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FAM111B MUTATION IS ASSOCIATED WITH INHERITED EXOCRINE PANCREATIC DYSFUNCTION

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Abstract

Objectives—Few genetic causes of exocrine pancreatic dysfunction have been described to date. We identified a family with multiple affected members manifesting exocrine pancreatic dysfunction. Additional associated features included facial rash, sparse hair, hypohidrosis, and swelling of the extremities. The transmission pattern of these clinical features was consistent with an autosomal dominant mode of inheritance. The two proband siblings also had transient elevated liver transaminases with hepatic steatosis early in life. This study identifies the genetic cause of exocrine pancreatic dysfunction in this family.

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Methods—Whole exome sequencing was performed to identify the genetic cause of exocrine pancreatic dysfunction.

Results—A heterozygous germline in-frame deletion in the gene *FAM111B* (*c.* 1261_1263delAAG, p.Lys421del) co-segregated with the phenotype: the variant was present in all affected relatives genotyped and absent in all unaffected relatives genotyped. The variant is also absent from public control sequence databases.

Conclusions—Our findings implicate *FAM111B* in autosomal dominantly inheritable exocrine pancreatic dysfunction.

Keywords

exocrine pancreatic dysfunction; inherited bone marrow failure syndromes; Shwachman-Diamond Syndrome

Introduction

Exocrine pancreatic dysfunction is characterized by impaired production or release of digestive enzymes. The most common cause of inherited exocrine pancreatic dysfunction is cystic fibrosis, followed by Shwachman-Diamond Syndrome.¹ These are autosomal recessive disorders caused by mutations in the *CFTR* and *SBDS* genes, respectively.^{2–5} Other causes of heritable exocrine pancreatic dysfunction are rare, and many remain genetically unidentified.

We evaluated a 3-year-old girl with exocrine pancreatic dysfunction referred to hematology clinic for suspected Shwachman-Diamond Syndrome. Her sister shared similar symptoms. Genetic testing failed to reveal mutations in the *SBDS* gene and immunoblotting showed normal SBDS protein levels. Exome sequencing analysis was performed to identify the genetic basis of disease in this family.

Materials and Methods

Patient Samples

Informed consent was obtained from all study subjects in accordance with a protocol approved by the institutional review boards (IRBs) of Fred Hutchinson Cancer Research Center and Seattle Children's Hospital. Bone marrow stromal cells and lymphoblasts were prepared and cultured as previously described.⁶

gDNA Processing

Genomic DNA was purified from patient blood samples using the AllPrep DNA/RNA Mini Kit (Qiagen, Venlo, The Netherlands). Saliva samples were collected with the Oragene Dx collection kit (DNA Genotek, Kanata, Canada). Genomic DNA was purified from samples using the prepIT-L2P reagent and protocol (DNA Genotek, Kanata, Canada).

Targeted Gene Capture Panel Sequencing and Analysis

Targeted gene capture panel sequencing and analysis was performed as previously described.⁷

Exome Sequencing and Analysis

Genomic DNA libraries were prepared using the KAPA Library Preparation Kit (KAPA Biosystems, Wilmington, MA) and then hybridized to the SeqCap EZ HGSC VCRome Design Library (NimbleGen Roche, Basel, Switzerland). Captured sequences were sequenced on an Illumina HiSeq2500 using 2×101bp paired-end reads (Illumina, San Diego, CA). Average depth of coverage was 120.3×, with 93.8% of targeted reads having >10× coverage. The reads were filtered for quality and then aligned to the exome using BWA against human genome assembly hg19. Single nucleotide and small indel (1–23 bp) variants were called using GATK and Pindel. Copy number variant analysis was performed as previously described.⁸ Variant annotation and classification was performed using custom Perl scripts. Variants were classified as either intergenic, intronic, 5' UTR, 3' UTR, splice site, or coding. Coding sequence variants were also classified as either nonsense, missense, frameshift, in-frame deletion/insertion, silent, or splice site alteration. The likelihood of damaging effect of missense variants was predicted using PolyPhen2 and SIFT as well as search of the published literature. Sequence conservation scores such as GERP were also used to inform the likelihood of the damaging effect of a variant.

Common variants were excluded by comparison to an in-house database of >1000 exomes, dbSNP v142, the 1000Genomes project, NHLBI Exome Variant Server (EVS), and the Exome Aggregation Consortium (ExAC) database. Variants found with frequency >1% in dbSNP, >5 in EVS, and >20 in ExAC were excluded. Missense variants with PolyPhen2 scores 0.90 and/or SIFT P >0.05 were excluded as candidate variants.

Sanger Sequencing

Direct sequencing was performed using BigDye v3.1 Terminator (Life Technologies/Applied Biosystems, Carlsbad, CA) on PCR template generated using primers flanking the variant sites. Sequence reads were collected via capillary electrophoresis with the 3130×1 Genetic Analyzer (Life Technologies/Applied Biosystems, Carlsbad, CA)

Immunoblotting

Protein lysates were prepared from patient-derived lymphoblasts as previously described.⁶ Immunoblotting was performed using antibodies against FAM111B (Sigma-Aldrich, St. Louis, MO) (1:250), SBDS (1:10,000)⁶, or γ -tubulin (Abcam) (1:10,000) and quantitated followed by the LI-COR two-color fluorescence detection system (LI-COR, Lincoln, NE).

Results

Clinical Features

Clinical features of affected members in the study family (Figure 1) are summarized in Table 1 and 2. The proband, patient V-2, was referred to Hematology Clinic at 5 years of age for diagnostic evaluation of possible Shwachman-Diamond Syndrome (SDS) after assessment

by pediatric gastroenterologists at several institutions since her first year of life for liver enzyme elevation and steatorrhea. Upon presentation at our center at age 3 years, AST was 178 IU/L (normal: 5–41), ALT 251 IU/L (normal: 5–40), GGT 407 IU/L (normal: 5–55), alkaline phosphatase 506 IU/L (normal: 95–380), triglycerides 182 mg/dL (normal: 60–135), albumin 4.9g/dL (normal: 3.8–5.4). Liver ultrasound and magnetic resonance cholangiopancreatography were unremarkable. Workup was negative for infantile sclerosing cholangitis and inborn errors of metabolism, including cholesterol-bile acid biosynthetic pathway defects or peroxisomal beta-oxidation of bile acid intermediates. Screening tests for alpha-1 antitrypsin deficiency, celiac disease, and cystic fibrosis (including sweat chloride testing) were negative. A percutaneous liver biopsy was performed at 2 years of age, and the histopathology revealed diffuse microvesicular steatosis without cholestasis or inflammation. At 3 years of age she developed poor weight gain, recurrent abdominal pain and frequent malodorous loose bowel movements typically 4–5 times per day. She was diagnosed with exocrine pancreatic dysfunction when fecal pancreatic elastase testing revealed < 50 µg elastase / g stool (normal: 200 – 500 µg elastase / g stool) on two separate occasions. Pancreatic enzyme replacement therapy resulted in improvement of her gastrointestinal symptoms. Serum trypsinogen (3.1 ng/mL; lower limit normal (LLN) 10 ng/mL) and pancreatic isoamylase (6 units/L; LLN 13 units/L) were decreased, consistent with ongoing pancreatic dysfunction.⁹ The findings of microvesicular steatosis on liver biopsy in conjunction with the pancreatic insufficiency prompted referral to hematology clinic for possible Shwachman-Diamond syndrome.¹⁰ During initial hematology evaluation at age 5 years, the complete blood count (CBC) was normal. Given the concerns about possible Shwachman-Diamond syndrome¹¹, a bone marrow aspirate and biopsy was performed, which was notable for hypocellularity for age (biopsy cellularity 50%), no dysplasia and no cytogenetic clonal abnormalities noted by metaphase analysis or by fluorescent *in situ* hybridization. No sideroblasts or vacuoles were noted to suggest Pearson syndrome. Telomere length analysis was normal.

Past medical history was significant for bilateral facial rash on cheeks, sparse hair particularly of the eyebrows and eyelashes, hypohydrosis with heat intolerance, and intermittent swelling of hands and feet. Additional history included vesiculoureteral reflux leading to recurrent urinary tract infections which resolved after bilateral ureteral implantation. She also had amblyopia, and one episode of cellulitis on her arm without apparent antecedent cause. Mild stiffness of her heel cords were noted on physical exam. Workup for a rheumatologic disorder was negative.

The patient's older sister, patient V-1, shared similar symptoms summarized in Table 1. She developed frequent emesis in early infancy and was diagnosed with gastroesophageal reflux treated with Prevacid. She had a dermatologic evaluation for severe eczema and photodistributed poikiloderma characterized by mid to lateral facial reticulate erythema, telangiectasia, mottled pigmentation (alternatively described as hyper and hypopigmentation) on her lower neck and trunk, and mild epidermal atrophy involving the nasal tip but otherwise sparing the central face. Xerosis and follicular papules were noted on her lower trunk. Poikiloderma and mild non-pitting edema were noted on the dorsum of both hands. No psoriasiform change noted. No erysipelas-like changes noted. No nail changes or sclerosis of the digits noted. Percutaneous liver biopsies at 1 and 3 years of age

revealed macrovesicular and microvesicular steatosis, with minimal local portal and lobular inflammation. At 7 years of age, pancreatic isoamylase was noted to be low (5 units/L; LLN 13 units/L) despite the absence of overt malabsorption symptoms. Patient V-1 also had a history of hypohidrosis, sparse eyebrows and eyelashes, and slow-growing scalp hair. Dermatologic history is also notable for intermittent spontaneous occurrence of hemorrhagic bullae on her hands and feet. Skin biopsies were non-diagnostic. Her past medical history was also notable for amblyopia and hyperopia.

Family history was notable for numerous paternal relatives (Table 1, 2 and Figure 1) similarly affected with poikiloderma, truncal pigmentary changes, lymphedema, hypohidrosis/heat intolerance, and exocrine pancreatic dysfunction. In addition, older affected family members had sclerosis of the digits, hand numbness or pain upon cold exposure, and lung disease.

Gene Discovery

Genetic testing for cystic fibrosis and Shwachman-Diamond Syndrome was negative: there were no mutations in the *CFTR* or *SBDS* genes. Furthermore, *SBDS* protein levels were comparable to that of healthy controls, arguing against promoter or regulatory mutations affecting *SBDS* expression levels (Supplementary Figure 1). Targeted gene capture panel followed by next-generation sequencing of genes causing bone marrow failure syndromes and myelodysplastic syndrome⁷ was performed in individuals IV-4, IV-5, V-1, and V-2 (Figure 1A) and did not reveal any candidate variants. Exome sequencing of family members IV-4, IV-5, V-1, and V-2 identified protein-altering variants in 5 genes, *FAM111B*, *PRF1*, *SMC4*, *TRIO*, and *WFDC12*, affecting evolutionarily conserved amino acids predicted to be deleterious and segregating with exocrine pancreatic dysfunction in an autosomal dominant pattern. After Sanger sequencing of these five mutations in additional family members, III-2, III-3, III-5, III-6, IV-1, IV-4, IV-5, IV-6, V-1, and V-2, co-segregation with the phenotype was noted for only one variant: a heterozygous germline *FAM111B* variant, c.1261_1263delAAG (NM_198947.3), encoding p.Lys421del (NP_945185.1), at chr11:58,892,831–58,892,833 (hg19). *FAM111B* missense mutations were recently described to cause a similar clinical phenotype, including poikiloderma, eczema, lymphedema of extremities, sclerosis of digits, hypohidrosis/heat intolerance, and restrictive pulmonary disease (Figure 2A).¹² Additional phenotypic features included muscle weakness and myopathies. The *FAM111B* p.Lys421del variant has not been reported in public databases dbSNP139, the Exome Variant Server, the 1000 Genomes Project, the ExAC database of 61,486 exomes, and is not present in our in-house exome database. The lysine residue at position 421 is highly conserved in primate species (Figure 2B). Direct Sanger sequencing was used to validate the mutation and to further genotype the extended family (Figure 1B, Supplementary Figure 2). Seven affected and three unaffected individuals in our kindred were genotyped and demonstrated co-segregation of p.Lys421del with the phenotype. Immunoblotting showed that *FAM111B* protein levels were similar across affected and unaffected family members (Figures 2C, 2D).

Discussion

We identified a familial *FAM111B* mutation co-segregating with exocrine pancreatic dysfunction, liver transaminase elevation early in life, and skin rash in an autosomal dominant fashion. The proband also had a hypocellular marrow but no peripheral blood cytopenias. Together, this clinical phenotype shares overlapping features with Shwachman-Diamond Syndrome which typically presents with exocrine pancreatic dysfunction due to pancreatic lipomatosis, marrow failure, as well as eczema and liver transaminase elevation early in life. The phenotypic similarities between our family and those previously reported with heterozygous variants in *FAM111B*¹² further supports the notion that mutations in *FAM111B* are responsible for producing the shared phenotype. Exocrine pancreatic dysfunction and bone marrow cellularity had not been previously evaluated in published reports of patients with *FAM111B* mutations. Of note, pancreatic lipomatosis was noted on autopsy in one previously reported affected individual harboring a *FAM111B* mutation.¹² Previously described *FAM111B* heterozygous missense mutations were all located within the linker region of the trypsin-like cysteine/serine peptidase domain of *FAM111B*, whereas the heterozygous deletion of the conserved lysine residue at amino acid position 421 in this study lies upstream of this conserved domain, which might contribute to the phenotypic variation (Figure 2A).

FAM111B is member B of the two-gene “Family With Sequence Similarity 111” gene family. The function of *FAM111B* is currently unknown. *FAM111B* contains a conserved trypsin-like cysteine/serine peptidase domain, which is split into two parts joined by a linker region 41 amino acids in length. Mutations in *FAM111B* produce a complex phenotype affecting many organ systems, including the exocrine pancreas, skin, liver, skeletal muscle, and lung.¹² Given the marrow hypocellularity noted in the proband, additional characterization of the hematologic features of this syndrome is of interest. The liver dysfunction in SDS typically presents with elevated liver enzymes early in life that then resolves.¹⁰ An increased risk of hepatic complications with stem cell transplant has been reported for patients with SDS.^{13,14} Since little is known about *FAM111B*, future investigations into the molecular and cellular function of this gene are of high interest, especially in the context of the phenotypic similarities with SDS.

Patients with *FAM111B* mutations may benefit from monitoring for potential long-term medical complications such as pulmonary fibrosis.¹² Mutations in *FAM111B* should be considered in the differential diagnosis of patients presenting with heritable exocrine pancreatic dysfunction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(A) Family Pedigree. Asterisks indicate individuals evaluated with whole exome sequencing. The arrow indicates the proband. The *FAM111B* variant allele (c.1261_1263delAAG; p.K421del) is indicated with a V, and wildtype alleles are indicated with a N. Clinical test results for exocrine pancreatic dysfunction were available for individuals III-2, IV-4, IV-6, V-1, and V-2. Marrow hypocellularity was evaluated in individual V-2. NA= not available

(B) Representative variant and wildtype Sanger sequencing traces.

Figure 2.

(A) *FAM111B* Mutations and Domains. The conserved trypsin-like cysteine/serine peptidase domain (light gray) is a two-part domain joined by a loop linker region (dark gray). Lines mark the positions of reported variants in this protein. All previously described variants are located in the loop linker region.¹² (B) Amino acid conservation across vertebrate species. Bold font indicates the lysine residue at position 421 of the human protein sequence. Seven amino acid residues flanking position 421 are shown. (UCSC Genome Browser). (C) Immunoblot against FAM111B. Protein lysates of EBV-immortalized lymphoblastoid cell lines from the indicated study subjects were evaluated by immunoblot for FAM111B together with γ -tubulin as an internal loading control. (D) Densitometry quantification of FAM111B immunoblot levels relative to healthy control. The results from 4 independent experiments are shown.

Table 1**Clinical Characteristics of Affected Individuals**

	I-2	II-1	III-2	III-3	IV-1	IV-2	IV-4	IV-6	V-1	V-2
Age at last contact	56 yr (deceased)	67 yr (deceased)	58 yr	57 yr	30 yr	27 yr	28 yr	31 yr	10 yr	8 yr
Congenital poikiloderma/red cheeks	NA	+	+	+	+	+	+	+	+	+
Truncal hypo/hyperpigmentation	NA	–	+	+	+	+	+	+	+	+
Hemorrhagic bullae hands/feet	NA	–	–	–	–	–	–	–	+	–
Eczema	+	+	–	–	–	–	+	+	+	–
Lymphedema/swelling of extremities	NA	+	+	+	+	+	+	+	+	+
Raynaud's or numbness/pain with cold exposure	NA	+	+	+	–	–	+	–	–	–
Sclerosis of digits/inability to straighten fingers/scleroderma	+	+	+	+	–	–	+	+	–	–
Hypohidrosis/heat intolerance (red in face with heat)	NA	+	+	+	+	+	+	+	+	+
Hypotrichosis (scalp, eyebrows, eyelashes)	NA	+	+	+	+	–	+	+	+	+
Lung disease	NA	+	NA	–	–	–	–	–	–	–

Key: – indicates not present, + indicates present; abbreviations used: NA = Not Available

Table 2

Gastrointestinal and Hematologic Features

	III-2	IV-4	IV-6	V-1	V-2
Abnormal hepatic biopsy	ND	ND	ND	+	+
Transaminitis	–	+	+	+	+
Low pancreatic isoamylase	+	+	+	+	+
Steatorrhea/diarrhea	+	+	–	–	+
Hypocellular marrow	ND	ND	ND	ND	+

Key: – indicates not present, + indicates present; abbreviations used: ND = Not Determined