Key Summary of Published Article

A *Grammastola spatulata* Mechanotoxin-4 (GsMTx4)-Sensitive Cation Channel Mediates Increased Cation Permeability in Human Hereditary Spherocytosis of Multiple Genetic Etiologies

Background

- Hereditary spherocytosis (HS) is a rare hemolytic anemia caused by mutations in the genes *ANK1*, *SLC4A1*, *SPTB*, *SPTA1*, and *EPB42*. These genes encode proteins that function in membranes of red blood cells.[1]
- Some of these mutations are associated with increased cation permeability in red blood cells, which is also seen in HS patients.[1,2]
- Previous reports on how mutations affect cation function have provided mixed results.[3,4]
- **Objective:** The investigators of this study identified mutations in red blood cells of HS patients and used on-cell patch clamp analysis to determine how those mutations affect activity of cation channels in red blood cells.

Methods

- DNA and RNA were isolated from whole-blood specimens of 13 patients with a clinical diagnosis of HS.
- cDNA was generated from the isolated mRNA for Sanger sequencing of *SLC4A1*.
- When an *SLC4A1* mutation was not detected, Sanger sequencing of *ANK1* and *SPTB* was performed on cDNA and/or genomic DNA (gDNA).
- Whole-exome sequencing (WES) was conducted on gDNA from blood specimens (n=6) that had been uninformative in the other mutational analyses.
  - Sanger sequencing was used to confirm any mutations detected by WES.
- On-cell patch clamp analysis was conducted on a subset of red cells from HS patients who had mutations identified; non-HS red cells were also analyzed as controls.

Results

- Among 13 patients with a clinical diagnosis of HS
  - 5 had previously identified pathogenic variants in *SLC4A1* (2 of 5 were siblings)
  - 7 had novel pathogenic variants identified in *SLC4A1* (1), *ANK1* (2), or *SPTB* (2)
    - 1 patient with a novel *ANK1* mutation also had a novel missense variant with suspected pathogenicity identified in *SPTA1*.
  - 1 had a novel likely pathogenic variant in *SPTB*
- Red blood cells from 6 HS patients with identified mutations (*SLC4A1*, *ANK1*, and *SPTB*) were used for on-cell patch clamp analysis. Cells from these patients had substantial cation channel activity (average unitary conductance of cation channels, 26+2.1 pS).
- The mechanosensitive cation channel inhibitor *Grammastola spatulata* mechanotoxin-4 almost completely blocked the increased cation channel activity; this and the above results are consistent with modulation a specific ion channel, PIEZO1, causing the channel activity.

Conclusions

- The findings of this study confirm that patients with variants that cause HS have increased cation channel activity.
- The increased activity may be largely caused by modulating PIEZO1.

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