Allelic background of LEPRE1 mutations that cause recessive forms of osteogenesis imperfecta in different populations

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Abstract

Biallelic mutations in LEPRE1 result in recessively inherited forms of osteogenesis imperfecta (OI) that are often lethal in the perinatal period. A mutation (c.1080+1G>T, IVS5+1G>T) in African Americans has a carrier frequency of about 1/240. The mutant allele originated in West Africa in tribes of Ghana and Nigeria where the carrier frequencies are 2% and 5%. By examining 200 samples from an African-derived population in Tobago and reviewing hospital neonatal death records, we determined that the carrier frequency of c.1080+1G>T was about one in 200 and did not contribute to the neonatal deaths recorded over a 3-year period of time in Trinidad. In the course of sequence analysis, we found surprisingly high LEPRE1 allelic diversity in the Tobago DNA samples in which there were 11 alleles distinguished by a single basepair variant in or near exon 5. All the alleles found in the Tobago population that were within the sequence analysis region were found in the African American population in the Exome Variant Project. This diversity appeared to reflect the geographic origin of the original population in Tobago. In 44 individuals with biallelic LEPRE1 mutations identified by clinical diagnostic testing, we found the sequence alterations occurred on seven of the 11 variant alleles. All but one of the mutations identified resulted in mRNA or protein instability for the majority of the transcripts from the altered allele. These findings suggest that the milder end of the clinical spectrum could be due to as yet unidentified missense mutations in LEPRE1.

Keywords
African, allelic diversity, allelic heterogeneity, LEPRE1, mutations, neonatal death, osteogenesis imperfecta.

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Introduction

Osteogenesis imperfecta (OI) is a group of disorders characterized by fractures with minimal or no trauma (Silchenko et al. 1979; Rauch and Glorieux 2004). The severity of OI ranges from perinatal lethality to severe skeletal deformities with mobility impairments and very short stature, and to, at the mild end of the spectrum, asymptomatic individuals with a mild predisposition to fractures, normal stature, and normal lifespan. More than 95% of disease-causing mutations for OI have been found in COLIA1 (MIM# 120150) and COLIA2 (MIM# 120160), which encode the chains of type I procollagen, the major protein of bone (unpublished data and database of OI mutations [http://www.le.ac.uk/ge/collagen/]). Most of the remainder have biallelic mutations in any of 11 additional genes (Forlino et al. 2011; Byers and Pyott 2012; Rohrbach and Giunta 2012; Pyott et al. 2013) or in IFITM5 which result in a dominantly inherited form of OI, OI type V (Cho et al. 2012). Mutations in LEPRE1, [MIM# 610339], which encodes prolyl 3-hydroxylase 1, account for close to half of individuals with recessively inherited OI (Dalgleish 1997, 1998; see http://www.le.ac.uk/ge/collagen/). On the basis of sequence analysis of a relatively small pool of individuals with perinatal lethal OI, estimate of the causative gene proportions are similar: 95% of infants are heterozygous for a mutation in COLIA1 or COLIA2 and the remainder had recessively inherited forms with mutations in other genes (Bodian et al. 2009). One LEPRE1 mutation (c.1080+1G>T, IVS5+1G>T) has a carrier frequency of

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about 1/240 in the African American population (Cabral et al. 2009, 2012) and usually results in a perinatal lethal form of OI. As a consequence, about a quarter of African American infants with the perinatal lethal forms of OI are estimated to be homozygous for that single sequence alteration. The c.1080+1G>T allele originated in Ghana and Nigeria in West Africa, where the current carrier frequency ranges from 1% to 2% to as high as 4–5% in individuals from some tribal groups (Cabral et al. 2012). In this study, we sought to determine the carrier frequency of LEPRE1 c.1080+1G>T in Trinidad and Tobago (T&T). By directed sequencing we discovered extensive allelic diversity in the African-derived Tobago samples studied. This led us to ask if other populations also had a predominant mutant allele and if we could determine the background sequence of normal alleles on which the alterations occurred. We characterized the allelic background on which LEPRE1 mutations occurred in 44 probands with OI, determined the background sequence on which they occurred, and compared it with a set of normal alleles we identified the 200 individuals from Tobago.

Materials and Methods

Consent

Waivers of research consent for human subjects were granted at the University of Washington and University of West Indies, St. Augustine Investigation Review Boards to use anonymized DNA samples from a previous study of newborn blood collected to measure the rate of hemoglobinopathies on Tobago and to review medical records of perinatal deaths.

Sample collection and sequencing

DNA was extracted from 200 stored newborn screening blood cards in Tobago by standard methods. The samples were collected in 2009 during a pilot test of newborn screening for hereditary hemoglobinopathies. To measure the frequency of the LEPRE1 c.1080+1G>T allele, a 488-bp fragment of LEPRE1 that included exon 5 and flanking intron regions was amplified (sense primer: 5’-AAGTAGCAGGCACCAGCTTGTT-3’; antisense primer: 5’-TTGAGGCTCCTGTTACTCCC-3’) and analyzed by automated sequencing (ABI 3130XL). The unique sequence of the amplicons was 445 bp (Chr1:43, 223, 265-43, 223, 710, hg19). The same primers were used for amplification and sequence determination. All variants in the region were recorded and the frequencies were measured.

Record review

All neonatal death records between 2006 and 2009 at Mt. Hope Hospital in St. Augustine, Trinidad were reviewed by one investigator (M. G. P.). Recorded data included: gestation at birth, cause of death, congenital anomalies, size relative to gestational age, limb length, fractures, radiograph reports, family history, and maternal health history when available.

Identification of mutations in LEPRE1 by diagnostic laboratory testing in individuals with OI

In most individuals in whom LEPRE1 diagnostic gene sequencing was completed in the Collagen Diagnostic Laboratory (CDL), Department of Pathology, University of Washington, the diagnosis of OI was clear on clinical grounds. The strategy of testing was as outlined by van Dijk et al. (2012) so that mutations were first excluded in the type I collagen genes and then mutations were sought in genes known to be associated with recessive forms of OI. The amplification and sequencing primers for LEPRE1 are available upon request. The sequences of the 445-bp fragment that included exon 5 and the flanking intronic regions from 70 diagnostic samples stored in the CDL were compared to the variants identified in the Tobagonian population. In addition, the homologous sequence of the related gene, CRTAP [MIM# 605497], was sequenced and allele frequencies were determined. The frequency of each allele among European Americans and African Americans was identified from the Exome Variant Server (http://evs.gs.washington.edu/EVS/).

Results

Neonatal deaths in Trinidad and Tobago

From January 2006 to October 2009, a total of 172 neonatal deaths were documented among an estimated 45,000 births in T&T. With a neonatal death rate of 27 per 1000 births (http://www.who.org) (Bassaw et al. 2001), we would expect to find records of roughly 350 deaths; one third of births occurring at Mt. Hope Hospital. Of 172 neonatal deaths reviewed, none had evidence of neonatal lethal OI.

Carrier frequency of c.1080+1G>T LEPRE1 allele in Trinidad and Tobago

Analysis of polymorphic DNA sites in a male population on Tobago determined that 94% are of African descent (Miljkovic-Gacic et al. 2005). In DNA from 200 newborns from Tobago, we identified one sample with a single copy of the LEPRE1 c.1080+1G>T allele.

Allelic variants in LEPRE1

By sequence analysis of the 445 bp region that encompassed exon 5 and parts of the flanking introns in the 200
Tobagonian newborn samples, we identified 11 LEPRE1 haplotypes (Fig. 1, Table 1). Each allele was defined by a single basepair variant on the background of a shared sequence. The variants that marked 5 of the alleles were present in the Exome Variant Server (http://evs.gs.washington.edu/EVS/) at about the same frequency in African Americans and in the Tobago population (Table 1). The remaining variants were 80 bp or more from the nearest intron/exon boundary and so were probably beyond the regions sequenced in the EVS sample. Three exonic variants and three intronic variants that we identified in the Tobago population were not present in the clinical diagnostic samples (see Table 1). Five Tobago variants were detected in European and African American populations, and one (c.1045G>A; p.Gly349Arg) was at high enough frequency in both to be consistent with a relatively ancient origin (see Table 1).

**Allelic variation in CRTAP**

We sequenced the same region of the related gene, CRTAP, which encodes CRTAP that binds to P3H1, to determine if the allelic variation was similar, given the relationship between the genes. We found only two variants in the Tobago population (c.1032T>G; p.Thr344Thr and c.1044G>A p.Ser348Ser).

**Characterization of and distribution of LEPRE1 mutations identified in individuals with recessively inherited forms of OI**

Through diagnostic testing, we identified 46 different LEPRE1 mutations in 44 individuals with recessively inherited OI (Fig. 2, and Tables 2 and 3). Outside of exon 5 and the flanking intronic regions, noncausative sequence variation in the domains we sequenced (exons and flanking intronic regions) was rare. In 29 individuals the identified mutations were homozygous. These included 12 who were homozygous for the c.1080+1G>T mutation, three who were homozygous for the c.1170+5G>C mutation (all of Vietnamese origin), and two who were homozygous for the c.2041C>T mutation who were Arabic, one from Palestine and the other from Saudi Arabia. Each remaining mutation was identified only once.

In the 23 individuals who were homozygous (12) or heterozygous (11) for the c.1080+1G>T mutation all had the sequence alteration on the “2” allele as defined in the Tobago population (Fig. 1, and Tables 2 and 3). In all but one of these the extended haplotype of sampled sequences throughout the gene was identical. In the outlier there was a single nucleotide change (c.941-52c>a) that was consistent either with a second mutation or with a crossover event that transferred the mutation to a “0” allele. Three Vietnamese infants shared the identical homozygous c.1170+5G>C mutation on the background of an identical ancestral “0” haplotype. The Arabic Palestinian mutation was on the background of the “0” allele.

All 44 individuals found by clinical testing to have LEPRE1-related OI were identified at birth or by prenatal ultrasound because of the presence of short bowed limbs and multiple fractures. The median age of laboratory diagnosis was 22 days with a range of 1 day to 25 years. The LEPRE1 mutation was identified later in childhood in about a quarter of the individuals studied although the clinical diagnosis of a moderate to severe form of OI was made in infancy. The delay in testing in those probably represented the restudy of individuals with OI after the recognition of the gene as a candidate for mutations that cause OI. All identified children living with LEPRE1-related OI were nonambulatory as a result of bone fragility, bone fracture, and deformity.

In almost all instances, the identified mutations led to premature termination codons and unstable mRNA as a result of nonsense codons, frameshifts, or splice site mutations. The absence of P3H1 that resulted from LEPRE1 homozygous null mutations was not always associated with a neonatal lethal phenotype (Table 2).
### Table 1. LEPRE1 gene alleles and frequency in different source samples.

| Allele | gbk | rs | Chromosome location (Hg19) | Minor allele/major allele | Variant description | Diagnostic samples (140 alleles) | Tobago (400 alleles) | European American | African American |
|--------|-----|----|--------------------------|--------------------------|----------------------|---------------------------------|---------------------|------------------|-----------------|
| 0      | gbk8957 | 111653864  | 1: 43223686 | c/g | c.941-93G>C | 111 | 200 | c = 15/g = 385 | n/a | n/a |
| 1      | gbk8998 | 72956932  | 1: 43223645 | c/a | c.941-52A>C | 2 | c = 3/a = 137 | 1 | 200 | c = 65/a = 335 | c = 7/a = 4611 | c = 336/a = 2318 |
| 2      | gbk9080 | 142954359 | 1: 43223563 | T/C | c.971C>T; p.Ala324Val | 3 | 125 | T = 2/C = 50 | 1 | 200 | T = 0/C = 8600 | T = 1/C = 4403 | T = 1/C = 4403 |
| 3      | gbk9087 | 74070022  | 1: 43223556 | T/C | c.978C>T; p.Thr326Thr | 4 | 125 | T = 2/C = 50 | 1 | 200 | T = 1/C = 4403 | T = 1/C = 4403 | T = 1/C = 4403 |
| 4      | gbk9087 | 6100157  | 1: 43223508 | T/C | c.1026C>T; p.Ala342Ala | 5 | 125 | T = 2/C = 50 | 1 | 200 | T = 1/C = 4403 | T = 1/C = 4403 | T = 1/C = 4403 |
| 5      | gbk9135 | 6700677  | 1: 43223489 | A/G | c.1045G>A; p.Gly349Arg | 6 | 125 | A = 8/G = 375 | 1 | 200 | A = 40/G = 360 | A = 354/G = 215 | A = 478/G = 3928 |
| 6      | gbk9269 | 6700677  | 1: 43223374 | c/t | c.1080+80T | 7 | 125 | c = 2/t = 375 | 1 | 200 | c = 24/t = 375 | n/a | n/a |
| 7      | gbk9304 | 7521929  | 1: 43223339 | g/a | c.1080+115A | 8 | 125 | g = 1/a = 139 | 1 | 200 | g = 24/a = 375 | n/a | n/a |
| 8      | gbk9336 | 7521929  | 1: 43223307 | a/g | c.1080+147G | 9 | 125 | a = 17/g = 139 | 1 | 200 | a = 14/g = 375 | n/a | n/a |
| 9      | gbk9341 | 7521929  | 1: 43223302 | a/g | c.1080+152G | 10 | 125 | a = 17/g = 139 | 1 | 200 | a = 14/g = 375 | n/a | n/a |

n/a, not identified in the Exome Variant Server (EVS) (http://evs.gs.washington.edu/EVS/).

### Discussion

Among the causative mutations that we identified: (a) the mutation in the African American population ([c.1354G>C; p.Glu449Glu]) that caused the same nucleotide change, (b) the last nucleotide of exon 8, a position that normally contributes to the function of the sno-miRNA (G) of exon 6 and is also predicted to affect sno-miRNA splicing. We did not have cells with the splicing donor site. However, we were able to perform a four nucleotide out of frame splice products, three of which contained four abnormal splice products, three of which contained an in-frame premature termination codon (U. Schwarze, unpubl. data) and the fourth was a minor product. The study of the carrier frequency of the West African population, studied by Bodian et al. (2009), is representative recessive forms of OI account for about 5%. Perinatal lethal OI is estimated to have an incidence of approximately 1/40-60,000 births (Sillence et al. 1979). If the region. Outside this region, the sequences were characterized in the course of clinical mutation detection, most of the mutations was on a distinct allelic background. We identified two additional groups and in each, the mutations were on the same individual. There was very little diversity. As a result, we could use the results in three additional regions in the region that included the first reported missense mutation (c.1170G>A, p.Pro390Pro), that changed the same nucleotide (c.1345G>A, p.Glu449Glu), the last nucleotide of exon 8, a position that normally contributes to the function of the sno-miRNA (G) of exon 6 and is also predicted to affect sno-miRNA splicing. We did not have cells with the splicing donor site. However, we were able to perform a four nucleotide out of frame splice products, three of which contained four abnormal splice products, three of which contained an in-frame premature termination codon (U. Schwarze, unpubl. data) and the fourth was a minor product.
This translates into an incidence of 1/800,000 births, consistent with an overall carrier frequency of about 1/450. As the carrier frequency in a population increases, the proportion of infants with recessively inherited OI type II due to homozygosity for one allele increases, as demonstrated in homozygosity for LEPRE1 c.1080G>T in West Africa and the presence of founder mutations in other distinct geographic endogamous groups.

African ancestry is estimated to be as high as 88% (39,000 of 44,000) on the island of Tobago (http://www.cso.gov/tt/statistics) and about 39% on Trinidad. Slave voyage records (http://slavevoyages.org/tast/database/search.faces) document that approximately 15,000 slaves from West Africa (Bight of Benin, Bight of Biafra and Gulf of Guinea islands [45%], Gold Coast [25%], Sierra Leone [15%], and nearby regions [15%]) disembarked on the island of Tobago. The measured carrier frequency of the LEPRE1 c.1080G>T mutation of one in 200 in Tobago is similar to that seen in the United States among African American individuals which is consistent with the similar African origins of the two populations. Given this carrier frequency, the expected incidence of perinatal OI due to homozygosity for this mutation would be 1 in 160,000 births; one infant with LEPRE1-related OI caused by this mutation once every 3–4 years in T&T. The absence of OI neonates in review of the death records is consistent with the rare nature of the disorder, in general, and the identified mutant LEPRE1 allele frequency in Tobago.

Allelic diversity identified

The variation seen (11 variants in this 445 bp region of the LEPRE1 gene) in this population is most consistent with accumulation of sequence alterations among a dispersed population in Africa, incorporation of a diverse population into this small geographic area through the slavery-based migrations, and little or no recombination in the region thereafter. Sequencing of the same region in a related gene (CRTAP which encodes CRTAP that interacts with and stabilizes prolyl 3-hydroxylase) found only two variants in the Tobago population leaving us without an explanation for the high sequence diversity in LEPRE1.

The LEPRE1 mutations we identified in diagnostic samples came from individuals of diverse geographic and ethnic backgrounds. Of the mutations we identified, 17 were not previously reported in the database of mutations in OI (http://www.le.ac.uk/genetics/collagen/) (Dalgleish 1997, 1998). Of these mutations, five were seen in the homozygous state and 12 were seen in the presence of a second allele of which 11 were the c.1080G>T allele. Given the relative rarity of the mutation bearing alleles, most affected individuals were homozygous for the same mutation and were from discrete ethnic populations. The mutations occurred on the background of four of the 11 alleles that we identified in the Tobago population (alleles 0, 2, 6, and 9). The 1080G>T mutation occurred on a single allele [2] with one exception. One infant with parents of SE Indian origin had the mutation on the background of the most prevalent allele [0], consistent with a single recombination event.

Clinical consequence of biallelic LEPRE1 mutations

Of the 46 different disease alleles we identified, all but four were splice site mutations, nonsense mutations, or led to
| ID  | i4-i5 Allele | Allele | Intron or exon | DNA change | Protein       | Type       | Mutation effect                                                                 | Ethnicity          | Last known age | Previous report |
|-----|-------------|--------|---------------|------------|---------------|-----------|---------------------------------------------------------------------------------|--------------------|----------------|-----------------|
| 1   | 9           | 1      | c.392C>T     | p.Ser131*  | Substitution  | Nonsense: PTC unstable mRNA – NMMD | Native American    | d. 1 day of age | Proband 7 |                |
| 2   | 0           | 1      | c.439_441delAAC | p.Asn147del | Deletion     | Deletion: deletion of single AA in tetratricopeptide region (stable mRNA product) | Somali             | 25 years       |                  |
| 3   | 0           | 2      | c.570_571delTG | p.Gly191Serfs*10 | Deletion     | Frameshift: PTC (exon 2) NMMD | Arabic             | 3 years        |                  |
| 4   | Data needed | 5i     | c.1080+1G>T  | p.Glu374*  | Substitution  | Splice Site: results in alternate splice isoforms PTC unstable mRNA NMMD (ref) | African American   | 1 day of age    |                  |
| 5   | 2           | 2      | c.1080+1G>T  | p.Glu374*  | Substitution  | Splice Site: results in alternate splice isoforms PTC unstable mRNA NMMD | African American   | 1 day of age    |                  |
| 6   | 2           | 2      | c.1080+1G>T  | p.Glu374*  | Substitution  | Splice Site: results in alternate splice isoforms PTC unstable mRNA NMMD | African American   | 1 day of age    |                  |
| 7   | 2           | 2      | c.1080+1G>T  | p.Glu374*  | Substitution  | Splice Site: results in alternate splice isoforms PTC unstable mRNA NMMD | African American   | 1 day of age    |                  |
| 8   | 2           | 2      | c.1080+1G>T  | p.Glu374*  | Substitution  | Splice Site: results in alternate splice isoforms PTC unstable mRNA NMMD | African American   | 1 day of age    |                  |
| 9   | 2           | 2      | c.1080+1G>T  | p.Glu374*  | Substitution  | Splice Site: results in alternate splice isoforms PTC unstable mRNA NMMD | African American   | 1 day of age    |                  |
| 10  | 2           | 2      | c.1080+1G>T  | p.Glu374*  | Substitution  | Splice Site: results in alternate splice isoforms PTC unstable mRNA NMMD | African American   | 1 day of age    |                  |
| 11  | 2           | 2      | c.1080+1G>T  | p.Glu374*  | Substitution  | Splice Site: results in alternate splice isoforms PTC unstable mRNA NMMD | African American   | 1 day of age    |                  |
| 12  | 2           | 2      | c.1080+1G>T  | p.Glu374*  | Substitution  | Splice Site: results in alternate splice isoforms PTC unstable mRNA NMMD | African American   | 1 day of age    |                  |
| 13  | 2           | 2      | c.1080+1G>T  | p.Glu374*  | Substitution  | Splice Site: results in alternate splice isoforms PTC unstable mRNA NMMD | African American   | 1 day of age    |                  |
| 14  | 2           | 2      | c.1080+1G>T  | p.Glu374*  | Substitution  | Splice Site: results in alternate splice isoforms PTC unstable mRNA NMMD | African American   | 1 day of age    |                  |
| 15  | 2           | 2      | c.1080+1G>T  | p.Glu374*  | Substitution  | Splice Site: results in alternate splice isoforms PTC unstable mRNA NMMD | African American   | 1 day of age    |                  |
| 16  | 0           | 6      | c.1120G>T    | p.Glu374*  | Substitution  | Nonsense: PTC unstable mRNA NMMD | Hispanic           | d. 1 day of age |                  |
| 17  | Data needed | 6i     | c.1170+2T>A  | p.Ser361_Pro390del | Substitution | Splice Site: stable product 90 bp deletion | First Nation Canadian | d. 5 years    | Proband 18 |                |
| 18  | 0           | 6i     | c.1170+5G>C  | p.Ser361_Pro390del | Substitution | Splice Site: stable product 90 bp deletion | Vietnamese         | 5 years       |                  |
| 19  | 0           | 6i     | c.1170+5G>C  | p.Ser361_Pro390del | Substitution | Splice Site: stable product 90 bp deletion | Vietnamese         | 5 years       |                  |
| 20  | Data needed | 6i     | c.1170+5G>C  | p.Ser361_Pro390del | Substitution | Splice Site: stable product 90 bp deletion | Vietnamese         | 5 years       |                  |

(Continued)
| ID | Allele 1 | Allele 2 | Intron or exon DNA change | Protein | Type | Mutation effect | Ethnicity | Last known age | Previous report |
|----|----------|----------|---------------------------|---------|------|-----------------|-----------|----------------|----------------|
| 21 | 0        | 8        | c.1345G>A                | p.Gly449Ser | Substitution | Missense/Splice Site – single AA last nucleotide of exon 8 unstable spliced mRNA products NMMD | Lebanese | 2 days of age |                |
| 22 | 6        | 8        | c.1345G>C                | p.Gly449Arg  | Substitution | Missense/Splice Site – single AA last nucleotide of exon 8 unstable spliced mRNA products NMMD | Finnish | 2 months     |                |
| 23 | 0        | 8        | c.1346-340_c.1473+36del   | p. (504 bp deletion with breakpoints in introns 8 and 9) | Deletion | Frameshift – PTC in exon 10 unstable mRNA NMMD | French–Canadian |                | Proband 17²    |
| 24 | 9        | 9        | c.1383_1389dup           | p.Lys464Glufs*19 | Duplication | Frameshift: PTC in exon 9 unstable mRNA NMMD | Asian Indian | 4 years |                |
| 25 | 0        | 11       | c.1656C>A                | p.Tyr552*    | Substitution | Nonsense: PTC results in unstable mRNA – NMMD | Pakistani | 16 years | Proband 16³    |
| 26 | 6        | 13       | c.1881_1882delTT         | p.Phe627Leufs*4 | Deletion | Frameshift: PTC in exon 13 unstable mRNA NMMD | Hispanic | d. 2 weeks | Proband 5³     |
| 27 | 0        | 14       | c.2014_2015insA          | p.Ile672Asnfs*21 | Insertion | Frameshift: PTC exon 15 stable mRNA but without terminus KDEL sequence for anchoring to ER membrane | African American | 18 years |                |
| 28 | 0        | 14       | c.2041C>T                | p.Arg681*    | Substitution | Nonsense – PTC P3H1 lacks last 55 AA unstable. (Portion of mRNA probably also unstable) | Palestinian | 21 months |                |
| 29 | Data needed | 14       | c.2041C>T                | p.Arg681*    | Substitution | Nonsense – PTC P3H1 lacks last 55 AA unstable. (Portion of mRNA probably also unstable) | Arabic | 5 months | Proband 9²     |
| 30 | 0        | Allele 1 | 1 c.232delC              | p.Gln78Serfs*30 | Deletion | Frameshift: PTC (exon 1) unstable mRNA NMMD | Unknown | Prenatal ultrasound |                |
| 31 | 2        | Allele 1 | 5i c.1080+1G>T           | Substitution | Splice Site (disruption of NS13 donor splice site) | outcome unknown | African American | Unknown |                |
| 2  | Allele 2 | 1        | c.95_99delTGGTGinsA      | p.Met32Lysfs*24 | Deletion/Insertion | Frameshift: PTC (exon 1) unstable mRNA NMMD | African American | Unknown |                |
| ID | Allele | i4-i5 | Intror or exon | DNA change | Protein | Type | Mutation effect | Ethnicity | Last known age | Previous report |
|----|--------|-------|----------------|------------|---------|------|----------------|-----------|----------------|-----------------|
| 32 | 2      | Allele 1 | 5i | c.1080+1G>T | Substitution | Splice Site: alternate splice isoforms PTC unstable mRNA NMMD | African American | Prenatal ultrasound |
| 0  | Allele 2 | 2   | c.618+1G>A | Substitution | Splice Site: (disruption of IVS2 donor splice site) | African American | d. 6 weeks | Proband 5 |
| 33 | 2      | Allele 1 | 5i | c.1080+1G>T | Substitution | Splice Site: alternate splice isoforms PTC unstable mRNA NMMD | African American | 7 months |
| 0  | Allele 2 | 3   | c.765C>A | p.Tyr255* | Substitution | Nonsense: PTC unstable mRNA NMMD | African American | 2 months | Moul et al. (2013) |
| 34 | 2      | Allele 1 | 5i | c.1080+1G>T | Substitution | Splice Site: alternate splice isoforms PTC unstable mRNA NMMD | African American | 5 months |
| 36 | 2      | Allele 1 | 4  | c.838C>T | p.Gln280* | Substitution | Nonsense: PTC unstable mRNA NMMD | African American | 3 months |
| 0  | Allele 2 | 10  | c.1554_1555delCT | p.Phe519Glnfs*63 | Deletion | Frameshift: PTC unstable mRNA NMMD | Asian; Indian | Prenatal ultrasound |
| 37 | 2      | Allele 1 | 5i | c.1080+1G>T | Substitution | Splice Site: alternate splice isoforms PTC unstable mRNA NMMD | Unknown | 1 month |
| 0  | Allele 2 | 1  | c.1881_1882delTT | p.Phe627Leufs*4 | Deletion | Deletion: frameshift PTC unstable mRNA NMMD | African American | 2 months |
| 0  | Allele 2 | 14  | c.1996delA | p.Arg666Glyfs*29 | Deletion | Deletion: frameshift PTC unstable mRNA NMMD | African American | Prenatal ultrasound |
Table 2. Continued.

| ID | Allele | Intron or exon | DNA change | Protein | Type | Mutation effect | Ethnicity | Last known age | Previous report |
|----|--------|----------------|------------|---------|------|----------------|-----------|----------------|-----------------|
| 41 | 2      | Allele 1       | 5i         | c.1080+1G>T | Substitution | Splice Site: alternate splice isoforms PTC unstable mRNA NMMD | African American | 1 month |                 |
|    | 0      | Allele 2       | 15         | c.2148_2149delCCinsA | Insertion | Insertion: frameshift PTC unstable mRNA NMMD |          |                 |                 |
| 42 | 0      | Allele 2       | 8          | c.1300G>T | Substitution | Nonsense: PTC unstable mRNA NMMD | European; Italian | Prenatal ultrasound |                 |
| 43 | 0      | Allele 2       | 6          | c.1370G>A | Substitution | Nonsense: PTC unstable mRNA NMMD | Unknown | 3 days of age |                 |
|    | 0      | Allele 2       | 9          | c.1459C>T | Substitution | Nonsense: PTC unstable mRNA NMMD | Unknown adopted | 10 years |                 |
| 44 | 0      | Allele 2       | 13         | c.1914+1G>A | Substitution | Splice Site: predicts disruption of IVS13 donor site (outcome unknown) |          |                 |                 |
Table 3. Compound heterozygous LEPRE1 mutations.

| ID | Allele | Intron or exon | DNA change | Protein | Type | Mutation effect | Ethnicity | Last known age | Previous report |
|----|--------|----------------|------------|---------|------|----------------|-----------|----------------|----------------|
| 30 | 0      | Allele 1       | c.232delC  | p.Gln78Serfs*30 | Deletion | Frameshift: PTC (exon 1) unstable mRNA NMMD | Unknown | Prenatal ultrasound |
| 0  | Allele 2 | c.1914+1G>A    | Substitution | Splice Site: (disruption of IVS13 donor splice site) outcome unknown |
| 31 | 2      | Allele 1       | c.1080+1G>T | Substitution | Splice Site: alternate splice isoforms PTC unstable mRNA NMMD | African American | Unknown |
| 2  | Allele 2 | c.95_99delTGGTGinsA | p.Met32Lysfs*24 | Deletion/ Insertion | Frameshift: PTC (exon 1) unstable mRNA NMMD |
| 32 | 2      | Allele 1       | c.1080+1G>T | Substitution | Splice Site: alternate splice isoforms PTC unstable mRNA NMMD | African American | Prenatal ultrasound |
| 0  | Allele 2 | c.618+1G>A     | Substitution | Splice Site: (disruption of IVS2 donor splice site) |
| 33 | 2      | Allele 1       | c.1080+1G>T | Substitution | Splice Site: alternate splice isoforms PTC unstable mRNA NMMD | African American | d. 6 weeks Proband 5^1 |
| 0  | Allele 2 | c.765C>A       | Substitution | Nonsense: PTC unstable mRNA NMMD |
| 34 | 2      | Allele 1       | c.1080+1G>T | Substitution | Splice Site: alternate splice isoforms PTC unstable mRNA NMMD | African American | 7 months |
| 2  | Allele 2 | c.765C>A       | Substitution | Nonsense: PTC unstable mRNA NMMD |
| 35 | 2      | Allele 1       | c.1080+1G>T | Substitution | Splice Site: alternate splice isoforms PTC unstable mRNA NMMD | African American | 2 months Moul et al. (2013) |
| 0  | Allele 2 | c.838C>T       | Substitution | Nonsense: PTC unstable mRNA NMMD |
| 36 | 2      | Allele 1       | c.1080+1G>T | Substitution | Splice Site: alternate splice isoforms PTC unstable mRNA NMMD | African American | 5 months |
| 0  | Allele 2 | c.1170+5G>C    | Substitution | Splice Site: disrupts IVS 6 donor splice site results in deletion of exon 6 90nts |
| 37 | 2      | Allele 1       | c.1080+1G>T | Substitution | Splice Site: alternate splice isoforms PTC unstable mRNA NMMD | Unknown | 1 month |
| 0  | Allele 2 | c.1554_1555delCT | p.Phe519Glnfs*63 | Deletion | Frameshift: PTC unstable mRNA NMMD |
| 38 | 2      | Allele 1       | c.1080+1G>T | Substitution | Splice Site: alternate splice isoforms PTC unstable mRNA NMMD | African American | 3 months |
| 0  | Allele 1 | c.1720+5G>A    | Substitution | Splice Site: (alternate splice isoforms PTC or exon 11 skip both unstable mRNA NMMD little or no P3H1) |

(Continued)
| ID | Allele | Allele | Intron or exon | DNA change | Protein | Type | Mutation effect | Ethnicity | Last known age | Previous report |
|----|--------|--------|----------------|------------|---------|------|-----------------|-----------|----------------|----------------|
| 39 | 0      | 1      | 5i             | c.1080+1G>T| Substitution | Splice Site: alternate splice isoforms PTC unstable mRNA NMMD | Asian; Indian | Prenatal ultrasound |
| 39 | 0      | 2      | 13             | c.1881_1882delTT | p.Phe627Leufs*4 | Deletion | Deletion: frameshift PTC unstable mRNA NMMD |
| 40 | 2      | 5i     | 1              | c.1080+1G>T | Substitution | Splice Site: alternate splice isoforms PTC unstable mRNA NMMD | African American | Prenatal ultrasound |
| 40 | 0      | 14     | 13             | c.1996delA | p.Arg666Glyfs*29 | Deletion | Deletion: frameshift PTC unstable mRNA NMMD |
| 42 | 2      | 5i     | 1              | c.1080+1G>T | Substitution | Splice Site: alternate splice isoforms PTC unstable mRNA NMMD | African American | 1 month |
| 42 | 0      | 10     | 15             | c.2148_2149delCCinsA | p.Glu719Asnfs*29 | Insertion | Insertion frameshift PTC unstable mRNA NMMD |
| 43 | 0      | 1      | 6              | c.1120G>T | p.Glu374* | Substitution | Nonsense: PTC unstable mRNA NMMD | European; Italian | Prenatal ultrasound |
| 43 | 0      | 2      | 8              | c.1300G>T | p.Glu434* | Substitution | Nonsense: PTC unstable mRNA NMMD |
| 43 | 0      | 1      | 6              | c.1170G>A | p.Pro390Pro last nucleotide of exon 6 - effect on mRNA splicing | Substitution | Nonsense: PTC unstable mRNA NMMD | Unknown | 3 days of age |
| 43 | 0      | 9      | 9              | c.1459C>T | p.Gln487* | Substitution | Synonymous: PTC Site: last nt of exon 6 (greatest effect on IVS6 splicing) |
| 44 | 2      | 1      | 8              | c.1244dup | p.Arg416Thrfs*40 | Duplication | Duplication: results in frameshift PTC unstable mRNA NMMD | Unknown adopted | 10 years |
| 44 | 0      | 13     | 13             | c.1914+1G>A | Substitution | Splice Site: predicts disruption of IVS13 donor site (outcome unknown) | Unknown adopted | 10 years |

1Baldridge et al. (2008).
frameshifts, and all of those were shown or predicted to result in loss of mRNA stability. Two of the remaining mutations likely did not affect mRNA stability but resulted in a protein that lacked the carboxyl-terminal rough ER (RER) localization signal (KDEL sequence). The consequence is functional haploinsufficiency through either rapid protein (P3H1) degradation or lack of P3H1 retention in the RER, or both. One mutation resulted in deletion of a single amino acid. The mRNA was stable, but the fate of the protein remained unclear. Some may have residual function, which could explain the milder phenotype of this individual (ID2, Table 2) relative to all individuals with biallelic 

**LEPRE1** mutations. The last mutation result in a single amino acid deletion (p.Asn147del). It is striking that of all the reported mutations, only one, true missense mutation (c.1466T>C, p.Leu489Pro) in an individual with a second mutation that disrupted a splice site (Zhang et al. 2012) has been reported. That individual was 24 years old and had a moderately severe form of OI; less severe than those with the other types of mutations in 

**COL1A1** and **COL1A2**. The mRNA was stable, but the fate of the protein remained unclear. Some may have residual function, which could explain the milder phenotype of this individual (ID2, Table 2) relative to all individuals with biallelic 

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**LEPRE1**. This observation suggests that as the search for recessive OI mutations is widened to include milder phenotypes, this missing class of mutations is likely to appear more frequently.

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**Conflict of Interest**

None declared.

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