Journal of Perinatology (2014) 34, 948–950

www.nature.com/jp

PERINATAL/NEONATAL CASE PRESENTATION

Clinical and genetic complexity of Mitchell–Riley/ Martinez–Frias syndrome

L Cruz[1], RE Schnur[2], EM Post[3], H Bodagala[1], R Ahmed[4], C Smith[5], LB Lulis[2], GE Stahl[6] and A Kushnir[6]

Mitchell–Riley syndrome/Martinez–Frias syndrome (MRS/MFS) is a rare, autosomal recessive disorder with multisystem involvement and poor prognosis. Most reported cases have been associated with homozygous or compound heterozygous mutations in the *RFX6* gene, a transcriptional regulatory factor for pancreatic morphogenesis. Given the limited number of reported cases, the syndrome may be under-recognized. When the particular phenotype of MFS includes a mutation on the *RFX6* gene and neonatal diabetes, it has been called Mitchell–Riley syndrome. Because of this, we propose that MFS/MRS is a symptom continuum or an *RFX6* malformation complex. We report an infant with all of the key clinical features of MRS/MFS without a definable mutation in *RFX6* gene, supporting the consideration of these features as a symptom complex, and raising the question of genetic heterogeneity.

Journal of Perinatology (2014) **34,** 948–950; doi:10.1038/jp.2014.162

INTRODUCTION

The combination of pancreatic hypoplasia, intestinal atresias, extrahepatic biliary aplasia or hypoplasia, with or without tracheoesophageal (TE) fistula has been reported as Martinez–Frias syndrome (MFS). Martinez and Frias described these features in siblings conceived by consanguineous parents,[1] while Anneren[2] and Mitchell[3] suggested that the syndrome was associated with autosomal recessive inheritance. Newer case reports referred to patients with features of MFS, neonatal diabetes and presence of *RFX6* mutation, but lacking TE fistula as having Mitchel–Riley syndrome (MRS).[4,5] Another emerging key feature is the presentation of neonatal hemochromatosis, as described by Martinovici *et al* [6] in 2010. Thus, there appears to be a continuum of symptoms between these two syndromes, suggesting MFS/MRS or an *RFX6* malformation complex. We present a case of an infant with congenital duodenal atresia, pancreatic insufficiency, neonatal diabetes, anemia in the setting of iron overload, and hepatic siderosis.

CASE

The patient was a product of *in vitro* fertilization, diagnosed with duodenal atresia on a 21-week prenatal ultrasound with normal chromosomes and microarray by amniocentesis. He was born at 37 5/7 weeks by vaginal delivery, APGARs of 9 and 9 and birth weight of 1752 g, to a 28-year-old G3P0020 mother with history of two miscarriages. The patient underwent successful surgical repair of duodenal atresia on day of life (DOL)#2. Hours after surgery he was found to be hyperglycemic while receiving parenteral nutrition with a glucose infusion rate of 4.8 mg kg^{-1} min^{-1}. Regular insulin drip was started on DOL#3, with brief transition to diluted glargine insulin[7] on DOL#14. Glargine did not provide adequate glucose control and regular insulin infusion was reinitiated within 8 h.[4] Enteral breast milk intake temporarily normalized glucose levels with periods of hypoglycemia (Table 1). Suspicion for neonatal diabetes arose and was supported with low C-peptide and a normal cortisol level (Table 1). The unusual presentation of both hyper- and hypoglycemia was in direct relation to parenteral nutrition versus enteral intake, respectively. Neonatal diabetes has been previously reported as a part of MRS/MFS, however, glucose fluctuation is a new and possibly unrecognized aspect of this disorder. The patient's newborn screen did not reveal any abnormality related to glucose or galactose regulation.

The patient manifested inadequate weight gain throughout the hospital course. Fat malabsorption and glucagon deficiency due to pancreatic insufficiency were hypothesized, as well as limited glycogenolysis due to hepatic dysfunction. Hepatic dysfunction was seen throughout the hospital course, as evidenced by elevated coagulation profile, elevation in gamma-glutamyl transpeptidase (GGT) and persistent cholestasis (Table 1). In MRS/MFS, the pancreas can be structurally normal with pancreatic dysfunction manifested by neonatal diabetes, or have anomalous structure with either hypoplastic or annular pancreas.[5] Our patient's pancreatic insufficiency manifested with loose, watery and pale colored stools, despite a structurally normal pancreas on abdominal ultrasound and as seen during duodenal atresia repair. The abnormal stooling pattern was most evident once the patient was tolerating full enteral intake. By the 3rd week of life, weight loss was observed with both enteral and parenteral nutrition. Medium chain triglyceride oil and pancreatic enzymes were added to compensate for presumed liver and/or pancreatic dysfunction, with minimal response. There were two state newborn screens which were abnormal for cystic fibrosis, with very elevated trypsinogen and normal sequencing of the cystic fibrosis transmembrane conductance regulator *(CFTR)* gene (Table 1), supporting the diagnosis of pancreatic insufficiency.

[1]Department of Pediatrics, Cooper University Health Care, Cooper Medical School of Rowan University, Camden, NJ, USA; [2]Division of Genetics, Cooper University Health Care, Cooper Medical School of Rowan University, Camden, NJ, USA; [3]Division of Pediatric Endocrinology, Cooper University Health Care, Cooper Medical School of Rowan University, Camden, NJ, USA; [4]Division of Pediatric Hematology, Cooper University Health Care, Cooper Medical School of Rowan University, Camden, NJ, USA; [5]Department of Clinical Pharmacology, Cooper University Health Care, Camden, NJ, USA and [6]Division of Neonatology, Cooper University Health Care, Cooper Medical School of Rowan University, Camden, NJ, USA. Correspondence: Dr A Kushnir, Division of Neonatology, Cooper University Health Care, Cooper Medical School of Rowan University, 1 Cooper Plaza, Dorrance 755, Camden, NJ 08103, USA.
E-mail: kushnir-alla@cooperhealth.edu
Received 19 March 2014; revised 15 July 2014; accepted 28 July 2014

Table 1. Clinical features and laboratory data

Medical problems	*Laboratory data*	*Imaging data*	*Medications*
Small for gestational age Duodenal atresia Failure to thrive	Newborn screen: abnormal for CF, normal sequencing of CTFR gene SNP chromosome microarray: normal	'Double bubble' on prenatal ultrasound	
Anemia	Ferritin: 852–966 ng ml^{-1} Hemoglobin: 8.1–11.9 g dl^{-1} MCV: 83.5–109.3 fl MCH: 30.0–36.5 p g Reticulocyte: 0.5–4.9% Total bilirubin: 3.3–16.2 mg dl^{-1}	MRI of abdomen with moderate to severe iron depositions in liver	
Hepatic iron deposition	Direct bilirubin: 0.6–8.3 mg dl^{-1} Prothrombin Time 14.5 s PTT 37.0 s INR 1.3 AST 19–31 U l^{-1} ALT 5–45 U l^{-1} GGT 76–134 U l^{-1}	MRI of abdomen with moderate to severe iron depositions in liver	
Neonatal diabetes Glucose dysregulation	Glucose: 38–425 mg dl^{-1} C-peptide: 0.12 ng ml^{-1} Cortisol: 5.9 ug dl^{-1}		Regular insulin 0.01–0.1 unit kg^{-1} h^{-1} (average 0.01–0.03 unit kg^{-1} h^{-1}) Glargine 0.3 units (0.15 units kg^{-1} per dose subcutaneously every12 h)
Pancreatic insufficiency Chronic diarrhea	Fecal fat: >100 FAD/High Power Field Pancreatic elastase: < 15 mcg g^{-1} Trypsonigen 184–357	Normal pancreas on postnatal ultrasound	Medium chain triglyceride oil (6 ml every 2 h as needed) Creon (0.25 capsules 4 times daily) Omega-3 Fish Oil (0.1 g kg^{-1} every 12 h) Multivitamins A, D, E, K (1 ml daily)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; GGT, gamma-glutamyl transferase; INR, international normalized ratio; PTT, partial thromboplastin time; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; MRI, magnetic resonance imaging; SNP, single nucleotide polymorphism.

Table 2. Literature review of clinical manifestations of MRS/MFS

Clinical features	*Martinez–Frias syndrome*	*Mitchell–Riley syndrome*	*Our patient*
Pancreatic anomaly or dysfuction	Yes[1,2]	Yes[3,5,6,9,11,14]	Yes
Duodenal atresia	Yes[1,2]	Yes[3,5,6,9,11,14]	Yes
Abnormal biliary tract	Yes[1,2,15,]	Yes[7,9]	No
Tracheoesophageal fistula	Yes[1,15]	No	No
Hypospadius	Yes[1,15]	No	No
Chronic diarrhea	None reported	Yes[3,11]	Yes
Anemia	No	Yes[6,11]	Yes
Siderosis	No	Yes[6,11]	Yes
Neonatal diabetes	No	Yes[3,5,6 9,11,14]	Yes
Insulin therapy	No	Yes[3,5,6,9,11,14]	Yes
RFX6 mutation	No	Yes[3,5,6,9,11,14]	No
Hepatic siderosis	Yes[6]	None reported	Yes
Abnormal newborn screen for cystic fibrosis	None reported	None reported	Yes
Glucose dysregulation	No	No	Yes
Dates of the published findings	1992–1999	2004–2014	2013–2014

Abbreviations: MFS, Martinez–Frias syndrome; MRS, Mitchell–Riley syndrome.

In the first week of life, the patient required two transfusions with packed red blood cells for anemia. Cholestasis, abnormal liver function, anemia, elevated ferritin level and suspicion of MRS/MFS (Table 2) resulted in evaluation for hemochromatosis in the 3rd week of life. Magnetic resonance imaging (MRI) of the abdomen demonstrated moderate to severe deposition of iron in the liver (Figure 1), concerning for neonatal hemochromatosis. A salivary

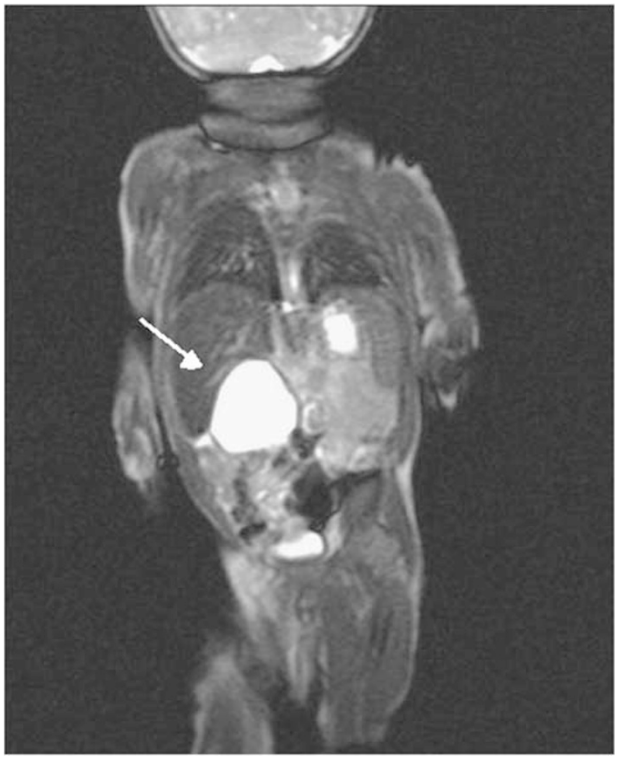

Figure 1. Magnetic resonance imaging with evidence of iron deposition in the liver. Liver measures 5.3 cm in height. There is no intrahepatic duct dilatation. The liver iron concentration is read as consistent with moderate to severe iron deposition and was based on the standards established by Yves Gandon at the University of Rennes, France.

gland biopsy to assess for extrahepatic siderosis using silver stain was done and was negative.[8,9] The combination of liver disease and extrahepatic siderosis is pathognomonic for neonatal hemochromatosis and can be considered diagnostic of neonatal hemochromatosis when seen together.[10] In our patient there was no evidence of iron deposition outside of the liver and thus neonatal hemochromatosis is unlikely.

Prenatal SNP-based chromosome microarray (Integrated Genetics, 2 695 000 genotyping targets) was reanalyzed using postnatal criteria with negative results. There were no detected dosage abnormalities and there was no increase in homozygosity. Sequencing and targeted dosage analysis of the *RFX6* gene did not reveal any mutations.

DISCUSSION

MFS was first reported to include pancreatic hypoplasia, intestinal atresias, extrahepatic biliary aplasia or hypoplasia, with or without TE fistula[1] and sometimes associated with consanguinity.[5,6,3] MRS has similar clinical presentation to 'classic' MFS, except for the presence of neonatal diabetes and *RFX6* mutation,[8] and absence of TE fistula. The earlier reported cases succumbed to very early death, often prior to recognition of neonatal diabetes. In addition, some of the cases were reported prior to *RFX6* being linked to the disorder. Our patient has all of the key clinical features of MRS/MFS, as well as early anemia, glucose dysregulation and hepatic siderosis.

Intestinal atresias may involve any portion of the gastrointestinal tract, with duodenal atresia being a typical presenting feature of MRS/MFS and reported in all the cases.[1,9] Duodenal atresia should be considered part of the minimal diagnostic criteria for this clinical phenotype, rather than the presence or absence of TE fistula.

Hepatic siderosis, cholestasis and severe anemia have been reported in previous cases of MFS and are seen in our patient.[6,3,11] While the patient mentioned by Martinovici *et al*,[6] had siderosis on liver biopsy, the other reports did not have iron assays, thus raising the possibility for undiagnosed iron overload disease. Neonatal diabetes had been documented in babies with MRS/MFS,[8] but the wide glucose fluctuation of our patient is a new and unrecognized aspect of this disorder.

Failure to thrive could be attributed to either pancreatic dysfunction or primary liver disease, both of which have been described in previous cases of MRS/MFS.[8] The pancreas in MRS/MFS may be absent, hypoplastic, annular or normal in size on gross morphology.[9] Malabsorption seen in our patient with enteral nutrition, which was unresponsive to enzyme supplementation, made parenteral nutrition necessary to maintain growth. There were, also unusually high trypsinogen levels on newborn screening for cystic fibrosis, with normal *CFTR* gene sequencing results, which is consistent with pancreatic insufficiency. This is the only reported case of MRS/MFS to have abnormal cystic fibrosis results.

RFX6 is a transcriptional regulatory factor that has been mapped to chromosome 6q22.2.[12,13] *RFX6* is primarily expressed in the pancreas and to a lesser extent in the liver. In mice, *RFX6* directs islet cell differentiation, and knockout mice are unable to produce normal islet cells with the exception of pancreatic polypeptide-producing cells.[12,13] In 2010 Smith[14] was the first to analyze *RFX6* as a gene candidate for probands possessing manifestations of MRS/MFS, with concurrent neonatal diabetes.[14] For mutation negative cases like ours, other possibilities might include genes that encode proteins that interact with *RFX6* or occult *RFX6* mutations in regulatory regions, including promoter regions, intronic regions, the 3′ end or processing defects. For these reasons, MRS/MFS has been considered a continuum of symptoms or an *RFX6* malformation complex. In addition, our and Smith's mutation-negative patients[14] suggest the possibility of genetic heterogeneity for the MRS/MFS phenotype. There was a recent report of a child with MFS features and neonatal diabetes with a novel homozygous missense mutation of K260T.[9] Further reports of patients with MRS/MFS phenotype and genetic testing may clarify the question of genetic heterogeneity.

CONCLUSION

MRS/MFS is a rare condition with a specific phenotypic presentation that includes being small for gestational age, duodenal atresia, pancreatic hypoplasia or insufficiency, hepatobiliary dysfunction, hepatic siderosis and neonatal diabetes. Most cases have been associated with homozygous or compound heterozygous mutations in the *RFX6* gene. We report a patient with all of the key phenotypic features of MRS/MFS, as well as with significant glucose dysregulation and elevated levels of trypsinogen on state newborn screens, which were not previously described. Despite fulfilling all of the proposed criteria for MRS/MFS, our patient lacks an *RFX6* mutation, suggesting the possibility of genetic heterogeneity. This case adds to the current knowledge of MRS/MFS.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1 Martinez-Frias ML, Frias JL, Galan E, Domingo R, Paisan L, Blanco M. Tracheoesophageal fistula, gastrointestinal abnormalities, hypospadias, and prenatal growth deficiency. *Am J Med Genet* 1992; **44**: 352–355.
2 Anneren G, Meurling S, Lilja H, Wallander J, von Dobeln U. Lethal autosomal recessive syndrome with intrauterine growth retardation, intra- and extrahepatic biliary atresia, and esophageal and duodenal atresia. *Am J Med Genet* 1998; **78**: 306–307.
3 Mitchell J, Punthakee Z, Lo B, Bernard C, Chong K, Newman C *et al.* Neonatal diabetes, with hypoplastic pancreas, intestinal atresia and gall bladder hypoplasia: search for the aetiology of a new autosomal recessive syndrome. *Diabetologia* 2004; **47**: 2160–2167.
4 Jeha GS, Venkatesh MP, Edelen RC, Kienstra KA, Karaviti L, Fernandes CJ. Neonatal diabetes mellitus: patient reports and review of current knowledge and clinical practice. *J Pediatr Endocrinol Metab.* 2005; **18**: 1095–1102.
5 Chappell L, Gorman S, Campbell F, Ellard S, Rice G, Dobbie A *et al.* A further example of a distinctive autosomal recessive syndrome comprising neonatal diabetes mellitus, intestinal atresias and gall bladder agenesis. *Am J Med Genet A* 2008; **146A**: 1713–1717.
6 Martinovici D, Ransy V, Vanden Eijnden S, Ridremont C, Pardou A, Cassart M *et al.* Neonatal hemochromatosis and Martinez-Frias syndrome of intestinal atresia and diabetes mellitus in a consanguineous newborn. *Eur J Med Genet* 2010; **53**: 25–28.
7 Barone JV, Tillman EM, Ferry RJ Jr. Treatment of transient neonatal diabetes mellitus with subcutaneous insulin glargine in an extremely low birth weight neonate. *J Pediatr Pharmacol Ther* 2011; **16**: 291–297.
8 Online Mendelian Inheritance in Man O. 2010: MIM Number 601346.
9 Concepcion JP, Reh CS, Daniels M, Liu X, Paz VP, Ye H *et al.* Neonatal diabetes, gallbladder agenesis, duodenal atresia, and intestinal malrotation caused by a novel homozygous mutation in RFX6. *Pediatr Diabetes* 2014; **15**: 67–72.
10 Whitington PF. Neonatal hemochromatosis: a congenital alloimmune hepatitis. *Semen Liver Dis* 2007; **3**: 243–250.
11 Spiegel R, Dobbie A, Hartman C, de Vries L, Ellard S, Shalev SA. Clinical characterization of a newly described neonatal diabetes syndrome caused by RFX6 mutations. *Am J Med Genet A* 2011; **155A**: 2821–2825.
12 Aftab S, Semenec L, Chu JS, Chen N. Identification and characterization of novel human tissue-specific RFX transcription factors. *BMC Evol Biol.* 2008; **8**: 226.
13 Online Mendelian Inheritance in Man O. 2010: MIM number 612659.
14 Smith SB, Qu HQ, Taleb N, Kishimoto NY, Scheel DW, Lu Y *et al.* Rfx6 directs islet formation and insulin production in mice and humans. *Nature* 2010; **463**: 775–780.
15 Gentile M, Fiorente P. Esophageal, duodenal, rectoanal and biliary atresia, intestinal malrotation, malformed/hypoplastic pancreas, and hypospadias: further evidence of a new distinct syndrome. *Am J Med Genet* 1999; **87**: 82–83.