Eur J Pediatr (1999) 158: 210–212
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IMMUNOLOGY/ALLERGOLOGY

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The complement component C4 in sudden infant death

Received: 8 June 1998 / Accepted in revised form: 7 September 1998

Abstract The aim of the present study was to compare partial deletions of the complement C4 gene in victims of totally unexplained sudden infant death (SID) ($n = 89$) and borderline SID ($n = 15$) with and without slight infections prior to death, in cases of infectious death ($n = 19$), and in living infants with and without infections ($n = 84$). The SID and borderline SID groups were pooled. In this total SID group slight infections prior to death was associated with deletion of either the C4A or the C4B gene ($P = 0.033$), and the SID victims with such infections had a higher deletion frequency than the controls ($P = 0.039$). There were no differences between the living infants with and without upper airway infections.

Conclusion The present study confirms that partial deletions of the C4 gene in combination with slight upper airway infections may be a risk factor in sudden infant death.

Key words C4 deficiency · Infections · Sudden infant death

Abbreviation *SID* sudden infant death

Introduction

Several reports describe immune stimulation in sudden infant death (SID) [9, 12, 13]. These observations may indicate that some SID victims are more vulnerable to such stimulation than other infants, making them incapable to cope with a simple infection. Previous studies [8, 11] indicated that one of the reasons for such a vulnerability might be partial deletions of the C4 gene. Thus both Schneider et al. [11] and we [8] have demonstrated that in SID victims slight infections prior to death are associated with partial deletions of either the C4A or the C4B gene. [7]

Complement is a system of interacting serum proteins which plays an important role in defence against microorganisms. The proteins are named from C1 to C9, factor B and D. The C4 gene consist of two loci giving rise to the two isotypes of C4; C4A and C4B [6], and is located on the short arm of chromosome 6 [14]. The C4 gene is closely linked to the steroid-21-hydroxylase gene.

The purpose of the present study was to compare partial deletions of the C4 gene in SID victims with and without slight infections prior to death, in cases of infectious death, and in living infants with and without infections.

Materials and methods

In the present study we investigated 89 cases of SID without explainable cause (median age 3.5 months, range 0.5–18 months), 15 cases of borderline SID (median age 4 months, range 1–36 months), 19 cases of infectious death (median age 5 months, range 0.5–22

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months) and a control group of 84 living infants (median age 4 months, range 0.5–12 months) being sampled for blood for paternity testing. Totally unexplainable SID cases and borderline SID cases were classified according to the pathological criteria for SID in the Nordic countries [3]. All subjects were from the eastern part of Norway.

In the SID group, 45 children had slight symptoms of upper airway infection prior to death while 44 had no such symptoms. In the borderline SID group, the distribution was seven cases with such symptoms and eight cases without. The information about the infants health was, in the SID and borderline SID cases, obtained from the parents, either by the doctor who first examined the infant or by using a questionnaire.

The paternity test infants were examined by the doctor who sampled them and the mothers were asked if the infant may had had colds or other symptoms of infections after birth. All information was made anonymous. In this group of living infants, 22 had symptoms of upper airway infection, either at the time of the blood sampling or earlier, while 62 had no such symptoms or had ever been ill. To be included in the control group with upper airway infection the infant should have had such symptoms more than once after birth.

The group with infectious deaths consist of nine cases of pneumonia, six cases of septicaemia, two cases of general virus infection and two cases of meningitis.

Identification of the C4 gene

DNA from spleen and blood was extracted either in a 340A nucleic Extractor (Applied Biosystem, Rotterdam, The Nederlands) or by hand, both using standard methods (phenol-chloroform extraction and ethanol precipitation) [5]. The DNA was digested to completion with the enzyme Taq I, and separated by electrophoresis in a 0.8% agarose gel (buffer system: 65 mM Tris-HCl, 37.5 mM boric acid, 1.25 mM EDTA, pH 8.8) for approximately 20 h at 48 V, until a 2 kb DNA fragment had migrated 12 cm. The DNA was transferred onto nylon membranes using a vacuum blotting system. The solutions used were denaturation (0.5 M NaOH, 1.5 M NaCl) for 20 min, neutralisation (0.5 M Tris-HCl, 3 M NaCl, pH 7.6) for 20 min and 20 × SSC (3 M NaCl, 0.3 M tri-sodium citrate) for 60 min.

The membranes were hybridised with a probe identifying the 5′ end of the C4 gene and a probe identifying the 5′ end of the steroid-21-hydroxylase gene [15]. The probe identifying the C4 gene was made by PCR. The oligonucleotide primers were 5′-CTCTCT-TGGATCCTCCAGCC and 5′-GCTGAGATCTTCCAGGTCCC. The reaction mixture described by Saiki et al. [10] was modified to 100 ng genomic DNA, 1.5 mM $MgCl_2$, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 0.01% gelatin, 200 pmol of each primer, 0.2 mM of each dNTP and 1 u Taq polymerase. The PCR thermal profile was 94°C for 10 s, 63°C for 45 s and 75°C for 90 s.

The probes were radioactively labelled with [α^{32}]dCTP according to the random labelling method [1]. Prehybridisation (3 h) and hybridisation (overnight) were carried out at 65°C in a solution containing 7% SDS, 1 g/l bovine serum albumin and 0.5 M Na_2HPO_4, pH 7.2. After hybridisation the filters were washed (at 65°C) first for 15 min in a 0.04 M Na_2HPO_4 solution pH 7.2 containing 1% SDS, then for 30 min in a solution containing 75 mM NaCl, 7.5 mM tri-sodium citrate and 0.01% SDS, and finally for 30 min in a solution containing 30 mM NaCl, 3 mM tri-sodium citrate and 0.01% SDS. The radiolabelled filters were exposed to X-ray films for 1–7 days at −70°C.

The C4A gene (22 kb size) was visualised as a 7 kb fragment, the short C4B gene (16 kb size) in combination with deletion of C4A gene as a 6.4 kb fragment, and the two remaining C4B genes (22 kb and 16 kb size) as 6 kb and 5.4 kb fragments respectively. The steroid-21-hydroxylane B gene was 3.7 kb and the steroid-21-hydroxylane A gene was 3.2 kb. Heterozygote deletions were detectable as different strength of the bands. Deletion of a C4 gene was reflected by deletion of an adjacent steroid-21-hydroxylane gene.

Statistical analysis

The chi-squared test was used for comparison between the different distributions of C4 restriction fragments and deletions. When the total number of cases were below 100, the Fisher exact test was used.

Results

There were no differences between the living infants with and without upper airway infections, neither with respect to deletions of the C4A gene ($P = 1.0$) nor the C4B gene ($P = 0.74$).

The distribution of the C4 genes in the totally unexplained SID cases and the borderline SID cases was not significantly different, these two groups were therefore pooled. In this total SID group 16.4% of the chromosomes had deletion of the C4A gene, while 1.9% had deletion of the C4B gene (Table 1). This was not significantly different from neither the infectious death group (C4A: $P = 0.93$, C4B: $P = 0.87$) nor the controls (C4A: $P = 0.22$, C4B: $P = 0.51$). There were no differences between the infectious death group and the controls.

In the SID group, slight infections prior to death was associated with deletion of either the C4A or the C4B gene (Table 2) ($P = 0.033$). The SID victims with such infections also had a significantly higher deletion frequency of the C4 A and the C4B genes than the controls (Table 3) ($P = 0.039$). The four SID cases with C4B deletion were all found in this group (when compared to SID victims without such infections $P = 0.13$, when compared to controls $P = 0.14$). There were no differ-

Table 1 Distribution of the C4 restriction fragments and deletions

Gene	Fragment (TaqI)	SID (%)	Infectious death (%)	Controls (%)
C4A	7.0 kb	174* (83.7)	32 (84.2)	148 (88.1)
	Deletion	34 (16.4)	6 (15.8)	20 (11.9)
		28** (26.9)	6 (31.6)	19 (22.6)
C4B	6.4 kb	34 (16.4)	6 (15.8)	20 (11.9)
	6.0 kb	117 (56.3)	24 (63.2)	92 (54.8)
	5.4 kb	42 (20.2)	7 (18.4)	44 (26.2)
	Deletion	4 (1.9)		1 (0.6)
		4 (3.8)		1 (1.2)
	Duplication	11 (5.3)	1 (2.6)	11 (6.5)
		11 (10.6)	1 (5.3)	11 (13.1)

* Number of chromosomes
** Number of cases

Table 2 C4 gene deletions in SID victims with and without infections prior to death

		Infection		
		Yes	No	*n*
C4 gene deletion	Yes	21	11	32
	No	31	41	72
	n	52	52	

Table 3 C4 gene deletions in SID victims with infections prior to death and controls

		SID	Controls	*n*
C4 gene	Yes	21	20	41
deletion	No	31	64	95
	n	52	84	

ences between the SID cases with infections and the group with infectious death ($P = 1.0$). Furthermore there were no differences between deletions of the C4A or the C4B gene in the SID cases without infections prior to death and the controls ($P = 0.54$) or the infectious death group ($P = 0.89$).

Discussion

The main finding of the present study is that SID victims with slight upper airway infections prior to death more often have deletions of either the C4A or the C4B gene than both the SID victims without such infections and the controls. In the SID group we found the same association between deletions of either the C4A or the C4B gene and upper airway infections prior to death as described in other studies [8, 11]. There were no differences between living infants who had been ill several times from upper airway infection and living infants who had remained healthy, neither with respect to deletion of the C4A nor the C4B gene. This observation indicates that it is only in the SID group that the combination of slight upper airway infection and partial deletions of the C4 gene may be dangerous.

Information about previous and present infections in the control infants in the control group was obtained from the mothers by the doctor who performed the blood sampling. This approach resembles the way information about infections prior to death was obtained in the SID cases. From the information collected there was no reason to believe that the SID infants had been more often or more severely ill than the living control infants with infections.

The two isotypes of C4 have different binding properties. C4A reacts more rapidly with amino groups forming amide bonds while C4B has a preference for hydroxyl groups and ester bond formation [6]. This means that for any infecting bacteria or virus, the composition of the surface may determine whether C4A or C4B is the component best suited. Individuals with complete deficiency of C4 demonstrate delayed complement activation and suboptimal immune response [2], but even a partial deficiency in one of the isotypes may give a situation in which neutralisation of the microorganism is impaired [4]. This is important when dealing with SID, since studies of the immune system in SID have shown immune stimulation in both pheriphal organs [12] and within the CNS [13].

The present study confirms that partial deletions of the C4 gene in combination with slight upper airway infections is a risk factor for SID. However, the study also indicates that there must be one or more additional factors and that coincidence of several factors is necessary to induce the fatal event.

Acknowledgement The study was supported by the Norwegian SIDS Society and Medinnova.

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