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# Deficiency of the sixth component of complement and susceptibility to *Neisseria meningitidis* infections: studies in 10 families and five isolated cases

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## SUMMARY

Complement component C6 deficiency (C6D) was diagnosed in 15 patients who presented, independently, with recurrent meningococcal infection. This condition is thus not particularly rare in the Cape. Ten of the patients belonged to multiplex families, and family studies led to the diagnosis of another 12 C6D cases among the siblings. Segregation analysis showed that C6D occurred more frequently among the siblings of affected individuals than would be expected for co-dominant inheritance. The possible reasons for this are discussed. We also observed that the 12 non-proband C6D siblings included only four with a history suggestive of meningococcal infection, and thus C6D individuals apparently differ in susceptibility to *Neisseria meningitidis* infection. We confirmed previous observations that primary infection occurs later in C6D individuals than amongst susceptible complement-sufficient individuals. Among 123 patients presenting with primary meningitis, one case of C6D was diagnosed. The data show that C6D is an important factor associated with susceptibility to meningococcal infection in the Cape.

## INTRODUCTION

The association between genetically determined deficiency of late-acting complement component C6 (C6D) and susceptibility to Neisserial infections is well established (Leddy *et al.*, 1974; Lim *et al.*, 1976; Ross & Densen, 1984). Affected individuals have tended to present as isolated cases in America and Europe. Ross & Densen (1984) assembled data from a number of complement-deficient patients from different centres, including 33 patients with C6D. They documented certain tendencies in patients with Neisserial infections secondary to late-acting component deficiencies. They report that *N. meningitidis* tends to be a more important complicating pathogen than *N. gonorrhoeae*, and that the first episode of meningococcal disease usually occurs at a later age in complement-deficient individuals than in susceptible complement-sufficient persons.

In the Cape, meningococcal disease is still endemic and C6D is an important predisposing cause of recurrent infection. Reported here are studies of 10 multiplex C6D families and five individual cases. These studies have shown that among C6D individuals there appears to be varying susceptibility to *N. meningitidis* infection, and have confirmed that the first episode of infection tends to occur later among complement-deficient than among susceptible complement-sufficient individuals. Moreover, our family studies show that C6D index cases have an unexpectedly high proportion of C6D siblings; this suggests that deficiency of late-acting complement components may, under certain circumstances, be beneficial.

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## MATERIALS AND METHODS

*Patients and family members*

Informed consent was obtained from C6D family members, or from their parents or guardians. Ascertainment was through referral of cases of recurrent meningococcal infection. Thus, all families were ascertained through affected children and, for the purpose of segregation analysis, the ascertainment was incomplete. Although four of the C6D siblings of index cases gave a history of previous meningitis, these four siblings were ascertained through their respective index cases and so, for each of the 10 families reported, there was only one proband. None of the marriages was consanguinous. The recurrent presenting episode in every case was bacteriologically confirmed meningitis. No case was ascertained because of *N. gonorrhoeae* infection; furthermore, none of the subjects gave a history indicative of this infection. Thirteen of the 15 index cases were Cape Coloured (mixed Caucasoid, South East Asian and Southern African [Botha, 1972]), and two were Black belonging to the Xhosa Tribe.

**Table 1.** Results for segregation analysis of the C6D families

| Family | No. of living sibs | No. of deceased sibs | No. of sibs tested | No. of sibs affected | Affected sibs | |
|---|---|---|---|---|---|---|
| | | | | | Mening. disease* | No mening. disease |
| (a) F1 | 3 | 0 | 3 | 2 | 1 | 1 |
| F2 | 3 | 1 | 3 | 2 | 1 | 1 |
| F3 | 4 | 0 | 4 | 3 | 3 (2) | 0 |
| F4 | 4 | 0 | 4 | 2 | 1 | 1 |
| F5 | 4 | 1 | 4 | 2 | 1 | 1 |
| F6 | 5 | 0 | 5 | 3 | 1 | 2 |
| F7 | 8 | 0 | 7 | 3 | 2 (1) | 1 |
| subtotal | 31 | 2 | 30 | 17 | 10 | 7 |
| (b) M1 | 3 | NK | 3 | 3 | 2 (1) | 1 |
| M2 | 3 | 1 | 2 | 1 | 1 | 0 |
| M3 | 4 | 3 | 3 | 1 | 1 | 0 |
| Total | 41 | NK | 38 | 22 | 14 | 8 |

In each family there was only one proband, and results for the probands are included with the others. (a) (Families F1–F7) includes families where both parents were available and had been shown to be C6 sufficient and thus presumptive heterozygotes. Genetic markers had been used to confirm family relationships. (b) (Families M1–M3) includes those families in which one or both parents were unavailable. Analysis of the data to determine whether the pattern of inheritance is compatible with co-dominant inheritance is given in the text.

NK, not known.

* A positive history of meningococcal disease. Numbers in brackets indicate number of non-proband-affected sibs with a positive history.

Of the patients with primary meningococcal disease, Series A consisted of 27 randomly selected cases sampled during non-epidemic times, and Series B consisted of 96 children with Group B infection sampled during an epidemic (Beatty, Ryder & Heese, 1986).

*Assays for C6 and C7*

Serum or plasma samples were used fresh or were stored at −70° until use. Samples were frozen in small aliquots so that repeated freezing and thawing was avoided. Serum C6 haemolytic activity was assayed as described elsewhere (Lachmann & Hobart, 1978). Briefly, 5-$\mu$l serum (or plasma) samples were inoculated into agarose gel that incorporated appropriately sensitized sheep red blood cells (EAC143b) (Orren, Preece & Dowdle, 1985) and C6D rabbit serum. Gels were incubated at 4° overnight. Diameters of the rings of lysis ranged from 6 to 10 mm and gave a semi-quantative measurement of the C6 present. C6D was diagnosed when samples produced no lysis. In addition, we confirmed the absence of antigenic C6 by rocket electrophoresis (Laurell, 1966) into gels containing an anti-C6 antiserum which had been raised by immunizing C6D rabbits with rabbit C6 (Lachmann & Hobart, 1978; Orren *et al.*, 1985). This assay provides a quantitative measurement of C6 concentrations. All C6D samples, except one, failed to produce rockets. The one sample that gave rise to a tiny rocket had a calculated C6 level less that 5% of the normal control. The obligative heterozygotes had C6 concentrations between 64% and 85% of the normal control. C6 allotyping was performed by iso-electric focussing with subsequent C6 indicator overlay (Hobart, Lachmann & Alper, 1975). C7 haemolytic activity was determined in C7 agarose indicator gels, as previously described (Orren, Lerch & Dowdle, 1983).

*Genetic markers*

HLA A, B and C typing was used to confirm stated family relationships. Typing was performed by micro-lymphocytotoxicity (Terasaki *et al.*, 1974), using a total of 180 antisera.

*Statistical methods*

The Mann–Whitney *U*-test (Siegel, 1956) and the adapted 'singles' method (Davie, 1979) were used where appropriate (see the Results and Discussion).

## RESULTS

Table 1 shows the data obtained from the multiplex C6D sibships. In the families listed in Table 1(a) (F1–F7) we were able to obtain blood specimens from both parents. These parents all had positive serum-C6 activity and were therefore presumptive heterozygotes. C6 allotyping showed all 14 possessed only one C6 allotype, and this provided supportive evidence of heterozygosity. In only one family were we unable to test all living sibs. Half siblings (who presented as such or who were recognized because of the genetic testing) have not been included in the table. Table 1(b) gives data for families investigated less fully in which one or both parents were unavailable (M1–M3).

The results show that a high proportion of C6D siblings were found within the affected families. Given that C6 is co-dominantly expressed and that C6D results from the presence of two C6 null genes (Glass *et al.*, 1978), the expected probability, $P$, of a child of a hetrozygous mating being homozygous affected is 0·25.

The data were analysed by the 'singles method', as adapted by Davie (1979). The estimated probability is:

$$\hat{P}=\frac{R-J}{T-J}$$

**Table 2.** List of all C6D individuals investigated

| Patient | P/NP* | Age at presentation (yr) | No. attacks M. disease | Age 1st attack (yr) | Age 2nd attack (yr) | Age subsequent attacks (yr) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| F1a | P | 16 | 2 | 15 | 16 | | | | | |
| F1b | NP | 10 | 0 | | | | | | | |
| F2a | P | 21 | 2 | 12 | 20 | | | | | |
| F2b | NP | 22 | 0 | | | | | | | |
| F3a | P | 14 | 2 | 8 | 14 | | | | | |
| F3b | NP | 17 | 1 | 12 | | | | | | |
| F3c | NP | 12 | 1 | 7 | | | | | | |
| F4a | P | 10 | 3 | 2 | 5 | 6 | | | | |
| F4b | NP | 3 | 0 | | | | | | | |
| F5a | P | 20 | 2 | 16 | 20 | | | | | |
| F5b | NP | 11 | 0 | | | | | | | |
| F6a | P | 15 | 2 | 13 | 14 | | | | | |
| F6b | NP | 16 | 0 | | | | | | | |
| F6c | NP | 7 | 0 | | | | | | | |
| F7a | P | 27 | 7† | 21 | 22 | 22 | 25 | 25 | 26 | 27 |
| F7b | NP | 30 | 2 | 21 | 23 | | | | | |
| F7c | NP | 21 | 0 | | | | | | | |
| M1a | P | 19 | 2 | 13 | 19 | | | | | |
| M1b | NP | 15 | 1 | 14 | | | | | | |
| M1c | NP | 21 | 0 | | | | | | | |
| M2a | P | 30 | 3 | 21 | 24 | 30 | | | | |
| M3a | P | 21 | 2 | 16 | 21 | | | | | |
| S1 | | 31 | 3 | 18 | 20 | 31 | | | | |
| S2 | | 13 | 3 | 5 | 9 | 12 | | | | |
| S3 | | 24 | 4 | 21 | 22 | 23 | 24 | 27 | | |
| S4‡ | | 0·75 | 3 | 0·75 | 1·25 | 41 | 1·5 | | | |
| S5 | | 22 | 2 | 14 | 22 | | | | | |

Family numbers are as in Table 1 and individuals in the families indicated by a, b, etc.

Individuals S1–S5 were individuals who either had no full sibs (S1–S4) or who had no available sibs (S5).

* Proband (P) or non-proband (NP).

† History difficult, only two of these attacks were bacteriologically confirmed.

‡ This child has, in addition to C6D, partial C2 deficiency. Data for this patient have therefore been omitted from the calculations using age of first attack or interval between attacks.

where R = total number of affected siblings; T = total number of siblings tested; and J = number of sibships with only one proband (in this case total number of sibships). Analysis of all the data in Table 1 results in the following: $\hat{P} = 0{\cdot}43$ [variance $\hat{P}$ (var $\hat{P}$) approximately 0·009, SE approximately 0·096, 95% confidence limits 0·25–0·61].

If data from only those families in which both parents were available are analysed (Table 1a) then $\hat{P} = 0{\cdot}435$ (var $\hat{P}$ approximately 0·0107, SE approximately 0·103, 95% confidence limits 0·23–0·64).

History suggests one child each in families F5 and M3 died of meningitis. These have not been included in the calculations.

Another result apparent in Table 1 is that a large proportion of C6D subjects had no history of meningococcal infection. Thus, of 12 non-probrand C6D individuals, only four had suffered clinical disease.

Table 2 lists all 27 C6D subjects, giving ages at investigation and the ages of all meningococcal infections. The most susceptible of the patients (S4) was found to have, in addition to C6D, partial C2 deficiency, and data from this patient has not been included in the analyses of the effect of age on susceptability to infection. For C6D patients the median age at first attack of meningococcal infection was 14 years (range 2–23 years); this was significantly ($P < 0{\cdot}001$, Mann–Whitney $U$-test higher than in 27 complement-sufficient patients (Series A) (median 1 year, range 1 month–58 years).

The frequency of C6D in patients presenting with primary meningococcal infection was investigated by testing total haemolytic complement or C6 activity in sera from patients presenting with primary disease. The 27 Series A patients and the 96 Series B patients were tested. Only one C6D patient was identified; she was a child of 26 months from Series B. She recovered from meningitis but was unfortunately unavailable for further follow-up.

Due to the possible association of C7 deficiency with C6 deficiency (Lachman, Hobart & Woo, 1978), sera from all C6D individuals were tested for C7 activity. No case of associated C7 deficiency was found.

## DISCUSSION

We report here results of studies of 15 independantly diagnosed

C6D cases and their family members. The total number of persons found to be C6D was 27; all lived within the Western Cape Province. The availability of this relatively large group of patients has enabled us to investigate various aspects of complement deficiency and susceptibility to Neisserial infections. Clinical data and methods used to prevent further infection will be reported elsewhere.

Table 1 and the accompanying analysis show that C6D occurred more frequently in sibships of C6D index cases than would be expected for co-dominant inheritance. The problem is to assess the significance of the observation. Davie (1979) showed that the 'singles' method of Li & Mantel (1968) could be used under incomplete ascertainment. The method assumes that families are only identified through affected children, that each sib has the same probability of being affected, and that any individual ascertained independently of his siblings is a proband. All these assumptions are true for the present study. The rationale of the method is that, amongst all individuals having a sibling who is a proband, a proportion, $P$, should be affected. The calculated value $\hat{P}$ (see the Results) is an estimate of this value. The value we obtained for $\hat{P}$ was 0·43, which is very much higher than the expected $P=0{\cdot}25$; on the other hand, the large variance means that the significance is borderline. However, the calculation of the variance is only an estimate (Davie, 1979); also the sib method, which is virtually identical to the one we have used, is recognized as giving a low value for $P$ (Emery, 1976). It therefore seems highly probable that the high $\hat{P}$ we obtained is truly different from the expected value. If this is so, an explanation is required. There is no reason to believe that expression of the C6 gene is other than co-dominant (Glass *et al.*, 1978); indeed, the results of C6 allotyping of our own patients were in accordance with this concept (data not shown).

Another factor to consider is the possible biological consequences of C6 deficiency. Various studies, including this one, have failed to demonstrate suceptability to non-Neisserial infections in patients with late-acting complement deficiencies. Therefore, it appears that these components are not essential in host-defence mechanisms against non-Neisserial organisms. Moreover, Ross & Denson (1984) have commented on the low mortality of meningococcal disease in complement-deficient individuals and suggested that perhaps the tissue damage that results from activation of complement by endotoxin is reduced in these people. It is logical to speculate that the consequences of serious infection with other gram-negative organisms may also tend to be less severe. Therefore, for non-Neisserial gram-negative infections, late-acting complement-deficient individuals may have an advantage in that they do not have increased susceptibility to infection while the possible consequences of infection are lessened. The survival factor arising from this advantage may explain the unexpectedly high number of C6D siblings found in the C6D families. If the reported deaths within the C6D families had tended to occur in C6-sufficient siblings, there would be a disproportionate number of C6D siblings in the survivors. Unfortunately it was not possible to get a full history of the deaths in families M1–M3. The better studied families, F1–F7, had two deaths, both in infancy, and one of these was probably due to meningitis. Another point is that serious infections with gram-negative pathogens in infancy and childhood frequently occur in the Cape. Possibly, in an environment such as this, the survival value of homozygous late-acting complement deficiency would be most likely to be manifest.

We have also shown that while certain C6D individuals were subject to repeated meningococcal infections, other affected individuals were apparently untouched. Of 12 non-proband individuals, only four gave a history of what was probably meningococcal infection. Our data are similar to those gathered by Ross & Densen (1984); their data shows that of nine non-propositous C6D individuals, only one (11%) had had meningococcal disease. In addition, we have confirmed that the first attack of meningococcal infection occurs significantly later in C6D individuals than in susceptible normal individuals. This might be, as proposed by Ross & Densen, because normal individuals have a 'window of susceptibility', from about 6–24 months. However, although this would explain the low age of first attack in normal children, it does not fully explain why complement-deficient individuals often only become infected later.

The data in Table 2 do suggest that individuals who have once been infected are more susceptible than C6D individuals who have never been infected. The median age of first attack (which may be regarded as the interval from birth to first attack) was 14 years, while the median interval between first and second attacks was three years. Comparison of the two sets of data shows a highly significant difference ($P<0{\cdot}001$, Mann–Whitney $U$-test). Therefore, if the time interval to subsequent infection can be regarded as an index of susceptability, those who have suffered their first infection are at greater risk than those potentially susceptible individuals who have not yet been infected. It may be that the development of certain antibodies is actually deleterious in persons without a complete complement cascade. IgA antibodies have been shown to 'block' serum bactericidal activity (Griffiss, 1975), and in complement-deficient patients they may also block serum opsonic activity. Work is in progress sto investigate the role of antibodies in the face of complement deficiency.

We attempted to determine the importance of C6D in primary meningococcal infection in our community and found one C6D individual in 123 cases. Unfortunately, Series A was small and the results from Series B may underestimate the true importance of C6D because patients were sampled during an epidemic.

In conclusion, we report here results of a relatively large study of C6D patients in the Cape. Although the patients show the expected increased susceptibility to meningococcal infection, our results also show that index cases had an unexpectedly high proportion of C6D siblings. This raises the possibility that there may be additional beneficial factors associated with complement deficiency.

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