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A missense mutation in the plasminogen gene, within the plasminogen kringle 3 domain, in hereditary angioedema with normal C1 inhibitor

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ABSTRACT

Hereditary angioedema (HAE) is a genetically heterogeneous disease that is characterized by recurrent skin swelling, abdominal pain attacks, and potentially life-threatening upper airway obstruction. The two classic types, HAE types I and II, are both caused by mutations in the complement C1 inhibitor (SERPING1) gene resulting either in a quantitative or a qualitative deficiency of C1 inhibitor. In so-called HAE type III, in contrast, patients show normal C1 inhibitor measurements in plasma ('HAE with normal C1 inhibitor'). As previously shown by us, one subgroup of 'HAE with normal C1 inhibitor' is caused by mutations of the coagulation factor XII (F12) gene. For the present study, following the exclusion of numerous candidate genes, we screened eight unrelated index patients representing eight 'HAE families with normal C1 inhibitor and no F12 mutation' for mutations in the plasminogen (*PLG*) gene. A rare non-conservative missense mutation was newly identified in exon 9 of the *PLG* gene. This mutation (c.1100A > G), encountered in three out of eight patients, predicts a lysine-to-glutamic acid substitution in position 311 of the mature protein (p.Lys311Glu). Using isoelectric focusing of plasma samples followed by an immunoblotting procedure we demonstrated that the presence of the mutation is associated with a dysplasminogenemia, namely the presence of an aberrant plasminogen protein. The predicted structural and functional impact of the mutation, its absence in 139 control individuals, and its co-segregation with the phenotype in three large families provide strong support that it causes disease. Extending a previously proposed gene-based alphabetic nomenclature for the various HAE types one may use the term 'HAE type C′ for the HAE entity described here.

1. Introduction

Angioedema, a localized and self-limiting edema of subcutaneous or submucosal tissue, is a commonly diagnosed symptom of various acquired and inherited disease entities. Hereditary angioedema (HAE) is a disease clinically characterized by recurrent skin swelling, abdominal pain attacks due to swelling of the bowel wall, and potentially life-threatening upper airway obstruction [1,2].

The classic types of hereditary angioedema, HAE types I and II, are both caused by mutations in the complement C1 inhibitor (SERPING1) gene. These mutations result either in a quantitative or a qualitative deficiency of C1 inhibitor, a serpin which is the main control element of the enzymatic activity of complement component C1, but also an important inhibitor of proteases of the kinin-forming cascade (kallikrein, activated coagulation factor XII) and other serine proteases involved in coagulation and fibrinolysis. The deficiency of the inhibitor - via an insufficiently controlled activation of these cascade systems - finally leads to the generation of vasoactive substances involved in edema development [2,3].

In 2000, a novel type of hereditary angioedema has been characterized [4,5]. Symptoms are similar to those seen in HAE types I and II; however, patients show normal C1 inhibitor concentration and activity in plasma ('HAE with normal C1 inhibitor'; 'HAE type III'; 'estrogen-dependent inherited angioedema') [4–7]. The molecular basis of this apparently heterogeneous entity has been elucidated only in part. For a subgroup of approximately 25% of HAE families with normal C1 inhibitor mutations of the coagulation factor XII (F12) gene have been established as the underlying molecular cause [2,8].

In the work presented here, we describe the identification of a

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rare missense mutation of the plasminogen (*PLG*) gene that - together with the corresponding dysplasminogenemia - apparently represents the molecular basis for the disease of a further substantial subgroup of patients with 'hereditary angioedema with normal C1 inhibitor'.

2. Materials and methods

2.1. Study subjects

Eight unrelated patients, all female and of German origin, were studied. All patients had experienced recurrent angioedema attacks, had one or more affected relatives, and showed normal C1 inhibitor measurements; further, all patients had been tested negative for the coagulation factor XII (F12) mutations found in 'HAE with normal C1 inhibitor' [2,8]. For several of the eight index patients, various numbers of family members could be included in the study; patients and family members were personally interviewed, medical histories, pedigree information, and venous blood samples for the preparation of genomic DNA and plasma were obtained. One hundred and thirty-nine unselected outpatients of a general practice served as controls; all were of German origin. Informed consent was provided by all subjects. The study was carried out in accordance with the principles of the Declaration of Helsinki.

2.2. Polymerase chain reaction (PCR) and sequencing of PCR products

Genomic DNA was isolated from venous blood samples by using QIAamp DNA Blood Mini Kit (Qiagen). Oligonucleotide primers were designed with Primer3 software, avoiding any known polymorphism within primer annealing sites. Further, care was taken to avoid accidental co-amplification of sequences of plasminogen-related genes. Standard polymerase chain reaction (PCR) was carried out on approximately 70 ng of genomic DNA by using twenty pairs of oligonucleotide primers to amplify the nineteen exons and the exon-intron boundaries of the *PLG* gene as twenty separate amplicons. Direct sequencing of PCR products was performed using the BigDye™ Terminator v3.1 cycle sequencing kit (Life Technologies/Thermo Fisher Scientific), usually with the same primers as were used in the PCR amplification, only in some instances with internal sequencing primers.

2.3. Analysis of plasminogen protein patterns in plasma samples

Plasminogen protein patterns in plasma were examined by isoelectric focusing (IEF) in polyacrylamide gels as well as by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

IEF was performed as described previously [9], with minor modifications. Prior to focusing, plasma samples were pre-treated with *Clostridium perfringens* neuraminidase (Sigma, type VI) to reduce pattern microheterogeneity [9,10]. The desialized samples were then subjected to IEF in thin-layer polyacrylamide gels containing 2.5% (w/v) carrier ampholytes (Pharmalyte®; 4 parts pH 5–8, 1 part pH 3–10), and 0.2 M taurine. The focused proteins were transferred onto a nitrocellulose membrane (Amersham; 0.2 μm) by press-blotting for 1 h. For the subsequent enzyme immunoassay for the specific detection of plasminogen, we used goat anti-human plasminogen/affinity-purified IgG (Affinity Biologicals) as the primary antibody, and peroxidase-conjugated rabbit anti-goat immunoglobulins (DAKO Denmark A/S) as the secondary antibody. Finally, peroxidase activity was visualized using the 'Super Signal®West Pico' kit (Thermo Scientific).

For SDS-PAGE, plasma samples prepared in reducing Laemmli buffer were applied to a 8% (w/v) polyacrylamide gel. Following transfer to a nitrocellulose membrane, plasminogen detection was achieved as described above.

3. Results and discussion

The 19 exons and splice junctions of the *PLG* gene [11] were screened in eight unrelated patients by PCR amplification and direct sequencing of PCR products. Aside from numerous known polymorphic variants, one new mutation was identified, namely a non-conservative missense mutation in exon 9 of the plasminogen gene (Fig. 1). This mutation (c.1100A > G), encountered in three unrelated patients, affects the first position of the codon (AAA) encoding amino acid residue 311 of the mature protein [11], a lysine residue, and results in a GAA triplet encoding a glutamic acid residue [p.Lys311Glu; corresponding to p.Lys330Glu if residue numbering is based on the primary translation product (GenBank accession number NP_000292.1)]. Thus, the positively charged residue of the wild-type sequence is substituted by a negatively charged residue. In accordance with the dominant inheritance pattern of the disease, patients are heterozygous for the mutation.

By sequencing of exon 9 we screened 139 control individuals derived from the same population as the affected families. The missense mutation p.Lys311Glu observed in the patients was not detected in this control panel. Thus, a missense mutation affecting Lys311 was seen in 3 out of 8 unrelated patients, but in none of 139 controls ($p = .00011$; Fisher's exact test). Further, the mutation was neither described in the Exome Variant Server (6503 individuals) [12] nor in the ExAC browser (60,706 individuals) [13]. In contrast, within ongoing studies, we identified an additional HAE family

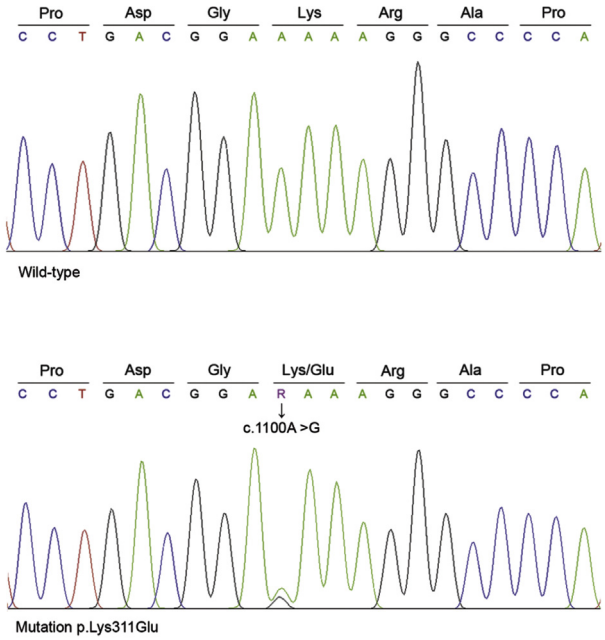

Fig. 1. Missense mutation of the plasminogen gene in hereditary angioedema with normal C1 inhibitor. Sequence chromatograms from exon 9 of the *PLG* gene showing the c.1100A > G transition observed in a patient [predicting a lysine-to-glutamic acid substitution in amino acid position 311 of the mature protein (p.Lys311Glu)], in addition to the wild-type sequence observed in a control individual [nucleotide numbering according to GenBank accession number NM_000301 (version NM_000301.3); c.1100A > G corresponds to c.988A > G if nucleotide numbering starts with the A of the ATG translation initiation codon; with respect to the genomic NCBI reference sequence NG_016200 (version NG_016200.1) the mutation affects position 21,538: g.21538A > G].