A deleterious mutation in the PEX2 gene causes Zellweger syndrome in individuals of Ashkenazi Jewish descent


Zellweger syndrome is known to be caused by numerous mutations that occur in at least 12 of the PEX genes. While phenotypes vary, many are severely debilitating, and death can result in affected newborns. Since the disease follows an autosomal recessive pattern of inheritance, carrier screening can be done for at-risk couples, but the number of potential mutations sites to screen can be daunting. Ethnicity-specific studies can help narrow this range by highlighting mutations that are present at higher percentages in certain populations. In this article, the carrier frequencies for two mutations causative of the severe Zellweger syndrome spectrum phenotype that occur in the PEX2 gene, c.355C>T and c.550del, were studied in individuals of Ashkenazi Jewish descent in order to advise on inclusion in existing carrier screening mutation panels for this population. The screening was performed for 2093 individuals through the use of TaqMan genotyping assays, real-time PCR, and allelic discrimination. Results indicated a carrier frequency of 0.813% (±0.385%) for the c.355C>T mutation and a carrier frequency of 0.00% (±0.00%) for the c.550del mutation. On the basis of these frequencies, we believe that the c.355C>T mutation should be considered for inclusion in carrier screening panels for the Ashkenazi population.

Conflict of interest
Nothing to declare.

Zellweger syndrome, which is a peroxisome biogenesis disorder, arises from mutations that occur in the PEX genes. Mutations in at least 12 PEX genes have been shown to be responsible for this disorder, although mutations in six genes are believed to account for 90% of all cases (1). The PEX2 gene was the first gene identified to cause Zellweger syndrome (2), with mutations causing peroxisomes to stop importing proteins. Accordingly, the PEX2 protein has been found to be an E3, RING finger peroxin, and functions as a protein–ubiquitin ligase (3). Phenotypic consequences of mutations in any of the PEX genes vary and can include dysmorphic features, liver dysfunction, hypotonia, decreased muscle tone, seizures, chondrodysplasia punctata, neurological impairment, and death in infancy.

Owing to the high mortality rate associated with PEX mutations, an algorithm for the detection of the causative PEX gene defects in patients with peroxisomal disorders has been developed for allelic mutations in six of the PEX genes (1). Two of these mutations, which both occur in the PEX2 gene, have been found in individuals belonging to two distinct sub-populations: the Ashkenazi Jews and the Karaite Jews. The Ashkenazi Jews, which compose one of the three major Jewish populations, originated in the Middle East and then migrated to Eastern Europe and the United States, while the Karaite Jews also originated
Fedick et al.

in the Middle East and then appeared in northeastern Europe between the 12th and 14th centuries. Since 47% of the Jews in Israel are Ashkenazi (4) and many of the Karaite Jews from Iraq immigrated to Israel in 1951 (5), two mutations responsible for causing Zellweger syndrome in each of the populations were further investigated on Ashkenazi samples to determine their prevalence in this population.

The first PEX2 mutation is a non-sense point mutation that causes Zellweger syndrome through the early termination of peroxisome assembly factor-1, resulting in a protein product that is truncated before the first transmembrane domain (6). This c.355C>T mutation (rs = 61752123) was first identified in a homozygous affected Japanese female who died at 8 months of age after inheriting the mutation from her parents in an autosomal-recessive manner (7). This same mutation was found in a cell line from Amsterdam University (8) and in two homozygous affected siblings of Ashkenazi Jewish descent who died in early infancy (6).

The second mutation is a one base pair deletion that causes a frameshift and the introduction of a termination codon downstream, resulting in a truncated protein product (9). This c.550del mutation (rs = 63545361) was first identified in a patient who was homozygous for the deletion and died at 2 months of age (9). This deletion was also identified as the cause of Zellweger syndrome in two Karaite Jewish infants, born to non-consanguineous parents, both of whom died within 2 months of age (10). An abstract (no. 664) from the 2007 American Society of Human Genetics meeting (Singer et al., http://www.ashg.org/genetics/ashg07s/f20637.htm) also identified this c.550del mutation as the cause for most of the neonatal Zellweger syndrome cases in the Karaite Jewish population.

Currently, screening for Zellweger syndrome is not included in mutation panels designed for individuals of Ashkenazi Jewish descent. The unexpected frequency of four Ashkenazi families reporting one or more children homozygous for the c.355C>T mutation in the last 5 years, however, implies that the c.355C>T mutation is ethnically prevalent and responsible for the rise in Zellweger syndrome observed in this population (Bonei Olam, personal communication). Additionally, the predominance of the c.550del mutation among the neighboring Karaite population allows for the possibility of this deletion to be present in the Ashkenazi population as well. Therefore, in order to investigate whether these two mutations should be included in Ashkenazi Jewish screening panels, carrier frequency studies were performed for both mutations on samples obtained from individuals of Ashkenazi Jewish descent.

Materials and methods
Ethics statement

The samples used in this study were obtained with written patient consent from self-identified Ashkenazi Jews enrolled in the carrier testing Dor Yeshorim program (11) to be used for research purposes. Consent form information included that patient material would be used for clinical testing and that excess material would be de-identified and used for research purposes to characterize single-gene disorders in the Ashkenazi Jewish population. The control samples were obtained in conjunction with clinical testing, and institutional review board permission was not required for the samples used in the carrier frequency study because all sample identifiers were removed prior to receipt by our lab (45 CFR part 46.101(b)(4)).

Assay design and validation

A TaqMan genotyping assay was designed for the c.550del mutation by using NCBI to search for the full sequence of the gene (FASTA). Roughly 200 base pairs upstream and downstream of the mutation site were selected and put into Repeat Masker (Institute for Systems Biology, Seattle, WA, USA) to mask the repeats. The original selected sequence was then put into the NCBI Blast site (SNP Flanks) so that SNPs could be masked. The assay was then made in File Builder software [Life Technologies (LTI), Carlsbad, CA, USA] with sequence-specific forward (F-GTATTCATTCTGTATTTTGGCATCTCAA) and reverse (R-GCCAGAACGTTCCATATTGTCTG) primers to amplify the polymorphic sequence, and VIC (AGCCAACTTCAGTTATGTT) and FAM (AAGACACTTCAGTTATGTT) labeled probes to detect the normal and mutant alleles, respectively. A predesigned TaqMan assay (C______5557_10) was commercially available for the c.355C>T mutation which was ordered from the Life Technologies Web site.

To validate the assays, a no template control (NTC) consisting of water, three wild-type samples, and one (for the c.550del mutation) or six (for the c.355C>T mutation) known heterozygous carriers samples were normalized to 5 ng/μl. The genotypes of the heterozygous carrier samples were confirmed using Sanger Sequencing prior to use as controls. The samples were plated in duplicate in a 384-well plate along with TaqMan Genotyping Master Mix (LTI) and the assay bringing the final volume to 5 μl. The plates were then centrifuged for 1 min, sealed, and run in duplex real-time PCR reactions followed by allelic discrimination on the ABI PRISM® 7900 HT Sequence Detection System using SDS 2.3 software (LTI).

Carrier frequency study

For the carrier frequency study, 2093 samples were plated in six 384-well plates. The gDNA samples were not normalized prior to plating, but almost all samples fell within the suggested range of 1 to 20 ng (LTI). 1 μl of gDNA and 3 μl of molecular grade biology water were plated per well. The plates were then dried, and TaqMan Genotyping Master Mix (LTI) and the assay were added, bringing the final volume to 5 μl. The plates were then run on the GeneAmp® PCR System 9700
A deleterious mutation in the *PEX2* gene

**Results**

The initial validation of the assays on a small scale yielded 100% genotyping accuracy. Figure 1a shows the successful genotyping of the two mutations on control samples specific for each mutation. Unique clusters indicative of different genotypes were formed based on the signal intensity ratio of the two probes being used (VIC and FAM), allowing for the successful assignment of genotypes.

After validating the TaqMan assays, carrier frequency experiments were performed for each of the mutations on a sample size of 2093 individuals. References consisting of the samples used in the initial validation were included in the analysis, and the results were analyzed using TaqMan Genotyper v1.1 software (LTI). As seen in Fig. 1b, despite the increased sample size, clear clusters were still formed, allowing for genotypes to be assigned. The carrier frequency result for the c.355C>T mutation was 0.813% (±0.385%), based on the presence of 2074 wild-type samples and 17 carriers. Two samples did not have genotyping calls assigned to them, with one sample not amplifying and one being called undetermined, and therefore were not included in the calculation. The carrier frequency result for the c.550del mutation was 0.00% (±0.00%), based on the presence of 2091 wild-type samples and no heterozygous carriers. Two samples did not have genotyping calls assigned because they did not amplify, and therefore were not included in the calculation.

All the samples identified as being heterozygous carriers of the c.355C>T mutation had their genotypes confirmed through Sanger Sequencing. The genotypes for all of the heterozygous carrier samples were concurrent between the TaqMan allelic discrimination and Sanger Sequencing methods.

**Discussion**

According to the American College of Medical Genetics (ACMG), a mutation should be considered for inclusion in an Ashkenazi screening panel based on the following: a good understanding of the natural history of the disorder, the potential for significant morbidity/mortality in the homozygous or compound heterozygous state, and either a detection rate of >90% or an allele frequency of ≥1% (12). While the 0.813% carrier frequency of the c.355C>T mutation does not reach the 1% mark, the history of Zellweger syndrome is well-understood, and the disorder has a significant morbidity/mortality rate in affected patients, with no standard treatment options currently available, and infants homozygous for the c.355C>T mutation not surviving beyond several months. Additionally, the carrier frequency of 0.813% is higher than several other mutations already included on ACMG screening panels. For instance, the disease Mucolipidosis IV is estimated to...
have a carrier frequency of 0.787% in this population (12), but due to its associated severe neurodegenerative phenotype, it has been recommended for screening.

Owing to the advent of high-throughput screening programs (13), it is now possible for potential parents to be screened for additional and/or rare mutations, with many commercial labs already offering screening panels that extend beyond the scope of mutations specifically recommended by the ACMG and other such organizations (14). The ACMG, along with the American College of Obstetricians and Gynecologists (ACOG), has acknowledged that individuals may want to be screened for additional disorders and recommends that educational material and/or genetic counselors should be made available to aid patients in making informed decisions (15, 16).

Although the inclusion of the c.355C>T mutation will not guarantee that parents of Ashkenazi Jewish descent are not carrying other mutations in the PEX2 gene that can cause Zellweger syndrome due to compound heterozygosity, or indicate if they are carrying mutations in the other 11 PEX genes that can cause a phenotype, it will help to reduce the risk that both parents are carrying the same mutation due to the prevalence of this mutation in this population.

It should be noted that the samples used in this study came from the ultra-Orthodox community. While there is little evidence for a difference between mutations found in this community and the broader Ashkenazi Jewish community, the carrier frequency may be higher in the ultra-Orthodox population due to individuals predominantly marrying within the community. Also, the lack of any carriers found for the c.550del mutation indicates that this mutation is not prevalent in individuals of Ashkenazi Jewish descent. It’s prevalence in individuals of Karaite Jewish descent, however, supports the idea that while Zellweger syndrome can result from a number of different mutations across 12 different genes, certain mutations are more prevalent in homogeneous populations. Therefore, by specifically looking at the c.355C>T mutation, the prevalence of Zellweger syndrome can be decreased without having to screen across many genes in the Ashkenazi Jewish population.

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References