

Insulin dependent diabetes mellitus (IDDM) and autoimmune thyroiditis in a boy with a ring chromosome 18: additional evidence of autoimmunity or IDDM gene(s) on chromosome 18

C Dacou-Voutetakis, A Sertedaki, M Maniatis-Christidis, C Sarri, G Karadima, M B Petersen, A Xaidara, M Kanariou, P Nicolaidou

Abstract

A 4 year 3 month old boy with insulin dependent diabetes mellitus (IDDM), autoimmune thyroiditis, slight mental retardation, facial dysmorphism, and a de novo ring chromosome 18 (deletion 18q22.3-18qter) is described. This unique association of defects could represent a chance association. Alternatively, the clinical features could be the result of the chromosomal aberration. If so, one could speculate that a gene or genes on chromosome 18 might act as a suppressor or activator of the autoimmune process by itself or in concert with other IDDM loci.

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The association of chromosomal aberrations with distinct phenotypic expression may have important implications for the localisation of genes responsible for certain types of morphogenesis and malfunction. Thus, we report the concurrence of a ring chromosome 18 with IDDM and hypothyroidism (two autoimmune disorders) in a young boy.

Case report

A boy, aged 4 years 3 months, was admitted to our clinic because 15 days previously he had shown polydipsia, polyuria, increased food intake, and fatigue. On physical examination the following were detected: his height was 100 cm (10th centile), weight was 15.5 kg (10th centile), and head circumference 48.9 cm (3rd centile). There was a depressed nasal bridge, hypertelorism, and high arched palate. The thyroid gland was moderately enlarged. A mild systolic murmur was audible over the praecordium. Evaluation by a cardiologist disclosed a small intraseptal defect. There was no evidence of hepatosplenomegaly. Examination of the external genitalia showed hypospadias. The testes were descended and measured 1.5 cm³. The general condition was good with 5% dehydration. The laboratory findings on admission were: K 4.4 mEq/l, Na 130 mEq/l, Ca 9.2 mEq/l, pH 7.18, haematocrit 38.2%. Ketonuria and glycosuria were detected and the blood glucose level was 580 mg/dl. The boy

was managed appropriately with fluids, electrolytes, and insulin and he was discharged home on diet and insulin injections with the diagnosis of IDDM.

PAST AND FAMILY HISTORY

He was born after an uneventful term pregnancy to a 24 year old mother, para 1. His father was 37 years old. The delivery was normal with cephalic presentation and the birth weight was 3150 g. He cried immediately and had no neonatal problems. He was late to achieve developmental milestones. At 17 months he had not walked and was admitted to our clinic for evaluation. Abdominal sonography showed that the urinary tract and other abdominal organs were normal, but lithiasis of the gall bladder was present. The blood amino acids were normal and antibodies against rubella, herpes simplex virus, and CMV, as well as the Dye test, were negative. The parents were normal and non-consanguineous. A younger brother, aged 5 months, was also normal.

SPECIAL STUDIES

The total triiodothyronine (T3) value was 91 ng/dl (normal 80-220), the total thyroxine value was 6.6 µg/dl (normal 5.5-12.5), and the thyrotrophin (TSH) value was 19 µU/ml (normal <5). The plasma cortisol was 23 µg/dl (normal 5-25) and ACTH 17 pg/ml (normal <50). Antithyroid antibodies were positive. More specifically the antithyroglobulin antibodies were positive 1:80 and the microsomal 1:1600. Antipancreatic antibodies (islet cell antigen, ICA) were also positive. The HLA antigens were A2, A26 (10)/B35B38, DR3, DR4. Two months following his discharge, the total thyroxine value was 5 µg/dl and the TSH greater than 100 µU/ml. The thyroid gland was further enlarged. Hypothyroidism caused by Hashimoto thyroiditis was diagnosed and he was started on thyroxine substitution therapy. One month later the TSH value was 0.3 µU/ml and the total T4 12.5 µg/dl. His blood glucose has been fairly well controlled on twice daily insulin injections. His developmental evaluation placed him at the level of 18 months.

INSULIN GENE AND HLA GENOTYPING

Genomic DNA was extracted from blood lymphocytes using standard methods. No perma-

National and Kapodistrian University of Athens School of Medicine, First Department of Paediatrics, Aghia Sophia Children's Hospital, Goudi 115 27, Athens, Greece
C Dacou-Voutetakis
A Sertedaki
M Maniatis-Christidis
A Xaidara
M Kanariou
P Nicolaidou

Department of Genetics, Institute of Child Health, Aghia Sophia Children's Hospital, Athens, Greece
C Sarri
G Karadima
M B Petersen

Correspondence to:
Dr Dacou-Voutetakis.

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nent cell line is available from this patient. The second exon of the HLA DQB1 gene was PCR amplified using the primers GH29 and DB130 and dot blotted onto nylon membranes which were hybridised with horseradish peroxidase labelled allele specific oligonucleotide probes as described previously.¹ Our patient was shown to be a homozygote for the DQB1*0201 allele (father: DQB1*0201/*0201, mother: DQB1*0201/*0303).

For the insulin gene 5' VNTR typing, genomic DNA was digested with the restriction enzyme *PvuII*, electrophoresed, Southern blotted, and hybridised with the probe phins310 containing the 5' insulin polymorphic region² (supplied by the UK HGMP Resource Centre). Our patient was shown to be a heterozygote for the class I alleles.

CYTOGENETIC STUDIES

Chromosome analysis was performed on standard PHA stimulated blood cultures. GTG banding was applied to air dried slides. The karyotype, 46,XY,r(18), showed the presence of a ring chromosome. Interpretation of the banding pattern showed possible breakpoints in p11.2 and q23 or p11.32 and q22.2 respectively (fig 1). A total of 165 cells in mitosis were analysed for the presence of the ring while 15 were fully analysed. They were all diploid and the ring was generally stable. Ninety-four percent of the analysed cells contained one closed ring, 2% contained one double ring, and 4% had 45 chromosomes with only one normal chromosome 18, while the ring 18 was absent. No normal cells were found. The karyotypes of the parents were normal.

MICROSATELLITE DNA ANALYSIS

For molecular analysis of the deleted region of the ring chromosome, we used microsatellite DNA polymorphisms from human chromosome 18 (table 1). Primers flanking microsatellites were as published elsewhere.³ Polymerase chain reaction (PCR) amplification of genomic DNA with end labelling of primer, polyacrylamide gel electrophoresis of the amplification products, and autoradiography were performed according to a previously published protocol.⁴ The order of markers was known from linkage analysis³ and their appropriate

Table 1 Microsatellite DNA analysis in a boy with ring chromosome 18

Locus	Genotypes		
	Proband	Father	Mother
D18S54	2/22	22	12
D18S63	34	13	24
D18S62	23	24	13
D18S53	23	13	23
D18S67	1/11	11	11
D18S64	1/11	11	11
D18S61	2/22	23	12
D18S58	3	14	23
D18S54	2	13	24
D18S70	3	12	23

The loci on chromosome 18 are ordered corresponding to their relative order based on linkage analysis.³ The numbers in the genotype columns represent the different alleles at a specific locus.

physical location was based on somatic cell hybrid mapping.⁵ The three distal microsatellites on 18q showed non-inheritance of the paternal allele (table 1), thus indicating a paternal deletion of 18q22.3-18qter. Non-deleted markers showed biparental inheritance of the two chromosomes 18. The most distal microsatellite on 18p (D18S54) was not informative for deletion.

FLUORESCENCE IN SITU HYBRIDISATION STUDIES

Chromosome painting was carried out to rule out the presence of a translocation of the deleted part of chromosome 18. FISH was carried out using commercially available biotin-dUTP labelled probes according to the manufacturer's instructions. Whole chromosome paint 18 (Oncor) was used. The biotin labelled probe was detected with FITC (fluorescein isothiocyanate) conjugated avidin (Vector Lab) and amplified by two rounds of biotinylated anti-avidin antibodies (Vector Lab). The chromosomes were counterstained with DAPI and propidium iodide. Preparations were mounted in antifading agent (Perma Fluor, Immunon) and observed under a fluorescence microscope (filter combination Zeiss 4302295). Fluorescence was detected only on the chromosome 18 homologues, showing that the deleted region has not been translocated to another chromosome. FISH analysis was also performed using the all human telomeres digoxigenin labelled probe (Oncor) for the detection of 18p and 18q telomeres. The method used was that recommended by the manufacturer but with some slight alterations. Telomeres were absent from the r(18) chromosome.

Discussion

Our patient, a 4 year old boy with a ring chromosome 18 and deletion of 18q22.3-18qter, has two autoimmune disorders, namely IDDM and autoimmune thyroiditis. This combination of autoimmune disorders is quite unusual in this age group and the events described could represent a chance association. Nevertheless, pertinent published data, which are briefly outlined below, point to a possible link between the chromosomal aberration and the autoimmune endocrinopathies.

Endocrine disorders have been reported, although rarely, in subjects with 18q-syndrome.⁶⁻⁸ These include hypothyroidism,

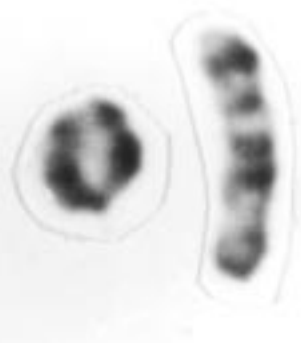


Figure 1 Partial karyotype of the proband with GTG banding.

hypoparathyroidism, and association of hypothyroidism and IDDM. More specifically, a 21 year old female with hypothyroidism, certain distinct anatomical features, IgA deficiency, and a deletion in the long arm of chromosome 18 has been reported.⁶ A 10 year old female with "congenital hypothyroidism" who had a de novo ring chromosome 18 has been reported.⁷ No TSH values were mentioned in the above two reports. The report of Henrot *et al*⁸ described an adolescent girl with primary hypothyroidism from the age of 13 years, who also had antiparietal and antimicrosomal antibodies. This patient also developed pernicious anaemia at the age of 16. She had a deletion of the long arm of chromosome 18 (46,XX,del(18)(q21.3)). The report of Gordon *et al*⁹ is quite distinct. The authors described a 36 year old female with mental retardation, dystonia, hypothyroidism, diabetes mellitus, and a q22.2 deletion of chromosome 18. It is not clear in this report what type of diabetes mellitus the patient had. The antinuclear antibodies were positive 1:320. No antipancreatic or GAD antibodies were detected.

A genome wide search for human IDDM susceptibility genes disclosed that the microsatellite locus D18S64, which has shown evidence of linkage to IDDM, lies in the same region of chromosome 18q12-q21 as the Kidd blood cell surface antigen (Jk) locus.¹⁰ More recently it was shown by allelic association dependent methods that IDDM6 resides on chromosome 18q21.¹¹

The phenotypic map of the 18q- syndrome, associated with deletions of the distal part of the long arm from 18q21.3 or 18q22.2 to 18qter, did not include diabetes mellitus.¹² This could indicate that a presumed diabetes susceptibility locus is not located in that region or that this locus is a minor IDDM locus which acts in tandem with the primary or secondary loci for IDDM susceptibility, namely IDDM1 and IDDM2. Our patient possesses the HLA DR3 and DR4 antigens. Furthermore, he is homozygous for the HLA DQB1 *0201 allele and heterozygous for the class I allele of the insulin gene 5' VNTR (IDDM1 and IDDM2 loci, respectively). It is known that subjects carrying these alleles exhibit increased susceptibility to IDDM. The incidence of IDDM in Greece for the age group 0-4.9 is 2.8/100 000/year.¹³ The relative risk for IDDM in the Greek population is 14 for the presence of HLA DQB1*0201 allele and 1.13 for the class I allele of the insulin gene 5' VNTR (A Sertedaki, C Dacou-Voutetakis, unpublished results). Additionally, the presence of HLA DR3/DR4 confers a relative risk of 8.4.¹⁴ Hence the presence of genes that confer susceptibility to diabetes mellitus in our patient constitutes strong evidence that the IDDM in

this boy could be connected to the IDDM1 and IDDM2 loci rather than to a presumed gene on chromosome 18. However, IDDM and autoimmune thyroiditis are rarely encountered in children younger than 5 years old. Hence, the concurrence of chromosome 18 deletion with two autoimmune disorders in a boy of such a young age constitutes evidence, albeit weak at this point, of a cause-effect relationship between the chromosome deletion and the autoimmune disorders. If this holds true, the mechanism of this association is quite difficult to explain. It has been widely accepted that IDDM is a multifactorial and polygenic disorder with a strong autoimmune basis. The strongest loci so far connected with IDDM are IDDM1 and IDDM2. One may speculate that a gene(s) on chromosome 18 also affects the autoimmune process. Deletion of this gene(s) could lead by itself or in concert with other loci to autoimmune disorders in general or IDDM in particular. The gathering of analogous clinical prototypes and further molecular studies of such cases might disclose the genes responsible, if any, on chromosome 18 and improve our understanding of susceptibility to autoimmune disorders in general and IDDM in particular.

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