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BRIEF REPORT—MUTATION REPORT

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A novel nonsense mutation at Glu-631 in a Spanish family with complement component 7 deficiency

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Abstract Deficiency of the seventh component of complement (C7D) is frequently associated with recurrent neisserial infections. We report in the present study the genetic basis for C7D in a Spanish family. We used exon-specific polymerase chain reaction (PCR)/single-strand conformation polymorphism (SSCP) analysis as a screening step for mutations, followed by direct sequencing of the target exon. The mutation in the proband was a homozygous G-to-T transversion at nucleotide 1957, the first nucleotide of the codon GAG for Glu-631, leading to a stop codon TAG (E631X). Our result provides further evidence that the molecular pathogenesis of C7D is heterogeneous.

Key words Nonsense mutation · Complement component 7 · Deficiency

Introduction

Complement component 7 (C7) is one of the five complement proteins that generate a large protein-protein complex called membrane attack complex (MAC), upon activation of the complement system. Assembly of the MAC on target cells leads to its gradual insertion into the lipid bilayer and the formation of a transmembrane channel, which eventually leads to the lysis of the target cells (Müller-Eberhard 1986). C7 is a single-chain polypeptide composed of 821 amino acid residues and is structurally similar to the other components of MAC; C6, C8α, C8β, and C9 (DiScipio et al. 1988; Hobart et al. 1995). The genes for C7, as well as those for C6 and C9, are located on chromosome 5p13 (Abbott et al. 1989). The *C7* gene spans about 80 kb of DNA and is encoded by 18 exons (Hobart et al. 1995).

Inherited deficiencies have been reported for all the components of MAC, and are frequently associated with recurrent systemic infections caused by *Neisseria meningitidis* or *Neisseria gonorrhoeae*, including meningococcal meningitis, meningococcemia, and disseminated gonococcal infection (Würzner et al. 1992). To date, five different molecular defects have been reported, two in the Japanese (Nishizaka et al. 1996b), two in the Irish, and one in Moroccan Sephardic Jews (Fernie et al. 1997). In the present study, we report a novel mutation of the gene in a Spanish family with C7 deficiency (C7D).

Subjects and methods

C7D Subject

The patient is a 25-year-old Spanish woman who had two meningococcal sepsis episodes in the past 1-year period. Total hemolytic activity (CH50) was undetectable in her serum. Subsequent analysis by radial immunodiffusion assay revealed no detectable C7 in the serum. Total hemolytic activity was reconstituted by the addition of purified C7.

Polymerase chain reaction (PCR)/single-strand conformation polymorphism (SSCP) analysis

PCR was carried out with exon-specific primers for exons 1-17 of the *C7* gene, as described (Nishizaka et al. 1996a, b). Genomic DNA was prepared from peripheral blood mononuclear cells (PBMC) as described (Horiuchi et al. 1989, 1990). PCR was performed with 50 ng genomic DNA as

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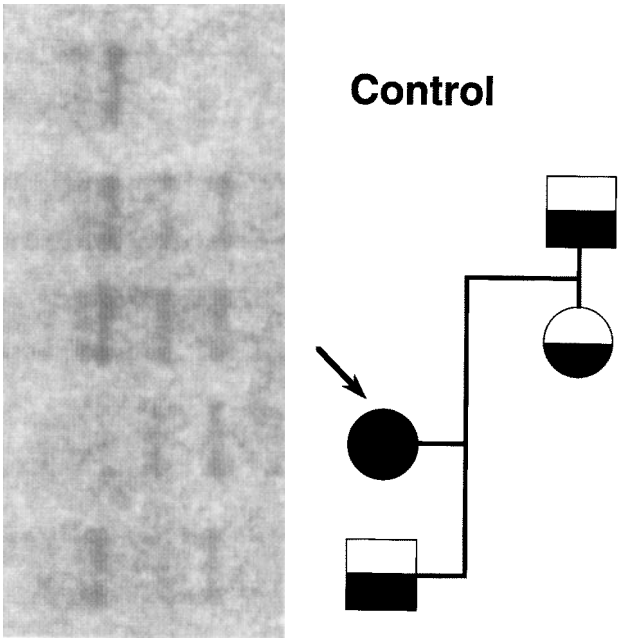

Fig. 1. Pedigree of the C7D family and polymerase chain reaction (PCR)/single-strand conformation polymorphism (SSCP) analysis of exon 14. *Black filled* (*arrowed*) indicates the proband. *Half-blackened symbols* represent family members heterozygous for the mutation. The PCR-SSCP patterns of exon 14 are shown on the left side of the symbols for each individual. Exon 14-specific PCR products of genomic DNA from the proband, her parents, her brother, and a C7-normal control were submitted to electrophoresis on 12% polyacrylamide gel containing 5% glycerol at 25 °C, followed by silver staining

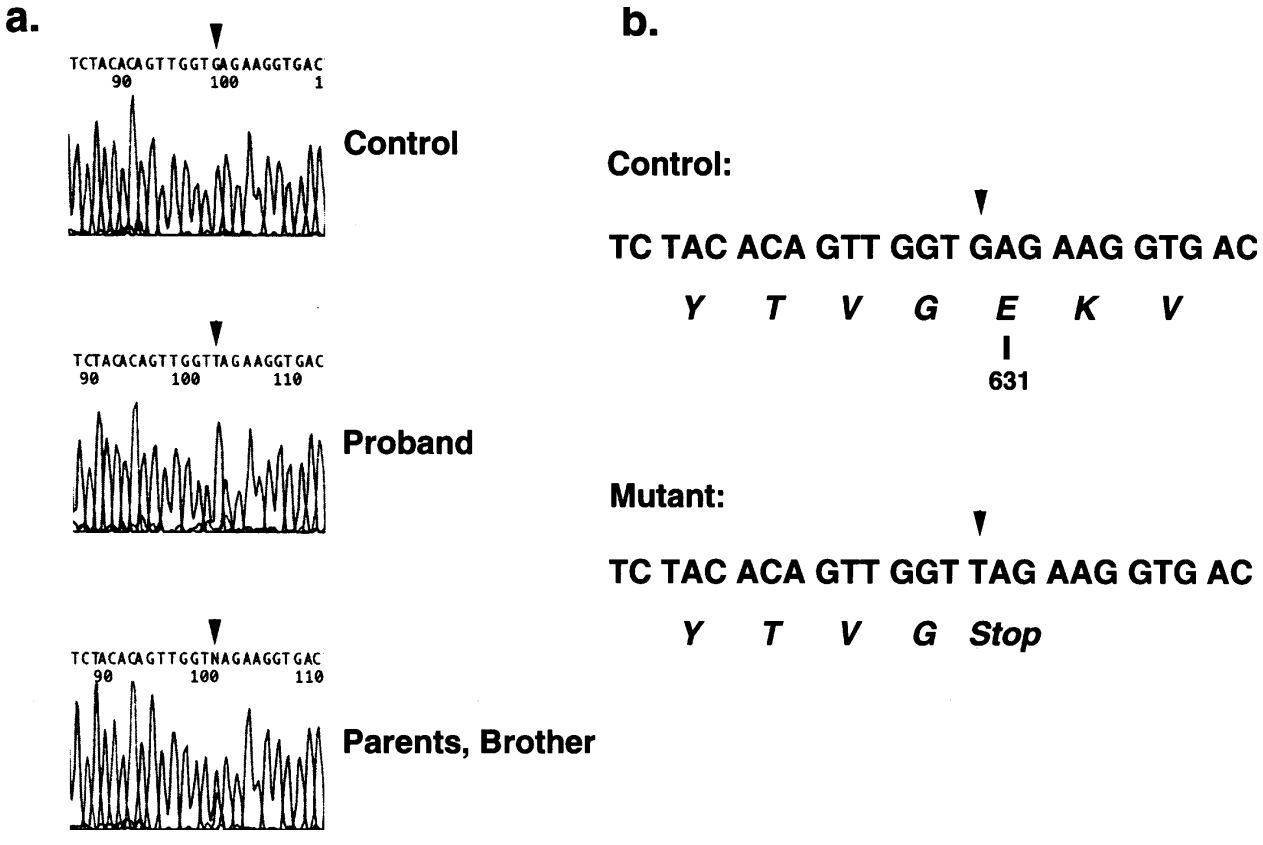

Fig. 2a, b. Definition of the mutation in exon 14. a Partial DNA sequence of genomic DNA for a C7-normal control, the proband and her parents and brother. b The nucleotide sequence and deduced amino acid sequence (*one-letter code*, in *italics*) around the mutation at amino acid 631. The arrow heads indicate the site of mutation at nucleotide 1957

template, 0.2 μM of each primer, 25 μM of dNTP, 0.125 U of Taq polymerase, and standard buffer supplied by the manufacturer, in a total reaction volume of 10 μl. Reactions were conducted for 30 cycles consisting of 1 min at 95 °C and 2 min at 60 °C, using thermal cycler PJ2000 (Perkin-Elmer, Norwalk, CT, USA). After denaturation at 80 °C for 5 min,

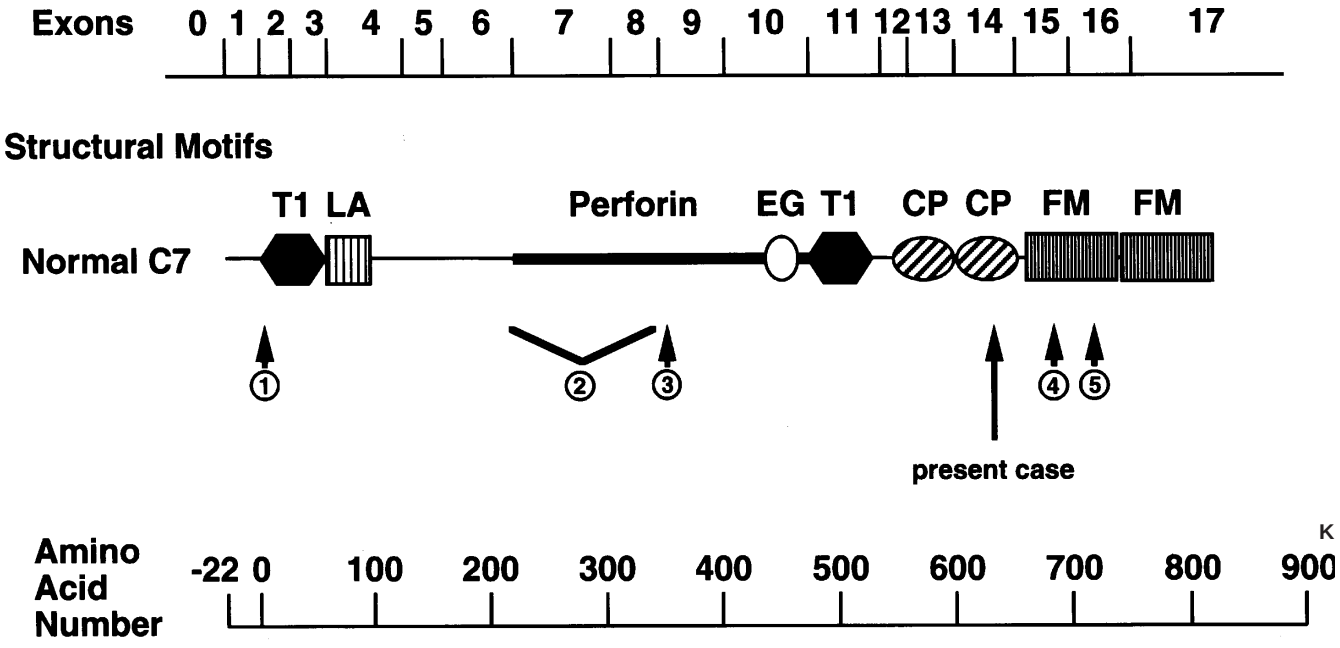

Fig. 3. Schematic diagram of the molecular structure of normal C7 (adapted from Hobart et al. 1995 with permission) and the position of mutations described to date (numbered from ① to⑤) as well as the novel mutation in our Spanish family. The mutations are; ①, a point mutation at the 3′ splice acceptor site of intron 1; ②, a deletion defect of exons 7 and 8, ③, a mis-sense mutation from Gly to Arg at amino acid 357, G357R; ④, a 2-bp deletion, 2137delTG/2138delGT/2139delTG; ⑤, a nonsense mutation of Cys at amino acid 728, C728X, and the novel nonsense mutation of Glu at amino acid 631 (E631X in our present case). Modules are designated according to the recommendations of a recent workshop (Doolittle 1995) as follows; T1, Thrombospondin type 1; *LA*, low-density lipoprotein (LDL) receptor type A; *EG*, epidermal growth factor-like; *CP*, complement control protein; and *FM*, complement factor I, membrance attack complex proteins

the PCR products were submitted to electrophoresis on 12% polyacrylamide gel at 25 ˚C with 5% glycerol at 13 V/cm, using 45 mM Tris-borate and 1 mM ethylenediaminere tetraacetic acid (EDTA) buffer, pH 8.3. DNA fragments were visualized by silver staining.

Nucleotide sequencing

The PCR products that showed abnormal patterns were fluoresceined with a BigDye terminator cycle sequencing kit (Perkin-Elmer) and were sequenced with an ABI PRISM 310 genetic analyzerm (Perkin-Elmer).

Results and discussion

PCR/SSCP analysis revealed aberrant bands in exon 14, while no other aberrant bands were detected in any other exons in the proband. As shown in Fig. 1, the PCR product in the proband displayed two bands migrating differently from those in the C7-normal control. Genomic DNA from the proband's parents and brother displayed four bands, two of which migrated with the same mobility as those of the proband and the other two as those of the C7-normal control. These results suggest that the proband was homozygous for the abnormality in exon 14, whereas her parents and brother were heterozygous for the abnormality.

The PCR product for exon 14 from the proband, as well as those from her parents and brother, were directly sequenced in their entirety. The nucleotide sequence in the proband was identical with that reported previously (DiScipio et al. 1988), except that the first nucleotide of the codon GAG for Glu-631 was changed to T (Fig. 2a). This homozygous G-to-T transversion at nucleotide 1957 resulted in the generation of the termination codon TAG, which led to the premature truncation of C7 protein (E631X) (Fig. 2b). As this truncated part contains domains that are important for the binding of C7 to C5b (Haefliger et al. 1989; DiScipio et al. 1992), the truncated C7 is not able to participate in the formation of MAC even if secreted. In the nucleotide sequence of the parents and brother, both the native nucleotide G and the mutated T were identified at nucleotide 1957 (Fig. 2a), indicating heterozygosity for the mutation.

Figure 3 schematically shows the mutation sites of the *C7* gene reported so far. The molecular bases were first described in two unrelated Japanese homozygous individuals (Nishizaka et al. 1996b). Both defects are small mutations and reside in the 3′ region of the gene, and represent either a nonsense mutation (C728X) or a 2-base pair (bp) deletion (2137delTG/2138delGT/2139delTG). Three mutations were subsequently reported in two Irish families (point mutation at the 3' splice acceptor site of intron 1, deletion of exons 7 and 8) and in the families of Moroccan Sephardic Jews (G357R), respectively (Fernie et al. 1997). In the

present study, we have demonstrated that a Spanish family carries a different molecular defect, E631X.

In conclusion, our results provide additional evidence that the molecular basis for C7D is heterogeneous, namely different families carry different molecular defects. This is in contrast to the case of the deficiency of C8b or C9, both of which are structurally and functionally similar to C7. In both cases, ethnic predominance is evident, and the cause in most of the patients is presumed to be a founder mutation (Kaufmann et al. 1993; Horiuchi et al. 1998).

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