

# Report on 2 Liver Damage Cases of Unknown Reason with *ANK1* Mutations

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## Case report

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# Abstract

**Background:** *ANK1* mutations are usually related to hereditary spherocytosis. Few studies show direct relationships between *ANK1* mutations and liver damage. Recent researches revealed that *ANK1* mutations may downregulate the expression of hepcidin, a key hepatic hormone important to iron homeostasis. Hepcidin deficiency can induce iron overload that do harm to liver cells and cause ferroptosis.

**Case Presentation:** In both cases, yellowing of skin and hepatomegaly were found. Lab results showed increased alanine aminotransferase (ALT) and aspartate aminotransferases (AST). Genetic tests confirmed de novo heterozygous mutations in *ANK1* gene. Liver biopsy of one child indicated mild liver damage. Prussian blue staining revealed iron accumulation.

**Conclusions:** Here we report 2 cases featuring *ANK1* mutations with liver damage of unidentified causes. We suspect that *ANK1* mutation is related to liver damage through decreasing hepcidin, which may cause iron overload and then ferroptosis.

## Background

*ANK1* is the coding gene for ankyrin-1 protein which is mostly expressed in red blood cells.<sup>1,2</sup> Mutations in *ANK1* are related to about 60% of hereditary spherocytosis (HS) cases.<sup>3</sup> HS is a type of hemolytic anemia disease that usually leads to jaundice and enlarged spleen.<sup>4</sup> Other complications of HS include gallstones and aplastic crisis. Iron is an essential element in the body playing a key role in many biological metabolism routes, of which liver is an important site for maintaining its homeostasis. When dysregulated, iron-related diseases, such as anemia and iron overload, will be caused.<sup>5</sup> To control iron regulation, hepcidin, a hepatic hormone, is of vital importance.<sup>6</sup> Its malfunction may result in excessive iron, producing toxicity of which the liver is the main target organ.<sup>7</sup> It is reported that in mice models, *ANK1* mutations may decrease the hepcidin expression and lead to iron overload, especially in kidney and liver.<sup>8</sup> In addition, there are evidences that hepcidin level positively correlates to the prognosis of acute liver failure patients<sup>9</sup> and hepcidin can be used as a biomarker of some liver disorders<sup>10</sup>.

Studies on the direct relationship between *ANK1* mutations and liver failure are still limited. Researches on children are even fewer. Here we report the clinical features, diagnosis, and treatment of two liver failure pediatric cases with *ANK1* mutations, hoping to provide more information to further understand the topic.

## Case Presentation

**Case A** was a 6-year-and-11-month-old Chinese boy presented with jaundice for more than a year. He has been diagnosed with liver failure, cholestatic liver disease, coagulopathy, spherocytosis type 1, abdominal pain with unknown cause and low T3 syndrome in the past. He experienced partial splenic embolization

surgery for spherocytosis. On admission, the boy showed mild yellowing of skin and hepatomegaly were found. The vital signs were unremarkable. During hospitalization, a liver biopsy was conducted, and the result indicated mild liver damage, with swelling of sinusoidal endothelial cells, increased number of lymphocytes, presence of Kupffer cells and the Scheuer score was G2S1. Prussian blue staining revealed slight iron deposition. The biopsy also found some enlarged mitochondria with irregular shape. Genetic test confirmed a de novo heterozygous mutation in *ANK1* gene, which is usually associated with HS. Multiple abdominal ultrasonography discovered an enlarged spleen. During hospitalization, laboratory tests showed increased prothrombin time (12.6 s, reference range 9–12 s) and activated partial thromboplastin time (41.1 s, reference range 25–35 s). The boy was found positive for herpes simplex virus IgM. In addition, his reticulocyte count increased by 14.37%. And free thyroxine level decreased to 9.48 pmol/L (reference range 12–31 pmol/L). Propionylcarnitine was high when tested on the 6th day of hospital stay. In addition, total bilirubin hit 43.14  $\mu\text{mol/L}$  with direct bilirubin of 10.20  $\mu\text{mol/L}$ . Alanine aminotransferase was 42 U/L (reference range 0–35 U/L) and aspartate aminotransferase was 41 U/L (reference range 0–35 U/L). Based on above signs and findings, the boy was diagnosed with cholestatic hepatitis, hereditary spherocytosis, and post splenic embolization. After admission and until the liver biopsy, treatment given include ursodiol P.O., vitamin K I.M., and preventive administration of oseltamivir. The boy also received plasma and albumin transfusion to further stabilize his condition, later followed by transferring back to the normal ward. The boy's condition was significantly improved with no signs or symptoms and thus he was discharged. Up to now, follow-ups shows a good outcome.

**Case B** is a 4-month-and-21-day-old boy brought to the hospital due to yellowing of skin and sclera for more than four months. The boy suffered low birth weight (caesarean section, 2400 g) and was a premature baby (35 weeks and 3 days). He was diagnosed with neonatal hyperbilirubinemia, neonatal anemia, neonatal pneumonia, neonatal thrombocytopenia, intraventricular hemorrhage (left), auditory pathway disorders (bilateral), and was at high risk of congenital syphilis (maternal syphilis). He was hospitalized for two weeks after birth and was discharged when the conditions improved. However, the yellowing of skin and sclera persisted despite the use of oral ursodiol. He was then hospitalized twice due to cholestasis (diagnosed by laparoscopic common bile duct exploration, cholangiography, and liver biopsy), convulsion, infant liver syndrome, bronchopneumonia, moderate anemia, as well as atrial septal defect (patent foramen oval). The treatment did not work well for his liver function. His liver condition and the yellowing of skin and sclera became worse. On physical examination, the boy's vital signs were unremarkable and there were no significant abnormalities except for the yellowing of sclera and skin. Imaging examinations found hepatomegaly, splenomegaly, peritoneal effusion in the lower abdomen, and mild inflammation in both lungs. Genetic tests confirmed a de novo heterozygous mutation c.3630-1G > C (p.?) in *ANK1* gene. This mutation does not exist in both parents. Significant laboratory results include increased level of lactic acid (3 mmol/L, reference range 0.67–1.8 mmol/L), increased white blood cell count ( $18.18 \times 10^9/\text{L}$ , reference range  $4.5\text{--}11 \times 10^9/\text{L}$ ), increased C-reactive protein (9 mg/L, reference range less than 5 mg/L), prolonged prothrombin time (17.7 s, normally 11–13 s), longer activated partial thromboplastin time (37.2 s, normally 25–35 s), higher international normalized ratio (1.55, normally no more than 1.1), and high fibrinogen level (2.24 g/L, reference range 1.5-4 g/L). In

addition, direct bilirubin (496  $\mu\text{mol/L}$ , reference range 0-5.1  $\mu\text{mol/L}$ ), total bilirubin (842.78  $\mu\text{mol/L}$ , reference range 5.1–20.5  $\mu\text{mol/L}$ ), alanine aminotransferase (1211 U/L, reference range 0–35 U/L) and aspartate aminotransferase (1655 U/L, reference range 0–35 U/L) were all greatly elevated. Other abnormal findings involve elevated level of serum type IV collagen (280 ng/ml), serum hyaluronic acid (219.79 ng/ml), serum laminin (237.68 ng/ml), and serum type III procollagen (250 ng/ml). Following above examinations, the boy was diagnosed with infant liver syndrome, acute liver failure, hyperbilirubinemia, hepatic encephalopathy, coagulopathy, hyperammonemia, hyperlactic acidemia, anemia, and bronchopneumonia. several medications including ursodiol and Transmetil were administered. Based on the condition and test results of the boy, he was transferred to PICU for special care and monitoring. After interventions including but not limited to oxygen inhalation, anti-inflammatory drugs, vitamin K, and transfusion of blood components, despite some improvements, the boy was still facing a predicted poor outcome. Therefore, keeping current treatment was no longer an option and he was transferred to another hospital for liver transplantation. Now the transplantation has finished, and the liver function is significantly improved while his anemia is still worrying. The condition is still in progress and to be followed-up.

## Discussion And Conclusion

Compared to classic liver failure scenarios, these two cases are associated with *ANK1* mutations and HS. Anemia, jaundice, hepatomegaly, and splenomegaly are the main features of HS<sup>11</sup>. Based on above clinical presentations and the finding of *ANK1* mutation, case A was previously diagnosed with HS and had taken partial splenic embolization surgery. However, his liver function did not improve, implying that HS may not be the only driving factor. As for case B, genetic diagnosis confirmed *ANK1* mutation and HS was suspected. Given the young age and the severity and emergence of his liver condition, liver transplantation was of higher priority. Since the liver condition of our cases cannot be solely explained by HS, we wanted to investigate if other connections between *ANK1* mutation and liver damage exist.

Some researches illustrated that *ANK1* mutations may cause hepcidin deficiency, then iron overload.<sup>8</sup> Liver, as the main source of hepcidin, is also the main target of iron toxicity.<sup>7</sup> Milic et al. have revealed the underlying mechanism, that the combination of excessive iron and reactive oxygen increases hydroxyl radical level, thus influences the normal properties of phospholipids, amino acid side chains, proteins and DNA strains.<sup>12</sup> Recent studies also showed that iron overload can trigger ferroptosis, which is an iron overload-induced nonapoptotic cell death.<sup>13</sup> According to Wang et al., mitochondria, the center of cellular metabolism, also serves as the core organelle of iron regulation.<sup>14</sup> The findings of iron deposition and swelled mitochondria during biopsy further aroused our suspicion of iron dysregulation. This may provide us with an insight into what happened to our cases, especially why case A, after splenic embolization, still suffered from worsening liver condition. Additionally, it is now clear that hepcidin has protective role against ferroptosis.<sup>15</sup> Therefore, further tests on iron metabolism are needed to see if the hypothesis is valid and try treatment for iron overload through adjusting hepcidin level. However, case A, after conservative treatment, has been showing improvements and seems not in need of such interventions.

According to our hypothesis, since the mutation in *ANK1* persists, he might experience relapse of liver damage. For case B, who had liver transplantation but is still showing liver abnormalities, further examinations on iron metabolisms and hepcidin levels are needed.

Our two cases indicated that the clinical features of HS and liver manifestations are closely linked. Some presentations (e.g. jaundice and hepatomegaly) can attribute to both HS and iron metabolism pathway.

Problems in our analysis include the lack of adequate cases, the halt of disease progression in one case, and limited evidence found during diagnosis and treatment. We are aware that this condition could be a coincidence. Therefore, *ANK1* mutation and iron metabolism situation, especially hepcidin level, deserve being taken into consideration when facing similar cases in future practices. For further investigation, we will continue to acquire more information through follow-ups and try diagnostic treatment if necessary.

### Take-away messages:

1. *ANK1* mutations are usually related to hereditary spherocytosis.
2. We report 2 cases featuring *ANK1* mutations with liver damage of unidentified causes.
3. We suspect that *ANK1* mutation is related to liver damage through decreasing hepcidin, which may cause iron overload and then ferroptosis.

## Abbreviations

| Abbreviations: | Full names:                   |
|----------------|-------------------------------|
| ALT            | Alanine Aminotransferase      |
| AST            | Aspartate Aminotransferases   |
| HS             | Hereditary Spherocytosis      |
| I.M.           | Intramuscular Injection       |
| PICU           | Pediatric Intensive Care Unit |
| P.O.           | Oral Administration           |

## Declarations

## Ethics approval and consent to participate

Written consents were obtained from the parents of the patients for the study and the publication of this case report. All personal information of both patients was excluded in this report. The study was approved by the Regional Ethical Review Board in Shanghai Children's Hospital.

## Consent for publication

Written consent forms for this case report publication were obtained from parents of both patients.

## Availability of data and materials

The data supporting the conclusion of this report is included within the article.

## Competing interests

The authors declare no competing interests.

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There is no supporting funding in this study.

## Authors' contributions

TZ, YX and DL acquired the clinical data, LY drafted the manuscript, YW edited the manuscript. All authors read and approved the final manuscript.

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