



Category: Clinical Genomics

First report of the mutational and phenotypic spectrum of Hereditary Spherocytosis in Indian patients by targeted resequencing

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Abstract

Hereditary spherocytosis (HS) is a common inherited hemolytic anemia characterized by the presence of microspherocytes. The pathogenesis involves defects in any of the several genes coding for membrane proteins that link the membrane skeleton to the overlying lipid bilayer. Membrane proteins include ankyrin, band 3, β - and α -spectrin and protein 4.2. We studied the molecular spectrum and genotype-phenotype correlation of HS in Indian patients.

Complete blood counts, incubated osmotic fragility test, antiglobulin test, eosin-5'dye-binding test were done in 76 cases from 50 families to diagnose HS. RBC membrane ghosts were prepared and were analysed on gradient gels (4-12%). Relative quantification of mRNA isolated from enriched reticulocytes was done by qRT-PCR. cDNA sequencing of *ANK1*, *SPTB*, *SLC4A1* and *EPB42* genes were done (ABI3130). TruSight One™ sequencing panel was used for preparing libraries in 11 cases that were sequenced on Miseq™ (Illumina). MiSeq Reporter™ and Variant Studio™ were used for analysis, characterization and annotation of variants. Possible pathogenic variants were validated by Sanger sequencing in cases and family members. G6PD-deficiency, α -thalassemia and *UGT1A1* polymorphism were studied as phenotype modifiers.

SDS-PAGE, qRT-PCR and cDNA sequencing were not contributory in deciphering molecular pathologies due to instability of mutated RNA and compensatory protein production by normal allele. NGS uncovered novel pathogenic mutations in *ANK1* and *SPTB* out of which 30% were splice site, 30% were indels and 40% were nonsense mutations in 10 patients. Inheritance was non-dominant in 50% and autosomal dominant in 30% cases. G6PD Mediterranean variant in four HS patients led to greater transfusion requirements. Genotyping for Gilbert syndrome showed homozygosity (TA₇/TA₇) for promoter variant of *UGT1A1* in 38% of the HS patients with significantly higher mean bilirubin level of 8.44 mg/dl and higher frequency of cholelithiasis (36%) (p<0.001).

This first ever study on the molecular spectrum of HS from India revealed predominantly sporadic and dominantly-inherited defects in *ANK1* and *SPTB* in patients. Most of the cases (70%) presented at an early age with jaundice and anemia. Co-inherited G6PD deficiency and Gilbert's syndrome led to phenotypic variability. NGS provided a sensitive, cost-effective and rapid tool for understanding the molecular pathology of HS patients.

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