ICF syndrome with variable expression in sibs

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Abstract

We describe a new familial case of ICF syndrome (immunodeficiency, centromeric instability, facial anomalies) in a woman of 29 years and in her brother of 30 years. The proband showed mental retardation, facial anomalies, recurrent respiratory infections, combined deficit of IgM and IgE immunoglobulin classes, and paracentromeric heterochromatin instability of chromosomes 1, 9, and 16. The brother had minor signs of the syndrome and had an apparently normal phenotype. Their parents were healthy and non-consanguineous. Chromosome anomalies consisted of homologous and non-homologous associations, chromatid and isochromatid breaks, deletions of whole arms, interchanges in the paracentromeric region, and multibranched configurations of chromosomes 1, 9, and 16. CD bands and fluorescence in situ hybridisation with alphoid DNA sequence probes specific for the centromeres of chromosomes 1 and 16 showed that the centromere was not directly implicated in the formation of multibranched configurations. These cases indicate the autosomal recessive mode of inheritance and the variable expressivity of the ICF syndrome.

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Case report

The proband, a female (fig 1), was the second child born to healthy, non-consanguineous parents. She was born in December 1960 and came to our attention at the age of 29 because of mental retardation and phenotypic malformations. She was the term product of a normal pregnancy. Delivery was by caesarean section because of podalic presentation. The fetal movements were reported to be normal. Developmental milestones were retarded. The child crawled at the age of 12 months, sat up at the age of 14 months, and started to walk with help at the age of 2 years. Initial speech development was delayed but improved with therapy so that by 3 years she was putting two or three words together although her speech was indistinct and poorly formed.

At the age of 6 years she was admitted to a 'special school' and evaluation of her psychomotor development showed an IQ of 90 with a primary lack of language. At the age of 2 years she presented palsy of the right facial nerve. From 4 years of age she suffered recurrent respiratory tract infections and diarrhoea. On clinical examination she had a peculiar face characterised by roundness, hypertelorism with epicanthic folds, an upturned, small nose with a flat nasal bridge, micrognathia, protrusion of the tongue, and macroglossia. Dermatoglyphics were unremarkable.

The older brother of the proband, born in 1959, does not have the phenotypic characteristics of ICF syndrome, but turned out to have the cytogenetic and immunological features.

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A syndrome characterised by combined immunodeficiency, instability of paracentromeric heterochromatin, and facial anomalies was reported by Tiepolo *et al*¹ and Hultén *et al*.² Another eight cases have since been reported by various authors.³⁻⁹ All these patients were ascertained because of recurrent respiratory infections and facial anomalies, and Maraschio *et al*⁶ proposed the acronym ICF (immunodeficiency, centromeric instability, facial anomalies) (McKusick No 242860).

No consanguinity has been reported. Two sibs with ICF syndrome were reported by Fasth *et al*.⁹ In the two families reported by Tiepolo *et al*¹⁰ and Valkova *et al*⁸ a brother of the probands died after recurrent respiratory infections and thus it was suggested that the ICF syndrome could have an autosomal recessive mode of inheritance.

In this paper we report a familial occurrence of ICF syndrome ascertained in a female and her brother, both adult, with variable expressivity in the phenotypic, cytogenetic, and immunological features.

Figure 1 The proband at the age of 29 years.

Table 1 Surface marker analysis of peripheral blood MNC from the patient and her brother.

Surface marker*	Proband (% positive cells)	Brother (% positive cells)
CD2	75	87
CD3	67	79
CD4	23.5	27
CD8	38.1	53
CD20	10.5	3
CD16	13	5
CD56	15	5
CD11b	25	11
HLA-DR	21	8
CD4/CD8	0.61	0.5

* As determined by flow cytometry on cells stained in indirect immunofluorescence.

IMMUNOLOGICAL INVESTIGATIONS

Peripheral blood mononuclear cells (MNC) were isolated on a Ficoll-Hypaque density gradient, washed twice with PBS, and stained with a battery of murine monoclonal antibodies (mabs), as previously reported.¹¹ The anti-T cell mabs OKT3-CD3, OKT4-CD4, OKT8-CD8, and OKT11-CD2 were from Ortho Pharmaceutical Co (Raritan, NJ). The anti-B cell mab B1-CD20 was from Coulter (Hialeah, FL). An anti-HLA-DR mab, OKIa1, was from Ortho. A mab that reacts with Fc receptors for IgG located on granulocytes and natural killer (NK) cells, Leu11b-CD16, was purchased from B-D (Sunnyvale, CA). Another NK cell specific mab, NKH-1-CD56, was from Coulter. A mab that reacts with monocytes-macrophages, NK cells, and a subpopulation of T cells, OKM1-CD11b, was from Ortho. Mouse mabs were detected by indirect immunofluorescence with fluorescein conjugated goat anti-mouse immunoglobulin (Ig) serum (polyvalent) (NEN, Florence, Italy). Cell suspensions stained with fluorescein conjugated antibodies were fixed with 1% paraformaldehyde and analysed by flow cytometry using a FACS analyser (Facs-Star, B-D). Control preparations consisted of unstained cells, cells stained with the labelled second reagent alone, and cells treated with unrelated antibodies of the same isotype as test mabs.

Serum IgG, IgA, and IgD were normal in the proband, her brother, and mother. Serum IgM and IgE were reduced in the proband, in particular IgM 15.5 mg/dl (normal range 77 to 280 mg/dl) and IgE < 2 kU/l (normal range > 9 kU/l). IgM and IgE were also reduced in the brother, in particular IgM 46 mg/dl and IgE < 2 kU/l. IgG subclasses were all normal.

The immunophenotype of the peripheral

blood MNC from the proband and her brother was investigated (table 1). MNC from both the proband and her brother were found to contain a reduced proportion of CD4+ T cells, with a CD4/CD8 ratio of 0.61 and 0.5, respectively; this finding was confirmed in a subsequent test performed after six months (not shown). Normal proportions of T, B, and NK cells with a normal CD4/CD8 ratio were detected in the peripheral blood of the mother.

Quantification of serum Ig at various intervals showed that both the proband and her brother had a selective deficiency of IgM and IgE, with normal concentrations of IgG, IgG subclasses, and IgA. In contrast, normal levels of all Ig classes and IgG subclasses were detected in the mother's serum.

Taken together, these findings indicate that the proband and her brother shared a number of immunological abnormalities related to both cell mediated and humoral responses.

CYTOGENETIC STUDIES

Chromosome analyses were performed on peripheral blood cultures by QFQ, GTG, and CBG banding. The first culture showed associations and interchanges, breaks and multi-branched configurations in the centromeric regions of chromosomes 1, 16, and, to a lesser extent, chromosome 9, suggesting the anomalies found in the ICF syndrome.

Six months later, a second sample of blood from the proband and her brother was analysed and a lymphoblastoid cell line was initiated from EBV transformed lymphocytes of the proband. At the same time fibroblast cultures were set up from a skin biopsy. Lymphocytes, fibroblasts, and lymphoblasts were treated with FUdR, 5-azacytidine, and excess of thymidine.

In lymphocytes we found that the overall frequencies of the chromosome anomalies ranged from 16.4% in untreated cultures to 26.6% in cultures treated with an excess of thymidine in the proband, and from 2.36% to 13.5% in her brother (table 2). No anomalies were detected in the mother and the father refused investigation.

Chromosome abnormalities consisted, principally, of interchanges in the centromeric regions among homologous or non-homologous chromosomes, deletions of whole arms, chromatid and isochromatid breaks in the centromeric regions, and multibranched configurations formed by a variable number of arms of the same or different chromosomes (fig 2).

Chromosome 1 frequently showed stretching of paracentromeric heterochromatin (fig 3A). CD bands showed the presence of an active centromere on the short arm of multibranched chromosomes 1 and 16 (fig 3C,D). The short arm of chromosome 1 and 16 were never duplicated in the multibranched configurations.

In the EBV transformed lymphocytes only two cells of the 100 analysed showed chromosome abnormalities, while skin fibroblasts showed only stretching of the paracentromeric

Table 2 Frequency of cells with abnormalities.

Culture medium	Cells scored (No)	Cells with abnormalities	Stretching only
		No of cells (%)	No of cells (%)
Proband			
Difco TC-chromosome medium	157	26 (16.5)	27 (17.2)
Difco + FUdR	71	12 (16.9)	17 (24.0)
Difco + thymidine	139	37 (26.6)	45 (62.5)
Difco + 5-azacytidine	140	23 (16.4)	11 (15.4)
Brother			
Difco TC-chromosome medium	127	3 (2.4)	18 (14.0)
Difco + FUdR	65	2 (3.1)	10 (15.4)
Difco + thymidine	74	10 (13.5)	37 (50.0)
Difco + 5-azacytidine	52	2 (3.8)	8 (15.4)

Figure 2 Chromosome abnormalities in ICF syndrome. (A) Multibranched configuration of two chromosomes 16 (16ppqqq). (B) Multibranched chromosome 1 (1pqq) and association of two chromosomes 16. (C) Multibranched chromosome 1 (1pqq) with a chromosome 16 associated or interchanged (QFQ banding).

Figure 3 Centromeric heterochromatin in ICF syndrome. (A) Stretching of heterochromatin of a chromosome 1. (B) In situ fluorescence hybridisation of an alphoid DNA sequence (pSD1-1); the signal (arrow) detects the centromere on the short arm of a multibranched chromosome 1 (1pqq). (C,D) CD bands show the presence of an active centromere (arrow) on the short arm of multibranched chromosomes 1 (1pqq) and 16 (16pqq). The large dot present on 16q is an artefact.

heterochromatin of chromosome 1 in the 42% of the cells examined.

FLUORESCENCE IN SITU HYBRIDISATION

Probes pSD1-1 (D1Z5) and pSE16-2 (D16Z2) (Oncor) detect specifically alphoid DNA sequences at the centromere of chromosomes 1 and 16.^{12,13} The probes were labelled by nick translation with biotin-16-dUTP (Boehringer, Mannheim, FRG). In situ hybridisation and fluorescence detection was performed according to Pinkel *et al.*¹⁴

Slides were treated with RNase (100 µg/ml in 2 × SSC) for one hour at 37°C, washed in 2 × SSC, dehydrated in an ethanol series, and air dried. Chromosomal DNA was then denatured in 70% formamide, 2 × SSC at 70°C

for two minutes. Hybridisation mixture contained 65% formamide, 2 × SSC, 10% dextran sulphate, sonicated salmon sperm DNA (100 µg/ml), and 15 ng of probe per slide. Hybridisation mixture was denatured at 70°C for five minutes and applied to prewarmed slides (37°C), sealed with a coverslip, and incubated in a humidified box at 37°C for 12 to 16 hours. Coverslips were removed and the slides washed in 65% formamide in 2 × SSC at 43°C for 20 minutes.

Detection of the biotinylated probes was achieved by incubation with fluorescein isothiocyanate (FITC) labelled avidin (5 µg/ml). The fluorescent signals were amplified by subsequent incubation with biotin linked anti-avidin antibody followed by fluorescein conjugated avidin. Chromosome preparations were counterstained with propidium iodide and DAPI, mounted in an antifade medium, and observed under an epifluorescence microscope. The hybridisation signal was always present, as a unique spot, in the centromeric region of chromosomes 1 (fig 3B) and 16. The duplicated long arms show no hybridisation signal of the alphoid DNA probes.

Discussion

Autosomal recessive inheritance of the ICF syndrome has been postulated because parents are normal, it is observed in both sexes, and two sibs in two published families died of recurrent respiratory infections. Recently two sibs with ICF syndrome belonging to a heavily inbred family were described by Fasth *et al.*⁹

In previous reports, patients were referred to clinicians for recurrent infections, facial anomalies, and mental retardation. Most of them were observed early in their life and some died in childhood.

Our proband was observed for the first time at 29 and her brother at 30 years of age. Unlike her brother, the proband suffered recurrent infections particularly viral.

Mental retardation and facial anomalies were present in the proband but not in her brother and chromosomal anomalies were more frequent in the proband than in her brother. Thus, it can be postulated that there is some correlation between the frequency of chromosomal anomalies, different levels of immunoglobulins, and phenotypic manifestations of the ICF syndrome.

Immunological studies showed that the proband and her brother had reduced proportions of circulating CD4+ T cells, with an inverted CD4/CD8 ratio, and decreased serum levels of IGM and IGE; these abnormalities were consistently detected on different occasions. In contrast, no evidence for T cell subset or Ig imbalances could be found in the mother. An inverted CD4/CD8 ratio may be found in certain lymphoproliferative diseases (Hodgkin's disease, T gamma lymphoproliferative disease), in transplanted patients receiving immunosuppressive treatment, and during the course of various viral infections, particularly the acquired immunodeficiency syndrome (AIDS). Our probands had no clinical or

laboratory evidence for any of the above diseases. It is tempting to speculate that the chromosomal rearrangements typical of the ICF syndrome affect predominantly or exclusively the CD4⁺ T cell subset, thus leading to a selective depletion of this lymphocyte subpopulation and, eventually, to an increased risk of infections caused by viruses and other intracellular pathogens. This issue is now being investigated in our laboratories.

Hypogammaglobulinaemia appears to represent a common feature of the ICF syndrome. The published cases indicate that hypogammaglobulinaemia may affect the three main classes (IgG, IgM, and IgA) or only some of them; furthermore, an IgE deficit is frequently observed. Both our proband and her apparently healthy brother had a reduced serum level of IgM and IgE.

In conclusion, the results of immunological studies suggest that abnormalities of both cell mediated and humoral responses may be involved in the pathogenesis of the immunodeficiency occurring in the course of the ICF syndrome. The reasons why the proband, but not her brother, had a lifelong history of respiratory infections can be attributed to the lower levels of immunoglobulins than her brother and thus to a different expression of the ICF syndrome. The molecular mechanisms underlying the immunodeficiency are so far obscure.

In our first report of a case of ICF syndrome we pointed out that chromosomal anomalies could be correlated with the presence of a fragile site in the centromeric heterochromatin that causes cross links during DNA replication or interchanges preventing the normal segregation of the chromatids.¹⁰ The more complex figures involving two or more of chromosomes 1, 9, and 16 could be explained by the centromeric regions of these chromosomes being characterised by the preferential location of satellite II DNA and being likely to be associated in interphase.

Fryns *et al.*³ pointed out that centric fission of chromosomes 1, 9, and 16 leads to the formation of functional centromeres of the short or long arms of these chromosomes and through successive duplications they can give rise to the observed multibranched configurations. In situ fluorescence hybridisation and CD bands showed that the centromeres of chromosomes 1 and 16, involved in multibranched configurations, were not split in two or more parts, as hypothesised by Fryns *et al.*³ In fact, a single centromere was always present on the short arm and absent on the long arm of chromosomes 1 and 16, as shown in fig 3B-D. Furthermore, in the present cases and in that

reported previously,¹⁰ only the long arms of chromosomes 1, 9, and 16, where the centromere was absent, were duplicated in the multibranched configurations.

These observations allow us to postulate that the centromeres are not directly involved in the origin of the multibranched chromosomes, but that paracentromeric heterochromatin of chromosomes 1, 9, and 16 was involved. An autosomal recessive mutation of a gene(s) that controls the normal process of condensation of part of the centromeric heterochromatin could be the cause of the chromosomal anomalies found in this syndrome, as postulated by Maraschio *et al.*¹⁵

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