Brief Genetics Report

Transmission of DQ Haplotypes to Patients With Type 1 Diabetes

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Autoimmune type 1 diabetes is a multifactorial autoimmune disorder characterized by destruction of insulin-producing β-cells in pancreatic islets (1). The major histocompatibility complex on chromosome 6 has been shown to contain one or more major genetic determinants of disease susceptibility (2). At least 40% of the familial aggregation of type 1 diabetes is accounted for by HLA genes, in particular, the HLA class II genes DQ and DR. In Caucasians, high-risk class II molecules include DQA1*0501-DQB1*0201 (associated with DR3) and DQA1*0301-DQB1*0302 (associated with DR4). Dominant protection is apparently conferred by the DQ molecule DQA1*0102-DQB1*0602, which is carried on DR2 haplotypes (3).

The presence or absence of aspartic acid (Asp) at position 57 of the DQ β-chain has been reported to play a key role in determining disease protection or susceptibility, respectively (4,5). However, the amino acid at DQβ position 57 alone is insufficient to fully explain the effect of HLA DQ. For example, the DQB1*0201 allele, which lacks Asp at position 57, is associated with susceptibility on DR3 haplotypes, but is neutral on DR7 haplotypes. Furthermore, Asp at position 57 of the DQ β-chain is not protective in the Japanese population, where DQB1*0401 and DQB1*0303 are the highest-risk DQ alleles (6). Several studies have led to the proposal that *cis* or *trans* encoded DQ molecules consisting of a DQ α-chain with arginine (Arg) at position 52 and a non-Asp DQ β-chain were most strongly associated with type 1 diabetes susceptibility (7,8).

The power to detect a high-risk, but uncommon, allele from patients and control subjects is significantly influenced by allele frequencies within the study population (9). The Human Biological Data Interchange (HBDI) type 1 diabetes repository has made it possible to minimize this problem by analyzing transmission of DQ haplotypes to affected and unaffected offspring from 574 parents from 289 families. Most of the families were ascertained on two affected siblings, except for 13 families (4.5%) that only had one affected child per family with diabetes. Both parents in 276 families were unaffected, while 8 families had an affected father and 5 had an affected mother. Among the 574 parents, 83 were homozygous for DQ haplotypes (34 with DQA1*03-DQB1*0302, 34 with DQA1*0501-DQB1*02, and 15 with other haplotypes) that prohibited the transmission test from being performed. Parents with the heterozygous genotype 03/0302, 0501/02 were also excluded (n = 116). HLA-DQA1 and DQB1 genotypes were determined on samples from both of the parents, affected children (n = 595), and unaffected children (n = 269) in all families with polymerase chain reaction sequence-specific oligonucleotide probes (10,11).

The transmission disequilibrium test (TDT) was used to assess the transmission of DQ haplotypes from parents to children with type 1 diabetes and their siblings (12). The TDT considers parents who are heterozygous for a particular DQ haplotype and evaluates the frequency with which that haplotype is transmitted to affected offspring; it also helps distinguish between association due to linkage and spurious associations that may be caused by population stratification. Under the null hypothesis of no-linkage, a haplotype is expected to be transmitted 50% of the time from a heterozygous parent to an offspring, regardless of diabetes status. For a haplotype that had a statistically significant transmission frequency among affected subjects, the direction of the risk can be observed. The TDT compares the observed transmission frequency against the expected 50%. A significant TDT result could be due to meiotic segregation distortion. If so, both affected and unaffected offspring would have this distortion. To rule out this possibility, the haplotype transmission frequencies to affected and unaffected offspring are compared by a 2×2 contingency χ^2 test. A P value <0.05 was considered statistically significant.

As expected, the strongly susceptible haplotypes DQA1*03-DQB1*0302 and DQA1*0501-DQB1*02 were transmitted to >80% of affected children from heterozygous parents with either DQA1*0501-DQB1*02 or DQA1*03-DQB1*0302 (87 and 82%, respectively) (Table 1). Parents heterozygous for DQA1*0501-DQB1*0201 and DQA1*0301-DQB1*0302 transmitted either haplotype ~50% of the time to affected offspring (DQA1*0501-DQB1*0201 41/81 vs. DQA1*0301-DQB1*0302 40/81). To analyze the relative contribution of the other haplotypes to the risk of diabetes, the transmission of a series of DQA1-DQB1 haplotypes

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HBDI, Human Biological Data Interchange; TDT, transmission disequilibrium test.

TABLE 1
Transmission frequencies of DQ haplotypes to affected and unaffected offspring from heterozygous parents

DQ haplotype in parent (DQA1/ DQB1)	Transmission frequency		Amino acid position	
	To affected offspring	To unaffected offspring	57 of DQ β-chain	52 of DQ α-chain
03/0302	352/406 (87)*	77/181 (43)†‡	A	R
0501/02	272/333 (82)*	81/169 (48)‡	A	R
0401/0402	27/33 (82)†	8/18 (44)§	D	R
0102/0502	15/19 (79)†	4/7 (57)	S	S
0101/0501	53/74 (72)†	15/32 (47)§	V	S
0102/0604	18/33 (55)	5/18 (28)	V	S
03/0303	6/11 (55)	2/6 (33)	D	R
03/0301	17/39 (44)	7/19 (37)	D	R
0501/0301	15/36 (42)	9/15 (60)	D	R
0201/02	14/37 (38)	11/18 (61)	A	H
0103/0603	8/28 (29)†	8/9 (89)†§	D	S
0102/0602	1/37 (2.7)*	12/20 (60)‡	D	S

Data are n (%). Using the TDT χ^2 test statistic: *$P < 0.00005$, †$P < 0.05$; using the 2 × 2 contingency χ^2 test: ‡$P < 0.00005$, §$P < 0.05$. D, aspartic acid; R, arginine; S, serine; V, valine; H, histidine; A, alanine.

to affected children from heterozygous parents with neither DQA1*03-DQB1*0302 or DQA1*0501-DQB1*02 was analyzed. The known protective DQ allele DQA1*0102-DQB1*0602 was transmitted to only 1 of 37 (2.7%) affected children from such heterozygous parents. In contrast, 60% of unaffected offspring received this same haplotype (Table 1).

Among the DQ alleles that have non-Asp at position 57 (alanine, valine, or serine) in the DQ β-chain, the transmission frequencies of DQA1*0102-DQB1*0502, DQA1*0101-DQB1*0501, and DQA1*0102-DQB1*0604 among affected children were 79, 72, and 55%, respectively. Interestingly, the DQA1*0401-DQB1*0402 haplotype that has Asp57 on the DQ β-chain was also frequently transmitted to affected offspring (82%) when the other haplotype in parents was neither DQA1*03-DQB1*0302 nor DQA1*0501-DQB1*02 (Table 1).

The null hypothesis of distorted segregation causing the differences observed for DQA1*03-DQB1*0302, DQA1*0501-DQB1*02, DQA1*0401-DQB1*0402, and DQA1*0101-DQB1*0501 was rejected by comparison of transmission to unaffected children, thus confirming the finding of linkage. However, we were unable to rule out a segregation disorder causing the difference in 0102/0502 transmission between affected and unaffected sibs ($P = 0.54$). Furthermore, the transmission frequencies of haplotypes DQA1*0102-DQB1*0602 and DQA1*0103-DQB1*0603 to affected offspring were significantly lower than that to unaffected offspring ($P < 0.00005$). The rank order of transmission to diabetic offspring did not directly correspond to the absence of Asp57 on the DQ β-chain or the presence of Arg52 on the DQ α-chain (Table 1). The two DQ haplotypes (DQA1*0103-DQB1*0603 and DQA1*0102-DQB1*0602) that were least often transmitted to affected offspring (29 and 2.7%, respectively) had DQβ Asp57 and non-Arg DQα52 (Table 1).

Among Norwegian patients with type 1 diabetes, a significant excess of DQA1*03-DQB1*0302/ DQA1*0401-DQB1*0402 heterozygotes has been reported (13,14). A similar DQB1 allele, DQB1*0401, which is positively associated with type 1 diabetes (15), is found on Japanese DR4 haplotypes. In this population, the DQB1*0401 allele is in linkage disequilibrium with the DQA1*0301 allele. The amino acid sequences of DQB1*0401 and DQB1*0402 differ only at residue 23. Thus, it follows that Caucasian DQA1*0301-DQB1*0302/DQA1*0401-DQB1*0402 heterozygotes and Japanese DQA1*0301-DQB1*0401 individuals may encode very similar DQαβ heterodimers: in Caucasians by DQA1*0301 and DQB1*0402 in the *trans* position, and in Japanese by DQA1*0301 and DQB1*0401 in *cis*. In Sardinia, the allele DQA1*0102-DQB1*0502 is more common among patients with diabetes and is in linkage disequilibrium with DR2 (16,17).

The DQB1 alleles DQB1*0401 and DQB1*0402 differ from all other DQB1 alleles by having leucine at position 56 rather than proline. Thus, all of the DQB1 alleles that have Asp57 (DQB1*0301, *0303, *05031, *0602, and *0603) have Pro/Asp at position 56/57 compared with Leu/Asp for DQB1*0401 and DQB1*0402. Lund et al (18) have reported that an amino acid substitution at position 56 in the β-chain of I-A^{g7} prevented the development of diabetes in NOD mice. This amino acid difference at position 56 may influence a conformational structure of the DQ β-chain in the region of Asp57, and thereby influence diabetes susceptibility similar to what is seen in I-A^k transgenic mice (19).

In conclusion, our findings suggest that future molecular modeling and hypotheses concerning diabetogenecity of class II alleles may need to consider a larger number of DQ molecules. Analysis of transmission of DQ haplotypes within a large collection of families, such as HBDI, aids in defining high-risk DQ alleles that are infrequent in the general U.S. populations but may be common in other populations. In particular, the diabetes-associated alleles identified in this study are similar to those found in other ethnic groups, and suggest that high-risk DQ diabetes haplotypes are similar in effect among multiple ethnic groups.

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REFERENCES

1. Eisenbarth GS, Ziegler AG, Colman PA: Pathogenesis of insulin-dependent (type I) diabetes mellitus. In *Joslin's Diabetes Mellitus*. 13th ed. Weir GC, Kahn CR, Eds. Philadelphia, Lea & Febiger, 1994, p. 216–239
2. Davies JL, Kawaguchi Y, Bennett ST, Copeman JB, Cordell HJ, Pritchard LE, Reed PW, Gough SCL, Jenkins SC, Palmer SM, Balfour KM, Rowe BR, Farrall M, Barnett AH, Bain SC, Todd JA: A genome-wide search for human type 1 diabetes susceptibility genes. *Nature* 371:130–136, 1994
3. Baisch JM, Weeks T, Giles R, Hoover M, Stastny P, Capra JD: Analysis of HLA-DQ genotypes and susceptibility in insulin-dependent diabetes mellitus. *N Engl J Med* 322:1836–1841, 1990
4. Morel PA, Dorman JS, Todd JA, McDevitt HO, Trucco M: Aspartic acid at position 57 of the HLA-DQ beta chain protects against type I diabetes: a family study. *Proc Natl Acad Sci U S A* 85:8111–8115, 1988
5. Todd JA, Bell JI, McDevitt HO: HLA-DQB gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. *Nature* 329:599–604, 1987
6. Yamagata K, Nakajima H, Hanafusa T, Noguchi T, Miyazaki A, Miyagawa J, Sada M, Amemiya H, Tanaka T, Kono N, Tarui S: Aspartic acid at position 57 of DQ beta chain does not protect against type 1 (insulin-dependent) diabetes mellitus in Japanese subjects. *Diabetologia* 32:762–764, 1989
7. Gutierrez-Lopez MD, Bertera S, Chantres MT, Vavassori C, Dorman JS, Trucco M, Serrano-Rios M: Susceptibility to type 1 (insulin-dependent) diabetes mellitus in Spanish patients correlates quantitatively with expression of HLA-DQα Arg 52 and HLA-DQβ non-Asp 57 alleles. *Diabetologia* 35:583–588, 1992
8. Khalil I, d'Auriol L, Gobet M, Morin L, Lepage V, Deschamps I, Park MS, Degos L, Galibert F, Hors J: A combination of HLA-DQ beta Asp 57-negative and HLA-DQ alpha Arg 52 confers susceptibility to insulin-dependent diabetes mellitus. *J Clin Invest* 85:1315–1319, 1990
9. Payami H, Khan MA, Grennan DM, Sanders PA, Dyer PA, Thomson G: Analysis of genetic interrelationship among HLA-associated diseases. *Am J Hum Genet* 41:331–349, 1987
10. Erlich H, Bugawan T, Begovich AB, Scharf S, Griffith R, Saiki R, Higuchi R, Walsh PS: HLA-DR, DQ, and DP typing using PCR amplification and immobilized probes. *Eur J Immunogenet* 18:33–55, 1991
11. Saiki RK, Bugawan TL, Horn GT, Mullis KB, Erlich HA: Analysis of enzymatically amplified beta-globin and HLA-DQalpha DNA with allele-specific oligonucleotide probes. *Nature* 324:163–166, 1986
12. Spielman RS, McGinnis RE, Ewens WJ: Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am J Hum Genet* 52:506–516, 1993
13. Horn GT, Bugawan TL, Long CM, Erlich HA: Allelic sequence variation of the HLA-DQ loci: relationship to serology and to insulin-dependent diabetes mellitus susceptibility. *Proc Natl Acad Sci U S A* 85:6012–6016, 1988
14. Ronningen KS, Gjertsen HA, Iwe T, Spurkland A, Hansen T, Thorsby E: Particular HLA-DQ αβ heteodimer associated with IDDM susceptibility in both DR4-DQw4 Japanese and DR4-DQw8/DQw8-DQw4 whites. *Diabetes* 40:759–763, 1991
15. Aparicio JMR, Wakisaka A, Takada A, Matsuura N, Aizawa M: HLA-DQ system and insulin-dependent diabetes mellitus in Japanese subjects: does it contribute to the development of IDDM as it does in Caucasians? *Immunogenetics* 28:240–246, 1988
16. Carcassi C, Trucco G, Trucco M, Contu L: A new HLA-DR2 extended haplotype is involved in insulin-dependent diabetes mellitus susceptibility. *Hum Immunol* 31:159–164, 1991
17. Cucca F, Muntoni F, Lampis R, Frau F, Argiolas L, Silvetti M, Angius E, Cao A, De Virgiliis S, Congia M: Combinations of specific DRB1, DQA1, DQB1 haplotypes are associated with insulin-dependent diabetes mellitus in Sardinia. *Hum Immunol* 37:85–94, 1993
18. Lund T, O'Reilly L, Hutchings P, Kanagawa O, Simpson E, Gravely R, Chandler P, Dyson J, Picard JK, Edwards A, Kioussis D, Cook A: Prevention of insulin-dependent diabetes mellitus in non-obese diabetic mice by transgenes encoding modified I-A beta chain or normal I-E alpha chain. *Nature* 345:727–729, 1990
19. Slattery RM, Kjer-Nielsen L, Allison J, Charlton B, Mandel TE, Miller JFAP: Prevention of diabetes in non-obese diabetic I-A^k transgenic mice. *Nature* 345:724–726, 1990

Author Queries (please see Q in margin and underlined text)

Q1: For consistency, “DQ β-chain” and “DQ α-chain”
OK throughout?
Q2: To consolidate parts of Table 1, are changes that have been
made OK? If not, please correct.
Q3: “29 and 2.7” correct here?
Q4: Change to sentence beginning “A similar…” OK?
If not, please reword for clarity.

Reference query: Refs. 2, 6, 7, 8, 10, 17, 18: Please list all authors.