

SCREENING FOR NOVEL MUTATIONS IN THE HPS1 AND HPS3 GENES IN PUERTO RICAN PATIENTS HETEROZYGOUS FOR FOUNDER MUTATIONS

Hermansky-Pudlak Syndrome (HPS) is a heterogeneous group of autosomal recessive disorders involving organelle biogenesis. HPS is very common in Puerto Rico, particularly in the Northwest part of the island, where type 1 HPS is found in approximately 1:1,800 individuals. Founder mutations have been identified in two HPS genes, a 16-base pair (bp) duplication in *HPS1* and a 3,904-bp deletion in *HPS3*. Researchers at the National Institute of Health (NIH) have identified new mutations on the *HPS1* and *HPS3* genes in Puerto Rican (PR) patients. We have identified patients with type 1 and type 3 HPS who are heterozygous for the founder mutations described above. In order to identify mutations in the other HPS gene alleles, we screened the *HPS1* gene exons 11 and 13 and exons 3 and 5 of the *HPS3* gene in the heterozygous PR patients, since these are the most frequently mutated exons in these genes in non-PR HPS patients. Mutation screening was done by exon screening using PCR and DNA sequencing using dye terminator chemistry.

In conclusion, an intron mutation was found in the intron 11 region of the *HPS1* gene in 8 patients, which may affect correct splicing due to its closeness with the exon-intron junction. A frameshift mutation in codon 321 was found in 1 patient, located in a mutation hotspot of the *HPS1* gene. A cytosine was deleted in a region containing a run of C's. This mutation causes a truncated protein product. The *HPS3* gene base changes detected in the exon 3 and 5 regions in the 2 patients screened do not appear to be pathological; hence, further screening is needed to detect the other mutated allele.

BACKGROUND

Hermansky-Pudlak Syndrome (HPS) [MIM #203300] is an autosomal recessive disorder characterized by oculocutaneous albinism, a bleeding tendency, and a ceroid-lipofuscin-like lysosomal storage disease, with progressive restrictive lung disease that leads to pulmonary fibrosis and/or granulomatous colitis. The basic biochemical defect is thought to involve a component of membranes in melanosomes, lysosomes and platelet dense bodies. HPS is regarded as the most common single gene disorder in the Puerto Rican population. HPS is caused by at least eight different genes in humans *HPS1*, *ADTB3A*, *HPS3*, *HPS4*, *HPS5*, *HPS6*, *DTNBPI* and *BLOC1S3*. At least two HPS genes have been found to cause this syndrome in Puerto Rican patients, *HPS1* in 10q23 (16-bp frameshift duplication,¹) and *HPS3* in 3q24 (3,904 bp) deletion.²

METHODS

Mutation Screening of the *HPS1* gene exon 11

PCR primers for exon 11 of the *HPS1* gene were those designed by Bailin et al.³ PCR Reactions were done in a MJ Research Thermal Cycler in a final volume of 50 μ L. Final concentrations were 10 ng of DNA, 0.20 μ M for each Primer, 0.20 mM dNTP's, 1.5 mM MgCl and 0.5 μ L of *Taq* DNA polymerase. A hot start step was performed for 4 minutes at 94°C, followed by a 30 cycles program of 94°C for 30 seconds, 57°C to 60°C for 30 seconds, 72°C for 45 seconds, fol-

Student Researcher: Nathalie Fuentes,
José Gautier Benítez High School
Mentor: Carmen Cadilla, PhD, University of Puerto Rico
School of Medicine

lowed by a 72°C extension step for 10 minutes. PCR products were analyzed in a 1.0% agarose gel at 100 V for one hour.

Mutation Screening of the *HPS3* gene exons 3 and 5

PCR Primers are for exons 3 and 5 of the *HPS3* gene were those designed by each exon, as described in Anikster et al.² Reactions were done in an MJ Research Thermal Cycler in a final volume of 50 μ L. Final concentrations were 10 ng of DNA, .2 μ M of each primer, .2 mM dNTP's, 1.5 mM MgCl and .5 μ L of *Taq* DNA polymerase. A hot start step was performed for 5 minutes at 95°C, followed by a 35 cycles program of 94°C for 30 seconds, 50°C to 52°C for 30 seconds, 72°C for 45 seconds, followed by a 72°C extension step for 10 minutes. PCR products were analyzed in a 1.0% agarose gel at 100 V for one hour.

Exons screening by Sequencing Analysis

DNA sequencing services using dye terminator chemistry were provided by the University of Puerto Rico Medical Sciences Campus RCMI Center for Molecular Genetics- Molecular Biology Core Facility and the UPR Río Piedras Campus Sequencing and Genotyping facility. Sequence chromatograms were visualized using the 4Peaks software and compared to the *HPS3* and *HPS1* gene references sequences using the Blast2 sequences software available at the NCBI website. ORF analysis was done using the OrfFinder software also available at NCBI (<http://www.ncbi.nlm.nih.gov>).

Human Subjects

This study group included heterozygous HPS Puerto Rican HPS patients who were heterozygous for the *HPS1* gene 16 bp duplication and the *HPS3* gene 3904 bp duplication founder mutations, as well as two normal Puerto Rican controls for comparison.

RESULTS

HPS1 gene mutation screening

8 patients had no mutations in exon 11 but had a T[°]C change in base 19746 of the *HPS1* gene, in the intron 11 region, 13 bases away from the exon-intron junction. One patient had a frameshift mutation at codon 321 (a hotspot for mutations in non-Puerto Rican patients), caused by a deletion of an A base.

HPS3 gene mutation screening

Two PR patients were screened and two PR patients were controls. One patient had a silent mutation in codon 326 caused by an A–G transition in the codon wobble position at exon 5. Both patients had a base change causing overlapping sequence reads after base 11630 in the intron 3 region, which were seen in one of the control samples. Hence, these base changes, since they occur after a run of T's, may be common polymorphisms in the intron 3 region.

CONCLUSION

An intron mutation was found in the intron 11 region of the *HPS1* gene in 8 patients, which may affect correct splicing due to its closeness to the exon-intron junction. A frameshift mutation

in codon 321 was found in 1 patient, located in a mutation hotspot of the *HPS1* gene. The *HPS3* gene base changes detected in the exon 3 and 5 regions in the 2 patients screened do not appear to be pathological; hence, further screening is needed to detect the other mutated allele.

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