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Prevalence of a Loss-of-Function Mutation in the Proton-Coupled Folate Transporter Gene (*PCFT-SLC46A1*) Causing Hereditary Folate Malabsorption in Puerto Rico

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Abstract

Objective—To determine whether subjects of Puerto Rican heritage are at increased risk for a specific mutation of the proton-coupled folate transporter (*PCFT*) causing hereditary folate malabsorption (HFM).

Study design—Three percent of the births in Puerto Rico in 2005, with additional regional oversampling, were screened for the prevalence of the c.1082G>A; p.Y362_G389 del *PCFT* gene mutation. Six new subjects of Puerto Rican heritage with the clinical diagnosis of HFM were also assessed for this mutation.

Results—Six subjects of Puerto Rican heritage with the clinical diagnosis of HFM were all homozygous for the c.1082G>A; p.Y362_G389 del *PCFT* mutation. Three heterozygote carriers were identified from the 1582 newborn samples randomly selected from births in Puerto Rico in 2005. The carrier frequency for the mutated allele was 0.2% island-wide and 6.3% in Villalba.

Conclusion—These findings are consistent with a common mutation in the *PCFT* gene causing HFM that has disseminated to Puerto Ricans who have migrated to mainland United States. Because prompt diagnosis and treatment of infants with HFM can prevent the consequences of this disorder, newborn screening should be considered in high-risk populations and physicians should be aware of its prevalence in infants of Puerto Rican ancestry.

Hereditary folate malabsorption (HFM), a rare autosomal recessive disorder first described in 1961, arises in early infancy and usually presents with failure to thrive and anemia.[1] Some subjects have hypogammaglobulinemia with immune deficiency and infections, such as *Pneumocystis jirovecii*. Developmental delays and a variety of neurologic defects are seen. Seizure disorders, particularly when diagnosis and treatment are delayed, can occur even many years after birth and can be refractory to treatment. [2-5] The major defects in HFM are

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impaired intestinal folate absorption and impaired transport of folates across the blood/choroid plexus/cerebrospinal fluid barrier. The molecular basis for HFM is loss-of-function mutations in the proton-coupled folate transporter gene *(PCFT-SLC46A1)*, the primary transporter responsible for intestinal folate absorption.[3,6] We have reported 10 unrelated families in which there has been at least one member with the clinical diagnosis of HFM and loss-of-function PCFT mutations.[3,6-10] Three additional families have been reported by others.[4,11,12]

All reported mutations associated with HFM have been novel except for two families of Puerto Rican origin, one living in mainland United States.[4,6] Both were homozygous for a c. 1082G>A; p.Y362_G389del mutation (position 5882, GenBank accession number DQ496103), located in the splice acceptor of intron 2 (intron 2/exon 3 boundary), causing skipping of exon 3 and resulting in a splice variant (Figure 1, A) and a protein with decreased stability and impaired trafficking to the cell membrane.[6] In this article we report on the identification of the same mutation in six additional unrelated families of Puerto Rican heritage along with the results of a random screen of 3% of the births on the island in 2005 along with oversampling in specific regions.

Methods

This study was approved by the Albert Einstein College of Medicine's Clinical Committee of Investigation and the University of Puerto Rico Medical Sciences Campus Institutional Review Board, in accordance with the Declaration of Helsinki. Informed consent was obtained as necessary from patients with the clinical diagnosis of HFM and their family members. Separate approval was obtained for the newborn screening component. These protocols have been approved yearly.

Identification of the PCFT Mutation in Subjects with a Clinical Diagnosis of HFM

Peripheral blood samples were collected, and genomic DNA was extracted with the Gentra Systems DNA purification kit (Gentra Systems, Inc, Minneapolis, Minnesota). The primers and conditions for polymerase chain reaction (PCR) were reported previously.[6] *PCFT* genomic fragments that contain exons and flanking introns were purified on agarose gels and sequenced on an ABI 3730 DNA analyzer (Applied Biosystems, Foster City, California) in the Albert Einstein Cancer Center Genomics Shared Resource.

Population Screening

Filter paper blood spots were received from the newborn screening program of the Puerto Rico Department of Public Health. Because the Puerto Rican patients reported previously were from the municipality of Villalba and a sector of the metropolitan San Juan area, an initial screening was performed of randomly selected regional samples that consisted of 12% of births in 2005 for the municipalities of Villalba (n = 48), Juncos (n = 66), Humacao (n = 105), and the Santurce sector of San Juan (n = 141). The two towns in Eastern Puerto Rico (Juncos and Humacao) were chosen for comparison, expecting those samples to be negative for the mutation if the allele frequency was highest in the regions where the patients were located (Villalba and Santurce). However, a carrier in the Juncos samples was also detected, thus a larger prevalence study was performed to determine the island-wide allele frequency and whether there were municipalities with a high allele frequency. For the island-wide study, random samples were collected consisting of 3% of the total births on the island in 2005 (n = 1582).

To obtain DNA from filter paper blood spots, sterile distilled water (100 μL) was added to each sample followed by heating to 95° C for 15 to 30 minutes; samples were vortexed

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every 2 minutes. The eluates were then spun in a centrifuge briefly at 13 200 *g*. PCR was performed with 5 μL of DNA template, AmpliTaqGold with GeneAmp (Applied Biosytems, Branchburg, New Jersey), and primers (biotinylated forward primer purified by high-performance liquid chromatography: [BioTEG]CCAGCCCCATTTTCCTGAT and reverse primer ACCAGCTTGGAGAGTTTAGCC). PCR conditions were as follows: cycle 1, 95° C for 5 minutes; cycle 2 (repeated 45 times), 95° C for 15 seconds, annealing temperature 55° C for 30 seconds, elongation temperature 72° C for 15 seconds; and cycle 3, 72° C for 5 minutes. PCR products were analyzed on a 2% agarose gel to confirm that a clear strong product (106 bp) was obtained. PCR products were processed at the Albert Einstein Cancer Center Genomics Shared Resource for analysis on the HS 96 Pyrosequencer (Qiagen, Valencia, California) (with pyrosequencing primer GAAAAGCAACCCATATC). All positive samples and a subset of negative samples were sent for validation and confirmation to the CLIA-licensed laboratory at the Division of Genetics, Newborn Screening Program, Biggs Laboratory, of the New York State Department of Health.

Validation by the Laboratory of Human Genetics, Newborn Screening Program, Genetic Testing Section, New York State Department of Health

Samples were amplified by PCR with intronic primers for exon 3, reported previously, with the addition of M13 sequencing tags.[6] PCR was performed in a total reaction volume of 25 μL, containing 4 μL of DNA template from blood spot extractions or 2 ng of purified genomic DNA, 0.5 μmol of each forward and reverse primer, 1.5 μmol $MgCl_2$, 0.2 μL Taq antibody (Clonetech 639251) and 2.5 μL Roche Hybridization 10× PCR Reaction Mix (Roche 2158825). PCR conditions were as follow: cycle 1 at 95° C for 5 minutes; cycle 2 (repeated 35 times), 95° C for 30 seconds, annealing temperature 63° C for 30 seconds, elongation temperature 72° C for 30 seconds; and cycle 3 at 72° C for 5 minutes. PCR products were separated by agarose gel electrophoresis to confirm amplification and purified with the ExoSap-IT reagent (USB 78205). Bidirectional sequencing was performed with the BigDye Terminator v3.0 cycle sequencing kit (Applied Biosystems), on an ABI 3130xl instrument. Sequence results were analyzed with SeqScape software (Applied Biosystems).

Results

Prevalence of the c.1082G>A; p.Y362_G389del Mutation among Patients with a Clinical Diagnosis of Hereditary Folate Malabsorption

The Table (available at www.jpeds.com) lists all published *PCFT* gene mutations along with their geographic areas of origin and location at the time of the current study. The Table includes two families of Puerto Rican heritage previously reported, along with six new unrelated cases confirmed in this report. Of 19 known affected families in which there is one member with both alleles of the *PCFT* gene mutated, eight are of Puerto Rican heritage; all are homozygous for the c.1082G>A; p.Y362_G389del mutation, which leads to the loss of exon 3 resulting in a truncated *PCFT* mRNA and a non functional protein (Figure 1, A).[6]

Prevalence of the c.1082G>A; p.Y362_G389del Mutation

The initial regional oversampling (12% of births in 2005) revealed a carrier frequency of 6.3% in Villalba and 1.5% in Juncos; no carriers from Humacao or Santurce were identified. Three heterozygote carriers were identified from the 1582 newborn samples randomly selected from one year's births (2005) in Puerto Rico. Figure 1, B and C, shows a representative pyrosequencing assay. No homozygotes were identified. The carrier frequency for the mutated allele island-wide was 0.2%. The identified carriers were born in the towns of Ponce, Bayamon, and Villalba. Hence, although the highest allele frequency was found in the central municipality of Villalba, carriers were detected in municipalities in other parts of the island (Figure 2).

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Discussion

Among the earliest reports of HFM were children of Puerto Rican heritage and the molecular basis for HFM was established in a Puerto Rican family.[2,6,13] The fact that all affected Puerto Rican families are homozygous for the same mutation is consistent with a common mutation on the island, with highest allele frequency in the municipality of Villalba. Additional genetic epidemiological studies will be necessary to confirm more definitively the site(s) of highest prevalence on the island. It is clear that this allele has disseminated and is now present in individuals of Puerto Rican heritage living in the United States.

Although c.1082G>A; p.Y362_G389del is the first mutation found in multiple subjects, in three instances two mutations have been detected at the same residues, as indicated in the Table. In a Turkish subject, a Ser was substituted for Arg113[3]; in an Israeli Arab, there was a Cys substitution at this residue.[11] These mutations resulted in a loss of function of the carrier.[3,11] In a Mexican subject with heterozygous mutations, Trp was substituted for Arg376 in one allele and, although expression of the protein at the cell surface was unchanged, there was a complete loss of function.[3] On the other hand, in a Chinese subject, glutamine was substituted at this residue in both alleles. This resulted in only a modest reduction in protein expression at the cell surface and some retention of function that was substrate-dependent, but insufficient to maintain adequate folate absorption or folate transport across the choroid plexus.[8] There have been multiple mutations within the first intron involving substitutions and deletions. Mutations at Cys66 resulted in a frame-shift[9] and stop codon.[7] This highly GC-rich region of the first exon (the GC content of residues 63 to 70 is 75%) is also the site of two other mutations, frame-shifts at Gly65[3] and Cys66.[12]

Other prevalent mutations associated with rare diseases have been identified among the population of Puerto Rico. Two separate founder mutations in the *HPS1* (10q23) and *HPS3* (3q24) genes associated with the Hermansky-Pudlak syndrome have been identified on the island.[14-16] The p.G47D (11q14) mutation associated with Type I-A oculocutaneous albinism in Puerto Rico appeared to have originated from a common founder.[17] In addition, the p.R457Q (11p11) substitution is a common mutation in Puerto Rico and is linked to the otherwise extremely rare genetic prothrombin deficiency disorder.[18]

On the basis of findings in this article, a case can be made that screening for the c.1082G>A mutation should be considered for high-risk regions of Puerto Rico, and infants of parents from this region living elsewhere, especially because diagnosis and treatment shortly after birth can prevent all the adverse consequences of this disorder. Of particular importance is the prevention of seizures, that can be very refractory to treatment, that occur when diagnosis and treatment are delayed.[2,5]

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Glossary

HFM Hereditary folate malabsorption

PCFT	Proton-Coupled Folate Transporter
PCR	Polymerase chain reaction

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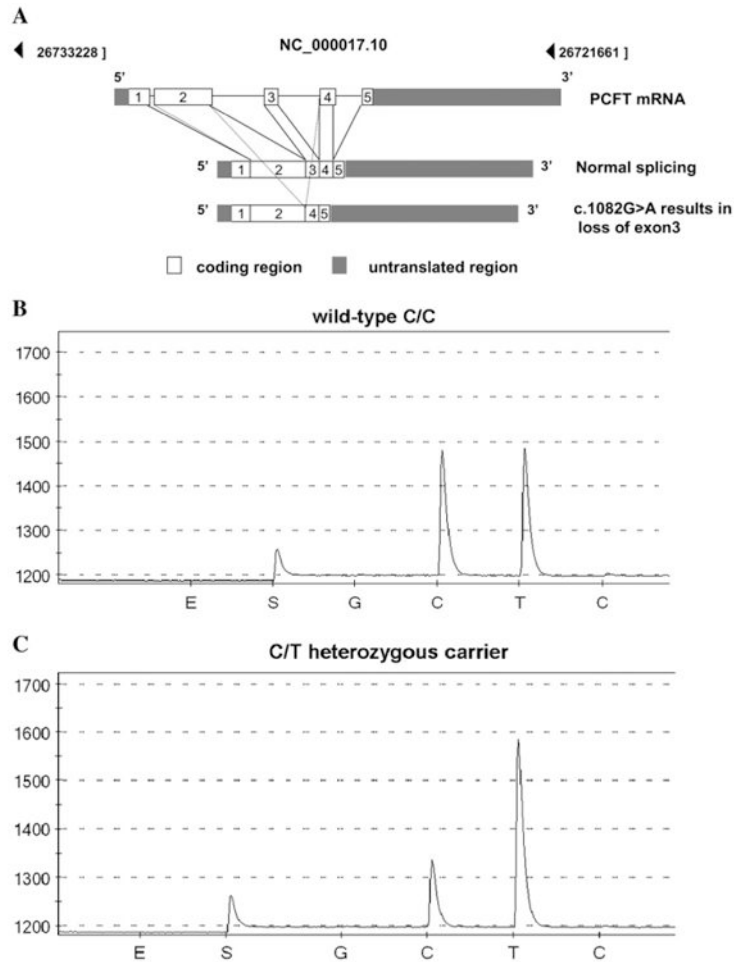

Figure 1.

A, The genomic organization and splicing of the *PCFT* mRNA along with the loss of exon 3 resulting in a truncated *PCFT* mRNA in patients of Puerto Rican ancestry with HFM. **B** and **C**, Representative pyrograms show the wild-type CC sequence and the CT carrier sequence identified from the 1582 newborn samples randomly selected from one year's births (2005) in Puerto Rico. The pyrogram shows a nucleotide sequence around the intron/exon boundary. The y-axis indicates the light emission and the x-axis indicates nucleotide binding. *E* and *S* indicate enzyme and substrate, respectively, followed by a negative control base *(G)*, the two peaks of interest *(C* and *T)*, and a negative control base *(C)*. For the C/T heterozygous mutation, a decrease in the height of the “C” peak and an increase in the height of the “T” peak are compared with the C/C wild-type sequence.

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Figure 2.

Map of the island of Puerto Rico indicating the regional distribution of common mutations that can cause rare autosomal recessive disorders. Municipalities of high carrier frequency (1.7% or higher) for the *HPS3* 3,904 bp gene deletion mutation are indicated with a grid pattern. Municipalities where the *HPS1* gene 16 bp duplication is prevalent are indicated in dark grey; towns where the carrier frequencies for both the *HPS1* and *HPS3* founder mutations are high are indicated in a checkered pattern. Municipalities where carriers of the *PCFT* gene c.1082G>A mutation were detected are shown in light grey; the town of Villalba, where carriers of both the *HPS3* gene deletion and the *PCFT* gene mutation have been identified, is indicated by the diagonal points.

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Table

Summary of published PCFT mutations in subjects with the clinical diagnosis of hereditary folate malabsorption

DNA Nucleotide Change	Protein amino acid change	Ethnicity	Location time of study
c.1082-1G>A[6]	p.Tyr362_Gly389del	Puerto Rican	Puerto Rico
c.194delG[3]	p.Gly65AlafsX25	African American	Pennsylvania
c.337C>A[3]	p.Arg113Ser	Turkish	Europe
c.439G>C[3]	p.Gly147Arg	Caucasian	Europe
c.1126C>T	p.Arg376Trp	Mexican	Texas
c.954C>G[3]	p.Ser318Arg		
c.1274C>G[3]	p.Pro425Arg	Arab	Israel
c.337C>T[11]	p.Arg113Cys	Arab	Israel
c.197_198delGCinsAA (c.197GC>AA)[7]	p.Cys66X	Portugese	New Jersey
c.1082-1G>A[4]	p.Tyr362_Gly389del	Puerto Rican	Boston, Massachusetts
c.194dupG[12]	p.Cys66LeufsX99	Pakistani	London
c.1127G>A[8]	p.Arg376Gln	Chinese	Australia
c.204 del CC[9]	p. Asp68LysfsX94	Turkish	Turkey
c.466G>T[10]	p.Asp156Tyr	Pakistani	Canada
c.1082-1G>A	p.Tyr362_Gly389del	Puerto Rican	Puerto Rico
c.1082-1G>A	p.Tyr362_Gly389del	Puerto Rican	Rochester, New York
c.1082-1G>A	p.Tyr362_Gly389del	Puerto Rican	Worcester, Massachusetts
c.1082-1G>A	p.Tyr362_Gly389del	Puerto Rican	Puerto Rico
c.1082-1G>A	p.Tyr362_Gly389del	Puerto Rican	Puerto Rico
c.1082-1G>A	p.Tyr362_Gly389del	Puerto Rican	Puerto Rico