medications including mycophenolate mofetil (MMF) for a renal graft secondary to lupus nephritis presented with congenital diaphragmatic hernia (CDH) and additional findings of microtia, esophageal atresia with tracheoesophageal fistula, cleft palate, congenital heart defect, digital anomalies, and dysmorphic facial features. Pulmonary hypoplasia resulted in death at day 2 of life. She was presumed to have Fryns syndrome based on diagnostic criteria established for this recessive disorder with prominent features including CDH, facial anomalies, and nail hypoplasia. In retrospect, this infant's findings are more likely the result of teratogenic exposure to MMF, as more recent data have emerged linking aural atresia, digital anomalies, and dysmorphic features to this drug. To date, this is the only human report of CDH in an infant with prenatal exposure to MMF, although the manufacturer's package insert alludes to animal studies with a broad spectrum of malformations, including CDH. Thus, a teratogenic exposure can mimic a known Mendelian genetic syndrome, and caution is urged in presuming a genetic etiology for infants with potential teratogenic exposure to relatively new drugs with limited published animal data.

Key words: mycophenolate mofetil; teratogen; Fryns syndrome; congenital diaphragmatic hernia; microtia; embryopathy

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INTRODUCTION

Mycophenolate mofetil (MMF) or CellCept® is an immunosuppressive drug developed in the mid-1990s that acts as a reversible inhibitor of inosine monophosphate dehydrogenase, thereby blocking de novo purine synthesis in lymphocytes [Sifontis et al., 2006]. Its use has largely replaced that of azathioprine in transplant recipients because of improved selectivity [Armenti et al., 2002]. Initially classified as a Pregnancy Category C drug (risk of fetal harm cannot be ruled out), the data regarding reproductive toxicity were limited until recently [Tendron et al., 2002; Armenti et al., 2003; Sifontis et al., 2006]. A number of publications have suggested that potential teratogenic effects include ear anomalies, cleft lip and/or palate, congenital heart defects, esophageal atresia, digital/nail hypoplasia, and facial dysmorphic features [Pergola et al., 2001; Le Ray et al., 2004; Kallen et al., 2005]. Similar findings can be seen in autosomal recessive Fryns syndrome, which often includes nail/distal digital hypoplasia, craniofacial dysmorphism, oral clefts, pulmonary hypoplasia, and polyhydramnios, in addition to the most prevalent anomaly, congenital diaphragmatic hernia (CDH) [Fryns et al., 1979; Slavotinek, 2004].

We describe a patient born after exposure to MMF with CDH and additional features of tracheoesophageal fistula, cleft palate,

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congenital heart defects, microtia, and hypoplastic toenails. Because she was born before an association between antenatal MMF exposure and congenital anomalies had been well established, the initial diagnosis was one of Fryns syndrome. We provide details of the case to illustrate that the effects of teratogens can mimic genetic syndromes, particularly when the data regarding embryopathy are limited.

MATERIALS AND METHODS

Clinical Report

This female infant was born at 35 weeks gestation to a 36-year-old Gravida 3, Para 1-2, SAB 1 mother and unrelated 40-year-old father. The pregnancy was complicated by the finding of a left large CDH at 27 weeks gestation with herniation of the stomach, intestinal contents, and liver into the left chest and displacement of the heart into the right chest. Polyhydramnios was present and her mother underwent six amnioreduction procedures in the third trimester. Amniocentesis revealed normal 46,XX female karyotype at the 400–450 band level without any aneuploidies by interphase FISH analysis. The mother had required immunosuppression for a cadaveric kidney transplant performed 3 years prior to delivery for lupus nephritis. She had been diagnosed with lupus/mixed connective tissue disorder at age 15 years of age. Oral antirejection medications included tacrolimus (3 mg in the morning and 2 mg at night), prednisone (5 mg daily), and MMF (250 mg twice daily). Additional medications during pregnancy included Norvasc, metoprolol, and Lasix for hypertension, as well as Epogen and prenatal vitamins. She took acyclovir in the first month for shingles. A renal biopsy 4 days prior to delivery for increased creatinine and hydronephrosis showed acute tubular injury without signs of rejection or evidence of lupus nephritis.

Family history was unremarkable. Parents were of mixed European, Eastern European and Mexican descent with no evidence of consanguinity. The couple had an early first trimester miscarriage and one healthy daughter born prior to the mother's renal transplant.

Birth was by spontaneous vaginal delivery with a birth weight of 2,163 g (10th centile). Head circumference was 32.3 cm (50–90th centiles). At birth, she was noted to be cyanotic but vigorous. She was difficult to intubate because of a cleft palate. Apgars were 4, 5, and 7 at 1, 5, and 10 min. In addition to a diaphragmatic hernia (Fig. 1D) and cleft palate, other anomalies included wide-spaced eyes with facial coarseness, low-set malformed ears without external auditory canals (Fig. 1A), a short webbed neck, and esophageal atresia with distal tracheoesophageal fistula (Fig. 1B). Echocardiogram suggested a VSD and possible coarctation of the aorta. Chest X-ray revealed bifid thoracic vertebra at T6 and T7 (Fig. 1C). There was a 2-vessel umbilical cord. Extremity examination showed short thumbs and fifth fingers with hypoplastic toenails (Fig. 1E,F).

She required increased ventilatory support with poor left-sided aeration secondary to the CDH. Because of the distal tracheoesophageal fistula, gas accumulated in the herniated abdominal contents, further compromising pulmonary function. She developed pulmonary hypertension. Findings on abdominal ultrasound included a dilated right kidney suggesting reflux and a large left extrarenal pelvis. The cranial ultrasound was normal. After discussion with the parents, supportive care was withdrawn, and the infant expired the day after birth.

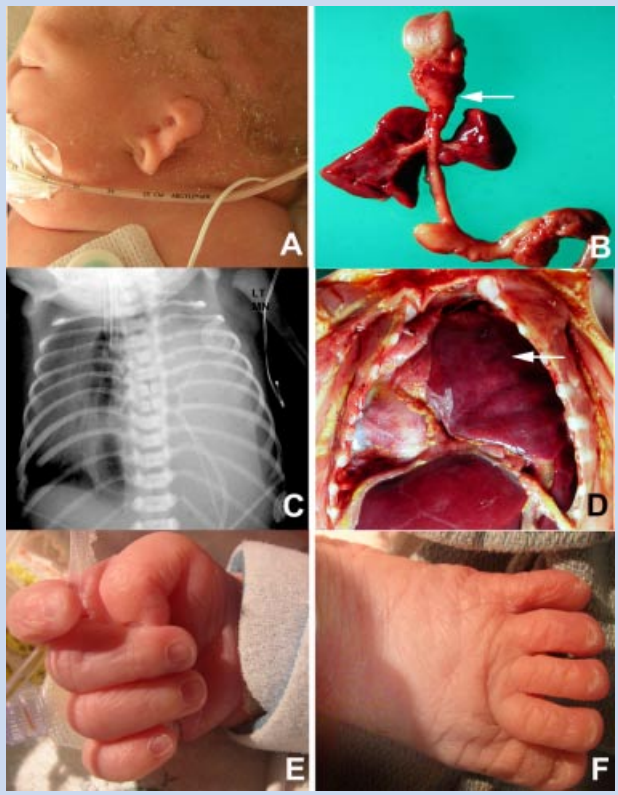

FIG. 1. A: Aural atresia with low-set, posteriorly rotated left ear and absent auditory canal. Short neck is also visible. B: Bilateral pulmonary hypoplasia worse on the left than the right accompanies esophageal atresia. The fistula is not well seen in this picture. The arrow is at the point of esophagus termination. C: Chest X-ray showing bifid T6 and T7 vertebral bodies. D: Open thorax and abdomen revealing the left chest to be occupied by the abdominal contents, with liver indicated by arrow. E: Right hand with short thumb and 5th digit. F: Left foot with hypoplastic toenails.

Postmortem Findings

On autopsy examination, the left-sided CDH was typical of those seen in sporadic cases, that is, a Bochdalek hernia. The CDH allowed an enlarged liver, stomach, entire small bowel, spleen, and one-third of the colon to occupy the left pleural cavity, resulting in hypoplasia of both lungs with the left being 10% of expected weight and the right 25% of expected weight (Fig. 1D). The heart was also shifted to the right and had a mildly hypoplastic (2/3 size) left ventricle with a small secundum atrial septal defect but no VSD or coarctation. There was absence of the brachiocephalic trunk, and the right carotid artery branched directly off the aortic arch. An adherent right subclavian artery had a retroesophageal path. The right umbilical artery was absent. The esophagus was atretic after 2.5 cm, and a tracheoesophageal fistula was present at the level of the

carina (Fig. 1B). Neuropathology showed, in addition to small hemorrhages in the subarachnoid space and a germinal matrix hemorrhage, small frontal lobes without other malformations or dysplasia. Although the bladder was enlarged and the renal pelves and ureters were dilated, no definite obstruction was seen to the outlet of the bladder, and renal dysplasia was lacking. The vertebra at T6 and T7 were bifid. The histology was noncontributory.

Craniofacial anomalies included ocular hypertelorism, broad nasal bridge, retrognathia, and short webbed neck. The auditory canals were atretic bilaterally with external ears ending in blind pits. The irises were hypopigmented. The palate was completely clefted in the midline exposing the nasal pharynx. In addition, the nipples were indistinct.

Laboratory Studies

Normal cytogenetic studies included a peripheral blood karyotype at the 600 band level, fluorescent in situ hybridization (FISH) for 22q11.2 deletion (velocardiofacial syndrome), and subtelomeric FISH. Her skin karyotype was normal at the 400–450 band level, without evidence of tetrasomy 12p. A research-based microarray comparative genomic hybridization (array CGH) study using the 100,000 single nucleotide polymorphism (SNP) platform (50K XbaI and 50K HindIII; Affymetrix; Santa Clara, CA) was performed after informed consent. Array data were analyzed using cut-off values of 20 or more consecutive SNPs together with a p value of $<10^{-8}$ for significance for possible copy number variations (CNVs) [Baross et al., 2007], and showed no copy number alterations consistent with deletions or duplications. An abnormality consistent with a small chromosome 12p11.21–12p11.22 duplication in a region with known CNVs was identified when lower stringency selection criteria were used, but the region is not specifically known to be associated with CDH [Holder et al., 2007] or other birth defects, although it is contained within the much larger 12p aberrations seen in Pallister–Killian syndrome. Parental studies were not undertaken in this family because of the lack of a significant *P*-value for this alteration.

DISCUSSION

At the time of initial evaluation of this newborn, a genetic syndrome was considered the most likely cause of her multiple malformations. While the changes associated with the CDH were indistinguishable from sporadic diaphragmatic hernia, the other anomalies, both internal and external, suggested Fryns syndrome [Fryns et al., 1979; Fryns, 1987]. In a review of 52 cases of Fryns syndrome, the most common manifestations were CDH, usually left-sided (96.1%), pulmonary hypoplasia (65.4%), nail hypoplasia (59.6%), brachyphalangy (49.6%), polyhydramnios (55.8%), anomalous ears (55.8%), cleft palate (50.0%), and VSD (40.4%) [Slavotinek, 2004]. Her craniofacial findings, particularly ocular hypertelorism and retrognathia, were suggestive of Fryns syndrome and are each seen in 36.5% of children with this diagnosis. The only finding typical of Fryns syndrome that she lacked was marked hypoplasia of the distal phalanges of the fingers. Although tracheoesophageal fistula is not a common finding in Fryns syndrome, it has been described in a few children with this diagnosis [Ayme et al., 1989; Slavotinek, 2004; Slavotinek et al., 2005]. In this patient, no significant genetic abnormalities were detected, including evaluations for chromosomal aberrations such as tetrasomy 12p (Pallister–Killian) which shows some overlap with Fryns syndrome [Enns et al., 1998], and research array CGH studies. Because the mortality in Fryns syndrome is so high secondary to pulmonary hypoplasia, and since those children who have survived beyond infancy have usually exhibited severe mental retardation [Slavotinek, 2004], the family chose to withdraw support. The parents were counseled regarding a probable 25% chance of recurrence in a subsequent pregnancy based on the autosomal recessive inheritance of Fryns syndrome.

At the time that this child was born in early 2005, there was limited information about the teratogenic risks of prenatal exposure to MMF. Some series reported normal outcomes of prenatal exposure to MMF, with no malformations identified in 15 infants whose mothers took the drug during pregnancy [Tendron et al., 2002]. However, unpublished studies on pregnant rats and rabbits suggested that there might be an increased risk of miscarriage and defects of the head and eyes in rodent fetuses exposed to doses equivalent to those used in humans [Sifontis et al., 2006; Mycophenolate mofetil package insert, 2008]. One case report described a child with a vascular abnormality, hypoplastic nails and short fifth fingers born to a woman who had a renal transplant at 6 weeks of pregnancy and was treated with MMF [Pergola et al., 2001]. Another early report described a fetus exposed to MMF during the first 13 weeks of gestation born with agenesis of the corpus callosum, cleft lip and palate, micrognathia, ocular hypertelorism, microtia with external auditory canal atresia, and a left pelvic ectopic kidney [Le Ray et al., 2004]. In the past several years, more publications have outlined the prenatal effects of MMF, with a recurring pattern of malformations including microtia or anotia, cleft lip and/or palate, congenital heart defects, facial anomalies including ocular hypertelorism and micrognathia, and rarely, digital hypoplasia [Pergola et al., 2001; Le Ray et al., 2004; Sifontis et al., 2006; Tjeertes et al., 2007; Ang et al., 2008; Carey, 2008; Perez-Aytes et al., 2008]. Many of these features overlap with those identified in our patient; in particular, the ear anomalies in our patient are very similar to those in recent case reports of MMF exposure [Ang et al., 2008; Perez-Aytes et al., 2008] and in retrospect, are more severe than typically described in Fryns syndrome. Based on the limited information available in 2005, the family was counseled that there was a possibility that her birth defects were related to her exposure to MMF during the pregnancy, and the family was referred to the National Transplantation Pregnancy Registry (NTPR). In fact, this case has been published in brief form in 2006 [Sifontis et al., 2006]. Thus far, this is the only published human occurrence of CDH in prenatal MMF exposure. Other novel findings in this case include vertebral clefts and complex vascular anomalies. The more extensive phenotype in this patient than that typically seen in MMF-exposed fetuses may suggest that multiple drug exposures, or a combination of genetic and environmental factors, contributed to the many malformations. Although data suggest that tacrolimus and prednisone are not associated with significant risks of birth defects in an exposed fetus [Mastrobattista and Katz, 2004], her mother was taking other immunosuppressive and antihypertensive medications, so an additive effect is possible.

A mechanism for the development of CDH in MMF exposure has not been proposed. One hypothesis is that MMF may have similar developmental effects as the gene defects that cause Fryns syndrome, thus resulting in similar birth defects. However, it is difficult to hypothesize how a drug that impairs purine metabolism in lymphocytes could produce such a broad spectrum of birth defects. Unfortunately, there are no published studies describing the effects of prenatal MMF exposure in animal models. The drug package insert states that at doses equivalent to those used in humans, there were increased rates of fetal loss and malformations; specifically, "in rat offspring, malformations included anophthalmia, agnathia, and hydrocephaly. In rabbit offspring, malformations included ectopia cordis, ectopic kidneys, *diaphragmatic hernia*, and umbilical hernia" (p. 16) [Mycophenolate mofetil package insert, 2008] (emphasis added by author). Of note, in October 2007, the Food and Drug Administration and the drug manufacturer revised the pregnancy category of MMF to a Pregnancy Category D drug (positive evidence of fetal risk), acknowledging the increased risk of first trimester pregnancy loss and congenital malformations [Ang et al., 2008].

Although a child born with multiple congenital anomalies may have findings similar to those of a known genetic syndrome, careful review of pregnancy exposures, particularly drugs with an unknown or incompletely determined teratogenic spectrum, may suggest an alternative etiology with markedly different recurrence risks. In this patient, the features suggestive of Fryns syndrome are more likely, in retrospect, due to prenatal MMF exposure. The findings in this patient suggest that MMF exposure can produce a phenocopy of a genetic syndrome, Fryns syndrome. Further information about the mechanism of action of MMF may yield clues as to the etiology of CDH and, potentially, genetic causes of Fryns syndrome.

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