Identification of a novel ANK1 c.856C>T nonsense mutation in two patients from a Chinese family with hereditary spherocytosis by NGS

Jie Yang[1], Chun-xue Yang[2], Yuan-yuan Wu[1], Deng He[1], Yu-qi Li[1], Ling Feng[1], Shao-shuai Wang[1], zhi-hui Rong[1], and Hai-Yi Liu[1]

[1]Huazhong University of Science and Technology
[2]Wuhan No. 1 Hospital

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Abstract

Hereditary spherocytosis (HS) is a common inherited heterogeneous hemolytic anemia that is characterized by the presence of spheroidal erythrocytes on the peripheral blood smear. Mutations in ankyrin gene (ANK1) is the most common cause of HS in Northern European populations and Chinese patients but is seen in only 5–10% of Japanese patients. The majority of them are familial mutations inherited in an autosomal dominant form. In this study, a heterozygous ANK1 c.856C>T mutation was identified in a 2-hour-old newborn with severe jaundice using targeted next-generation sequencing (NGS) and Sanger sequencing, and was confirmed to be inherited from his mother.

Introduction

Hereditary spherocytosis is a common inherited disorder that is characterized by the presence of spheroidal erythrocytes on the peripheral blood smear. Its prevalence ranging from 1:2,000 to 1:5,000 in north European and 1:100,000 in Chinese individuals [1, 2]. The clinical manifestations of HS are variable, ranging from an asymptomatic condition to severe hemolysis. Typical HS patients present anemia, jaundice, splenomegaly and reticulocytosis [3]. In the neonatal period, the major clinical presents are jaundice and anemia. Splenomegaly and spherocytes are rarely observed [4]. Previous researches have shown that mutations of five genes that encode the erythrocyte membrane proteins are associated with HS, including ANK1, SPTB, SPTA1, SLC4A1, and EPB42, which result in a loss of membrane deformability and lead to surface area loss and an increased number of peripheral blood spherocytes [1]. Approximately 75% of HS cases are inherited in an autosomal dominant manner and a subset of patients show autosomal recessive inheritance or may carry a de novo mutation [5]. Blood transfusion and splenectomy are the main treatment to correct anemia and reduce clinical symptoms for HS patients.

The ANK1 gene is located at chromosome 8p11.2 and encodes several alternative splices. ANK1 mutations have been implicated in approximately half of all patients with hereditary spherocytosis (HS) [6], causing both dominant and recessive disease that can range from clinically mild and severe. ANK1 contains 42 exons that encode an 1,881 amino acid protein (referred to NM_000037.4), with three distinct domains: a N-terminal domain containing multiple ankyrin repeats; a center region with a spectrin-binding domain, and a C-terminal regulatory domain [7]. By linking β spectrin to band 3, ANK1 protein leads to spectrin assemble on the membrane and stabilize the blood cell membrane. NGS provides a comprehensive and cost-effective approach to molecular diagnosis of hereditary hemolytic anemia, including HS [8].

In this study, a heterozygous ANK1 c.856C>T mutation was identified by NGS and Sanger sequencing in a

2-hour-old newborn and his mother whom had been suffered anemia, jaundice and splenomegaly during her young time and received a splenectomy at the age of 12.

Results .

Clinical history

The affected male newborn presented with jaundice without other pathological symptoms or signs since 2 hours after birth. Then, the routine blood examination showed that he suffered from mild hemolytic anemia with neonatal hyperbilirubinemia and sphere-shaped erythrocytes on peripheral blood smear (Figure 1B). And his Hb values decreased sharply over the subsequent 9 days (Table.1). The erythrocyte osmotic fragility test was positive while both glucose-6-phosphate dehydrogenase(G-6-PD) screening test and Coombs' test were negative. Hemoglobin electrophoresis analysis using for thalassemia preliminary screen was negative. High bilirubin susceptibility gene (5 mutation sites of UGT1A1) mutation test using Sanger sequencing were also negative. The male newborn took treatment in hospital for 2 weeks, including twice blood transfusions.

Therefore, we traced the family history again and found that the mother had undergone a total splenectomy for severe hemolysis and jaundice when she was 12 years-old. The proband's farther and sister were clinically and hematologically normal. This potentially suggested a dominant inheritance of disease phenotype from mother to the proband. This was another HS indicator, as HS patients with severe symptoms often get better after a splenectomy. The family tree is shown in Figure 1A, and the laboratory tests are summarized in Table 1.

Identification of ANK1 mutation

A heterozygous ANK1 c.856C>T mutation was identified using NGS. Sanger sequencing of the ANK1 c.856C>T mutation was performed on all family members. The mutation is a substitution of C>T, at the 46 nucleotide in exon 9. Both the patient and his mother were heterozygous for the ANK1 c.856C>T mutation, while his father had a wild-type ANK1 allele. The genetic analysis for his sister was not available (Figure 1C). This mutation was not found in the gnomAD, 1000G, ExAC and HGMD database, and confirmed as a novel mutation. The mutation was a heterozygous mutation.

Computational analysis

We used Swiss-model to visualize 3-D representations of the protein. The variation caused a substitution from Arginine acid to a premature stop at codon 286 and get a truncated ANK1 protein (Figure 2).

Genetic and phenotype association analysis

Genetic tests showed a heterozygous ANK1 c.856C>T mutation in this family, and both patients carried this mutation. HS associated clinic symptoms are observed in the patients. Consistent with this genetic finding, the mother who received a splenectomy in childhood, is now asymptomatic.

Discussion

ANK1 Mutation is the most common cause of HS. ANK1 mutations is inherited in both AD (autosomal dominant) and AR (autosomal recessive) patterns in HS and the majority is de novo mutation [1]. These mutations locate the entire ANK1 gene, throughout the promoter region and coding exons [12, 13]. To date, a total of 62 mutations are included in the HGMD public database. Missense/nonsense and small deletions have been identified in approximately 70% of all mutations in the ANK1 gene. Seven splicing mutations, occurring in IVS1,16,20,22,28 and 38, are included in the HGMD database. In this study, we described a Chinese family with a proband and his mother affected by HS. A de novo mutation (c.856C>T p.R286*) causing a premature stop codon in exon 9 of ANK1 was found by investigating this family through NGS followed by Sanger sequencing to certify the relationship between the ANK1 mutation and HS.

ANK1 plays a pivotal role in the stabilization of the membrane, providing the main membrane binding site for the spectrin-based membrane skeleton. ANK1 consists of three structural domains: a multiple repeats N-terminal domain(89kD), a spectrin-binding center region(62kD) and a regulatory C-terminal domain(55kD)

[7, 14, 15]. Mutations in the spectrin-binding domain and regulatory C-terminal domains result in the most severe anemia compared with those located in the other domains [14, 16]. In this family, a ANK1 c.856C>T mutation occurred in the N-terminus region. This mutation was not found in the gnomAD ,1000G, ExAC and HGMD database, and confirmed as a novel mutation. According to bioinformatics analyses, this point mutation generates a premature translation termination codon resulting in a truncated ANK1 protein, losing the important spectrin-binding and regulatory C-terminal domains and leaving only partial of the N-domain, which might cause HS. Moreover, consistent with the genetic diagnosis, the symptom of the mother was ameliorated after splenectomy, further supporting the genotype-phenotype relationship.

NGS provides a comprehensive and cost-effective approach to molecular diagnosis of hereditary hemolytic anemia, especially in cases where biochemical testing is unreliable due to multiple transfusions. In previous research [17], a female individual presented with a yellow complexion, jaundice and splenomegaly without other pathological symptoms or signs on the second day after birth. And then the girl who was clinically diagnosed with HS and under laparoscopic splenectomy because of splenomegaly and hemolysis when she was 6-year-old. Finally, using NGS detected a de novo nonsense ANK1 mutation (c.796G > T, p. Glu266X), which caused a substitution from glutamic acid to a premature stop at codon 266 and may lead to HS. But no mutation was detected in her parents. Thus, it is efficient and important for us to using NGS to identify inherited, rare gene mutations which are associated with a high risk for the development of diseases. If diagnosed earlier, fewer newborn infants would develop severe anemia and hazardous hyperbilirubinemia and it would remind pediatricians to closely monitoring of infants with HS during the first 6 months of life that is important for appropriate clinical management.

In summary, our results demonstrated an ANK1 c.856C>T mutation may lead to a premature stop at codon 286 and may be responsible for HS in two patients from a Chinese family. Identifying the underlying genetic cause not only helps with the management of the patients, it also facilitates accurate genetic counselling and helps in guidance regarding the predicted severity of clinical phenotype.

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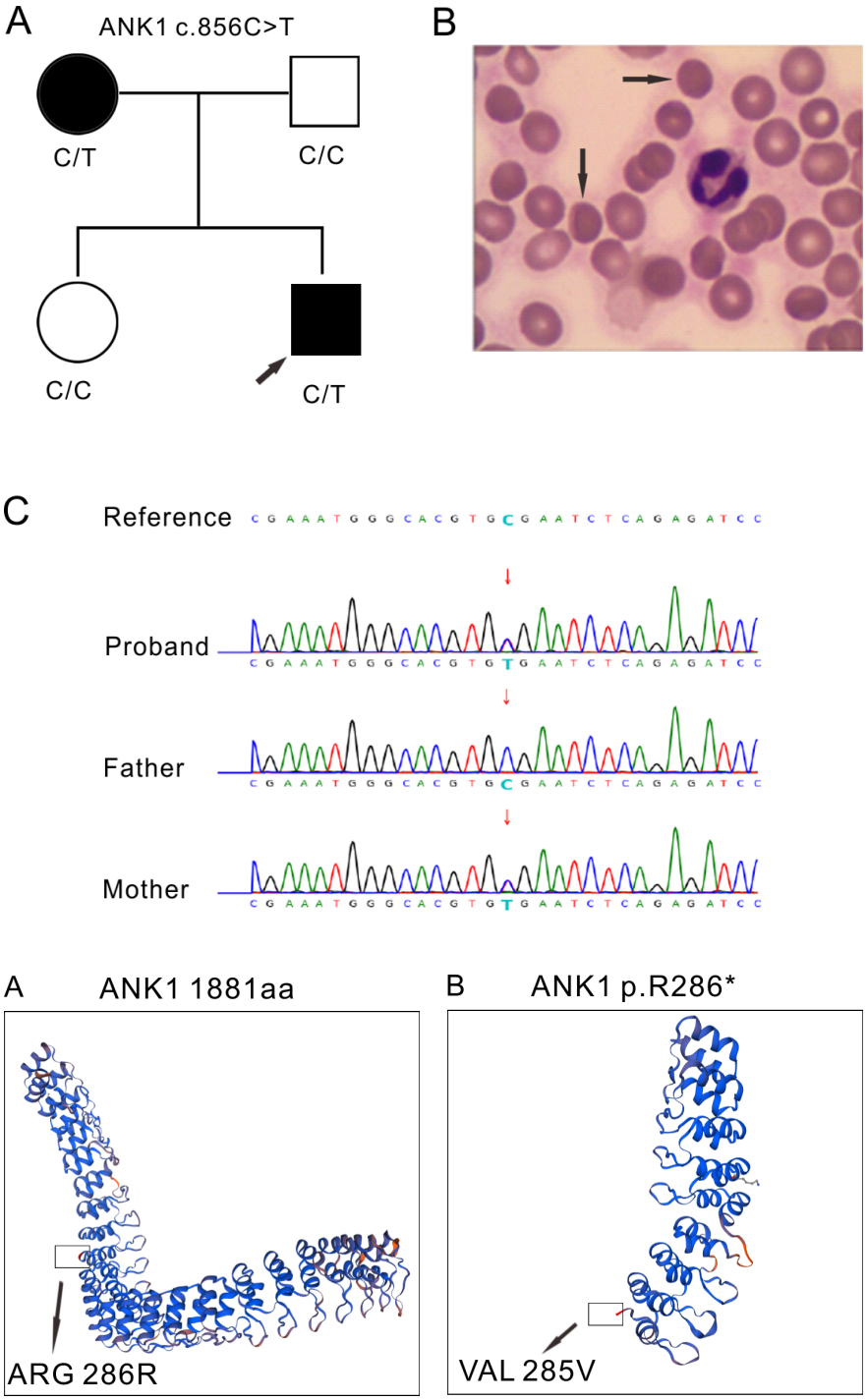
Figure legend

Figure 1. Identification of a novel ANK1 c.856C>T nonsense mutation in two patients from a Chinese family with hereditary spherocytosis. (A)Pedigree of Chinese family with HS. Squares indicate male and circles indicate female. Black symbols denote patients with HS. A black arrow indicates the Proband. (B)Peripheral blood smear. A black arrow indicates spherical-shaped erythrocytes. (C)Sanger sequencing identified an ANK1 c.856C>T mutation in the patients. A red arrow indicates the mutation site.

Figure 2. Protein spatial structure analysis. (A) Intact protein structure of ANK1. (B) Nonsense ANK1 mutation (c.856C>T, p.R286*), caused a substitution from Arginine acid to a premature stop at codon 286. A truncated ANK1 protein, losing the important spectrin-binding and regulatory C-terminal domains and leaving only partial of the N-domain.



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`Table 1.docx` available at https://authorea.com/users/728523/articles/709566-identification-of-a-novel-ank1-c-856c-t-nonsense-mutation-in-two-patients-from-a-chinese-family-with-hereditary-spherocytosis-by-ngs