#425

Molecular Diagnosis for Hereditary Angioedema (HAE) Using a 77-gene Next Generation Sequencing Panel

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Background

Hereditary Angioedema (HAE) is a rare autosomal dominant genetic disease frequently caused by mutations in the C1 inhibitor gene *SERPING1*, resulting in dysregulated kallikrein–kinin system (KKS) and overproduction of bradykinin. Despite a myriad of pathogenic mutations identified in *SERPING1* and several other genes in the KKS pathway, genetic diagnosis of some patients with unknown HAE causing mutations but clear HAE clinical presentations are elusive. Genetic modifiers that may contribute to disease severity remain to be investigated. We designed a custom 77-gene Next Generation Sequencing (NGS) panel to encompass genes in the coagulation, complement and tissue-kallikrein pathways. We validated the panel according to ACMG guidelines for NGS sequencing and carried out molecular diagnosis for a patient cohort.

Methods

Genomic DNA was extracted from peripheral blood and screened for mutations using a custom 77-gene NGS panel. Samples were processed through a semi-automated library prep pipeline and sequenced using Illumina technology. Read assembly and variant calling were performed using cloud-based commercial software. Large duplications/deletions were identified using multiplex ligation-dependent probe amplification (MLPA) or Sanger sequencing. The impact of variants was evaluated using commercial and publicly available computational tools.

Table 1. Patient demographics

Age	9-77 years old (mean 45.9)
Males	33
Females	59
HAE type I	41
HAE type II	18
Non-HAE diagnosis	33
Total samples sequenced	**92**

Summary Statement

We designed and clinically validated an HAE molecular diagnosis workflow that has the potential to improve clinical diagnosis and assist in treatment option selections.

Results

Among the 92 samples sequenced, we found:

- Pathogenic *SERPING1* mutations in 59 (100%) of the 59 HAE samples
- A gross heterozygous deletion in *SERPING1* of exons 1-6 using MLPA
- A novel familial 56bp deletion in *SERPING1* exon 6 using Sanger sequencing.
- Two nl-C1-INH patients with novel variants in factor XII (*F12)*

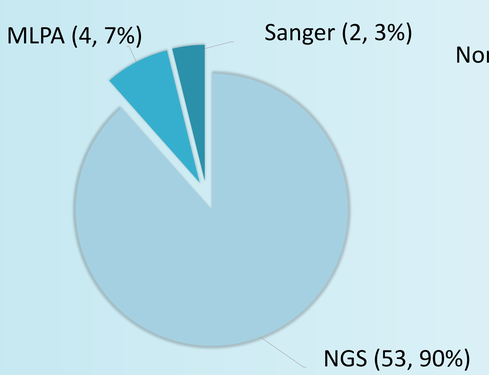


Figure 1. Method of *SERPING1* variant detection.

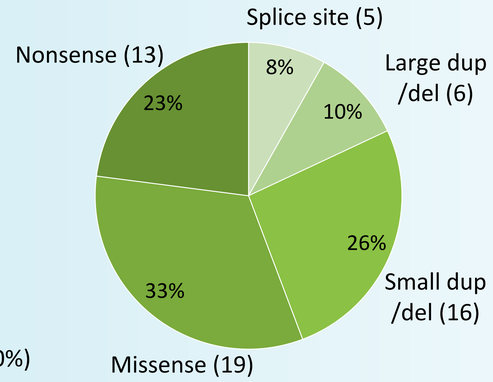


Figure 2. Types of *SERPING1* variants detected.

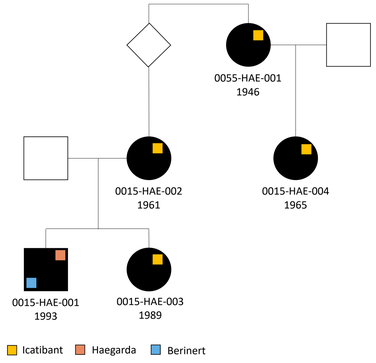
Two large families with potential modifiers

Additional variants identified with our NGS panel meeting computational and segregation analysis criteria were identified as potentially impacting genotype-phenotype in two large families. These variants of unknown significance suggest possible HAE disease-modifying genes that correlate with phenotypic severity.

Family 1

***SERPING1* c.1480C>T p.(Arg494*)**

Family 0015 HAE with Relative 0055



Potential modifiers

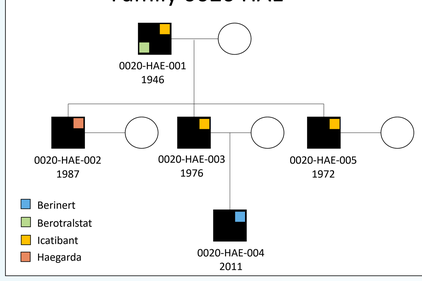
0015-HAE-001		
ADIPOQ	VOUS	missense
CFH	VOUS	donor splice site
FGA	VOUS	missense
KLK3	VOUS	missense
0015-HAE-002		
ADIPOQ	VOUS	missense
CFH	VOUS	donor splice site
F5	VOUS	missense
FGA	VOUS	missense
MYOF	VOUS	missense
0015-HAE-003		
F5	VOUS	missense
KLK13	VOUS	missense
MASP1	VOUS	missense
MYOF	VOUS	missense
0015-HAE-004		
ADIPOQ	VOUS	missense
CFH	VOUS	donor splice site
HS3ST6	VOUS	missense
0055-HAE-001		
ADIPOQ	VOUS	missense
CFH	VOUS	donor splice site
ITIH4	VOUS	missense

Family 1 Variants in the *MYOF* and *HS3ST6* genes have been previously linked to HAE7 (OMIM:619366) and HAE8 (OMIM:619367) respectively. SpliceAI (Broad Institute) predicts a donor splice site loss for the *CFH* variant. *CFH* deficiency results in inappropriate activation of the alternative complement pathway.

Family 2

***SERPING1* c.868_870del p.(Leu290del)**

Family 0020 HAE



Potential modifiers

0020-HAE-001		
F5	VOUS	missense
KLK14	VOUS	missense
0020-HAE-002		
F5	VOUS	missense
KLK14	VOUS	missense
XPNPEP2	VOUS	missense
0020-HAE-003		
no other variants found		
0020-HAE-004		
SERPINA4	VOUS	missense
0020-HAE-005		
XPNPEP2	VOUS	missense

Family 2 0020-HAE-001 and 0020-HAE-002 present with more frequent and severe attacks. Variants in the *F5* gene and the *KLK14* gene segregate with disease severity. *F5* combines with *F10* to activate *F2* (Thrombin) and the extracellular fibrin matrix while the *KLK14* gene is activated by plasmin to initiate fibrin clot remodeling and wound repair.

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