

Genetic Determinants of C1 Inhibitor Deficiency Angioedema Age of Onset

Panagiota Gianni^a Gedeon Loules^b Maria Zamanakou^b Maria Kompoti^a
Dorottya Csuka^d Fotis Psarros^c Markus Magerl^e Dimitru Moldovan^f
Marcus Maurer^e Matthaïos G. Speletas^a Henriette Farkas^d
Anastasios E. Germeris^a

^aDepartment of Immunology and Histocompatibility, Faculty of Medicine, School of Health Sciences, University of Thessaly, and ^bCeMIA SA, Larissa, and ^cDepartment of Allergology, Navy Hospital, Athens, Greece;

^dHungarian Angioedema Center, 3rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary; ^eDepartment of Dermatology and Allergy, Charité – Universitätsmedizin Berlin, Berlin, Germany;

^fUniversity of Medicine and Pharmacy, Tirgu Mures, Romania

Keywords

Age of disease onset · C1 inhibitor deficiency · *F12*-46C/T polymorphism · Hereditary angioedema · *KLKB1*-S143N polymorphism

Abstract

Background: In view of the large heterogeneity in the clinical presentation of hereditary angioedema due to C1 inhibitor deficiency (C1-INH-HAE), great efforts are being made towards detecting measurable biological determinants of disease severity that can help to improve the management of the disease. Considering the central role that plasma kallikrein plays in bradykinin production, we investigated the contribution of the functional polymorphism *KLKB1*-428G/A to the disease phenotype. **Methods:** We studied 249 C1-INH-HAE patients from 114 European families, and we explored possible associations of C1-INH-HAE clinical features with carriage of *KLKB1*-428G/A, combined or not with that of the functional *F12*-46C/T polymorphism. **Results:** Carriers of the G allele of the *KLKB1*-428G/A polymorphism exhibited a significantly delayed disease onset (i.e., by 4.1 years [$p < 0.001$], depending on the zygosity status), while carriers of both the

KLKB1-428G/A and the *F12*-46C/T polymorphism displayed an 8.8-year delay in disease onset ($p < 0.001$) and a 64% lower probability of needing long-term prophylactic treatment ($p = 0.019$). **Conclusions:** These findings support our initial hypothesis that functional alterations in genes of proteins involved in bradykinin metabolism and function affect the clinical phenotype and possibly contribute to the pathogenesis of C1-INH-HAE. Given that an earlier onset of symptoms is inversely correlated with the subsequent course of the disease and, eventually, the need for long-term prophylaxis, these polymorphisms may be helpful prognostic biomarkers of disease severity.

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Introduction

Hereditary angioedema due to C1 inhibitor deficiency (C1-INH-HAE) is a rare autosomal dominant disorder that manifests with recurrent incapacitating attacks of edema localized in subcutaneous or mucosal tissues, spontaneously developing in any body compartment. In C1-INH-HAE patients, numerous *SERPING1* variants

are responsible for C1-INH deficiency or dysfunction, which results in an uncontrolled activation of various plasma enzyme cascades leading to an increased production of bradykinin [1]. A most intriguing feature of C1-INH-HAE is the large inter- and intrafamilial heterogeneity with which the disease presents in terms of the age at disease onset, the severity and frequency of the attacks, triggers of acute episodes, localization of edema, and prodromal signs and symptoms [2, 3]. Thus, great efforts are being made to detect measurable biological determinants of disease severity in order to better manage the disabling and potentially fatal manifestations of C1-INH-HAE. Actually, a biological relationship with disease severity was first demonstrated 10 years ago [4].

To this aim, we hypothesized that functional variants of genes encoding for other proteins involved in bradykinin metabolism might be associated with C1-INH-HAE clinical severity. Plasma kallikrein (PK), a serine protease encoded by the *KLKB1* gene (on chromosome 4) is integral to the contact activation pathway on vascular endothelial cells and it is primarily inhibited by C1-INH. C1-INH deficiency results in uncontrolled generation of PK and subsequent proteolysis of high-molecular-weight kininogen which leads to excessive bradykinin production [5–7]. Most of the reported *KLKB1* polymorphisms are located in noncoding regions. The *KLKB1*-428G/A polymorphism (rs3733402), however, is located in exon 5, where it leads to the amino acid substitution Asn124Ser [8, 9], and strong evidence indicates that this mutation is linked to functional consequences [10, 11].

Thus, the *KLKB1*-428G/A polymorphism was chosen to investigate its association with clinical descriptors of C1-INH-HAE severity. The combined effect of the concomitant carriage of this polymorphism and another functional polymorphism that we had previously shown to be associated with age at disease onset, i.e., the *F12*-46C/T polymorphism (rs1801020) [12], was also examined.

Patients and Methods

In this study, 249 patients (116 males and 133 females, mean age: 36 years, range 1–82) with C1-INH-HAE (type I, $n = 221$; type II, $n = 28$) from 114 unrelated families (37 Greek, 12 German, 19 Romanian and 46 Hungarian) were enrolled. All patients were previously genotyped for *SERPING1* mutations and the *F12*-46C/T polymorphism [12–14]. Patients' medical records were reviewed and data regarding disease onset, treatment modalities, and the major clinical manifestations were recorded. The mean patients' age at disease onset was 9.8 years (range 1–70). The frequency of attacks was not considered as a variable of disease severity due to

the inclusion of patients under long-term prophylactic treatment (LTP). The local institutional review boards approved this study, and written informed consent was obtained from each individual or an accompanying relative.

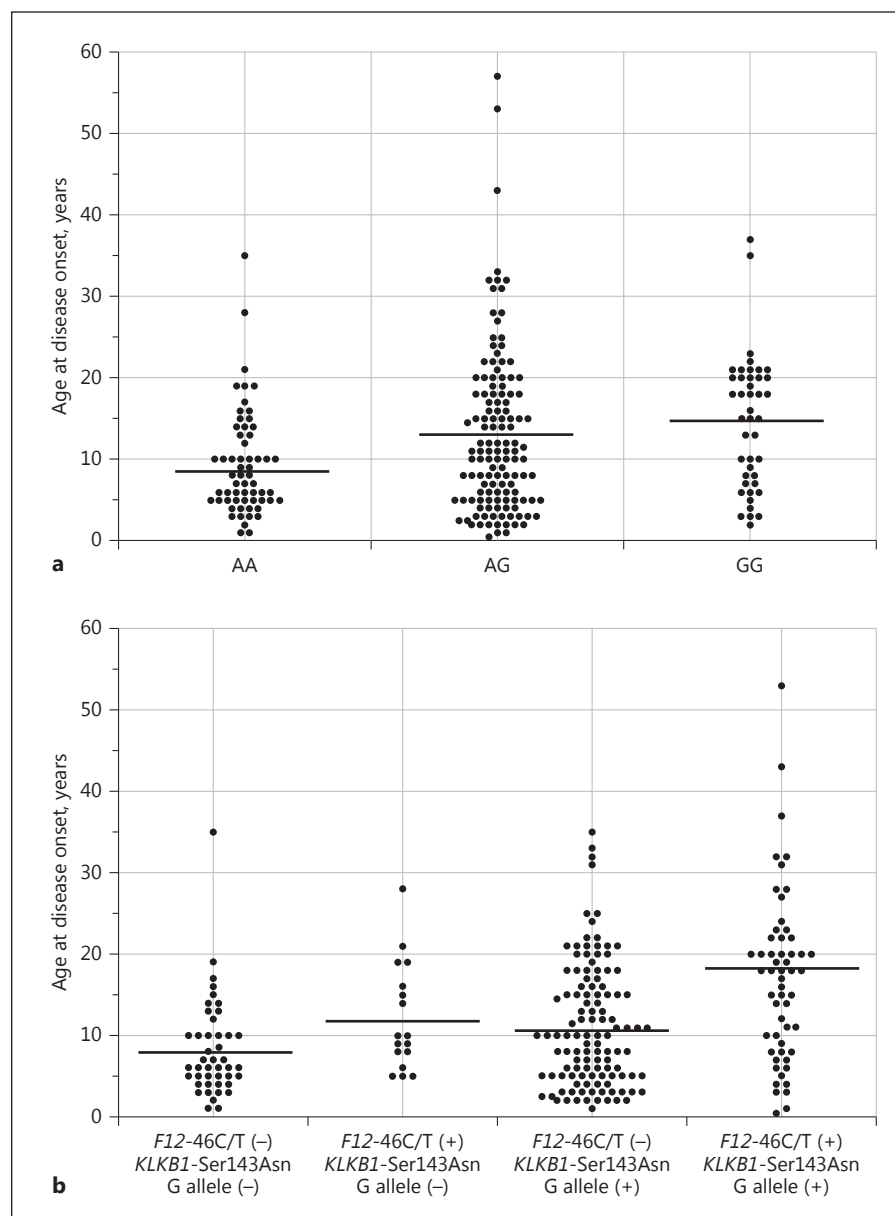
Detection of the *KLKB1*-428G/A polymorphism was performed by PCR in combination with Sanger sequencing. Briefly, a total of 100–200 ng of gDNA was amplified by PCR in a 30- μ L reaction mixture using 200 μ M of each deoxynucleoside triphosphate, 30 pmol of each primer, 1.5 mM MgCl₂ and 1.0 U Taq polymerase (Invitrogen, Thermo Fisher, USA) in a 10 $\times$ buffer supplied by the manufacturer. The PCR primers consisted of the forward primer 5'-AACCCAAATGGTAGTGGGTA-3' and the reverse primer 5'-AGCGCGACTCCATCTCAATA-3', and PCR conditions were: 5 min at 95°C, followed by 35 cycles of 95°C for 30 s, 55°C for 1 min, and 72°C for 1 min and 5 min at 72°C after the last cycle. All PCR reactions were carried out in the PCR engine apparatus Veriti 96-Well Thermal Cycler (Applied Biosystems, Thermo Fisher, USA). The emerging PCR products were purified using the USB ExoSAP-IT PCR Product Cleanup purification system (Thermo Fisher Scientific) and they were directly sequenced using a 3730 DNA analyzer (Applied Biosystems, Thermo Fisher) and a BigDye Terminator DNA sequencing kit (Applied Biosystems, Thermo Fisher).

With regard to the analysis of the results, categorical variables were analyzed with Fisher's exact test. The normality of continuous variables was assessed using the Kolmogorov-Smirnov test. Normally distributed data were analyzed with Student's *t* test and one-way ANOVA as appropriate. Skewed data were analyzed using nonparametric methods (Mann-Whitney test or Kruskal-Wallis test as appropriate). Given the fact that our patient population consisted of correlated subjects, generalized estimating equations (GEE), an extension of the generalized linear model that accounts for the within-subject correlation, was implemented. GEE was used to model the association of response variables (age at disease onset and need for LTP) with explanatory variables (race, gender, genotype, C1-INH-HAE type, and frequency of laryngeal and abdominal attacks). Age at disease onset was modeled as a continuous variable in linear GEE models and the need for LTP was entered as a binary variable in logistic GEE models. In all GEE models an unstructured correlation structure was used and the quasi likelihood information criterion was used for model selection. Data analysis was performed with SPSS 17.0 (IBM Corporation, New York, NY, USA). For all analyses, α was set at 0.05 (2-tailed). Linkage disequilibrium was assessed using standard methods.

Results

Among the 249 C1-INH-HAE patients we examined, 138 were heterozygotes and 47 were homozygotes for the G allele of the *KLKB1*-428G/A polymorphism (allele frequency: 46.58%). The prevalence of the polymorphism did not differ significantly between examined patient groups from different countries. In a GEE linear regression model with age at disease onset as the dependent variable, presence of the G allele was significantly associated with a delay in disease onset by 4.1 years ($p < 0.001$)

Fig. 1. a Differences in age at disease onset between wild-type (AA), heterozygote (AG), and homozygote (GG) C1-INH-HAE patients for the *KLKB1*-428G/A polymorphism ($p < 0.001$). **b** Differences in age at disease onset between patients not carrying *KLKB1*-428G/A or *F12*-46C/T, carriers of one of them, and carriers of both polymorphisms.



adjusted for sex and ethnicity. Compared to wild-type patients (AA), heterozygotes (AG) had a 3.3-year delay in disease onset ($p = 0.002$) while in homozygotes (GG) the delay was 4.3 years ($p = 0.003$) (Fig. 1a). When the combined effect of *KLKB1*-428G/A and *F12*-46C/T polymorphism carriage was examined, patients carrying both polymorphisms exhibited an 8.8-year delay in disease onset ($p < 0.001$), while those carrying only one of them had a 3.3-year delay ($p = 0.006$) and a 3.9-year delay ($p = 0.03$), respectively, compared to the carriers of the corresponding wild types (Fig. 1b). The 2 polymorphisms were not in significant linkage disequilibrium.

No significant association was noted between the kind of *SERPING1* mutation or C1-INH-HAE type and the age at disease onset. Moreover, patients carrying the *KLKB1*-428G/A and *F12*-46C/T polymorphisms had a 64% lower probability to need LTP (OR = 0.36; 95% CI 0.15 – 0.85, $p = 0.019$). Associations between the *KLKB1*-428G/A polymorphism, alone or in combination with *F12*-46C/T, were not detected with the frequency of either abdominal or of laryngeal attacks.

Discussion

Accumulating evidence indicates that hereditary angioedema presents a much greater genetic complexity than was initially considered. Beyond *SERPING1* alterations leading to C1-INH deficiency, alterations associated with normal C1-INH angioedema have been detected in the *F12* gene [15, 16] and, recently, in the *ANGPT1* gene encoding for angiopoietin-1 that targets key mechanisms contributing to maintenance of the endothelial barrier function [17] as well as in the plasminogen gene [18, 19]. On the other hand, with regard to the clinical expression of C1-INH-HAE, we have shown that carriage of missense *SERPING1* mutations [13, 14] or the functional promoter polymorphism *F12*-46C/T (rs1801020) is associated with a less severe clinical course [12].

On this basis, we hypothesized that functional alterations in genes of other proteins involved in bradykinin metabolism and function may affect the clinical phenotype and possibly contribute to the pathogenesis of C1-INH-HAE. The results of our study confirmed this hypothesis, showing that carriers of the *KLKB1*-428G/A polymorphism exhibit a significantly delayed disease onset by 4.1 years depending on the zygosity status, while carriers of both the *KLKB1*-428G/A and the *F12*-46C/T polymorphism displayed a longer delay in disease onset and a significantly lower probability of needing LTP.

The above mentioned associations of the *KLKB1*-428G/A polymorphism can be attributed to its functionality. As is well documented, this mutation in Apple domain 2 of the heavy chain results in a reduced formation of the PK-HMWK complex, a plasma and cell membrane-binding protein that interferes with optimal PK activation and bradykinin formation. Moreover, given that PK converts prorenin to renin, albeit in low pH [20], this variant has been shown to be linked to disruption of enzymatic cascade events leading to a diminished forma-

tion of active renin [10]. Finally, this variant exhibits a strong association with pre-pro-endothelin-1 and pre-pro-adrenomedullin [21], and with a reduced history of cardiovascular disease [11]. With regard to the functionality of the *F12*-46C/T polymorphism, located in the promoter region of the *F12* gene at nt46, it is known that it creates a new initiation codon (ATG) for transcription of the mRNA and a frameshift that produces a truncated protein. The T allele destroys Kozak's consensus sequence (GCCAGCCATGG), resulting in decreased translation efficiency and low plasma levels of FXII activity and antigen possibly affecting bradykinin production [22–24].

If appropriately evaluated in large enough patient cohorts, *KLKB1*-428G/A and *F12*-46C/T polymorphisms may be found to be useful predictive genetic biomarkers of age of disease onset in a symptom-free offspring carrying the same *SERPING1* mutation as their parent suffering from C1-INH-HAE. Moreover, given that an earlier onset of symptoms is inversely correlated with the subsequent course of the disease and, eventually, the need for LTP [25, 26], these polymorphisms may prove to be helpful prognostic biomarkers of disease severity, a topic attracting the interest of current angioedema research [27]. In any case, the validity of association studies, like this one, as well as any other attempt to detect biomarkers of C1-INH-HAE severity is faced with the problem of a widely accepted determination and an objective measurement of disease severity that is still lacking. Finally, our findings provide one more piece of evidence on the functionality of the *KLKB1*-428G/A polymorphism that is worthy of being further investigated.

Disclosure Statement

The authors declare that they have no conflict of interests with regard to this publication.

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