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Case Report

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Two novel mutations in the ankyrin-1 gene associated with hereditary spherocytosis

Abstract

Mutations in the ankyrin-1 gene (*ANK1*) underlie half of the cases of Hereditary Spherocytosis (HS), and of these, two thirds are due to mutations inherited in a dominant pattern, while the others are due to sporadic mutations. Here we report two novel *ANK1* mutations responsible for HS (c.1800+1G>A and c.1196_1196delC) and propose possible mechanisms of pathogenicity for each mutation described.

Keywords: hemolytic anemia, red cells, membrane disorders, molecular biology, pathogenicity, genotyping

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Abbrevations: ANK1, ankyrin-1 gene; HS, hereditary spherocytosis; CBS, center of biological sequence analysis; BC, bio cruces; NGS, next generation sequencing

Introduction

Hereditary spherocytosis (HS) is the most common inherited red cell membrane disorder with one case out of 2000-3000 individuals and probably even higher prevalence due to under diagnosis of minor or moderate forms of HS.[1] In this heterogeneous disorder abnormalities of red blood cell structural proteins lead to loss of erythrocyte membrane surface area, resulting in spherical-shaped, hyperdense, poorly deformable red blood cells with a shortened life span.[2] Mutations in the Ankirin-1 gene (*ANK1*) accounts 50% of the HS cases, which two thirds are inherited in a dominant pattern and the remaining cases are due to sporadic mutations; to date, more than seventy mutations have been described.[1,3,4] which demonstrate the high level of allelic heterogeneity in *ANK1* mutations so that makes necessary the search of new variants in this gene to establish the biological mechanisms of pathogenicity. Here we report two novel mutations in *ANK1* found in patients diagnosed with HS.

Patients and methods

Clinical and laboratory data from the two cases are summarized in Table 1.

Table 1 Clinical and laboratory data

	Case 1:ANK1:c.1800+1 G>A	Case 2:ANK1: c.1196_1196 delC
Age at diagnosis	1month	2months
Sex	Male	Male
Hemoglobin (g/dL)	7,9	10,4
Reticulocyte count (%)	310.000/mm^3 (11,8%)	522.500/mm^3 (12,9%)
Mean corpuscular volume (fL)	82.9	75.4
Mean corpuscular hemoglobin concentration (g/dL)	36.1	34.1
Serum lactate dehydrogenase (U/L)	266	327
Total Bilirubin mg/dL	1.8	0.9
Eosin-5'-maleimide test	Positive	Positive
Cryohemolysis	Positive	Positive
Osmotic frailty	Elevated	Elevated
Inheritance	De novo	Dominant

Genetyc analysis

The Euskadi Research Ethics Committee approved the study protocol in accordance with the principles of the Declaration of Helsinki (PI2014160). Informed consent was obtained from all of the adult subjects or from the parents of the children for genetic testing. We performed a targeted sequencing of genes that encode membrane proteins on a Next Generation Sequencing (NGS) platform as previously described[5] and identified two new mutations in *ANK1* (NM_000037) in heterozygous state related with pathogenesis of HS. These newly identified variants were no found in the Human Gene Mutation Database,[6] ClinVar,[7] 1000 Genomes Project dataset,[8] Exome Aggregation Consortium (ExAC,[9]) and the UCSC SNPs database and after a comprehensive review of current literature.

Case description

Case 1 was a male baby who presented with jaundice, severe anemia and extravascular hemolysis. His parents, no-consanguineous had no medical history of HS. Sequencing revealed a change of a Guanine to an Adenine in intron 16-17 at position c.1800+1 in *ANK1* (c.1800+1G>A). The consulted splicing prediction software (Center of Biological Sequence Analysis (CBS);[10]) indicates that this mutation can affect the mRNA transcription with a high coefficient of confidence (0.95), resulting in a different transcript, 315 base pairs longer than the canonical sequence, and this corresponds to the addition of 105 amino acids to the protein, which would imply changing its structure; nevertheless, it would be necessary to conduct mRNA analysis to confirm this. The inheritance pattern revealed that it is a "de novo" mutation, genotyping of the parents not showing the variation. Previously, seven other mutations that affect splicing in *ANK1* have been identified as causative of HS, leading to exon skipping or intron inclusion.[11,12]

Case 2 was a child diagnosed with hemolytic anemia few weeks after birth. His mother and uncle were diagnosed with HS during her childhood and splenectomized, but there was no data from molecular studies. Genotyping done in the child and his mother showed a deletion of cytosine at position 1196 in *ANK1*. This frameshift mutation determines a displacement of the reading frame causing the occurrence of a premature stop codon obtaining a peptide of only 406 amino acids instead of 1880 (c.1196_1196delC; p.Ala399Glyfs*7). The new truncated protein would cause disturbance of the structure of the erythrocyte membrane, due to lacking of both spectrin-binding domain and C-terminal regulatory domain.[13] Actually, nineteen small-deletions and five small-insertions mutations in *ANK1* have been reported as pathogenic.[4]

Conclusion

In summary, we identified two novel *ANK1* mutations responsible for HS and demonstrated their genotype/phenotype correlation and we propose a possible mechanisms of pathogenicity for each described mutation. Nowadays, the increasing use of NGS technologies as a genetic diagnostic tool in congenital hemolytic anemia will lead us to know many novel mutations implicated in this disease which will conduct to a better understanding of the biology of HS.

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Conflict of interest

The author declares no conflict of interest.

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