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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

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Summary

Complete complement component 6 deficiency (C6Q0) is a co-dominant genetic disease presenting as increased susceptibility to invasive *Neisseria meningitidis* infections. Affected individuals have two affected alleles which can be homozygous or compound heterozygous for the particular gene defects they carry. This disorder has been diagnosed relatively frequently in Western Cape South Africans. Affected patients are prescribed penicillin prophylaxis. In 2004 we commenced a clinical follow-up study of 46 patients. Of these, 43 had family age-matched C6 sufficient controls. Participants were classified as either (i) well, or (ii) having a serious illness (SI) or died (D). An SI was a long-term illness that did not allow the performance of normal daily activities. Among 43 patients, 21 were well and 22 were SI/D, while among 43 matched controls, 35 were well and eight were SI/D. This difference is highly significant. Among all 46 C6Q0 patients, those who had had recurrent infection had significantly more SI/D than those who had suffered none or one infection. Thus, this work demonstrates the long-term serious outcome of repeated meningococcal disease (MD) episodes. We investigated the frequencies of four C6Q0 pathogenic mutations known to affect Cape patients (828delG, 1138delC, 821delA and 1879delG) in 2250 newborns. A total of 103 defective alleles (2·28%) and three affected C6Q0 individuals were detected. For all defects combined, 5·24 affected subjects (C6Q0) are expected among 10 000 individuals. What is still unknown is the number of C6Q0 individuals who suffer MD or other infectious diseases.

Keywords: C6, complement deficiency, genetic defects, *Neisseria meningitidis*, recurrent meningitis

Introduction

Increased susceptibility to invasive infections with *Neisseria meningitidis* and *N. gonorrhoea* in individuals with genetic deficiencies of terminal complement proteins was described initially in approximately 1979 [1]. The terminal complement components comprise complement components five to nine. These combine sequentially to form the molecular attack complex (MAC); the absence of any one component leads to failure to form MAC and of serum complement bacteriolytic activity. There have now been several comprehensive reviews of genetically determined terminal complement component deficiencies (TCCD) [2–4].

At Groote Schuur Hospital, Cape Town, during the 1980s and 1990s, a number of patients were referred to us who had suffered recurrent meningococcal disease (MD). All patients were either South African Coloureds or Blacks, who were mainly Xhosa and originally from the Eastern Cape. Immunological analyses showed them to have genetically determined deficiency of the sixth complement component (C6) [5]. Functional haemolytic C6 assays showed that serum C6 was absent, and hence they were referred to as suffering C6 quantitatively zero (C6Q0), as opposed to subtotal C6 deficiency (SD). Six affected SA Western Cape individuals from two families plus four individuals from Europe/the United States with C6SD (low levels above 0·03 μg/ml) have

been described [6]. Among these, none had suffered MD, although there is an earlier report of death from MD of a patient with a C6 level of 4·8 μg/ml [7]. Numbers are small; nevertheless, for the optimal management of individuals with C6 deficiency it is important to be able to determine C6 levels accurately, or to determine the C6 genetic defects responsible for either C6Q0 or C6SD. The four defects determined in the present study have all been shown to be responsible for C6QO.

To protect the C6Q0 patients from further episodes of MD, patients were prescribed long-term monthly injections with long-acting penicillin (bicillin) [8]. Patients were also provided with charts where the administration of prophylaxis was recorded. However, patients faced numerous logistic and commercial problems when attending for prophylaxis; moreover, the injections themselves are very painful, thus compliance was often poor, and this is a major problem in the management of C6Q0 patients in the Cape.

Susceptibility to the meningococcus remained a major problem for the C6Q0 patients. In addition, they could suffer other complications both because of the absence of a complete terminal complement pathway and the sequelae of the MD episodes they had suffered. Therefore, in 2004 a follow-up study was started in order to determine the progress of the patients over the years since diagnosis. Four molecular defects responsible for C6Q0 in Western Cape have been described [9,10]. Affected individuals have two affected alleles and can be homozygous or compound heterozygous. None of the almost 50 C6Q0 patients we had tested had gene mutations which were not accounted for one or two of these four mutations. As a disease, C6Q0 appears much more frequently in South Africa (SA) than has been reported elsewhere [3,4]. However, no information on frequencies of these four gene defects in the local populations has been available. Without this information it is not possible to determine the impact of C6Q0 on MD frequency in the Western Cape.

This paper demonstrates that the long-term outcome for the C6Q0 patients who suffer recurrent MD is often very serious, and steps need to be taken to prevent further infections. However, among siblings of C6Q0 patients who were diagnosed because of recurrent MD, we have observed that not all adult or near-adult C6Q0 individuals have ever suffered MD. Also, we have no information about how many C6Q0 individuals are never diagnosed. It is evident that the increased susceptibility to MD is not uniform, and there may be additional controllable factors which affect susceptibility. It is only by identifying C6Q0 patients and investigating other susceptibility factors that the best means of prophylaxis can be determined.

To investigate the gene frequencies of the four local C6Q0 defects we used blood collected previously from the umbilical chords of babies born 2002–03 in the Cape Town area. DNA was prepared and used to investigate the frequencies of these defects in 2250 newborn Cape children.

Materials and methods

C6Q0 patients for the clinical study

The clinical follow-up study was carried out from 2003 to 2005. There were 46 C6Q0 patients numbered P1–P46. The average age of the patients in 2005 was 35 years. Nearly all index cases had been ascertained because of recurrent meningococcal infections, and their C6Q0 siblings were diagnosed subsequently through the family studies. Six adult or near-adult C6Q0 patients were identified as never having been diagnosed as suffering a meningococcal infection. Three C6Q0 cases were diagnosed as a result of investigations of individuals with a single episode of infection. The majority of the C6Q0 patients had been diagnosed originally in the period from 1983 to 1997, and these subjects have been included in earlier reports [5,8].

Prophylaxis was long-acting penicillin injections [benzathine penicillin G (Penilente LA bicillin), Biotech Laboratories, Midrand, South Africa, 2·4 million units monthly for adults, and appropriately less for children] given at a convenient day hospital. Patients were provided with record sheets to be completed by the nurse administering the injection. For the first 4 years, this treatment was successful when followed [8]. However, with time patients tended to default (Table 1). There are problems with the use of bicillin; one young boy had lost both legs from disseminated intravascular coagulation (DIC) due to MD at 2 years of age. There was no reasonable place on his body to receive a large painful injection. Therefore he was given azithromycin (Sandos, Johannesburg, South Africa) 250 mg (half a tablet) weekly. He continued prophylaxis for about 8 years but then was without prophylaxis for the next 5 years. He suffered no further episodes of MD. A problem with azithromycin is the expense, and it is not provided by the SA health service.

Vaccination was considered but not used, mainly because it was not available in SA at that time. Also, the most prevalent meningococcal serogroup was group B, and no vaccine is produced. It is possible that the infections themselves lead to some immunity, and certainly some patients had high levels of diverse antibodies [8,11]. However, there was no evidence that these antibodies were protective.

The study was approved of the University of Cape Town Ethics Committee and patients and controls gave signed informed consent to participate in the study. The study of frequency of genetic mutations is an extension of the earlier clinical study. Approval in 2003 was REC REF 282/2003 and in 2007 REC REF 259/2007. Approval for collection of blood spots from the umbilical chords of newborns was obtained in 2002 REC REF 093/2002.

Controls

In order to assess the long-term consequences of C6Q0 and the effects of recurrent MD we used one matched control for

Table 1. Clinical details of the 46 C6Q0 subjects who participated in this study.

C6Q0 subject	Family	Well, seriously ill or died	Age 2005 years	Episodes of MD and ages when they occurred	Prophylaxis	Complications and probable sequelae MD	Other illnesses not directly attributable to MD	School leaving	Employment/ occupation
P1	Family 1 index case	Well	41	2 at 14 and 20 years	Good. Long-acting penicillin	None	None	Std 10	Computer operator
P2	Family 1 brother	Well	43	0	No	Not applicable	None	Std 9	Fitter and turner on mines
P3	Family 2 Mother	Well	64	0	No	Not applicable	High blood pressure. Some arthritis with ageing	n.a.	Domestic work
P4	Family 2 index case	Well	39	5 at 13, 14, 14, 24 and 31 years	Very intermittent	None	Accident cut off most of 4 fingers but still does woodwork. Some alcohol excess	Std 9	Joiner
P5	Family 2 brother	Died MD	Died 27	7 episodes	None	Intellectual slowing, death at final infection	N/A	Std 4	Worked easy jobs (cleaning)
P6	Family 2 sister	Well	41	1 at 17	No, but antibiotic tablets available	None	None	Std 8	Dental receptionist
P7	Family 2 brother	Serious illness	40	2 at 35 years, 38 years (DIC)	No	After DIC amputation of toes and severe contracture L foot. Walking difficult. Short-term memory poor after 2nd MD. DG. SI	Alcohol, drugs, SI	Std 7	Disability grant Not working
P8	Family 3 index case	Died. Stabbed by neighbour	Death at 16	3 at 2, 2 and 12 years	Intermittent	Optic atrophy in left eye	n.a.	n.a.	Scholar until death
P9	Family 3 brother	Well	22	None	No		Aggressive, some drug use, cannot keep jobs	Std 9	Intermittent driver
P10	Family 4 index case	Serious illness	47	Approximately 10	Intermittent Allergic to penicillin and macrolides used	Slow thinking, Slow speech, social difficulties lives rough	Polycythaemia Dx 1984. Needed venesections, continued some years. Excess alcohol. Pain in legs prevent working, possible peripheral neuritis. Chest infections 3 or 4 times per year, incl. *H. influenza*. Applied for DG. Lives rough	Std 6	Dropout, lives rough does not work
P11	Family 4 brother	Serious illness	49	3 at 21, 23, 33 years	Intermittent-allergic to penicillin	Headaches, memory poor and thinking slower after 2nd attack meningitis	Polycythaemia Dx 1984; required venesections, continued some years. Now moved, cannot get venesections, but Hb normal. DG because of work accident causing back pain. Back operation ± 1990	Std 6	Salesman/storeman 15 years. Now grows vegetables on small-holding. DG due to back pain
P12	Family 4 brother	Well	40	1 at 15 years	No	None	No	Std 8	Yes Truck driver
P13	Family 5 index case	Serious illness	34	4 at 4, 10, 14 and 17 years	Intermittent prophylaxis and suffered MD and rheumatic fever during lapses	None	RF 1st ± 1978 again 1987 after C6Q0 Dx. Diastolic mitral murmur. Back pain1989 now improved. Severe psoriasis over trunk and limbs Rx by dermatology SI	Std 10	6 years SA Defence Force, now truck driver

Table 1. *Continued*

C6Q0 subject	Family	Well, seriously ill or died	Age 2005 years	Episodes of MD and ages when they occurred	Prophylaxis	Complications and probable sequelae MD	Other illnesses not directly attributable to MD	School leaving	Employment/ occupation
P14	Family 5 sister	Died TB	Age at death 32	0	No	None	Repeatedly c/o headaches. Investigated for amenorrhoea. Died TB 2002	Std 8	Worked, but type of work not specified
P15	Family 5 brother	Well	26	1 at 16 years	Intermittent injections. They cause problems at his work	None	None	Std 10	Mostly employed, unloading trucks
P16	Family 6 index case	Well	27	1 at 2 years	No	None	Recurrent tonsillitis once or twice a month, allergic rhinitis and conjunctivitis, frequently needs antibiotics. Throat swab culture +ve for Streptococcus once	Matric, technical college, paralegal diploma	Police
P17	Family brother	Well	20	0	No	No infections	None	Matric, Computer training at tech. college	Student
P18	Family 7 index case	Serious illness	36	3 at 16, 23 and 36 years	No	Mentally slow. Tends to fall asleep. SI	Severe asthma/ chronic reduced lung function. SI	Std 6	Delivery van or taxi driver
P19	Family 8 index case	Well	44	2 at 22 and 30 years	No	None	Subject of domestic violence. Overweight. High BP 180/160 and headaches. Taking BP tablets 2004	Std 8	Domestic servant
P20	Family 9 index case	Serious illness	23	4 before 10 years	Yes in childhood. Now intermittent as interferes with work	Severe bilateral deafness, went to school for the deaf. SI	None	Std 10	Hairdressing and jewellery cleaning
P21	Family 10 index case	Died about 1992 alcoholic stupor and inhalation problems	About 33 at death	Many (>5), 1st at approximately 11 years	Intermittent and poor compliance	Developed chronic meningococcal septicaemia 1985. Aggressive anti-social behaviour but this also found in members of his community and linked to alcohol	Polio at 8 years. Chronic URTI. Alcohol excess. Deformed right hand and fused cervical vertebrae. He had 10 half-sibs not C6Q0; 8 half-sibs had had violent or alcohol/drug-induced deaths	n.a.	Mostly unemployed. Had had a prison episode
P22 No Control	Family 11 index case	Well	24	1 at 7 years	No	None	None. However, attack was severe and unable to get out of bed during the hospitalization of 3 months	Std 10 and Technikon studying quantity surveying	Student
P23	Family 12 index case	Serious illness	46	4 at 21 years, 27 years, 31 years and 32 years	Stopped 2001 and restarted 2003 good	No specific sequalae of MD. Does have an alcohol problem	TB Dx & Rx 2003–2005. Leg pains possibly due to peripheral neuritis. Limited movement left arm, hands and back following injury. Back pain result of stab in spine Rx anti-inflammatory medicines. Anti-centromere antibody in autoantibody screen in 2004. Again positive in 2008, but no rheumatological disease found	Std 8	DG for TB and back pain

Table 1. *Continued*

C6Q0 subject	Family	Well, seriously ill or died	Age 2005 years	Episodes of MD and ages when they occurred	Prophylaxis	Complications and probable sequelae MD	Other illnesses not directly attributable to MD	School leaving	Employment/ occupation
P24	Family 13 index case	Serious illness	34	2	C6Q0	Learning difficulties. Recommended for sheltered employment. Occasional Epilepsy. DG	None	Left school. Std 2 after 1st attack	DG. 1990 had a job as a packer. Lost job after 2nd MD
P25	Family 14 index case	Serious illness	36	3 at 15 years, 16 years and 19 years	Started 1985.Soon stopped. Restarted 2004 with AIDS Dx	None	AIDS, recent TB Rx. Obtaining AIDS Rx	Std 7	Sometimes with food canning industry. Work availability very poor
P26	Family 14 sister	Well	30	1 at 22 years	No	None	None but overweight	Std 6	Little work availability. Some seasonal on fruit farms
P27	Family 15 index case	Well	39	2 at 14 years and 17 years	Yes	About 1987 Dx hyperprolactinaemia. Possible pituitary damage due to MD. Rx Bromocriptine 1987. Now 3 children	Bronchopneumonia as a child	Std 9	15 years working at dry-cleaners Usually working
P28	Family 15 brother	Serious illness	30	4 at 12 years, 14 years, 15 years and 27 years	Intermittent. Problem with gang attacks at the clinics	Severe headaches. Difficulty working. Probable drug problem	Joint pain occasionally and pain lower back. C/o a lot of bruising on legs when he plays soccer	Std 10	Difficulty finding and keeping a job
P29	Family 16 index case	Serious illness	20	3 at 8 years (DIC), 16 years and 17 years	Yes after Dx. MD attacks were all before Dx	Severe contracture L foot result of DIC during 1st MD. Causes lameness and tends to become infected. DG grant	None	Std 7 continuing at evening school	Difficult keeping a job because prophylaxis DG for foot
P30 No Control	Family 16 sister	Well	29	0	No	None	Gets tired easily, listless	Std 9	Unemployed, 2 young children
P31	Family 17 index case	Well	39	2 at 13 years and 18 years	Pen injections. Good for 5 years. Stopped 1990	None	None	Std 6	Contract building, labourer
P32	Family 17 brother	SI. Lame from polio and severe respiratory problems	35	1 at 13 years	No	None. His only MD was at 13 years; therefore leaving school early was not a result of MD	Polio at 3 months residual foot disability. Rx and operation at orthopaedic hospital, Walks with a limp. Overweight. Back pain. Chronic reduced lung function and asthma	Std 3	Drives forklift truck 10 years
P33	Family 17 sister	Well	44	0	No	None	None	Std 8	Domestic work
P34	Family 18 index case	SI. Physically well, serious behaviour problems	21	2 at 13 years and 18 years	No	Possible aggression expelled from school and hurt in street fighting	Stabbed in chest. Possibly penetrated to lung, 4 days in hospital	Std 7	Expelled from school, sells peanuts on the streets
P35	Family 19 index case	Well	51	3 at 21 years, 24 years and 30 years	Yes	None	Rheumatic fever 12–13 years	Std 2	Worked in factory from 18 years, now in domestic service

Table 1. *Continued*

C6Q0 subject	Family	Well, seriously ill or died	Age 2005 years	Episodes of MD and ages when they occurred	Prophylaxis	Complications and probable sequelae MD	Other illnesses not directly attributable to MD	School leaving	Employment/ occupation
P36	Family 20 index case	Well	44	1 at 28 years	No	None. Diagnosed by testing for C6Q0 in MD patients	None	Std 3	Municipal worker parks and forests
P37	Family 21 index case	Well	36	2 at 8 years and 14 years	No	None	Recurrent tonsillitis 89–90	n.a.	Working
P38	Family 21 sister	Well	40	1 at 12 years	No	None	Recurrent sore throats as a child, gets flu quite easily T/S as a child Strep A + ve	Std 10	Works in a shop
P39	Family 21 sister	Well	34	1 at 6 years	No	n.a., but family report she is well	Recurrent sore throats in youth, T/S beta haem. Strep A + ve	n.a.	n.a.
P40	Family 22 index case	Serious illness	46	5 at 9 years 21 years, 25 years, 29 years and 29 years	Intermittent	Slow thinking. Catnaps and problems remembering recent events Severe headaches	Asthma diagnosed about 2000	Std 10	Textiles, pattern weaver
P41	Family 23 index case	SI. Deaf	40	2 at 16 years 18 years	Yes	Bilateral deafness SI	Dyspepsia	Sub B left school prior to MD	DG, not working
P42	Family 24 index case	SI. Severe asthma and required special schooling	33	4 at 5 years, 9 years, 9 years and 12 years	Intermittent	Special school for learning difficulties as a child, but normal as an adult	Severe asthma	Std 5 then training at a garage	Fruit factory, but sprays cause problems with asthma
P43	Family 25 index case	Serious illness	12	1 at about 2 years	Azithromycin 1/2 tablet weekly	Bilateral leg amputations because of DIC	None	Std 4 2005 doing well, will continue	Scholar
P44	Family 25 sister	Well	14	1 at 6 years	Azithromycin weekly	None	In 2003 severe impetigo and scabies Rx at clinic	In school Std 6 2004	Scholar
P45	Family 25 brother	Well	17	1 at 10 years	Yes	None	2004 presented abdominal pain, fever. Dx viral mesenteric adenitis. Some headaches	In school Std 8 2004	Scholar
P46	Family 26 index case	Serious illness	36	2 at 16 years 18 years	Yes, good	Learning difficulties/ mental slowing	Eclampsia 2001 with fits. Epilepsy fits after childbirth	Std 4 left prior MD	DG. Occasionally sheltered employment

BP: blood pressure; DIC: disseminated intravascular coagulation; DG: disability grant; Dx: diagnosed: MD meningococcal disease; n.a.: not available; SI: serious illness; Strep: Streptococcus; TB: tuberculosis; T/S: throat swab (for culture); Rx: treated with; URTI: upper respiratory tract infection; Std: Standard. All prophylaxis was with Bicillin unless indicated otherwise. School years in South Africa until mid-2000s. Sub A (for 5- and 6-year-olds), then Sub B and Standards 1–10. Matric was written at end of Standard 10.

each case. No controls were available for patients P2, P22 and P30. Controls had similar ages and ethnic backgrounds to the cases, and lived in similar social and environmental conditions. In order to achieve this, family members, preferably siblings, were chosen. The studies of C6Q0 families had been identified as C6-sufficient as well as C6Q0 individuals. All potential controls were C6 tested and confirmed C6 sufficient. We also needed to ensure that controls who had died or were seriously ill (SI) were not omitted. We used the beginning of our first study in 1984 as the starting-point for the present study, and death was counted only if it occurred after 1 January 1984.

When there was no sibling control available, a C6-sufficient cousin (or in one case an uncle) was used, provided that he/she was close in age to the patient. None of the controls had suffered meningococcal disease. When cousins were required we chose preferably those who were children of the maternal sibling closest in age to the mother. From the children of this sibling, the cousin used as a matched control was the one closest in age to the C6Q0 patient, again not excluding any who had died. If the cousin closest in age to the patient was unavailable, but alive, the next available cousin (the second closest age-match to the patient) was used as a control.

Spouses or long-term partners of C6Q0 patients were not included in the study.

Fifty controls were contacted; their mean age was 36·2 years and there was no significant difference between the ages of the patients and controls. Controls were interviewed with the same questionnaire used for the patients and where possible blood was collected for laboratory investigations. The first primary control was the control nearest in age to the case, and only primary controls were used in the case–control clinical follow-up. The secondary controls were used only to provide additional samples for the biochemical/immunological *in-vitro* tests.

Interviews

Most cases and controls were interviewed in person using a standardized questionnaire. However, a few lived too far away and these were interviewed telephonically. Patients and controls were questioned about episodes of MD. None of the controls had a positive history of MD. Cases and controls were asked in detail about suffering the recognized sequelae of MD, including physical disabilities such as limb loss or loss of function due to DIC, learning and cognitive difficulties, deafness, vision impairment and debilitating headaches. Enquiries included the level of education achieved (see explanatory note to Table 1), employment or occupation history, hobbies and sports, serious infections including tuberculosis (TB) and other respiratory or chest infections. A detailed allergy history was obtained. We did not ask routinely about rheumatic fever (RF) or other streptococcal diseases. At the end of the interview we assessed the ability of patients and controls to comprehend what we explained, whether they could interact normally in conversation, and in their own living environment. Thus we assessed whether there was any cognitive dysfunction. Histories of patients who had died were obtained from relatives. They were asked if the person had coped normally with their living environment. All patients were asked about prophylaxis.

On the basis of the interview, patients were classified as (i) 'well', (ii) having an 'SI' or who had 'died'. An SI was a long-term illness that interfered with expected daily life of the subject and prevented them from carrying out appropriate normal daily occupations such as work, school or child-rearing. Any individual receiving a disability grant (DG) was also considered to have a SI. TB was counted as an SI only if the interview was within 2 years of diagnosis. We could not test for acquired immune deficiency syndrome (AIDS) without permission of the patient and did not test unless the history indicated we should. We tested two subjects, and one was positive.

Laboratory investigations

These were carried out on all samples from C6Q0 patients and controls who were available to give blood: 36 C6Q0 patients and 33 controls. All serum samples were stored at −80°C.

As part of our earlier studies, C6 assays had been performed in haemolytic agarose gel plates [5]. Assays performed from 2003 onwards were carried out almost exclusively on controls who had not been investigated in the early studies. They were performed in microtitre plates using an adaptation of the method described by Morgan [12], using human C6Q0 serum instead of C6 depleted serum.

Serum total and specific immunoglobulin (Ig)E levels were measured using the Pharmacia Diagnostics UniCAP® system (Uppsala, Sweden). Specific IgEs were measured against (a) house dust mite (*Dermatophagoides pteronyssinus*), (b) grass pollen mix (*Cynodon dactylon*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*, *Sorghum halepense*, *Paspalum notatum* and (c) mould mix (*Penicillium notatum*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Alternaria alternata*).

Autoantibodies were determined using the Pharmacia Diagnostics Varelisa ReCombi ANA screen, which qualitatively determines the presence of antibodies against one or more of a range of eight nuclear antigens (dsDNA, RNP, Sm, SS-A/Ro, SS-B/La, Scl-70, Centromere and Jo-1) in a single microwell. Subsequently, if the ANA screen was equivocal or positive the ReCombi ANA profile was used to determine specifically which antibodies were raised. The assays were performed according to the manufacturer's instructions and specifications.

Samples and genetic methods for the study of the frequency of gene mutations leading to C6Q0 among 2250 newborn neonates

Blood samples obtained from umbilical chords of babies born at maternity clinics in the Cape Town area in 2002–03 were used to make blood spots on Whatman 3MM filter paper and then stored dry and sealed at −20°C. Blood spots from 1500 South African Coloured neonates and 750 South African Black neonates were used for DNA preparation for the present study. Race classification was based only on the race of the mother. The 2001 census had shown approximately twice as many South African Coloureds living in the Western Cape as Blacks [13]. It was therefore appropriate to analyse twice as many blood spots from South African Coloureds as from Black South Africans.

The C6 gene nomenclature was discussed by Parham *et al*. [10], where it was stressed that authors need to make clear what system they are using. For this manuscript we are now using the nomenclature that abides by the Human Genome Variation Society (HGVS) recommendations of den Dunnen and Antonarakis [14,15]. The new and original names for the defects are included in Table 3. It should be noted that in the old nomenclature 879delG referred to the deletion of the 5′ nucleotide in a run of seven Gs, whereas using the new nomenclature 828delG refers to the 3′G in the same run of seven Gs.

DNA was extracted from the blood spots using MagZorb DNA extraction kit from Promega Corporation (Madison, WI, USA) and stored at −20°C. DNA samples were tested for four C6 gene mutations known to be responsible for C6Q0 in South Africa [9,10], namely 821delA, 828delG, 1138delC and 1879delG. To detect the 821delA and 828delG mutations, primers were designed to create a BseL1 restriction site. DNA samples were amplified, digested with BseL1 [Fermentas Life Sciences (part of Thermo Fisher Scientific) Vilnius, Lithuania] and visualized on a 3·5% agarose gel using ethidium bromide.

Amplification-refractory mutation system (ARMS) primers were designed to detect the mutant sequences for the 1138delC and 1879delG mutations in exon 7 and 12, respectively, and visualized on a 2% agarose gel using ethidium bromide. All positive samples were confirmed by sequencing using BigDye Terminator version 3·1 from Applied Biosystems (Life Technologies Corporation, Carlsbad, CA, USA).

Statistical analysis

Conditional logistic regression was used to compare the risk of SI or death in the patients to those in the matched controls, while adjusting for the relatedness/matching, We did not adjust this analysis for the further dependence created by the fact that several of the cases were related to one another.

In patients, we used mixed-effects logistic regression to compare the risk of SI or death in the patients who had no or one episodes to those who had more than one episode, while adjusting for the relatedness between individuals as random effect and age at diagnosis as fixed effect.

The binomial distribution (assumption of Hardy–Weinberg equilibrium) was used to estimate the number and frequency of mutation homozygotes. Confidence intervals were calculated using the so-called Wilson exact method [16,17].

R [18] and R packages were used for all analyses. Further information on the statistics is available from the fourth author.

Results

Results of clinical follow-up of C6Q0 patients and matched controls

Results of the interviews with C6Q0 patients, taken together with clinical information obtained over the years when patients attended the immunology clinic, are shown in Table 1. There were 46 patients from 26 families. Patients were classified into two broad groups according to their state of health; either (i) well or (ii) suffering from an SI or had died. Human immunodeficiency virus (HIV) infection could not be tested for unless the subject gave permission, and we only asked permission if there were indications of possible AIDS. Only two subjects were tested; in one the diagnosis was positive, and she was classed as SI. However, the other 44 were untested and none had overt symptoms of AIDS.

Table 1 shows repeated meningococcal infections had a devastating effect on many patients. Patient illnesses are listed either under ‘complications and sequelae of MD’ or ‘illnesses not directly attributable to MD’. However, the latter includes effects of alcohol/drugs abuse, including aggressive behaviour, which were found in both patients and controls.

We had matching information for the 43 controls. Therefore, in analyses where controls are compared with C6Q0 patients only data from the 43 patients who have controls are used. Otherwise, all 46 patients are discussed. Patients and controls were categorized according to whether they were (i) well or (ii) suffered an SI or D.

Control results are not tabulated, as they are less complex and the controls suffered less SI and D. Thirty-five of the controls were also classified; six had an SI and two had died. One death was from violence: that individual suffered from substance abuse (SA) prior to death; and one death was from AIDS. Four controls had recent or current tuberculosis, three had learning and cognitive difficulties and three had substance abuse that prevented them from living normal lives. One control suffered severe depression.

When we compared the number of individuals who had SI/D between the patient and control groups, it yielded a *P*-value of 0·006. The odds ratio is 0·17, so the odds of being

Table 2. Results for the clinical outcome of 43 case–control pairs well *versus* serious illness/died (SI/D) ($P = 0{\cdot}006$).

	Patient well	Patient SI/D	Total
Matched control well	18	17	35
Matched control SI/D	3	5	8
Total	21	22	43

well as a patient is only 17% of what it would be as a control (see Table 2).

As indicated in the Introduction (Table 1), the socio-economic circumstances of the C6Q0 patients meant that many failed to keep up regular bicillin injections. We believe that this study is not suitable for demonstration of the efficacy of bicillin. However, three patients with good injection records remained MD-free for 25 years.

Particular C6Q0 individuals illustrate the seriousness of the diseases. In family 2 there were four affected siblings and the C6Q0 mother, P5, had died, but history details were available from his family. He had seven MD attacks and died of the last attack at age 27 years. During life his MD attacks affected his mental ability and later he could perform only simple jobs. P7 suffered DIC during one attack, and had had toes amputated and developed a contracture in the same foot. Walking was difficult, and in addition he developed loss of short-term memory. In contrast, the other two affected siblings (P4 and P6) were both 'well', despite five attacks in P4.

In family 3, following one episode of MD, P8 had developed optic nerve atrophy in one eye. At the age of 16 he was stabbed and killed by a neighbour. It is not possible to know if he suffered from aggressive behaviour which led to this. Family 4 was unusual in that two affected brothers, P10 and P11, suffered polycythaemia as well as C6Q0 and recurrent episodes of MD; they were classified as SI because of cognitive disability, alcohol excess and accidental injury. The third C6Q0 brother P12 did not suffer polycythaemia, had one episode of MD and was classified as well. P13 had had four episodes of MD and was SI because he had suffered RF in the past and at interview suffered from rheumatic heart disease. In addition, he had very severe psoriasis over the trunk and limbs. RF was reported at some time in two patients and Group A Streptococcus (GAS) had been cultured in four who suffered from recurrent sore throats. Unfortunately, we did not ask some patients and or any controls about a history of RF.

Several C6Q0 patients had severe problems with alcohol or drugs and aggression. P34 in particular was physically well, but his aggressive behaviour had resulted in expulsion from school to a life on the streets. Learning and cognitive difficulties were present in nine patients.

P23 had numerous problems, not all necessarily sequelae of MD. He had had physical injuries to an arm and his spine. He had some alcohol abuse and complained of severe leg pains. His anti-nuclear antibody screen in 2004 showed positive for anti-centromere antibody, this was confirmed in 2008. This suggested the possibility of autoimmune disease and the patient was referred for further investigations; however, no reason for this antibody was found. P27 is informative because, at 21 years, subsequent to two MD attacks, she complained of amenorrhoea and infertility. She was diagnosed as having hyperprolactinaemia, due possibly to a pituitary adenoma and treated with bromocriptine. This helped, and she now has three children. No pituitary adenoma was ever confirmed, but meningococcal meningitis can affect the hypothalamic region, and it is possible that her infertility was due to hypothalmic damage during a meningococcal infection. P28 complained of severe recurrent headaches and gaining and keeping employment was difficult; he is classed SI. Several patients and controls complained of headaches and assessing the disability caused is difficult. In summary, the most common causes of SI in patients were cognitive difficulties, mental slowing and aggressive behaviour as result of MD. Twelve patients were severely compromised by the effects MD had on their brain function.

Asthma associated with measurable reduction in lung function was identified in three patients. P16 had had a history of severe allergy from early childhood. Allergy symptoms were classed as 1, none; 2, mild symptoms not normally treated; 3, moderate symptoms which required treatment; and 4, severe symptoms which need prolonged treatment. Although both the control group and C6Q0 patients had patients with allergy, the difference observed was that, in the four patients mentioned above, the symptoms were worse than in any of the controls. The possibility of a link between C6Q0 and severe long-term consequences of allergy, particularly with respiratory problems, cannot be excluded.

TB diagnosed within the previous 2 years was present in four controls and two patients, and one patient had died from TB. One control died from AIDS and one died from violence.

The results of the immunological and biochemical tests showed few differences between patients and their controls. Specimens for these tests were available from 36 C6Q0 patients and 33 controls. There was a wide range of IgE levels, but this was true in both groups and there was no significant difference between them. Also there were no significant differences in the number of specific IgE measurements, which would indicate allergy to common allergens such as house dust mite, grass pollen mix and mould mix.

To assess the contribution of recurrent meningococcal disease to the SI suffered, we divided the C6Q0 patients into two groups: (i) those who had no history of MD, together with those who had suffered only one MD episode, and (ii) those who had a history of two or more episodes of MD. The same criterion for SI was used as in Table 1. The data are illustrated in Fig. 1, which shows that the patients with recurrent disease were indeed clearly more prone to SI than those with no or one episode of MD. We realize, and show

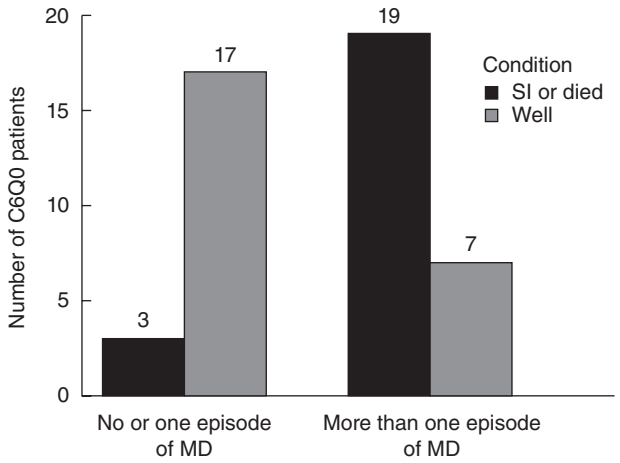

Fig. 1. Bar-graph demonstrating that C6Q0 patients who had suffered more than one episode of meningococcal disease (MD) were much more likely to have a serious illness or to have died than those C6Q0 patients who had suffered no or one episode of MD ($P = 0{\cdot}008$).

here, that SI can be brought about by the first episode of MD. However, until the risk of MD to C6Q0 individuals is understood more clearly, we are advocating screening all cases presenting with MD for C6Q0, which will mean that no prophylaxis can be given before the first episode.

Results of the gene frequency study

Seven hundred and fifty Black and 1500 South African Coloured samples were analysed for the four common defects in the C6 gene. Table 3 gives the results obtained. Nineteen alleles were positive for 821delA, 51 for 828delG, 26 for 1138delC and seven for 1879delG, so in total 103 defective alleles were detected from 4500 alleles (2·28%). In summary, 96 samples were heterozygous for one of the mutations, while one sample was homozygous for 828delG and another two samples were compound heterozygotes (821delA/828delG and 828delG/1879delG). All mutations are one base pair deletions, causing out-of-frame translation from that point onwards, and hence early termination of C6 protein synthesis. We know from clinical studies that all four defects result in the non-secretion of the C6 protein [5,10].

The observed homozygote or affected compound heterozygote mutation frequencies for South African Coloureds was three of 1500 and for Blacks was none of 750. Therefore, the combined affected C6Q0 samples were three of 2250 = 1·33 affected in 1000.

Table 4 shows the estimated homozygote (mm) frequencies for each defect and confidence intervals per 1000, using observed carrier frequencies. The last row shows the estimated number of samples needed to examine to detect a

Table 3. Genotype counts of the four C6Q0 genetic defects observed data.

Current nomenclature Original nomenclature	821delA 878delA	828del G 879delG	1138del C 1195delC	1879del G 1936delG	Collective Collective
Black ($n = 750$)					
mm	0	0	0	0	0
Mm	7	9	11	1	28
MM	743	741	739	749	722
South African Coloured ($n = 1500$)					
mm	0	1	0	0	1+2*
Mm	12	40	15	6	71–2*
MM	1488	1459	1485	1494	1428
South African Coloured plus Black ($n = 2250$)					
mm	0	1	0	0	1+2*
Mm	19	49	26	7	99–2*
MM	2231	2200	2224	2243	2150

*There are three affected individuals: one homozygous 828delG; and two compound heterozygotes: 821delA/828delG and 828delG/1879delG. The two compound heterozygotes, being affected, are counted in the collective data as 'mm'. m, Defective allele; M, normal allele.

Table 4. Estimated mm (affected) frequencies and 95% confidence intervals per 10 000 individuals using observed carrier frequencies.

	Black	South African Coloured	Combined
821delA	0·22 (0·05–0·92)	0·16 (0·05–0·49)	0·18 (0·07–0·43)
828delG	0·36 (0·10–1·29)	1·96 (1·08–3·56)	1·28 (0·74–2·21)
1138delC	0·54 (0·17–1·71)	0·25 (0·09–0·68)	0·33 (0·16–0·71)
1879delG	0·00 (0·00–0·14)	0·04 (0·01–0·19)	0·02 (0·01–0·10)
Compound heterozygotes	3·48 (1·68–7·21)	6·25 (4·00–9·75)	5·24 (3·58–7·66)
Number to examine*	2874 (1387–5952)	1600 (1026–2500)	1909 (1305–2797)

*The frequencies and number needed to examine in the 'combined' column were estimated assuming that the ratio of Black to South African Coloured people in the population for which these estimates are generated, are the same as their ratio, 1:2, in this study group.

single affected case. The 828delG is the most frequent defect both for the South African Coloureds and the combined group, as there are 1·28 homozygotes expected per 10 000 samples. However, for the Blacks the most common defect is 1138delC, as there are 0·54 homozygotes expected per 10 000 Blacks. For all groups and defects combined, 5·24 affected homozygous or compound heterozygous C6Q0 results are expected per 10 000 samples.

Regarding the estimated mm frequencies, the collective data are clinically relevant because C6Q0 is found in compound heterozygote individuals, and among the three affected individuals there are two affected compound heterozygotes (Table 4).

Discussion

This study has shown that the C6Q0 patients suffer SI or death significantly more often than matched controls. Our patients and controls had a mean age in their mid-30s and most of the SI or death of patients could be ascribed directly to invasive MD or its complications, such as a fatal acute MD episode (one instance), loss of limbs or digits from DIC (three instances) or bilateral deafness (two instances). However, MD can also be a major factor in severe alcohol/drug problems (five patients affected including one death) and learning and cognitive difficulties (nine patients affected). Accident or violence was responsible for two patients' deaths and two patients' deformities, and also responsible for one control death. Three patients had aggressive behaviour, and in two of these it was classed as an SI. Many serious consequences of MD, such as learning difficulties, are not exclusive to patients with terminal complement component deficiency.

In the Western Cape there were approximately 317 cases of MD reported for the 3 years mid-1999–mid-2002 [19]; the highest incidences were with Group B infections and also in children under 1 year of age. This is in contrast to the considerably higher age of first infection we reported previously for C6 deficient individuals in the Cape [5].

Another important question is whether it is susceptibility only to *Neisseria* infections that is increased in C6Q0 individuals. The infectious diseases which cause the most serious problems in South Africa are AIDS and TB. AIDS was not tested for routinely in this study, and therefore it is not informative about AIDS. Conversely, we were only really clinically concerned about AIDS in two patients, and only one was positive. Four controls and three C6Q0 patients had had TB within the previous 2 years and therefore C6Q0 would appear not to be a susceptibility factor.

There is some evidence that C6Q0 or other TCCDs may lead to some increased susceptibility to other bacterial infections besides *Neisseria* infections. Figueraro and Densen [3] discuss that *Haemophilus influenzae*, like *N. meningitidis*, possesses short surface lipopolysaccharide chains which make the cell walls vulnerable to complement killing in non-immune sera. However, in the long list of infections they reported in complement-deficient patients there is just one case of *H. parainfluenzae* meningitis in a C7-deficient patient compared to numerous cases of MD. In the present report one of the C6Q0 patients, P10, had multiple attacks of *Haemophilus*-species pneumonia; however, he had poor sleeping conditions and was an alcoholic, and thus very exposed. Three additional C6Q0 patients, but no controls, had asthma and serious lung infections, and another had repeated asthma attacks with repeated throat and tonsil infections including Streptococcus infections. These problems were not found in controls. Other problems were RF and/or positive throat culture with GAS, which were reported in five patients. This was not foreseen when the study started, and thus was not investigated routinely in either patients or controls. However, it is certainly possible that lack of terminal complement pathway function could compromise the host to infections with organisms other than *Neisseria*. Evidence is now accumulating of a complex relationship between GAS and the complement system and that the organisms develop complex evasion strategies to avoid complement attack [20].

The high rate of SI in the patients who suffer recurrent MD (see Fig. 1) makes it important to prescribe long-term antibiotic prophylaxis for vulnerable C6Q0 or other TCCD individuals [8]. Prevention of recurrent disease in C6Q0 individuals can be aimed for when all patients with invasive MD are tested for C6Q0. What would be extremely valuable for assessing how patients should be treated would be a way of determining which patients remain susceptible to further infections. There is no doubt that vulnerability differs between individuals; seven individuals listed in Table 1 reported no episodes of MD and all had reached the age of at least 20 years. Also, each one had a C6Q0 sibling who was the index case in the family, so it is very unlikely that they had no exposure.

In a much earlier publication [5] we reported on data from families with C6Q0 children. We used the ' "singles" method of Davie' [21] to determine whether there were an excess of homozygote C6Q0 siblings of the index cases. Unfortunately, the small numbers meant that the significance was borderline. Moreover, the data we present here on the long-term outcome for C6Q0 individuals are drawn largely from members of the same families. The data from the genetic screen show three homozygous C6Q0 individuals, which is indeed more than would be expected in the 1250 tested, but not enough to be significant.

The gene screening results for South African Coloureds clearly show 828delG to be the most frequent defect. This defect was first reported in 1998 by Hobart and co-workers [9] in this same group of South African C6Q0 patients. We found it most frequently in the South African Coloureds; however, it was also relatively frequent in Black patients. South African Coloureds are descended from Khoisan, Bantu (Black), European, Indian and South East Asian

ancestors, with a large maternal Khoisan input [22]. Therefore it is possible, but certainly not proved, that 828delG originated in the Khoisan people. Interestingly, the other three defects were first found in blacks living in North America or Europe, and defects very probably arrived in America with the slaves from West Africa. We show now that these defects also appear to have spread a long way within the African continent itself.

We do not have data for South Africans of European descent. C6Q0 is reported relatively rarely in white individuals living in Europe or North America, and therefore the defects are probably rare in South African Whites.

The results of this genetic screen show that C6Q0 in South Africa is not a rare genetic condition. In the Western Cape in 2001 there were approximately $2{\cdot}5 \times 10^6$ South African Coloureds and $1{\cdot}2 \times 10^6$ Blacks [13]. The population has probably increased markedly since then. Using our observation that one in 1909 (between 1305 and 2797, with 95% confidence) Western Cape Black and Coloured South Africans combined will be affected by C6Q0 (Table 4), we conclude there could be approximately 2000 C6QO Black or Coloured individuals living in this region.

However, we have only screened neonates and do not know what the susceptibility of young C6Q0 children is to other infections, particularly Gram-negative infections. It is relatively easy to determine the number of C6Q0 mutations among patients with MD. The risk of complement deficiency among MD patients has been reported as about 10% [3] in the United States. What is more difficult to determine is the risk of MD in C6Q0 individuals and also the risk of other bacterial infections.

The striking finding of very high serious morbidity in patients with C6Q0 due to meningococcal infections requires several proactive steps. The first would be to prevent subsequent infections with regular effective antibiotic prophylaxis in subjects already diagnosed and to provide support to those already disabled or disadvantaged (e.g. employment, occupational therapy and rehabilitation). It is also important that further studies of the gene frequency in the broader Southern Africa population are conducted. Early identification of C6Q0 cases when they have their first attack of meningitis could take place if all patients in Southern Africa with meningococcal meningitis were screened using polymerase chain reaction (PCR) for the C6Q0 genetic defects, resulting in early implementation of antibiotic prophylaxis, until vaccines are proven to be effective in these patients.

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Disclosure

None.

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