Identification of a novel mutation in the C6 gene of a Han Chinese C6SD child with meningococcal disease

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Abstract. Deficiency of the sixth complement component (C6D) is a genetic disease associated with increased susceptibility to *Neisseria meningitides* infection. Individuals with C6D usually present with recurrent meningococcal disease (MD). According to the patients' C6 levels, C6D is divided into complete genetic deficiency of C6 and subtotal deficiency of C6 (C6SD). The present study reported on a Han Chinese pediatric patient with MD, in whom further investigation revealed a C6SD genetic lesion. A heterozygote nonsense mutation (c.1062C>G/p.Y354*) in the C6 gene was identified by Sanger sequencing. The mutation alters the tyrosine codon at position 354 to a termination codon and results in a truncated protein. In conclusion, the genetic lesion of a pediatric patient with C6SD who was diagnosed due to having MD was investigated and a novel pathogenic mutation in the C6 gene was identified. The study confirmed the clinical diagnosis for this patient with C6SD and also expanded the spectrum of C6 mutations.

Introduction

The terminal complement components, which comprise complement components 5 (C5) to C9, participate in the assembly of the membrane attack complex (MAC) (1). The MAC is responsible for the lytic action of complement, which is essential in both the innate and adaptive immune responses against invading pathogens (2). Genetic deficiencies of any of the terminal complement components lead to failure to form the MAC and susceptibility to *Neisseria meningitides* (Nm) infections, the so-called terminal complement component deficiencies (TCCD) (3).

As one of five terminal complement components, the sixth complement component (C6) is a constituent of the MAC. Deficiency of C6 (C6D; Online Mendelian Inheritance in Man #612446) is associated with increased susceptibility to Nm infections and patients with C6D usually present with recurrent meningococcal disease (MD) (4). According to patients' C6 levels, C6D is divided into complete genetic deficiency of C6 (C6Q0) and subtotal genetic deficiency of C6 (C6SD). Patients with C6Q0 present with a complete lack of functional C6, as opposed to C6SD patients, in whom a small amount of C6 activity remains (5).

Similar to most other complement protein deficiencies, C6D results from a mutation in the C6 gene, which maps to chromosome 5p13. The C6 gene spans 80 kbp and is composed of 18 exons (4). To date, only 15 mutations of the C6 gene have been identified in patients with C6D. However, the genetic factors of C6D remain largely unknown and the detailed molecular mechanisms involved require to be further investigated.

Materials and methods

Subjects. The patient who participated in the present study was diagnosed at the Third Xiangya Hospital of Central South University (Changsha, China) in September 2019. The diagnosis of C6SD was made according to the standard formulated previously (6). Blood was collected from the patient and the patient's parents. A total of 200 unrelated healthy subjects were collected from the general population of Hunan in China as control subjects to exclude polymorphisms. The healthy controls (males/females, 100/100; age, 36.7±8.6 years) lacked C6D diagnostic features. The baseline characteristics of these 200 healthy controls were reported in a previous study by our group (7).

Biochemical analyses. The levels of CH50 (cat. no. NK095.OPT), C5 (cat. no. RN027.3), C6 (cat. no. RN102.38), C7 (cat. no. RN103.3), C8 (cat. no. RN089.3), C9 (cat. no. RN028.3)

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were measured by radial immunodiffusion assay (RID) kits (BINDARID™; The Binding Site Group Ltd.) according to the manufacturer's instructions.

DNA extraction. Genomic DNA was prepared from peripheral blood of the patient and all other participants using a DNAeasy Blood & Tissue kit (Qiagen GmbH) on the QIAcube automated DNA extraction robot (Qiagen GmbH).

PCR. Each exon of the C6 gene was amplified by PCR as previously described (7). The sequences of the primers we used were obtained from Hobart *et al* (8). All of the coding exons and exon-intron boundaries of the C6 gene were amplified by PCR in a 25-μl reaction mixture, which consisted of 0.3 mM deoxyribonucleotide triphosphates, 1X PCR buffer (10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.1% Triton X-100 and 0.01% w/v gelatin), 2.0 mM $MgCl_2$, 0.5 μM of each primer (forward and reverse), 1.5 U of Taq polymerase and 50 ng of genomic DNA. The thermal cycling consisted of an initial denaturation at 95˚C for 4 min, followed by 35 cycles of amplification consisting of denaturation at 95˚C for 1 min, primer annealing at desired temperature as previously described (7) for 30 sec and primer extension at 72˚C for 1 min. A final extension step was performed at 72˚C for 7 min.

Mutation sequencing. The sequences of the PCR products of C6 (NM_001115131) from the patient and other subjects, including the parents of the patient and the 200 controls, were determined with the ABI 3100 Genetic Analyzer (Applied Biosciences; Thermo Fisher Scientific, Inc.) as previously described (7).

Bioinformatics analysis. MutationTaster software (http://www.mutationtaster.org/) was used to predict the effect of the mutation on the function of the protein. SWISS-MODEL software (https://swissmodel.expasy.org/interactive) was used to determine the function of the mutation. The multiple C6 protein sequences across species were aligned using the program MUSCLE (http://www.ebi.ac.uk/Tools/msa/muscle/).

Results

Clinical features. In the present study, a Chinese trio family with C6SD was enrolled. The proband (II-1), a 6-year-old male from Hunan Province in China, was admitted to the Third Xiangya Hospital of Central South University (Changsha, China) due to high-grade fever. The patient had generalized muscle pain and had developed a purpuric rash on the lower extremities. The cerebrospinal fluid and the blood culture were positive for Nm. To provide protection against Nm, the patient was treated with ceftriaxone for 10 days, resulting in complete resolution of the fever. The patient's muscle pain resolved fully on day 3. Repeated blood cultures were negative. The patient's skin returned to normal within a month. Medical history investigation revealed that the patient had no history of previous meningococcal infection. Since the immunodeficiency workup suggested that all of the immune parameters were normal except for a low level of serum C6 (17.7 mg/dl, far below the mean normal level of 45 mg/dl; Table I), the patient was primarily diagnosed with C6SD (6). No other malformations were observed in this patient. After the patient's diagnosis, complement screening of the proband's father (I-1) revealed that the latter had a low level of C6 (25.2 mg/dl). However, the patient's father had never been diagnosed as meningococcal infection. C6 levels of the patient's mother (I-2) were in the normal range (56.0 mg/dl). Information on other members of the family was not available (Fig. 1A).

Table I. Immunological findings in the patient.

Item	Value	Reference range
CD3+ (%)	71	57-81
CD4+ (%)	35	26-48
CD8+ (%)	28	20-42
CD16+56+ (%)	23	8-28
CD20+ (%)	11	10-27
In vitro response to PHA (%)	59.1	65.8±9.2
IgG (mg/dl)	1,069	745-1,804
IgA (mg/dl)	197	57-282
IgM (mg/dl)	222	78-261
Anti B titer	1/64	>1/10
CH50[a]	Present	Present
C5[a] (mg/dl)	115	90-172
C6[a] (mg/dl)	17.7	45-95
C7[a] (mg/dl)	81	55-85
C8[a] (mg/dl)	121	112-172
C9[a] (mg/dl)	237	125-265

[a]Measured by radial immunodiffusion assay (BINDARID™; The Binding Site Group Ltd.). PHA, phytohemagglutinin; CH50, total hemolytic complement; C, complement.

Genetic analysis. Sanger sequencing analysis of the C6 gene was performed and a heterozygote nonsense mutation (c.1062C>G/p.Y354*) was detected (Fig. 1B). Segregation analysis suggested that the mutation was also present in the patient's father, while it was excluded in the patient's mother. This newly identified c.1062C>G mutation was not present in the 200 ethnically matched, healthy controls and was also not present in the dbSNP144 (http://www.ncbi.nlm.nih.gov/projects/SNP/index.html) and Exome Variant Server (EVS; http://evs.gs.washington.edu/EVS/) databases. Alignment of C6 amino acid sequences from humans, chimpanzees, monkeys, cats, mice, globefish and zebrafish revealed that the affected amino acid was evolutionarily conserved (Fig. 1C). The MutationTaster tool predicted that the p.Y354* variant is a disease-causing mutation. Analysis with SWISS-MODEL software for exploring the spatial configuration of the protein suggested a loss of more than half of the total protein in the mutated C6 protein compared with that in the wild-type protein (Fig. 1D).

Discussion

In the present study, the genetic lesion of a Han Chinese pediatric patient with C6SD who was diagnosed due to

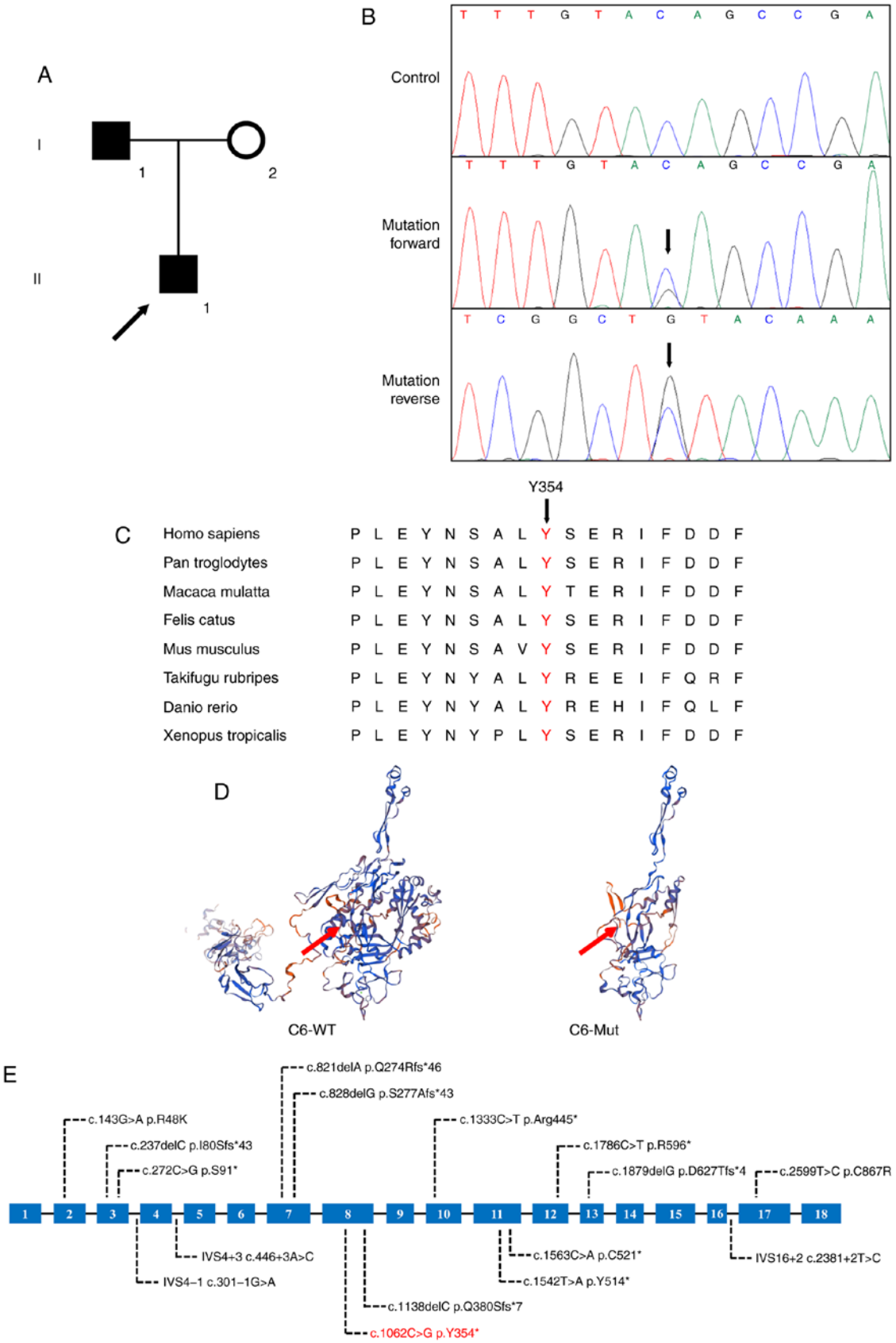

Figure 1. Sequencing data and analysis of the C6 mutation. (A) The pedigree of the trio family. Squares indicate male family members and circles female members. Filled and unfilled symbols indicate affected and unaffected members. The arrow indicates the proband. (B) Sequencing results of the C6 mutation. The sequence chromatogram indicates a C to G transition of nucleotide 1,062. (C) Alignment of multiple C6 protein sequences across species. The affected amino acid (Y354) is located in the highly conserved amino acid region in different mammals (from Ensembl). Letters in red indicate the Y354 site. (D) Structure prediction of the mutant protein. The protein structure of C6-WT and C6-Mut was predicted by the SWISS-MODEL online software. The red arrow points at the truncated start site of the mutated protein compared with the WT protein. (E) Overview of all known and novel C6 mutations. The C6 gene is presented, with all known C6 mutations (black letters) and the novel mutation (red letters). Blue rectangles indicate exons. Introns are not displayed to scale. WT, wild-type; Mut, mutant; del, deletion.

having MD was investigated. A heterozygote nonsense mutation (c.1062C>G/p.Y354*) of C6 was identified by Sanger sequencing. The nonsense mutation is located in exon 8 and alters the tyrosine codon at position 354 to a termination codon, leading to a truncated protein. Although the dysmorphic C6 protein still has certain residual activity (9), the truncated C6 protein in the present study is expected to fail to produce a functional MAC, as it lacks more than half of the normal protein. Thus, it was indicated that the nonsense mutation (c.1062C>G/p.Y354*) identified in the C6 gene may be a potential candidate factor for the development of C6D, which is consistent with previous findings (4,10).

The terminal complement system is essential for fighting MD. As one of the terminal complement components, C6D predisposes individuals to infection with Nm (4). Most patients with MD in the setting of C6D have undetectable levels of C6. Nm infection in C6SD has rarely been described (9,11). Multiple family studies have demonstrated examples of individuals with C6 <50% the normal levels who had no meningococcal infection (12). The present study reported on a trios family diagnosed as C6SD. The proband presented with a recent history of meningitis. A heterozygote mutation (c.1062C>G/p.Y354*) in C6 was identified. Of note, the patient's father harbored the same variant but had no meningococcal infection, which is consistent with the previous study. Although the numbers are small, patients with C6SD and MD have also been described. Compared to normal individuals, the remaining C6 protein in a patient with C6SD is not sufficient to resist the invasion of bacteria, having certain bactericidal activity but to a lesser extent (5,12). The same is suspected in the proband of the present study. A single episode of MD may result in serious long-term sequelae and studies indicated that 73% of C6Q0 patients who suffered recurrent MD developed serious illness or died (3). To protect patients with C6Q0 from further episodes of MD, patients were prescribed long-term monthly injections with long-acting penicillin (bicillin) and given a conjugated meningococcal vaccination (3). The susceptibility to MD or other infectious diseases between C6Q0 and C6SD is not uniform. There may be additional controllable factors, such as infections and accidents, affecting the susceptibility of patients with C6SD. No clear information is available with respect to the treatment and prognosis of C6SD. It is only by identifying C6SD patients and investigating other susceptibility factors that the best means of treatment and prophylaxis may be determined. Likewise, prophylactic treatment and/or vaccination are recommended for patients at risk of MD (13,14). It is crucial to test C6 levels accurately and determine the C6 genetic defects responsible for either C6Q0 or C6SD, which contributes to the optimal management of patients with C6D (3). As part of the management of C6D, the pediatric patient of the present study was advised to immunize against meningococci ACYW and B. Prophylactic oracillin was considered. The present study not only proved the diagnosis of C6SD for this child, but also further confirmed the association of meningococcal infections with TCCD.

Previous research has indicated that homozygosity, or compound heterozygosity of C6 defects, resulted in C6Q0 (3). However, heterozygous carriers of C6D have always been described as C6SD (11). The pediatric patient with a heterozygote nonsense mutation in the present study had a low level of C6, which is consistent with heterozygous C6 sufficiency/deficiency. To date, approximately 15 mutations of C6 have been reported in patients with C6D. All of these known mutations of the C6 gene are briefly summarized in Fig. 1E, which may be conducive to the genetic diagnosis of C6D and counseling of such patients. Of note, the mutation (c.1062C>G/p.Y354*) identified in the present study has not been previously published and is therefore considered novel.

The incidence of C6D varies considerably within populations. C6D has demonstrated a prevalence in Western Cape South Africans and it has been diagnosed much more frequently in South Africa than elsewhere (15). Furthermore, affected individuals in Irish families were also reported in countries where C6D appears to be sporadic and rare (4). C6D also appears to be sporadic and rare in East Asia. Population data on the prevalence of C6D are only available for Japan, where it was determined to be 2-7 per 100,000 (16). No data are currently available for China. In the present study, a Han Chinese pediatric patient with C6D was described. To the best of our knowledge, the present study was the first to investigate the role of the C6 gene in Han Chinese patients with C6D, and further studies are essential to evaluate the association between the C6 gene and C6D in the Han Chinese population.

In conclusion, the present study identified a novel nonsense mutation (c.1062C>G/p.Y354*) in the C6 gene as a possible cause of C6SD in a Han Chinese pediatric patient who was diagnosed due to having MD. The present study not only expanded the spectrum of C6 mutations but also confirmed the clinical diagnosis for this patient as C6SD.

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Availability of data and materials

The datasets for this article are not publicly available due to concerns regarding participant/patient anonymity. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

AQZ designed the study. YXL performed the molecular analysis and participated in manuscript writing. JYJ recruited and examined the family, collected blood samples and extracted DNA. CYW performed the bioinformatics analysis. DBX and LLF analyzed the data, participated in manuscript preparation and acquired funding for the study. YXL and LLF checked and confirmed the authenticity of the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Third Xiangya Hospital of Central South University (Changsha, China) and performed in accordance with the principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all of the adult participants and legal guardians of minor participants.

Patient consent for publication

The patients/participants provided written informed consent to participate in this study. Written informed consent was obtained from the individuals for the publication of any potentially identifiable images or data included in this article.

Competing interests

The authors declare that they have no competing interests.

References

1. DiScipio RG and Hugli TE: The molecular architecture of human complement component C6. J Biol Chem 264: 16197-16206, 1989.
2. Merle NS, Noe R, Halbwachs-Mecarelli L, Fremeaux-Bacchi V and Roumenina LT: Complement system part II: Role in immunity. Front Immunol 6: 257, 2015.
3. Orren A, Owen EP, Henderson HE, van der Merwe L, Leisegang F, Stassen C and Potter PC: Complete deficiency of the sixth complement component (C6Q0), susceptibility to Neisseria meningitidis infections and analysis of the frequencies of C6Q0 gene defects in South Africans. Clin Exp Immunol 167: 459-471, 2012.
4. Parham KL, Roberts A, Thomas A, Wurzner R, Henderson HE, Potter PC, Morgan BP and Orren A: Prevalence of mutations leading to complete C6 deficiency (C6Q0) in the Western Cape, South Africa and detection of novel mutations leading to C6Q0 in an Irish family. Mol Immunol 44: 2756-2760, 2007.
5. Wurzner R, Orren A, Potter P, Morgan BP, Ponard D, Spath P, Brai M, Schulze M, Happe L and Gotze O: Functionally active complement proteins C6 and C7 detected in C6- and C7-deficient individuals. Clin Exp Immunol 83: 430-437, 1991.
6. Ikinciogullari A, Tekin M, Dogu F, Reisli I, Tanir G, Yi Z, Garrison N, Brilliant MH and Babacan E: Meningococccal meningitis and complement component 6 deficiency associated with oculocutaneous albinism. Eur J Pediatr 164: 177-179, 2005.
7. Xiang R, Fan LL, Huang H, Cao BB, Li XP, Peng DQ and Xia K: A novel mutation of GATA4 (K319E) is responsible for familial atrial septal defect and pulmonary valve stenosis. Gene 534: 320-323, 2014.
8. Hobart MJ, Fernie BA, Fijen KA and Orren A: The molecular basis of C6 deficiency in the western Cape, South Africa. Hum Genet 103: 506-512, 1998.
9. Orren A, Wurzner R, Potter PC, Fernie BA, Coetzee S, Morgan BP and Lachmann PJ: Properties of a low molecular weight complement component C6 found in human subjects with subtotal C6 deficiency. Immunology 75: 10-16, 1992.
10. El Sissy C, Rosain J, Vieira-Martins P, Bordereau P, Gruber A, Devriese M, de Pontual L, Taha MK, Fieschi C, Picard C and Frémeaux-Bacchi V: Clinical and genetic spectrum of a large cohort with total and sub-total complement deficiencies. Front Immunol 10: 1936, 2019.
11. Wurzner R, Hobart MJ, Fernie BA, Mewar D, Potter PC, Orren A and Lachmann PJ: Molecular basis of subtotal complement C6 deficiency. A carboxy-terminally truncated but functionally active C6. J Clin Invest 95: 1877-1883, 1995.
12. Rosenfield L, Cvetkovic A, Woodward K and Quirt J: Late presentation of subtotal C6 deficiency in a patient with recurrent Neisseria meningitides infections. Ann Allergy Asthma Immunol 120: 432-433, 2018.
13. Westra D, Kurvers RA, van den Heuvel LP, Wurzner R, Hoppenreijs EP, van der Flier M, van de Kar NC and Warris A: Compound heterozygous mutations in the C6 gene of a child with recurrent infections. Mol Immunol 58: 201-205, 2014.
14. Owen EP, Leisegang F, Whitelaw A, Simpson J, Baker S, Wurzner R, Potter P and Orren A: Complement component C5 and C6 mutation screening indicated in meningococcal disease in South Africa. S Afr Med J 102: 525-527, 2012.
15. Ram S, Lewis LA and Rice PA: Infections of people with complement deficiencies and patients who have undergone splenectomy. Clin Microbiol Rev 23: 740-780, 2010.
16. Nishizaka H, Horiuchi T, Zhu ZB, Fukumori Y, Nagasawa K, Hayashi K, Krumdieck R, Cobbs CG, Higuchi M, Yasunaga S, *et al*: Molecular bases for inherited human complement component C6 deficiency in two unrelated individuals. J Immunol 156: 2309-2315, 1996.