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Report

The Transmission/Disequilibrium Test for Linkage on the X Chromosome

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The transmission/disequilibrium test (TDT), which detects linkage between a marker and disease loci in the presence of linkage disequilibrium, was introduced by Spielman et al. The original TDT requires families in which the genotypes are known for both parents and for at least one affected offspring, and this limits its applicability to diseases with late onset. The sib-TDT, or S-TDT, which utilizes families with affected and unaffected siblings, was introduced as an alternative method, by Spielman and Ewens, and the TDT and S-TDT can be combined in an overall test (i.e., a combined-TDT, or C-TDT). The TDT statistics described so far are for autosomal chromosomes. We have extended these TDT methods to test for linkage between X-linked markers and diseases that affect either males only or both sexes. For diseases of late onset, when parental genotypes are often unavailable, the X-linkage C-TDT may allow for more power than is provided by the X-linkage TDT alone.

The transmission/disequilibrium test (TDT) detects linkage between marker and disease loci in the presence of linkage disequilibrium (Spielman et al. 1993). The original TDT (here denoted as "TDT") requires families in which the genotypes are known for both parents and for at least one affected offspring, and this limits its applicability for diseases with late onset. The sib-TDT, or S-TDT, which utilizes families with affected and unaffected siblings, was introduced as an alternative method (Spielman and Ewens 1998). An attractive feature of the TDT is that data from families that meet the requirement criteria for either or both methods can be combined in an overall test (i.e., a combined-TDT, or C-TDT) (Spielman and Ewens 1998). The TDT statistics described so far are for autosomal chromosomes. We have expanded these TDT methods to test for linkage between X-linked markers and diseases that affect either males only or both sexes.

Consider an X-linked marker in which there are only two alleles—M_1 and M_2—or in which allele M_1 is of particular interest and all other alleles are grouped as M_2. For a disease that can affect only males (e.g., sex-linked diseases such as prostate cancer), nuclear families with at least one affected son qualify to be used for either the X-linked form of the TDT or the S-TDT if they can be classified into one of the following two types: (1a) the mother is genotyped and heterozygous for allele M_1; and (2a) the mother's genotype is unknown, but there is at least one unaffected son in the family, not all sons have the same genotype, and at least one son has allele M_1. Nuclear families that do not fit into either category will be excluded. For an X-linked disease that can affect both males and females, nuclear families with at least one affected child qualify to be used for these X-linked tests if they are of the following types: (1b) for female affected offspring, both parents are genotyped and the mother is heterozygous for allele M_1, or, for male affecteds, the mother is genotyped and is heterozygous for allele M_1; and (2b) the parent's genotypes are unknown, or, for female affecteds, one parent's genotype is unknown, but there is at least one affected and one unaffected child of the same sex in the family, and not all children of the same sex have the same genotype. The TDT is applied to family types 1a and 1b, whereas S-TDT is the appropriate method for family types 2a and 2b. Some of the families in type 1a or type 1b may also fulfill the requirements for the S-TDT (i.e., the genotype of at least one unaffected offspring is known), but the TDT is the preferable method. This is because, in large samples, the TDT is generally more powerful than the

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S-TDT (Spielman and Ewens 1998); also, for late-onset diseases, the affection status of the unaffected offspring may be questionable.

The X-linkage TDT statistic in the case of male or female affected offspring, following the terminology of Spielman and Ewens (1998), uses the number of transmissions of allele M_1 observed in the n pairs of heterozygous (M_1M_2) mothers and their affected children, denoted as "X," summing X over all families of type 1, in which families with multiple affected children contribute multiple pairs to n (e.g., a family with three affected children contributes three mother-child pairs to n). Under the null hypothesis of no linkage between marker and disease, X has a binomial distribution with mean $n/2$ and variance $n/4$. The test statistic is then the usual Z-score with a continuity correction: $Z_1 = [|X - n/2| - .5]/\sqrt{n/4}$.

For a nuclear family with a affected and u unaffected offspring, the total number of offspring is $t = a + u$. In the autosomal case, summing over all families, Spielman and Ewens (1998) define r to be the number of offspring with two M_1 alleles, s to be the number of offspring with only one M_1 allele, and Y to be the count of M_1 alleles among the affected offspring. For an X-linked locus, a son will never contribute to r, but he will contribute to s if he has the M_1 allele. A daughter will contribute either to r, if she is homozygous for M_1, or to s, if she is heterozygous.

When both males and females can be affected with the X-linked disease, the male and female members of a sibship must be considered as separate subfamilies and must be analyzed separately. Those subfamilies that are of type 2b qualify for use in the S-TDT. When the qualifying male subfamilies are considered, $r = 0$. In this situation, Y, s, and u are then summed over the male offspring in all families of type 2b. Table 1 gives the expectation and variance of Y_m—denoted as "A_m" and "V_m," respectively—under the null hypothesis of no linkage between the disease locus and the X-linked marker. The test statistic of the S-TDT for male subfamilies is then given by the equation $Z_{2m} = (|Y_m - A_m| - .5)/\sqrt{V_m}$, a standard Z-score with continuity correction. When the qualifying female subfamilies are analyzed, Y_f, r_f, s_f, and u_f are summed over all the female offspring. In this case, the expectation, A_f, and the variance, V_f, under the null hypothesis are the same as those given by Spielman and Ewens (1998) for an autosomal locus. The test of significance is the same as that for male subfamilies, with a simple substitution of Y_f, A_f, and V_f for Y_m, A_m, and V_m, respectively. Table 1 shows these formulas for the male and female subfamilies. The null hypothesis of no linkage for the S-TDT is rejected if either Z_{2m} or Z_{2f} departs significantly from 0, as judged on the basis of standard Z tables.

Table 1

***A* and *V* for the S-TDT**

	Family Type 2	
	Male Subfamilies	Female Subfamilies
A	$\frac{\Sigma sa}{t}$	$\frac{\Sigma(2r+s)a}{t}$
V	$\frac{\Sigma aus(t-s)}{t^2(t-1)}$	$\frac{\Sigma au[4r(t\text{-}r\text{-}s)+s(t\text{-}s)]}{t^2(t\text{-}1)}$

In the case of a sex-limited X-linked disease, then, there will never be any informative female subfamilies. For families of type 1a, then, the same test statistic, Z_{2m}, described above for male subfamilies, will be used.

As in the autosomal case (Spielman and Ewens 1998), to combine the TDT and S-TDT into a C-TDT (and to combine male- and female-subfamily S-TDTs), the natural test statistic is W, the sum of X and Y. Under the null hypothesis, W has mean $A_{comb} = (n/2) + A$ (or $A_{comb} = (n/2) + A_m + A_f$) and $V_{comb} = (n/4) + V$ (or $V_{comb} = (n/4) + V_m + V_f$), and the test of significance is a Z statistic of the form $Z = [|W - A_{comb}| - .5]/\sqrt{V_{comb}}$. The null hypothesis that disease and marker are unlinked is rejected if, as judged on the basis of standard Z tables, Z departs significantly from 0. This combined test is expected to be more powerful than the individual tests.

As in the autosomal case (Spielman and Ewens 1998), the X-linked TDT, S-TDT, and C-TDT can be assumed to be valid tests of association only when families with the "minimal" configuration are used: for the TDT, these are families with one affected child and the mother heterozygous for the marker; for S-TDT, these are families with exactly one affected and one unaffected sib, of the same sex but with different marker genotypes (Note that this implies that, for the C-TDT to be a valid test of association, the male and female subfamilies from a single family should not be combined in the C-TDT.) Otherwise, these are tests of linkage.

This method extends existing TDT, S-TDT, and C-TDT tests to X-linked loci. The X-linked C-TDT may allow for more power than does the X-linked TDT alone, for diseases of late onset, for which parental genotypes are often unavailable. Knapp (1999) recently introduced a new, reconstruction-combined TDT for autosomal chromosomes that employs parental-genotype reconstruction from genotypes of the offspring and corrects for the biases resulting from such reconstruction. This method, if extended to the X chromosome, may improve the power of the C-TDT that we have described here.

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