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CLINICAL UTILITY GENE CARD

Clinical Utility Gene Card for hereditary angioedema with normal C1 inhibitor (HAEnC1)

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1. DISEASE CHARACTERISTICS

1.1 Name of the disease (synonyms)

Hereditary angioedema type III (HAE-III)
Estrogen-related hereditary angioedema
Hereditary angioedema with factor XII mutations (FXII-HAE)
Hereditary angioedema of unknown origin (U-HAE)

1.2 OMIM# of the disease

610618

1.3 Name of the analysed genes or DNA/chromosome segments

Coagulation factor XII; Hageman factor; F12; chromosome 5q.35.2-q35.3

1.4 OMIM# of the gene(s)

610619

1.5 Mutational spectrum

To date, four disease-causing variants in *F12* have been reported (ClinVar database https://www.ncbi.nlm.nih.gov/clinvar/).

The missense variants NG_007568.1(NM_000505.3):c.983C>A;p.Thr328Lys and NG_007568.1(NM_000505.3):c.983C>G;p.Thr328Arg affect the threonine residue at position 328 of the protein.[1,2] The p.Thr328Lys variant accounts for the large majority of variant-positive cases reported to date. p.Thr328Lys has been described in patients/families from various ethnic backgrounds. These ethnicities include Arabian;[3,4] Australian;[5] Brazilian,[6] British;[7] French;[1,8,9] German;[1,2,10] Italian;[11-13,,] Jewish;[3] and Spanish.[14-16,,] Haplotype studies of German, French, British, Italian, and Brazilian families have suggested a common founder.[1,6,7,13] The p.Thr328Lys variant has been reported in a single German family.[2] Functional studies have demonstrated that the mutant proteins are defective in terms of mucin-type Thr309-linked glycosylation.[17] In these experiments, the loss of glycosylation led to increased contact-mediated autoactivation of zymogen FXII, resulting in excessive activation of the bradykinin-forming kallikrein-kinin pathway. In contrast, FXII-driven coagulation and the ability of C1-esterase inhibitor to bind and inhibit activated FXII were unaffected. Recent studies confirmed these findings, and showed that the change in protein glycosylation introduced new sites for potential cleavage.[18] The variants caused accelerated FXII activation by plasmin, thereby interrupting the balance between activation and inhibition of the contact system in solution. As a result, the FXII-mutants escaped C1inh inactivation during activation by plasmin. These results suggest that: (i) the plasminogen system and the contact activation system are functionally linked; and (ii) in HAEnC1 patients, an increased potential of plasminogen activation can prime plasma for excessive bradykinin production.

In addition to the two missense variants, one deletion and one duplication have been reported in single families. The NG_007568.1(NM_000505.3):c.971_1018+24del;p.Lys324_Ala340delinsThr variant is a deletion of 72 base pairs (bp).[19] This deletion causes a loss of 48 bp of exon 9 (coding amino acids 324 to 340) and 24 bp of intron 9. The deletion includes the authentic donor splice site of exon 9. The variant NG_007568.1(NM_000505.3):c.894_911dup;p.(Gln300_Thr305dup) is a duplication of 18 bp.[20] This duplication causes the repeat of 6 amino acids. As with the missense variants, the duplication and the deletion affect the proline-rich region, which suggests similar functional consequences.[21] For the deletion variant, the anticipated functional consequences have been demonstrated experimentally.[18] For the duplication, no functional investigations have yet been performed.

1.6 Analytical methods

A diagnosis of HAEnC1 is assigned on the basis of: (i) the clinical symptoms of angioedema, in the absence of significant wheals; (ii) the presence of a HAEnC1 causing variant or the existence of affected family members; and (iii) normal C1 inhibitor activity and C1 inhibitor protein levels in plasma.

The main strategy for the detection of *F12* variants is the direct sequencing of an amplified PCR product of exon 9 from the patient's genomic DNA (EDTA blood), including the flanking intronic sequences. In patients with no variant in exon 9, sequencing of the remaining exons and multiplex ligation-dependent probe amplification (MLPA) analysis can be considered.

1.7 Analytical validation

Direct sequencing of PCR products is performed in both forward and reverse directions. When a new variant is found, the pathological relevance should be demonstrated by familial segregation analysis, confirmation of the evolutionary conservation of the affected amino acid(s), assessment of pathogenicity by mutation prediction software, and/or functional studies.

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1.8 Estimated frequency of the disease

(Incidence at birth ("birth prevalence") or population prevalence. If known to be variable between ethnic groups, please report):

Clinical experience suggests that population prevalence is low, although no systematic data are available. Haplotype studies of the most prevalent p.Thr328Lys variant in families from various countries have suggested a common founder, and this may well result in population differences. However, no data are available concerning prevalences in specific populations.

1.9 Diagnostic setting

	Yes	No
A. (Differential) diagnostics	☒	☐
B. Predictive testing	☒	☐
C. Risk assessment in relatives	☒	☐
D. Prenatal	☒	☐

Comment: Mutation analysis is mainly used to confirm a clinical diagnosis and in relation to genetic counseling. In principle, prenatal diagnosis is possible. However, due to the episodic nature of the illness, the substantial clinical variability, the reduced penetrance, and the availability of therapeutic options, prenatal diagnosis is typically not requested. To date, prenatal diagnosis of HAEnC1 has not been reported.

2. TEST CHARACTERISTICS

	Genotype or disease		A: True positives	C: False negatives
	Present	Absent	B: False positives	D: True negatives
Test				
Positive	A	B	Sensitivity:	A/(A+C)
			Specificity:	D/(D+B)
Negative	C	D	Positive predictive value:	A/(A+B)
			Negative predictive value:	D/(C+D)

2.1 Analytical sensitivity

(proportion of positive tests if the genotype is present)

If one of the four known variants in exon 9 of *F12* is present, the sensitivity for Sanger sequencing of genomic DNA is almost 100%. To date, sequencing of patients with no variant in exon 9 has failed to identify variants in other regions of the gene.[13] However, deep intronic variants, large deletions, and duplications would not be detected using this approach. Linkage studies have identified families with no linkage to the *F12* locus,[1] and thus other causative gene(s) must exist. However, no other causative gene has yet been identified.

2.2 Analytical specificity

(proportion of negative tests if the genotype is not present)

Close to 100%.

2.3 Clinical sensitivity

(proportion of positive tests if the disease is present) The clinical sensitivity can be dependent on variable factors such as age or family history. In such cases a general statement should be given, even if a quantification can only be made case by case.

Clinical sensitivity is dependent on clinical presentation and family history. In investigations of large cohorts of patients with family-history positive HAEnC1,[22] approximately 25–33% of patients carried a variant in *F12*.[12,13,23]

2.4 Clinical specificity

(proportion of negative tests if the disease is not present)

The clinical specificity can be dependent on variable factors such as age or family history. In such cases a general statement should be given, even if a quantification can only be made case by case.

Close to 100%.

2.5 Positive clinical predictive value

(life time risk to develop the disease if the test is positive)

For *F12* variant carriers, the estimated penetrance is $<10\%$ in males and around 60% in females.[9,15,24]

2.6 Negative clinical predictive value

(Probability not to develop the disease if the test is negative). Assume an increased risk based on family history for a non-affected person. Allelic and locus heterogeneity may need to be considered.

Index case in that family had been tested:

If the index case has been tested and a variant in *F12* has been identified: 100%

Due to allelic and locus heterogeneity, the relatives of an index patient with no identified *F12* variant remain at increased risk.

Index case in that family had not been tested:

If the index case has not been tested, a negative test result in a non-affected relative does not exclude the presence of HAEnC1, since an undetected *F12* variant may exist, and the possibility of locus heterogeneity[12,13,23] and reduced penetrance remain.

3. CLINICAL UTILITY

3.1 (Differential) diagnostics: The tested person is clinically affected

(To be answered if in 1.9 "A" was marked)

3.1.1 Can a diagnosis be made other than through a genetic test?

No	☐ (continue with 3.1.4)	
Yes	☒	
	Clinically	☒
	Imaging	☐
	Endoscopy	☐
	Biochemistry	☒
	Electrophysiology	☐
	Other (please describe)	family history

Genetic testing helps to confirm the diagnosis. A clinical diagnosis of HAEnC1 is assigned on the basis of: (i) the clinical symptoms of angioedema, in the absence of significant wheals; (ii) the presence of a HAEnC1 causing variant or the existence of affected family members; and (iii) normal C1 inhibitor activity and C1 inhibitor protein levels in plasma.

3.1.2 Describe the burden of alternative diagnostic methods to the patient

The biochemical investigation of C1 inhibitor status requires blood sampling. No other invasive procedures are required to establish a clinical diagnosis.

3.1.3 How is the cost effectiveness of alternative diagnostic methods to be judged?
Clinical investigation, evaluation of family history, and biochemical assays are used routinely in the diagnostic evaluation of patients, which is necessary in order to determine appropriate management and follow-up. Genetic testing helps to confirm the diagnosis and enables the testing of relatives.

3.1.4 Will disease management be influenced by the result of a genetic test?

No	☒
Yes	☐
	Therapy (please describe)
	Prognosis (please describe)
	Management (please describe)

Comment: No controlled studies of the clinical management of HAEnC1 have been published, although the presumed pathophysiology suggests potential treatment options[22] and off label use has been reported.[9,25] No data are available concerning differences in clinical management, treatment response, or prognosis between patients with variants in *F12* (FXII-HAE) and variant-negative patients (U-HAE).

3.2 Predictive setting: The tested person is clinically unaffected but carries an increased risk based on family history (To be answered if in 1.9 "B" was marked)
If an *F12* variant has been demonstrated in an affected family member, unaffected relatives can be tested for the presence of the variant.

3.2.1 Will the result of a genetic test influence lifestyle and prevention?
If the test result is positive (please describe). In an unaffected female relative, a positive genetic test suggests that the subject will be sensitized to known triggering factors, such as pregnancy or the use of oral contraceptives or hormone replacement therapy. In such cases, alternative contraceptive and hormone stabilization strategies should be considered. In men, the genetic test result has less influence on lifestyle and prevention, since >90% of men with a positive genetic test remain asymptomatic, and no trigger factors have been identified in the small number of male patients reported to date.[15]

If the test result is negative (please describe). In women, a negative test result will also impact lifestyle, since no episodes of angioedema are anticipated in such cases. A negative result will therefore provide psychological relief for the female family members of affected patients.

3.2.2 Which options in view of lifestyle and prevention does a person at-risk have if no genetic test has been done (please describe)?
For the first-degree relatives of a confirmed *F12* variant carrier, the *a priori* risk of carrier status is 50%. Women in particular may consider adapting their lifestyle to their potential at-risk status as a preventive measure.

3.3 Genetic risk assessment in family members of a diseased person (To be answered if in 1.9 "C" was marked).
3.3.1 Does the result of a genetic test resolve the genetic situation in that family?
Yes.

3.3.2 Can a genetic test in the index patient save genetic or other tests in family members?
Yes, if no established or novel variant in the *F12* gene is detected in the index patient.

3.3.3 Does a positive genetic test result in the index patient enable a predictive test in a family member?
Yes.

3.4 Prenatal diagnosis (To be answered if in 1.9 "D" was marked)
3.4.1 Does a positive genetic test result in the index patient enable a prenatal diagnosis?
Yes.

4. IF APPLICABLE, FURTHER CONSEQUENCES OF TESTING
Please assume that the result of a genetic test has no immediate medical consequences. Is there any evidence that a genetic test is nevertheless useful for the patient or his/her relatives? (Please describe)
Not applicable.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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