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The molecular basis of the Kidd blood group polymorphism and its lack of association with type 1 diabetes susceptibility

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The Kidd blood group locus encodes a urea transporter which is expressed on human red cells and in the kidney. This gene is located on chromosome 18q12, and evidence for linkage and association with type 1 diabetes mellitus has been reported. To investigate this further, the genetic basis for the blood group Jk^a/Jk^b polymorphism was first determined by sequencing reverse-transcribed reticulocyte RNAs from Jk(a+b–) and Jk(a–b+) donors. The Jk^a/Jk^b polymorphism was caused by a transition (G838A), resulting in a Asp280Asn amino acid substitution and an *MnlI* restriction fragment length polymorphism (RFLP). Using the *MnlI* RFLP, we found that the Jk^a/Jk^b polymorphism was not in linkage disequilibrium with type 1 diabetes in 228 multiplex UK and US families tested.

INTRODUCTION

The Kidd blood group system (Jk) is defined by two alleles, Jk^a and Jk^b (frequency 0.51 and 0.48 in Europeans), whose products were first identified with alloantibodies responsible for haemolytic disease of the newborn or transfusion reactions. There are three common phenotypes Jk(a+b–), Jk(a–b+) and Jk(a+b+) and a rare null phenotype, Jk(a–b–) (1).

Since red cells from Jk(a–b–) individuals which lack Jk antigens exhibited an increased resistance to lysis in aqueous 2 M urea (2) and showed a defect in urea transport (3), it was suggested that both phenotypes could be carried by a single polypeptide. This hypothesis was fully confirmed by the molecular cloning of the urea transporter expressed in human erythrocytes (clone HUT11) (4,5). The gene encoding the Kidd/HUT11 urea transporter polypeptide has been assigned to chromosome 18q12–q21 by *in situ* hybridization (5), where the Kidd blood group gene locus has been mapped (6). The Kidd/urea transport protein is present on human red cells as well as in the kidney, particularly on the endothelial cells of the vasa recta in the inner and outer medulla, but is not present in renal tubules (7).

Type 1, or insulin-dependent, diabetes mellitus is a childhood disease affecting 0.4% of children of Caucasian descent. Disease, caused by autoimmune destruction of insulin-producing pancreatic β-islet cells, results from a combination of genetic and environmental factors. The major histocompatibility complex (MHC) locus on chromosome 6p21 is a primary genetic determinant (*IDDM1*) (8). Disease susceptibility is also conferred by the variable number of tandem repeats (VNTR) polymorphism immediately upstream of the insulin gene on chromosome 11p15 (*IDDM2*) (9). Recent genome scanning and other candidate gene studies have provided evidence for the existence of at least five further loci (*IDDM4*, *IDDM5*, *IDDM6*, *IDDM8* and *IDDM12*) that contribute to type 1 diabetes susceptibility (10–13).

Historically, the Kidd blood group locus has been implicated in susceptibility to type 1 diabetes. In 1982, a genome screen by Hodge *et al.* (14) with 27 polymorphic markers revealed linkage of the Kidd blood group locus, in addition to the MHC region, with type 1 diabetes in a US data set of 71 families (parametric lod score = 2.8 under a recessive model of inheritance). Despite a report of linkage disequilibrium of the Kidd blood group Jk^b allele with type 1 diabetes (15), evidence supporting linkage of Jk with disease was not extended when the US data set of Hodge and co-workers was extended to 100 families (16), nor was linkage replicated in a separate data set from the US (17). However, in 1994, evidence of linkage of the chromosome 18q21 region with type 1 diabetes was obtained in 93 UK affected sib pair families (18).

Therefore, in this report, we determine the molecular basis of the historical *Jk* polymorphism and directly test its association with type 1 diabetes by designing a PCR assay for the polymorphism and by analysing a large number of diabetic families.

RESULTS

Cloning and sequencing of Jk alleles

Reticulocyte RNAs from Jk(a+b–) or Jk(a–b+) blood samples were used as templates to amplify by hemi-nested PCR the entire Jk^a and Jk^b coding sequences, as shown in Figure 1A. Sequence analysis of the 1234 bp product from each allele revealed that the

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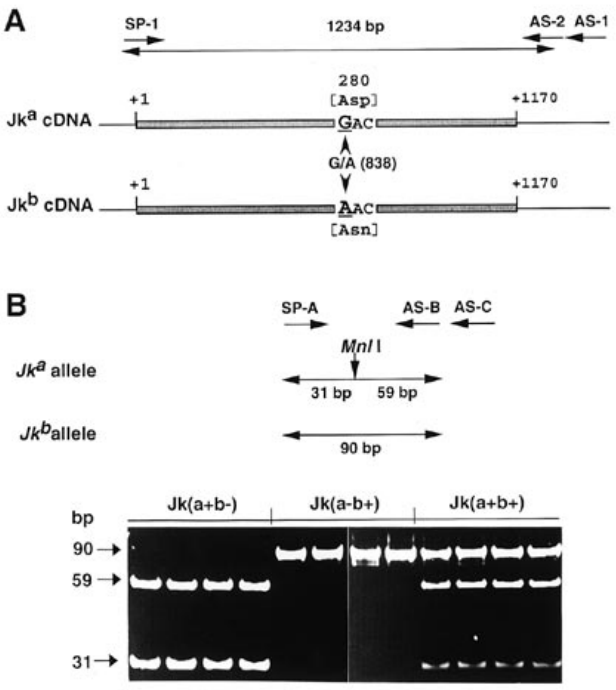

Figure 1. Characterization of Jk^a and Jk^b alleles. (**A**) Schematic representation of the Jk^a and Jk^b coding sequences (solid bar) amplified from reverse-transcribed reticulocyte RNA of Jk(a+b–) and Jk(a–b+) donors, respectively, by hemi-nested PCR using primers SP-1, AS-2 and AS-1. The Jk^a and Jk^b alleles differ by the single base substitution G838A (arrowhead) changing Asp to Asn at position 280 in the polypeptide. nt +1 is taken as the first nucleotide of the initiation codon of the Jk^a/Jk^b alleles (HUT clones deleted of one set of Val–Gly codons). (**B**) Jk^a/Jk^b DNA genotyping by PCR-RFLP. The 90 bp fragment encompassing the polymorphic nt 838 (see A) was amplified by hemi-nested PCR, using the SP-A, AS-B and AS-C primers, from four unrelated individuals of each of the three common phenotypes [Jk(a+b–), Jk(a–b+) and Jk(a+b+)] and digested with *Mnl*I. The digested fragments were separated on a 15% polyacrylamide gel and stained with ethidium bromide. Size of fragments (bp) are given on the left.

Jk^a and Jk^b cDNAs differed at only one position by a transition G838A, resulting in the amino acid substitution Asp280Asn (Fig. 1A), located in the fourth predicted extracellular loop of the human urea transporter (4). Sequence comparison of the HUT11 clone encoding the human urea transporter isolated previously(4) and *Jk* alleles revealed two further differences which were not related to the Jk polymorphism since they are shared by both the Jk^a and Jk^b alleles. One change occurred in codon 44 (transition A→G resulting in a Lys44Glu substitution) and a second corresponded to a hexanucleotide deletion that did not modify the reading frames and resulted in the absence of the dipeptide Val–Gly at amino acid positions 225 and 226. Allele-specific PCR and PCR-restriction fragment length polymorphism (PCR-RFLP) analysis confirmed that these differences were not allelic to the *Jk* locus (data not shown). As the G838A substitution was correlated with the presence or absence of an *Mnl*I restriction site, a hemi-nested PCR-RFLP assay for DNA genotyping of *Jk* was developed (Fig. 1B).

Testing *Jk* for contribution to type 1 diabetes susceptibility

The transmission disequilibrium test (TDT) (19) was used to test whether the *Mnl*I RFLP, which determines the serological specificity of the Kidd Jk^a/Jk^b polymorphism, also encodes susceptibility to type 1 diabetes mellitus. This test for linkage disequilibrium is family-based so it distinguishes between association due to linkage and association that may arise in the absence of linkage, such as that owing to population stratification. The 228 UK and US families were genotyped with the *Mnl*I RFLP. The frequency of the Jk^b allele, which previously had been reported to be associated with type 1 diabetes, was 0.45 in parents. Transmission of this allele from heterozygous parents to affected children was assessed (Table 1). There was no significant bias in transmission of the Jk^b allele to diabetic offspring when the data sets were analysed separately or together. Previously, association of the Kidd blood group locus with type 1 diabetes was observed only when the data set was partitioned according to genotype at *IDDM1*—only those type 1 diabetics with zero or one high risk HLA class II alleles (these genotypes are DR 3/X, 4/X and X/X, where DRX is not DR3 or DR4) showed association of the Jk^b allele with disease [relative risk = 2.5, P <0.025 (15)]. In this study, there was no significant bias in transmission of the Jk^b allele to diabetics with zero or one high risk *IDDM1* alleles (Table 1).

Linkage of the *Mnl*I RFLP to diabetes was also tested by the affected sib pair method. In the UK data set, the maximum lod score (MLS) was 0.0, the MLS was 0.1 in the US and was 0.0 in the total 225 families. Thus there was no evidence supporting linkage of the Jk^a/Jk^b polymorphism to type 1 diabetes in this data set.

Table 1. Testing for linkage disequilibrium (LD) of the Jk^a/Jk^b polymorphism with type 1 diabetes in UK and US family data sets

Data set	Unstratified			*IDDM1*—0,1 high risk alleles		
	T	NT	*P* value	T	NT	*P* value
UK	106	96	ns	39	41	ns
US	120	133	ns	49	51	ns
Total	226	229	ns	88	92	ns

Testing for linkage disequilibrium was done (see Materials and Methods) by the transmission disequilibrium test (TDT) (19). Transmissions (T) and non-transmissions (NT) of the Jk^b allele from heterozygous parents are shown to all affected offspring (unstratified), and affected offspring with zero or one of the alleles that confer a high relative risk for type 1 diabetes development at *IDDM1* (24) (type 1 diabetics with HLA-DR3/X, -4/X or X/X where X is not 3 or 4). ns = not significant.

DISCUSSION

This study provides two results. Firstly, the historical Jk^a/Jk^b polymorphism is due to an Asp280Asn amino acid substitution in the fourth extracellular loop of the red blood cell urea transporter. The *Mnl*I PCR-RFLP assay could be applied in the diagnosis of the severe haemolytic disease of newborns that is caused by antibodies to the Jk antigen.

Secondly, we have shown that the Jk^b allele, previously associated with type 1 diabetes, does not show either linkage or linkage disequilibrium with disease in 228 families. Recently we have provided evidence for linkage disequilibrium of the chromosome 18q12 region to type 1 diabetes, but to a locus 13 cM telomeric of the *Jk* locus (12). Using linkage disequilibrium mapping, the type 1 diabetes susceptibility locus *IDDM6* has been located near the *D18S487* marker. The 13 cM interval between *IDDM6* and the *Jk* locus is compatible with linkage of the *Jk* locus to type 1 diabetes observed previously (14), since regions of

linkage to polygenes can be broad, encompassing 20 cM (10,11). The results presented here indicate that the *Jk* locus is not *IDDM6*.

Nevertheless, more polymorphic markers in and around *Jk* would have to be analysed in multiple different ethnic groups before it could be firmly concluded that the *Jk* locus is not involved in type 1 diabetes. It is likely that many common variants in multiple genes will contribute to autoimmune diabetes, and the frequencies of these variants will in some cases vary considerably in different populations.

MATERIALS AND METHODS

Blood samples and reagents

Blood samples were collected on EDTA, and the Jk^a/Jk^b typing was determined by Antigen-Profil DiaMed-ID Micro Typing System (Diamed SA. Morat, Switzerland).

Diabetic families

All families used in this study were Caucasian with two affected siblings per family and both parents included. The 95 families recruited from the UK were restricted to those with grandparents born in the UK, and were affected sib pair families in which at least one sibling was diagnosed with IDDM under age 17 years and the other under age 29 years (20). The 133 US families were selected according to the same age-of-onset criterion and were from the Human Biological Data Interchange (HBDI) repository of type 1 diabetic families (21).

Reverse transcription-polymerase chain reaction

Total reticulocyte RNA (5 µg) extracted by the acid–phenol–ammonium method (22), was used to produce first cDNA strands using the commercial kit from Pharmacia (Uppsala, Sweden). The coding region of the Kidd/urea transporter (clone HUT11) was enzymatically amplified using one-sixth of the cDNA products, by a hemi-nested PCR carried out first between primers SP-1 (sense, nt –9 to + 15) and AS-1 (antisense, nt 1266–1243), as follows: 30 cycles of 30 s at 94°C, 30 s at 60°C and 1.5 min at 72°C. The second PCR was performed with 1/25 of the first PCR products in the same conditions, using primers SP-1 and AS-2 (antisense, nt 1231–1205). Final PCR products were subcloned into the *Eco*RV-digested pT7TS vector (provided by P. Krieg, Austin, TX). Nucleotide sequences of three independent clones from each phenotype were determined on both strands by the dideoxy chain termination method (Sanger) with the Sequenase kit version 2.0 (US Biochemical Corp., Cleveland, OH). For primer designation, nt +1 was taken as the first nucleotide of the initiation codon of HUT11 cDNA clone (4).

Restriction analysis

Determination of the Jk^a/Jk^b polymorphism by PCR-RFLP was carried out by a hemi-nested PCR reaction [30 cycles of 30 s at 94°C, 30 s at 66°C and 10 s at 72°C in the optimized buffer Tris-HCl 300 mM, $(NH_4)_2S0_4$ 75 mM, $MgCl_2$ 1.5 mM, pH 8.5], using 40 ng of leukocyte DNA. The first amplification was performed between primers SP-A (sense, 5′-tcctgtcttaacagGACT-CAGTC-3′, exonic nt 818–826) and AS-B (antisense, nt 952–933) and the second PCR between the primers SP-A and AS-C (antisense, nt 893–870), except that annealing was done at 55°C. For routine genotyping, the 90 bp PCR products were digested with 5 U of *Mnl*I restriction enzyme, and electrophoresed on a 4% (w/v) agarose gel stained with ethidium bromide.

Statistical analyses

Maximum lod scores were calculated as described by Risch (23). Transmission disequilibrium testing was performed as described previously (19)—the test statistic has a χ^2 (1df) distribution.

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