A Novel Type II Complement C2 Deficiency Allele in an African-American Family

Zeng-Bian Zhu, T. Prescott Atkinson and John E. Volanakis

*J Immunol* 1998; 161:578-584; ;
http://www.jimmunol.org/content/161/2/578

---

**References**

This article cites 44 articles, 28 of which you can access for free at: [http://www.jimmunol.org/content/161/2/578.full#ref-list-1](http://www.jimmunol.org/content/161/2/578.full#ref-list-1)

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: [http://jimmunol.org/subscription](http://jimmunol.org/subscription)

**Permissions**

Submit copyright permission requests at: [http://www.aai.org/About/Publications/JI/copyright.html](http://www.aai.org/About/Publications/JI/copyright.html)

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: [http://jimmunol.org/alerts](http://jimmunol.org/alerts)
A Novel Type II Complement C2 Deficiency Allele in an African-American Family

Zeng-Bian Zhu,* T. Prescott Atkinson,† and John E. Volanakis2§

A 9-yr-old African-American male presenting with severe recurrent pyogenic infections was found to have C2 deficiency (C2D). Analysis of his genomic DNA demonstrated that he carried one type I C2D allele associated with the HLA-A25, B18, DR15 haplotype. Sequencing all 18 exons of the C2 gene by exon-specific PCR/single-strand conformation polymorphism indicated abnormal bands in exons 3, 7, and 6, the latter apparently caused by the 28-bp deletion of the typical type I C2D allele. Nucleotide (nt) sequencing of the PCR-amplified exons 3 and 7 revealed a heterozygous G to A transition at nt 392, causing a C111Y mutation, and a heterozygous G to C transversion at nt 954, causing a E298D mutation and a polymorphic MaeII site. Cys111 is the invariable third half-cystine of the second complement control protein module of C2. Pulse-chase biosynthetic labeling experiments indicated that the C111Y mutant C2D was retained by transfected COS cells and secreted only in minimal amounts. Therefore, this mutation causes a type II C2D. In contrast, the E298D mutation affected neither the secretion of C2 from transfected cells nor its specific hemolytic activity. Analysis of genomic DNA from members of the patient’s family indicated that 1) the proband as well as one of his sisters inherited the type I C2D allele from their father and the novel type II C2D allele from their mother; 2) the polymorphic MaeII site caused by the G954C transversion is associated with the type I C2D allele; and 3) the novel C111Y mutation is associated in this family with the haplotype HLA-A28, B58, DR12. The Journal of Immunology, 1998, 161: 578–584.

D eficiency of the second component of complement (C2D)3 has been studied extensively. It is inherited as an autosomal recessive trait and is the most common genetic deficiency of a complement component among individuals of European descent, with an estimated frequency of the null allele (C2Q0) of approximately 1% (1, 2). More than half of C2D individuals present with systemic lupus erythematosus, systemic lupus erythematosus-like syndromes, glomerulonephritis, or various forms of vasculitis (1, 3). About one-third of C2D individuals have recurrent pyogenic infections (reviewed in Ref. 4).

Two distinct molecular mechanisms were recently shown to cause C2D (5). In type I C2D, a 28-bp deletion removes 9 bp of the 3′ end of exon 6 and 19 bp of the 5′ end of the adjoining intron of the C2 gene. This deletion causes skipping of exon 6 during RNA splicing, resulting in a shift of the reading frame and a premature termination codon (6). The putative primary translation product of the mutant message would contain 222 amino acid residues, but cannot be detected by biosynthetic labeling of fibroblasts from C2D individuals (5). Type I C2D is in strong linkage disequilibrium with the MHC haplotype HLA-A25, B18, C2Q0, BfS, C4A4, C4B2, Drw2 (7). This extended haplotype occurs in >90% of C2D individuals. In contrast, individuals with type II C2D are rare, representing about 7% of all cases of C2D, and are characterized by a selective block of C2 secretion, leading to the retention of a full-length C2 polypeptide in the intracellular compartment (5). Two published examples of type II C2D result from two distinct missense mutations of amino acid residues apparently critical for proper folding of the C2 polypeptide (8). One missense mutation (C566T) located in exon 5 results in a Ser189→Phe substitution and is associated with the HLA-A11, B35, DRw1 haplotype. The other (G1930A) is located in exon 11, results in a Gly444→Arg substitution, and is found in association with the HLA-A2, B5, DRw4 haplotype.

Here we report a 9-yr-old African-American male with severe recurrent pyogenic infections and unetectable serum C2 protein and total hemolytic complement activity (CH50). Analysis of his genomic DNA demonstrated that he carried two distinct C2D gene alleles. One was a type I C2D allele and was inherited from his father; the other was inherited from his mother and harbored a novel G392A transition in exon 3, causing a Cys111→Tyr substitution. As shown by biosynthetic labeling experiments using COS cells transfected with mutant C2 cDNA, the C111Y mutation results in an almost complete block of C2 protein secretion. Thus, the novel C111Y mutation, which in this family is associated with the haplotype HLA-A28, B58, DR12, leads to type II C2D.

Materials and Methods
Case report

A 9-yr-old African-American male was referred for evaluation of his complement system because of his third serious bacterial infection. He had developed meningitis at 4 and 6 yr of age caused by Hemophilus influenzae and Streptococcus pneumoniae, respectively. His most recent hospitalization was for septic arthritis of the right shoulder, again due to S. pneumoniae. All three infections had responded well to antibiotics. Laboratory evaluation had demonstrated undetectable total serum hemolytic complement activity (CH50).
ELISA for C2 and factor B

The concentration of C2 was measured by a solid phase ELISA using the IgG fraction of a rabbit anti-C2a serum as first Ab and the anti-C2b mAb 3A3.3 (9) as second Ab. The assay was developed with affinity-purified alkaline phosphatase-conjugated goat anti-mouse IgG (Southern Biotechnology Associates, Birmingham, AL), followed by Sigma 104 phosphatase substrate (St. Louis, MO). Color development was measured at 405 nm by using a Vmax kinetic microplate reader (Molecular Devices, Menlo Park, CA). The concentration of factor B was measured by a similar ELISA, using the IgG fraction of rabbit anti-Bb serum and the anti-Ba mAb H4A-1A (10) as the first and second Abs, respectively. A standardized serum was used to construct standard curves for both assays.

Analysis of genomic DNA for type I C2D

Genomic DNA was isolated from the buffy coat fraction of blood samples collected in EDTA (11). The genomic DNA from each member of the proband’s family (J. C. kindred) was subjected to PCR using the oligonucleotide pair, 5'-GCTTGGGGCGATACTGACGCAC-3' and 5'-GCA CAGGAAAGCCCTCTGCTGCA-3', which was designed to amplify exon 6 of the C2 gene and its downstream boundary. Agarose gel electrophoresis of PCR products was conducted at 20 watts and 25°C.

95°C, 1-min annealing at different temperatures depending on the melting temperature (Tm) of each oligonucleotide primer, 200 μM of each dNTP, 2 μCi of (α-32P)dCTP (Amersham, Arlington Heights, IL), and 0.5 U of Taq polymerase (AmpliTaq, Perkin-Elmer/Cetus, Norwalk, CT) in a final reaction volume of 25 μl. Samples were overlaid with 25 μl of mineral oil to avoid evaporation and were subjected to 30 amplification cycles using a Tempcycler (Coy Laboratory Products, Ann Arbor, MI). Each cycle consisted of 1-min denaturation at 95°C, 1-min annealing at different temperatures depending on the melting temperature (Tm) of each primer, and 1- to 1.5-min extension at 72°C.

Electrophoresis of the PCR products was conducted at 20 watts and 25°C for 4 to 4.5 h in 6% non-denaturing acrylamide gels containing 5% glycerol or at 4°C without glycerol, using 45 mM Tris-borate/1 mM EDTA buffer, pH 8.3. The single-stranded DNA fragments were visualized by autoradiography.

Nucleotide (nt) sequencing

PCR products of 280 and 241 bp were amplified from exons 3 and 7, respectively, using the oligonucleotide pair, 5'-ATCCAGTCCTATATCAGAGAT-3' and 5'-AGGTCTCCAGGAGCACCAC-3' for exon 3 and 5'-TCCCCCTTGCTTACGAGGCC-3' and 5'-AGAGGTTGGCTACCTT-3' for exon 7. PCR products were purified by electrophoresis in 2.5% low melting point agarose gel (Sea Plaque Agarose, FMC, Rockland, ME) and subcloned into the pCR II vector using the TA cloning kit (Invitrogen, San Diego, CA). Recombinant plasmids were purified and alkaline denatured to produce templates. The nt sequencing was performed by the dyeode chain reaction termination method, using modified bacteriophage T7 DNA polymerase (13). The complete nt sequences, including boundaries, of exons 3 and 7 of the C2 gene were determined in both orientations. Sequence data were analyzed using the National Biotechnology Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Foster City, CA), according to the manufacturer’s instructions.

Construction and expression of mutant C2 cDNA

The C2 cDNA C2SacR (14) cloned into the expression vector pcDNA3 (Invitrogen) was used to construct mutant cDNA C2SacR. C2SacR was derived from the full-length C2 cDNA C2HLS-5-3 (15) by deletion of the first 352 bp from the 5' untranslated region and by elimination of the internal EcoRI site by site-directed mutagenesis to facilitate transfer between vectors. Since the mutation is silent, C2SacR is considered wild-type (wt) cDNA. Two mutant C2 cDNAs, termed C2-G392A and C2-G954C, were constructed by site-specific mutagenesis (16), using the mutagenic oligonucleotides 5'-GTTGGGGGCTACTGAGCACC-3' and 5'-GTCGTAT CACGTCAGCTAT-3', respectively. Both mutations were verified by nt sequencing.

Ten micrograms of pcDNA3 containing wt or mutant C2 cDNA was transfected into 5 × 10⁶ COS cells using lipofectin (Life Technologies, Gaithersburg, MD). Transfected COS cells were grown in serum-free DMEM (Mediatech, Washington DC) at 37°C for 5 h. The medium was then replaced with fresh DMEM supplemented with 10% heat-inactivated FCS ( Irvine Scientific, Santa Ana, CA), 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin, and cell culture was continued at 37°C in 6% CO2. After 48 h, supernatants were collected and stored at −90°C until assayed. The C2 concentration was measured by ELISA, and the hemolytic activity was determined using EAC14 cells prepared as previously described (17).

Pulse-chase biosynthetic labeling and immunoprecipitation

COS cells (3.0 × 10⁵) were transfected by electroporation with 20 μg of the indicated recombinant plasmid. Transfected cells were divided equally into seven 60-mm petri dishes and cultured at 37°C for 72 h. The cells were then washed and incubated in methionine-free DMEM for 10 min. Biochemical labeling was performed by incubating the cells in 1 ml of methionine-free DMEM containing 125 μCi/ml [35S]methionine (DuPont-New England Nuclear, Boston, MA) for 15 min at 37°C in 6% CO₂. Cells were washed and chased in 1 ml of complete medium (DMEM with 10% heat-inactivated FCS) for 0, 15, 30, 60, 120, 240, and 480 min at 37°C. At each time point, culture supernatant was collected from one culture dish, and the cells were washed with Dulbecco’s PBS (Mediatech) twice and lysed in 1 ml of Dulbecco’s PBS, pH 7.2, containing 0.5% sodium deoxycholate (Boehringer Mannheim, Indianapolis, IN), 1% SDS, 1% Triton X-100 (Sigma), 10 mM EDTA, 2 mM PMSF (Sigma), and 1 μg/ml each of leupeptin, aprotinin (Boehringer Mannheim), and pepstatin A (Sigma; lysate buffer). Lysates and supernatants were cleared of cellular debris by centrifugation and were stored at −90°C until used. Immunoprecipitation, SDS-PAGE, and autoradiography were conducted exactly as described previously (18). Autoradiograms were scanned by using a Scanjet 3C/T (Hewlett Packard, Wilmington, DE), and the data were analyzed with the National Institutes of Health Image program (version 1.58, Bethesda, MD).

Analysis of the novel MacII polymorphic restriction site in exon 7

A 241-bp DNA fragment containing exon 7 of the C2 gene was amplified from genomic DNA and digested with MacII. Resulting fragments were separated on 4% agarose gels and visualized with ethidium bromide. In the absence of the novel polymorphic MacII site, fragments of 205 and 36 bp were detected, while in its presence the 205-bp fragment was split into 155- and 50-bp fragments.

Results

Pedigree analysis

Figure 1 shows the HLA haplotypes and serum concentrations of C2 and factor B of all living members of the proband’s family. Laboratory normal ranges (mean ± 2 SD) for serum C2 and factor B are 11 to 35 and 74 to 286 μg/ml, respectively. As the progenitor’s father was deceased, his HLA haplotypes were deduced from those of his offspring. The progenitor (II.2) and an asymptomatic female sibling (II.6) inherited haplotype a from their father (I.1) and haplotype c from their mother (I.2); both had undetectable serum C2, while their factor B levels were at the low range of normal. Haplotype a, HLA-A25, B18, DR15, is identical with that associated with type I C2D (7). The progenitor’s mother (I.2) and another female sibling (II.1) who had inherited haplotype c from the mother had low serum C2. All other siblings had normal C2 and factor B levels. These results indicated that haplotypes a and c carry C2D alleles, that heterozygosity for haplotype c, but not a, is associated with low serum C2, and that compound heterozygosity for C2D is associated with low normal factor B levels. In contrast to haplotype a, which is frequently associated with C2D, no such association has been reported for haplotype c. It thus seemed likely that haplotype c harbored a novel C2D allele.

Screening for type I C2D alleles

To confirm that haplotype a carried the type I C2D allele, genomic DNA from each family member was subjected to PCR using the...
oligonucleotide pair designed to amplify exon 6, and its downstream boundary and the size of the resulting PCR products were assessed by agarose gel electrophoresis. As shown in Figure 2, all family members who had inherited haplotype \( a \) from the father (II.2, II.4, II.5, and II.6) had, in addition to the 174-bp fragment amplified from normal C2 alleles, a 146-bp fragment, which is amplified from alleles carrying the 28-bp deletion that causes type I C2D. Thus, the propositus is heterozygous for a novel C2 mutation, C111Y. To verify that the C111Y mutation is associated with haplotype \( c \), PCR-amplified exon 3 from all family members carrying haplotype \( c \) (I.2, II.1, II.2, and II.6) and that from a control sibling (II.5) were subjected to nt sequencing. All four individuals carrying haplotype \( c \) had double A and G nt peaks at position 392, indicating heterozygosity for the C392A mutation. The control sequence had a single G nt peak at the same position. These data confirm that the novel C111Y mutation is associated with haplotype \( c \).

Translation and secretion of C111Y C2
To assess the functional consequences of the C111Y mutation, we performed pulse-chase biosynthetic labeling experiments using COS cells transfected with C2-G392A cDNA. Cells were pulsed with \(^{35}\)S]methionine for 15 min 72 h after transfection, then chased over an 8-h period. The results of a representative experiment are shown in Figure 4. In cells transfected with wt cDNA, C2 protein was detected as a single intracellular band of about 97 kDa. The band’s intensity increased during the first 30 min of chase and subsequently decreased, with <20% of peak levels detected intracellularly after 4 h of chase. Substantial secretion started at 60 min with the appearance of an approximately 99-kDa band in the extracellular fluid and was essentially complete at the end of the 8-h chase. In contrast, C111Y C2 was not secreted in detectable amounts in the extracellular medium, although very faint bands were visible after exposure of the film for 3 days, indicating minimal secretion. For the first 30 min of chase, mutant C2 was detected intracellularly as a single band of a size equal to that of wt C2. The band’s intensity reached peak levels at 30 min of chase and subsequently decreased slowly, so that after 8 h about 65% of the mutant C2 was still present intracellularly. An additional band of about 93 kDa appeared within the intracellular fraction after 60 min of chase, possibly representing a proteolytic degradation fragment of the mutant C2.

A new polymorphic site G954C in exon 7 of the C2 gene
Exon 7, which also displayed mobility shifts on PCR/SSCP, was amplified from the proband’s genomic DNA and subjected to nt sequencing. The results demonstrate that in this family the type I C2D allele is associated with HLA haplotype \( a \).
haplotype sis. As shown in Figure 6, all living family members carrying HLA II, and the digests were analyzed by agarose gel electrophoresis of the proband’s family. The PCR products were digested with the C2 gene was amplified from genomic DNA from each member the G954C mutation is associated with haplotype positus is heterozygous for the mutation. To investigate whether substitution at amino acid residue 298 (E298D). Apparently, the propositus (C2D, II.2) also had the same sequences as clones from a C2-sufficient individual used as a control. The G954C mutation generates a MaeII restriction site and causes a Glu for Asp substitution at amino acid residue 298 (E298D). Apparently, the propositus is heterozygous for the mutation. To investigate whether the G954C mutation is associated with haplotype a or c, exon 7 of the C2 gene was amplified from genomic DNA from each member of the proband’s family. The PCR products were digested with MaeII, and the digests were analyzed by agarose gel electrophoresis. As shown in Figure 6, all living family members carrying HLA haplotype a (II.2, II.4, II.5, and II.6) also had the MaeII RFLP, while none of the other family members did. Therefore, in this kindred the G954C mutation and the resulting polymorphic MaeII site of exon 7 are associated with haplotype a and the type I C2D allele. Subsequent studies have demonstrated that type I C2D alleles from additional unrelated individuals also carried the MaeII polymorphic site. The possible functional effects of this mutation were investigated by constructing the G954C mutant C2 cDNA and testing it in transfection experiments in comparison with wt C2. Transiently transfected COS cells secreted about equal amounts of mutant and wt C2. Moreover the specific hemolytic activity of E298D C2 (4.43 U/ng) was almost identical with that of wt C2 (4.39 U/ng). Therefore, the E298D mutation is functionally silent.

Discussion

Hereditary C2D is the most common complement component deficiency among individuals of European descent, but is relatively rare among other ethnic groups. Here we report a case of C2D in an African-American family. The proband of this family and his asymptomatic youngest sister were compound heterozygotes for type I and type II C2D. The type I C2D allele was inherited from their father, and the type II C2D allele was inherited from their mother. The former was associated with the HLA-A25, -B18, -DR15 haplotype and carried the 28-bp deletion in exon 6, which characterizes the common type I C2D seen among Europeans. The type II C2D allele carried a novel G392A mutation in exon 3 of the C2 gene, which led to a Cys111→Tyr substitution. Cys111 is the invariable third half-cystine of the second CCP module of C2, and its substitution with Tyr was shown to be responsible for blocking the secretion of the C2 polypeptide by COS cells transfected with mutant cDNA.

The mechanism leading to type II C2D was originally defined using L cells transfected with two different type II-associated C2Q0 genes from unrelated individuals (5, 8). One of these was due to a single missense mutation, C566T, located in exon 5 and resulting in a Ser189→Phe substitution, while the other was due to a different missense mutation, G1930A, located in exon 11 and leading to a Gly444→Arg substitution. These amino acid substitutions were apparently directly responsible for a marked inhibition of secretion of the respective mutant C2 proteins, but the molecular mechanism of inhibition was not investigated further. However, it seems likely that these two mutations as well as the one described in this report lead to misfolding of the polypeptide chain, resulting in its retention in the endoplasmic reticulum (ER) and eventual degradation. Thus, type II C2D appears to be another example of a growing number of human diseases caused by protein folding defects (reviewed in Ref. 19).

Classic examples of diseases caused by protein folding defects are cystic fibrosis and α1-antitrypsin deficiency. In the most common form of cystic fibrosis, deletion of a single phenylalanine at residue 508 of the cystic fibrosis transmembrane conductance regulator causes retention in the ER and inadequate expression of the
protein in the cell membrane (20). A common form of emphysema-related α1-antitrypsin deficiency is the result of a Glu342→Lys substitution (21), which has been shown to cause a folding defect apparently responsible for ER retention (22). Other examples of presumed misfolding leading to deficiency are missense mutations causing type IIA von Willebrand factor disease (23, 24) and several different missense and nonsense mutations of the A subunit of factor XIII causing deficiency of the enzyme and bleeding disorders (25, 26).

A generally accepted model for the folding and processing of membrane-associated and secreted proteins has emerged from studies on the intracellular folding of a small number of proteins (27–29). Initial cotranslational folding involving secondary and some tertiary structure occurs during translocation of the polypeptide chain into the cisternal space of the ER. Addition and initial processing of N-linked glycans and formation of disulfide bonds occurs next, and native conformation is attained. Proteins are translocated to the Golgi apparatus, further processed, and then are either translocated to the cell surface or secreted (19). ER chaperones, including BiP (30), calnexin (28, 31), and calreticulin (32), play a key role in the process by facilitating protein folding, oligomerization, and translocation (reviewed in Ref. 33). In addition to facilitating folding and maturation of polypeptides, ER molecular chaperones constitute an important component of the ER “quality control” system, which ensures that incompletely or incorrectly folded proteins are retained in the ER until they fold correctly or are degraded (34). Indeed, prolonged association with chaperones constitutes a signal for degradation of aberrant proteins. A number of recent reports have indicated that degradation of misfolded proteins takes place in proteasomes in the cytoplasm of some, but not all, cases, proteasome digestion is preceded by ubiquitination.

We have previously shown that C2Δ(17), the product of one of several naturally occurring alternatively spliced C2 transcripts (39), which is missing the region encoded by exon 17, is not secreted by transfected COS cells but is retained in the ER, apparently because it is misfolded (18). We have also demonstrated that both C2Δ(17) and wt C2 are associated with calnexin, which seems to be responsible for the ER retention of the deletional mutant (40). Misfolding and calnexin-mediated retention in the ER are the likely causes of the inhibition of secretion of the C111Y mutant described here, although other chaperones may participate as well. As mentioned, Cys111 is the third of four invariable half-cystines of the second of three CCP modules of C2. In native C2, Cys111 is thought to be disulfide linked to Cys69. CCP modules are widely distributed and occur in multiple copies in many complement receptors and regulatory proteins as well as other complement and noncomplement proteins, such as β₃-glycoprotein I and clotting factor XIII (41). Solution structures of CCP modules of factor H (42, 43) and vaccinia virus CCP (44) have shown a common structural fold that is probably shared by all CCP modules. The framework of a CCP module consists of a β sandwich in which β strands surround a compact hydrophobic core. The structure is stabilized by a pair of disulfide bonds between Cys1-Cys3 and Cys2-Cys4. The present data indicate that at least the first of these two disulfide bonds is necessary for the correct folding of the CCP module and/or for maintaining its native conformation. The structural importance of the CCP module disulfides was also indicated by the finding that mutation of Cys430 within the seventh CCP module of the b subunit of clotting factor XIII caused ER retention of the recombinant protein (45).

FIGURE 5. A new polymorphic site G954C in exon 7 of the C2 gene. Partial nt sequence of an exon 7 clone of the C2 gene showing a G to C transition at nt 954. PCR-amplified exon 7 products derived from genomic DNA of the propositus (C2D, II.2) and an unrelated C2-sufficient individual (C2S) were cloned and subjected to nt sequencing. The sequences depicted are those of the sense strands. The changed nt is boxed, and the substituted Asp for Glu at position 298 is in italics.

FIGURE 6. The G954C transversion generates a MaeII restriction site in exon 7 and is associated with the type I C2D allele. A 241-bp DNA fragment containing exon 7 of the C2 gene was amplified from genomic DNA from each member of the proband's family. The PCR products were digested with MaeII, and the resulting fragments were separated on a 4% agarose gel and visualized with ethidium bromide. The novel polymorphic MaeII site caused by the G954C transversion splits the 205-bp fragment into 155- and 50-bp fragments. All family members carrying HLA haplotype a also have the novel polymorphic site. Therefore, the polymorphic MaeII site of exon 7 is associated with the type I C2D allele of this kindred.
retention of the polypeptide and protein deficiency (45). Similarly, mutations leading to substitution of two different cysteine residues within CCP modules of the control protein factor H also resulted in ER retention of the mutant polypeptides and protein deficiency (46).

Disulfide bonds are important for stabilizing the tertiary structure of secreted proteins. They are also thought to play a significant role in the folding process per se (19). This proposal is based on in vitro studies of the folding of bovine pancreatic trypsin inhibitor (47) and on the intracellular folding of the β subunit of human chorionic gonadotropin (48, 49). In both cases, it was observed that during folding, transient disulfide forms, which are not present in the native protein, but represent important intermediates in the folding pathway. These findings indicate that disulfide rearrangement takes place during maturation of the protein and imply that failure to form certain transient disulfides during folding could result in trapping of the polypeptide at an intermediate stage of the folding pathway. In modular proteins such as C2, individual modules are believed to fold autonomously. Thus, it would be expected that the C111Y mutation would influence only the folding and/or tertiary structure of the second CCP module. However, a more widespread effect on the polypeptide cannot be excluded. Misfolding of C2 could also cause the secretory defect associated with the two previously described mutations, S189F and G444R, that lead to type II C2D (8). Ser189 is within a 30-residue segment that connects the third CCP to the von Willebrand factor type A module to the serine protease domain (12). It seems more likely that defective overall folding and/or conformation of C2, rather than a conformational defect localized in the connecting segments, is the immediate cause of the ER retention of these two mutants.

The second missense mutation, E298D, revealed by the PCR/SSCP analysis of the proband’s genomic DNA, was functionally silent, as would be expected from the conservative nature of the amino acid substitution. This mutation is associated with a MaeII polymorphic site of the type I C2D allele of this family. Our preliminary screening data indicate that this polymorphic restriction site is also present in type I C2D of other unrelated kindreds. It therefore provides a simple screening test for type I C2D. Additional testing is necessary to establish the association between the MaeII polymorphic site and other genetic polymorphisms linked to the typical type I C2D allele (50).

Acknowledgments

We are grateful to Dr. Robert Benak for referral of this patient, and to Mrs. Paula Kiley for assistance in preparing the manuscript.

References