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# Report

# Application and Interpretation of Transmission/Disequilibrium Tests: Transmission of HLA-DQ Haplotypes to Unaffected Siblings in 526 Families with Type 1 Diabetes

Benedicte A. Lie,[1] Kjersti S. Rønningen,[2] Hanne E. Akselsen,[1] Erik Thorsby,[1] and Dag E. Undlien[1]

[1]Institute of Immunology, The National Hospital, University of Oslo, and [2]Department of Population Health Sciences, National Institute of Public Health, Oslo

## Summary

**It is widely believed that, if a genetic marker shows a transmission distortion in patients by the transmission/disequilibrium test (TDT), then a transmission distortion in healthy siblings would be seen in the opposite direction. This is also the case in a complex disease. Furthermore, it has been suggested that replacing the McNemar statistics of the TDT with a test of heterogeneity between transmissions to affected and unaffected children could increase the power to detect disease association. To test these two hypotheses empirically, we analyzed the transmission of HLA-DQA1-DQB1 haplotypes in 526 Norwegian families with type 1 diabetic children and healthy siblings, since some DQA1-DQB1 haplotypes represent major genetic risk factors for type 1 diabetes. Despite the strong positive and negative disease associations with particular DQ haplotypes, we observed no significant deviation from 50% for transmission to healthy siblings. This could be explained by the low penetrance of susceptibility alleles, together with the fact that IDDM loci also harbor strongly protective alleles that can override the risk contributed by other loci. Our results suggest that, in genetically complex diseases, detectable distortion in transmission to healthy siblings should not be expected. Furthermore, the original TDT seems more powerful than a heterogeneity test.**

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Address for correspondence and reprints: Dr. Benedicte A. Lie, Institute of Immunology, The National Hospital, N-0027 Oslo, Norway. E-mail: b.a.lie@labmed.uio.no


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Family-based association tests have become popular for identification of genes involved in complex diseases, because associations due to population stratification are avoided. These tests also have the potential for discovery of associations in cases of tight linkage, when linkage itself is hard to detect (Spielman et al. 1993). It is therefore generally believed that association studies of families will become important in the genomewide mapping of disease genes (Risch and Merikangas 1996).

The most widely applied family-based association test is probably the transmission/disequilibrium test (TDT), in which trios of parents and proband are used (Spielman et al. 1993). Occasionally, data on transmissions to unaffected siblings are also included in this type of study, but the interpretation of such data varies. Some investigators have used these data merely to ensure that no segregation distortion has occurred (Spielman et al. 1993; Copeman et al. 1995; Nistico et al. 1996; Merriman et al. 1997, 1998; Lie et al. 1999). In other studies, the transmission to probands has been compared with transmission to healthy siblings, instead of to the randomly expected 50% (Reed et al. 1997; Nakagawa et al. 1998), where a transmission distortion is anticipated for unaffected siblings that is in the direction opposite to that for affected siblings. Boehnke and Langefeld (1997) have suggested that the McNemar statistics of TDT could be replaced by a $2 \times 2$ table of heterogeneity, to increase the power to detect disease-involved genes. Thus, for a disease-associated marker, it is not only widely expected but also theoretically logical that transmission to healthy siblings would show a trend opposite to that for transmission to probands.

Type 1 diabetes (MIM 142857) is a complex disease for which much effort has been devoted to identifying the predisposing genes. A large number of genomic

## Table 1

**TDT of DQA1-DQB1 Haplotypes in Probands or Healthy Siblings in 526 Norwegian Families with Type 1 Diabetes**

| Haplotype | Probands | | | Healthy Siblings | | | Heterogeneity |
|---|---|---|---|---|---|---|---|
| DQA1-DQB1 | No. (%) T | NT | $\chi^2$ [a] | No. (%) T | NT | $\chi^2$ [a] | $\chi^2$ [b] |
| 0301–0302 | 342 (84) | 65 | 189 | 261 (49) | 271 | 0 | 123 |
| 0501–0201 | 247 (76) | 78 | 88 | 174 (43) | 228 | 7.3 | 78 |
| 0301–0303 | 15 (54) | 13 | .1 | 13 (36) | 23 | 2.7 | 2 |
| 0102–0502 | 3 (50) | 3 | 0 | 2 (40) | 3 | .2 | .1 |
| 0401–0402 | 33 (49) | 34 | 0 | 43 (51) | 40 | .1 | .1 |
| 0102–0604 | 32 (44) | 40 | .9 | 43 (55) | 35 | .8 | 1.8 |
| 0101–0501 | 47 (29) | 115 | 29 | 110 (52) | 101 | .4 | 20 |
| 0301–0301 | 16 (25) | 48 | 16 | 38 (49) | 39 | 0 | 8.8 |
| 0201–0201 | 15 (21) | 56 | 23 | 41 (51) | 40 | 0 | 14 |
| 0103–0603 | 12 (14) | 74 | 45 | 65 (59) | 45 | 3.6 | 41 |
| 0501–0301 | 7 (13) | 47 | 30 | 32 (53) | 28 | .3 | 21 |
| 0201–0303 | 2 (8) | 22 | 17 | 14 (47) | 16 | .1 | 9.4 |
| 0101–0503 | 0 (0) | 12 | 12 | 8 (67) | 4 | 1 | 12 |
| 0102–0602 | 0 (0) | 164 | 164 | 123 (57) | 94 | 3.9 | 137 |

[a] $\chi^2 > 8.5$ provides a corrected $P < .05$.

[b] Heterogeneity between affected and unaffected offsprings was tested by 2 x 2 contingency tables. $\chi^2 > 8.5$ provides a corrected $P < .05$.

regions have been suggested to contribute to the pathogenesis (Delepine et al. 1997; Denny et al. 1997; Reed et al. 1997). That environmental factors trigger the disease has been implicated by the low penetrance of the disease (Todd 1991). The major disease locus, *IDDM1,* comprises the HLA complex and contributes most (~50%) to the familial clustering observed in type 1 diabetes (Davies et al. 1994). However, a number of minor disease loci (*IDDM2–IDDM13,* and *IDDM15*) have also been assigned (partly reviewed in a study by Todd [1997]) and later been supplemented by others (Davies et al. 1994; Merriman et al. 1997). Some fine-mapping studies of these minor loci presented TDT data on both affected and unaffected siblings. The transmissions to unaffected siblings showed trends the same or opposite to that observed for probands, but a deviation significantly different from 50% was only rarely observed (Spielman et al. 1993; Copeman et al. 1995; Nistico et al. 1996; Merriman et al. 1997, 1998; Reed et al. 1997; Nakagawa et al. 1998).

We wanted to investigate empirically the behavior of parental transmission of disease-associated genetic factors to healthy siblings. Some HLA-DQA1-DQB1 haplotypes represent the most pronounced genetic risk factors known (She 1996) and should therefore possess the highest potential to detect an opposite trend. Therefore, we studied the transmission of DQ haplotypes in families with type 1 diabetes, using data from 526 Norwegian families with one affected child (and 12 families with two affected children), their parents, and unaffected siblings (0–12 sibs in each family) (Undlien et al. 1995*a*). The transmissions of DQ haplotypes, both to probands and to unaffected children, were analyzed by the extended TDT (ETDT) (Sham and Curtis 1995). The overall ETDT statistics for distortion in transmission to probands provides $\chi^2 = 527$ (13 df; $P << .00001$), whereas for transmission to healthy siblings $\chi^2 = 22$ (13 df; $P = .06$) is obtained.

The TDT analyses of individual DQ haplotypes requires $\chi^2 > 8.5$ in order for statistical significance ($P < .05$) to be obtained after correction for multiple tests. As can be seen in table 1, all degrees of associations with type 1 diabetes were observed for the DQ haplotypes, in a range of predisposing ($T > 50\%$) to protective ($T < 50\%$). As expected, a strong positive association was observed for DQA1*0301-DQB1*0302 ($T = 84\%$) and DQA1*0501-DQB1*0201 ($T = 76\%$), whereas a remarkably strong negative association was observed for DQA1*0102-DQB1*0602 ($T = 0\%$). However, no significant bias in the transmission to healthy siblings was observed for any DQ haplotype. Only a slight and insignificant tendency, in the opposite direction, for the transmission of the strongly associated DQA1*0301-DQB1*0302 ($T = 48\%$), DQA1*0501-DQB1*0201 ($T = 43\%$), DQA1*0102-DQB1*0602 ($T = 57\%$) haplotypes was detected. Thus, despite the very strong positive and negative disease associations to particular DQ haplotypes, no statistically significant deviation from 50% was observed for the transmission to healthy siblings. These observations are in agreement with another study on transmission of DQ haplotypes in families with type 1 diabetes (Kawasaki et al. 1998), in which transmission to unaffected offspring was presented.

Several factors, both genetic and environmental,

are needed to develop the disease. Notably, all genetic risk factors identified to date (e.g., the DRB1*03-DQA1*0501-DQB1*0201 and DRB1*04-DQA1*03-DQB1*0302 haplotypes) are present at high frequencies in the general population. Thus, the low penetrance of the disease in individuals having these haplotypes may explain why no significant distortion in transmission to healthy offspring was observed. In addition, for both loci that have been fine mapped to a certain extent (*IDDM1* and *IDDM2*), some alleles have shown strong protection in an almost dominant fashion (Bennett et al. 1995; Undlien et al. 1995*b;* Thorsby 1997), and this might also be the case for other IDDM loci. One could hypothesize that many healthy individuals are dominantly protected by one or more IDDM loci and that a distortion in the transmission at another locus would therefore be unexpected.

A comparison between the McNemar statistics of the TDT and the heterogeneity test (which compares transmission to probands and unaffected siblings) indicates that the former provides greater power (table 1). Therefore, little may be gained by replacing the original TDT with a heterogeneity test, even though the transmission to healthy siblings shows the opposite trend. The inclusion of unaffected children may increase the rate of misclassified individuals, since this classification is true only at the time of sample collection. The healthy siblings may actually introduce more random noise into the data set, rather than additional information. Hence, the empirical data presented here do not support the advice to abandon the original TDT and, instead, test the heterogeneity of transmission between affected and unaffected offspring.

In conclusion, our data, together with those from other studies, suggest that, in genetically complex diseases such as type 1 diabetes, it will be hard to detect distortion in transmission to healthy siblings, even though, theoretically, one might expect a transmission pattern opposite to that observed in probands.

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## Electronic-Database Information

The accession number and URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for type 1 diabetes [MIM 142857])

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