Letters to the Editor

Am. J. Hum. Genet. 64:1473, 1999

Mutation Rate in Human Microsatellites

To the Editor:

It is common knowledge that mutation rates of DNA (minisatellite and microsatellite) loci can differ by orders of magnitude. Obviously, the mutation rate is closely related to the degree of polymorphism, which in turn is expressed by the respective heterozygosity rate.

In their article, Brinkmann et al. (1998) confirmed the observation that mutation events in the male germ line may be significantly more frequent than mutation events in the female germ line. They reported that the ratio of paternal to maternal mutations is an impressive 17:3. Because a similar "behavior" was already known for many DNA minisatellites, this trend could have been expected for highly polymorphic short tandem-repeat loci as well (Henke et al. 1993; Olaisen et al. 1993; Henke and Henke 1995). If this is taken into consideration, the compilation of mutations in table 1 gives rise to following questions: Why do Brinkmann et al. (1998) compile the overall number of meioses with respect to the number of mutations? If one takes into consideration that their data are extremely important in parentage testing, would it not be more meaningful to produce a table that unambiguously shows the frequency of mutations in paternal and in maternal meioses? Mutation rates that we found in a recent study are given in table 1.

Table 1

No. of Maternal and Paternal Mutations and Meioses

	No. of Mutations/ No. of Meioses	
Locus	Maternal	Paternal
CSF1PO	0/237	0/165
D13S317	0/258	0/178
D18S51	0/286	2/205
D21S11	1/267	3/189
D3S1358	0/257	0/176
D5S818	0/258	0/178
D7S820	0/256	2/176
D8S1179	0/213	0/149
FGA	0/307	3/218
ACTBP2	0/402	5/315
THO1	0/394	0/301
TPOX	0/240	0/167
VWA	1/258	0/178

Jürgen Henke and Lotte Henke
Institut für Blutgruppenforschung
Köln, Germany

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Address for correspondence and reprints: Dr. Jürgen Henke, Institut für Blutgruppenforschung, Hohenzollernring 57, Postfach 1904 20, 50501 Köln, Germany

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Reply to Henke and Henke

To the Editor:

From published data we could not derive "common knowledge" that microsatellite mutation rates differ by orders of magnitude. The authors seem to confuse "minisatellite" and "microsatellite" mutations and seem to extrapolate from observations in minisatellites, although it is known that the predominant mutation processes are not the same (Jeffreys et al. 1994). Furthermore, al-

though an influence of the degree of polymorphism exists, there is no "close relation." As we tried to show in our article (Brinkmann et al. 1998), the correlation is not that simple.

We also disagree with the statement that the sex ratios were to be expected from minisatellite loci. The mutation process is different, and other structures and loci are involved. In fact, the sex ratios seem to differ grossly among minisatellites—for example, 1:1 for D1S7 and 1:8 for D7S22 (Henke and Henke 1995). It is therefore unclear which of these ratios should be chosen for extrapolation to microsatellites.

We agree with the authors that application to biomedical or forensic calculations would require sex-specific mutation rates. Therefore, their data are a valuable contribution, especially if the same criteria have been met. We pooled our data for individual loci to focus on the correlation of the mutation rates with the mean length of the affected structure. The data set was too small to address the question of locus-specific ratios.

Apart from sex-specific mutation rates, further factors need to be considered when Henke and Henke's data and ours are used to determine mutation rates applicable to paternity probability calculations.

First, the mutation rates of individual alleles at a given locus vary according to size, and, as we described in our article (Brinkmann et al. 1998), longer alleles often exhibit higher mutation rates than shorter ones. Our current enlarged mutation database indicates that, at the locus FGA, the mutation ratio of short versus long alleles seems to be close to an order of magnitude (fig. 1).

Second, the complexity of the allele sequence seems to exert an influence on the mutation rate. For example, we showed that, for ACTBP2, the longer alleles exhibit a lower mutation rate because of the interruption of the AAAG repeat by an AA dinucleotide.

Third, the number of undetected mutations depends on the paternity case—that is, whether one parent or both parents are examined and whether the putative father is included or excluded. It also depends on the genotype distribution in the specific population. For example, in the trio mother 16/17; child 16/17; father 16/18, a paternal 16→17 or 18→17 mutation would be undetected.

Fourth, paternal age may affect the mutation rate. We demonstrated that the mean age of fathers in whom mutations occurred was higher than the mean age of all fathers in our database. This would mean that the mutation rate should also be classified according to age.

Finally, it needs to be stressed that ~10% of the mutation events in our study are of unknown origin. These mutations cannot be used directly for the calculation of a sex-specific mutation rate without further assumptions and elaborations.

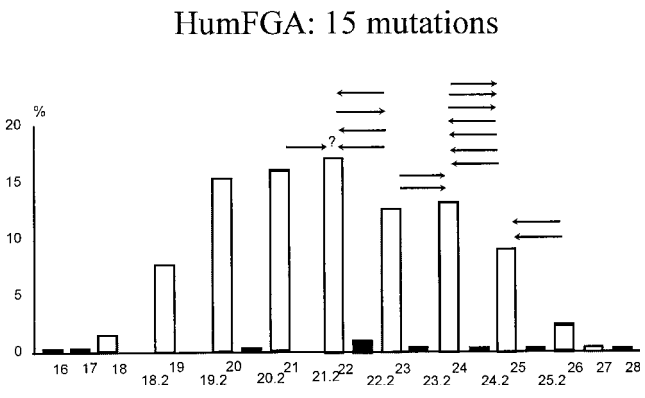

Figure 1 Allele frequencies and mutation events at the FGA locus.

It is clear, then, that a correct mutation rate would require knowledge of many more mutation events than we have observed in our laboratory or Henke and Henke have seen. The currently available data at least allow the definition of an overall ratio of mutations that occur in male and female gametes. We would like to have our considerations understood as an incentive to others to report their mutation events. As more data become available, more certainty will be gained for the biostatistical calculation of paternity probability in cases of single-locus exclusions due to mutations.

B. Rolf and B. Brinkmann
Institut für Rechtsmedizin
Westfälische Wilhelms Universität
Münster, Germany

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Address for correspondence and reprints: Dr. B. Brinkmann, Institut für Rechtsmedizin, Westfälische Wilhelms Universität, Von Esmarch Strasse 62, D-48149, Münster, Germany. E-mail: brinkma@uni-muenster.de

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Recurrent Williams-Beuren Syndrome in a Sibship Suggestive of Maternal Germ-Line Mosaicism

To the Editor:

Williams syndrome (WS; MIM 194050) is a multisystem disorder characterized by mental retardation and an outgoing personality, distinctive facial features, infantile hypercalcemia, and supravalvular aortic stenosis (Williams et al. 1961; Beuren et al. 1962). A deletion encompassing the elastin (Ewart et al. 1993), replication factor subunit 2 (Peoples et al. 1996), LIM kinase-1 (Frangiskakis et al. 1996; Tassabehji et al. 1996), wnt receptor *Drosophila* frizzled homologue FZD3 (Wang et al. 1997), WBSCR1 (Osborne et al. 1996), and syntaxin 1A (Osborne et al. 1997*b*) genes at the 7q11.23 locus almost always is found in patients with WS. Earlier reports have indicated that a high frequency of deletions associated with WS result from unequal meiotic recombination, as shown by the common concurrence of these deletions with recombination between markers proximal and distal to the deletion (Dutly and Schinzel 1996; Baumer et al. 1998). Several independent studies that demonstrated duplicated genes flanking the deleted region in WS have provided a structural basis for the high frequency of unequal crossover events (Robinson et al. 1996; Osborne et al. 1997*a*; Perez Jurado et al. 1998). The recurrence risk of an interchromosomal rearrangement in the sibship of a proband with WS is usually negligible and thus is likely to account for the sporadic occurrence of almost all cases of WS. A minority of interstitial 7q11.23 deletions could, however, result from an intrachromosomal rearrangement, occurring during or before parental meiosis. In case of a premeiotic intrachromosomal recombination, the theoretical possibility of gonadal mosaicism for the WS deletion has been suggested recently by Dutly and Schinzel (1996). In agreement with this hypothesis, we report here the recurrence of a 7q11.23 deletion in two siblings with WS whose parents did not carry this rearrangement in their somatic cells.

The first affected proband, a male, was the third offspring of unrelated parents aged 26 years (mother) and 32 years (father) at the time of delivery. Both elder sisters and parents were healthy. Unilateral kidney agenesis and growth retardation were detected in the fetus during the third trimester of pregnancy. The child was delivered at 40 wk of gestation by cesarean section. Birth weight, length, and head circumference were 2,020 g (<10th percentile), 43 cm (<10th percentile), and 31 cm (<10th percentile), respectively. No hypercalcemia was noted. Supravalvular aortic stenosis and peripheral pulmonary arterial stenosis were detected at age 3 mo. Psychomotor development was markedly delayed, walking started by age 25 mo, and language developed by age 4 years. At the current age of 8 years, the proband's facies is characteristic of WS, with a narrowed forehead, strabismus with hypermetropia, periorbital fullness, malar hypoplasia, and a large open mouth with an everted lower lip (fig. 1). IQ is markedly reduced, and behavior is characteristic of WS, with attention deficit, loquaciousness, and hypersensitivity to sound. The second affected child, a boy currently aged 3 years, has a clinical picture similar to the one reported in the first affected proband, except for the absence of supravalvular aortic stenosis (fig.1).

Informed consent was obtained from the probands' parents prior to implementation of the genetic studies reported here. FISH was done with biotin-labeled DNA kits (Oncor), according to the protocols provided by the manufacturer. ELN and D7S427 (7q36) probes (Oncor) were used for testing the 7q11.23 locus and for chromosome 7 identification, respectively. The 7q11.23 deletion was found in both affected siblings, whereas it was not detected in either parent (data not shown). To our knowledge, this is the first report of the recurrence of full-blown WS secondary to a 7q11.23 deletion in nontwin sibs whose parents are unaffected. The familial cases of WS with the typical 7q deletion reported so far have involved either offspring of an affected individual (Ewart et al. 1993, 1994; Morris et al. 1993*a,* 1993*b*) or MZ twins (Castorina et al. 1997). Recurrence of WS in nontwin sibs with unaffected parents has earlier been reported (Burn 1986), but has never been established in cases with proven elastin deletion.

Both affected siblings, their healthy sisters, and their parents were genotyped with DNA $(CA)_n$ polymorphisms, from centromere to telomere, as follows: D7S645-D7S2415-D7S672-D7S653-D7S489B-ELN

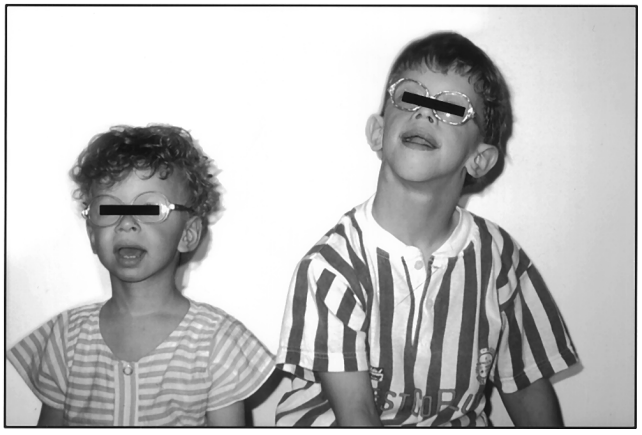

Figure 1 Photograph of the affected siblings

(intron 18 of the elastin gene)-D7S1870-D7S2470-D7S675-D7S669-D7S634-D7S660 (Généthon; Foster et al. 1993; Gyapay et al. 1994; Gilbert-Dussardier et al. 1995; Dib et al. 1996; Robinson et al. 1996). DNA samples from peripheral leukocytes were analyzed according to procedures published elsewhere (Gilbert-Dussardier et al. 1995). Both probands failed to inherit a maternal allele at the elastin and D7S1870 loci, thus indicating that the 7q11.23 deletion was maternal in origin (fig. 2). Moreover, they shared a single maternal haplotype, outside the deletion, with their eldest sister, who did not carry the WS deletion in her leukocytes, as indicated by microsatellite analysis. These data were highly suggestive of maternal mosaicism. In the probands' mother, heterozygosity at both elastin and D7S1870 loci, consistent with the absence of deletion of chromosome 7 in her leukocytes, by FISH, indicated that mosaicism was likely to be restricted to germ cells.

We could not rule out conclusively that the deletion in the present cases had occurred as a result of unequal crossover between homologous chromosomes 7 during maternal meiosis, because DNA of the maternal grandparents was not available. However, our comparison of haplotypes, using markers centromeric and telomeric to the deletion region, failed to suggest any recombination event within this region in any of the four siblings (fig. 2), making the possibility of a meiotic crossover very unlikely. On the basis of a female recombination distance of 6 cM between the first informative markers centromeric (D7S653) and telomeric (D7S675) to the deletion (Dib et al. 1996), the probability of a double recombination event in both affected sibs was very low (~1/7.5×10^4). Thus, the 7q11.23 deletion observed in both probands most likely resulted from a premeiotic intrachromosomal event responsible for a gonadal mosaicism in the probands' mother.

Apart from a gonadal mosaicism, the presence of a constitutive structural defect of the maternal chromosome 7 could theoretically account for the recurrence of the elastin deletion in sibs. A cryptic 7q11.23 rearrange-

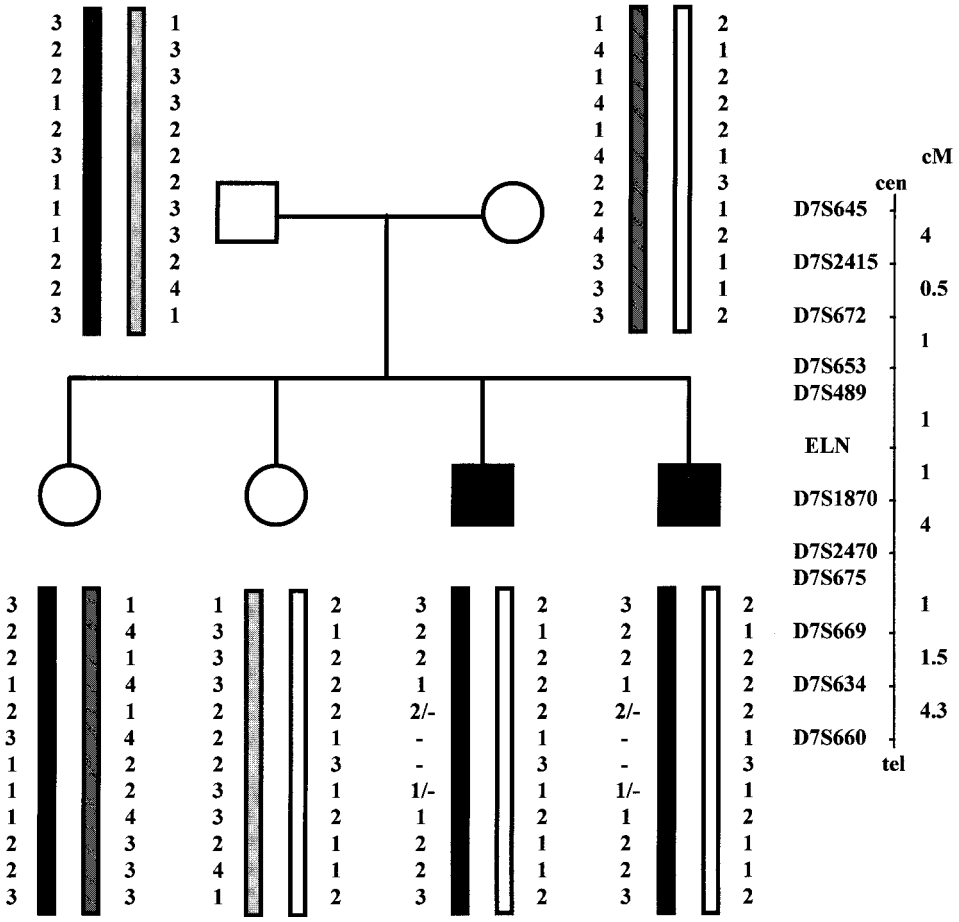

Figure 2 Segregation of microsatellite polymorphic markers within and surrounding the elastin gene on chromosome 7q in the nuclear family. The markers used and approximate genetic distances are given in the inset (sex average). The $(CA)_n$ repeat, which occurs in the elastin gene intron 18; marker D7S489B; and marker D7S1870 are known to be deleted in typical WS.

ment in a maternal homologue, such as an inversion or an insertion, could indeed interfere with meiotic pairing, thus leading to recurrence of unequal crossover in distinct meiosis and greatly enhancing the possibility of a deletion. However, high-resolution chromosome banding at the 500–850 bands level failed to detect any chromosomal abnormality in maternal leukocytes, and DNA haplotyping data were irrelevant to an interchromosomal crossover, thus arguing against this hypothesis. Whereas germ-line mosaicism in this particular family clearly results in an increased risk of WS in subsequent pregnancies, it would be necessary to determine the frequency of gonadal mosaicism in a large cohort of families with WS to assess the potential impact of this phenomenon on the overall recurrence risk of WS.

Ali Kara-Mostefa,[1] Odile Raoul,[2] Stanislas Lyonnet,[2] Jeanne Amiel,[2] Arnold Munnich,[2] Michel Vekemans,[2] Suzel Magnier,[3] Batool Ossareh,[2] and Jean-Paul Bonnefont[1,2]

[1]*Biochemical Genetics Unit and* [2]*Department of Genetics, Hôpital Necker Enfants Malades, and* [3]*Paediatric Cardiology Unit, Hôpital Robert Debré, Paris*

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Généthon, ftp://ftp.genethon.fr/pub/Gmap/Nature-1995/data/data_chrom7 (for sequence-tagged site used for chromosome 7 genotyping)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for WS [MIM194050])

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Address for correspondence and reprints: Dr. Jean-Paul Bonnefont, Biochemical Genetics Unit and Department of Genetics, Hôpital Necker-Enfants Malades, 149 rue de Sèvres, 75743 Paris cedex 15, France. E-mail: Bonnefont@necker.fr

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Localization of a Gene for Bitter-Taste Perception to Human Chromosome 5p15

To the Editor:

Some people perceive the taste of phenylthiocarbamide and its chemical relative propylthiouracil (PROP) as intensely bitter at low concentrations, whereas others are unable to detect them, even at high concentrations. This taste blindness is an inherited trait (Snyder 1931). Although inheritance is thought to be recessive, other possibilities have been suggested, such as multiple genes (Boyd 1950; Olson et al. 1989), incomplete dominance (Johnson et al. 1966; Bartoshuk et al. 1994; Reed et al. 1995), or multiple alleles of a single gene (Rychkov and Borodina 1973). This trait is among the most-studied in human genetics, but the relevant gene has not been characterized. Therefore, we conducted a genomewide scan by using 98 nuclear families and 356 markers spaced at ~10-cM intervals.

Three hundred ninety-three adults and their parents participated as research subjects. The 98 families were originally recruited as part of an ongoing study of the genetics of body weight at the University of Pennsylvania's Behavioral Genetics Laboratory, and the details of family collection have been published elsewhere (Price et al. 1998). The protocol was approved by the Committee of Studies Involving Human Beings at the University of Pennsylvania.

To phenotype the subjects, filter paper was soaked in a saturated PROP solution, dried, and cut into strips. Subjects were asked to place the paper in their mouths and to rate the bitterness of taste. The scale used by the subjects to rate the taste intensity utilized descriptive words and is referred to as a "labeled-magnitude scale" (LMS; Green et al. 1993). Because the LMS is continuous, it prevents the loss of information associated with categoric scales and therefore provides the type of data essential for quantitative linkage analysis. In addition, the LMS minimizes ceiling effects and is better at dis-

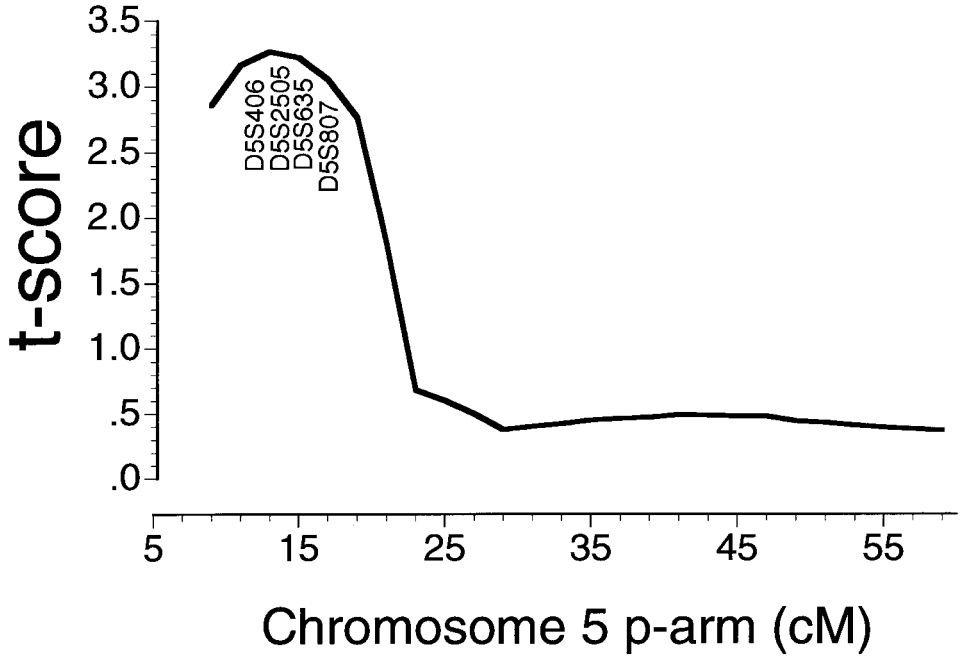

Figure 1 Multipoint results for chromosome 5

criminating sensitive tasters from nontasters than the classic nine-point scale (Lucchina et al. 1998*b*). The scale is labeled as follows (with numeric values assigned to each level of taste intensity): barely detectable (0), weak (6), moderate (17), strong (35), very strong (54), and strongest imaginable (100). The scores from the LMS were used as phenotypes for the quantitative linkage analysis.

The LMS is also a valid instrument to classify individuals as "nontasters" or "tasters." Studies scaling the suprathreshold bitterness of PROP with magnitude estimation demonstrate that psychophysical functions for nontasters and tasters diverge (Bartoshuk et al. 1994). The LMS produces suprathreshold functions equivalent to magnitude estimation (Green et al. 1996). Because the LMS is easier for naïve subjects to use, it is replacing magnitude estimation in studies of PROP (Snyder et al. 1996; Intranuova and Powers 1998; Lucchina et al. 1998*a,* 1998*b*; Schwartz et al. 1998; Prutkin et al., in press). Thus, the LMS provides a convenient, reliable, and reasonable choice for a large-scale gene-mapping study.

The mean rating of suprathreshold taste intensity was 31.2 ± 29.3 units, near the label "strong." As expected, the distribution of scores was kurtotic (−0.454) and skewed (.73). There was no relationship between subjects' ratings of PROP and height, weight, or body-mass index ($P > .05$).

We genotyped microsatellite markers spaced ~10.1 cM apart by using methods described by Lee et al. (1999). All half-siblings were eliminated prior to analysis. Computation of descriptive statistics and correlation coefficients were conducted with SPSS (6.1.1.). Quantitative trait loci analysis was conducted with the computer program MAPMAKER/SIBS version 2.0 (Kruglyak and Lander 1995). For analysis of transmission disequilibrium, the quantitative data were dichotomized into taster and nontaster categories, with all tasters reporting that suprathreshold concentrations of PROP tasted "strong," "extremely strong," or "strongest imaginable" ($n = 180$; 45.8%). Nontasters rated PROP as "barely detectable" or "weak" ($n = 115$; 29.3%). Subjects giving intermediate responses were excluded from analysis ($n = 98$; 25%). These cut-off values are conservative. Transmission of alleles from heterozygous parents to nontaster offspring was computed with TDTLIKE version 2.1 (Terwilliger 1995), which corrects for multiple-allele testing.

The telomeric portion of 5p gave the strongest evidence for linkage (t-score = 3.28, $P = .0005$; fig. 1), with the peak score near D5S2505. The linkage peak spanned ~10 cM, from D5S406 to D5S2081, and was the only region of the genome that had a t score ≥ 3.0. No candidate genes are apparent in 5p15. Markers from the telomeric portion of chromosome 5 were then examined for transmission disequilibrium. There was significant distortion in transmission of alleles from heterozygous parents to nontaster children for markers from the linked region, with D5S2505 being the most significant (D5S406, $P = .031$; D5S2505, $P = .007$, D5S635, $P = .017$; D5S807, $P = .034$; D5S2081, $P = .012$). These results are consistent with the hypothesis that a gene that confers the ability to taste PROP lies on the telomeric region of human chromosome 5p.

In addition to chromosome 5, there was a suggestion of linkage on chromosome 7, ~35–40 cM centromeric to the KELL locus, with a maximum t-score of 2.34 ($P = .008$) near D7S1789 and D7S796. Initial linkage studies suggested a locus was near KELL on chromosome 7 (Chautard-Freire-Maia 1974; Conneally et al. 1976), but later reports were unable to replicate this finding (Spence et al. 1984). The results of the current study suggest that a region on chromosome 7 may also influence the taster phenotype.

Acknowledgments

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DANIELLE R. REED,[1] ELIZABETH NANTHAKUMAR,[2] MICHAEL NORTH,[2] CALLUM BELL,[2] LINDA M. BARTOSHUK,[3] AND R. ARLEN PRICE[1]

[1]*Center for Neurobiology and Behavior, Department of Psychiatry, University of Pennsylvania;* [2]*Axys Pharmaceutical, La Jolla, CA; and* [3]*Yale University School of Medicine, New Haven, CT*

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Address for correspondence and reprints: Dr. R. Arlen Price, Center for Neurobiology and Behavior, University of Pennsylvania, 415 Curie Boulevard, CRB135b, Philadelphia, PA 19104 E-mail: arlen@bgl.psycha.upenn.edu

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Am. J. Hum. Genet. 64:1480–1484, 1999

Sperm Chromosome Analysis in a Man Heterozygous for a Paracentric Inversion of Chromosome 14 (q24.1q32.1)

To the Editor:

Paracentric inversions are rarely reported, since they can only be detected by the use of banding procedures (Pettenati et al. 1995). The incidence is estimated to be .09–.49/1,000 (van Dyke et al. 1983; Ferguson-Smith and Yates 1984; Hook et al. 1984; Fryns et al. 1988). Some investigators have suggested that paracentric inversions in man are generally harmless (Madan 1995); however, recombinant chromosomes have been observed in 17 cases, and the risk of viable recombinants has been estimated to be 3.8% (Pettenati et al. 1995). Because the frequency of spontaneous abortions may be increased in carriers of paracentric inversions (Mules and Stamberg 1984), chromosomally unbalanced conceptions may be lost early in pregnancy. A direct analysis of chromosomes in gametes would overcome this potential loss of information. In 1986, I reported the first analysis of sperm chromosomes in a man heterozygous for a paracentric inversion of chromosome 7 (Martin 1986). The present report represents the second study of sperm karyotypes in a paracentric-inversion carrier.

A paracentric inversion of chromosome 14 (q24.1q32.1) was ascertained at amniocentesis performed because of advanced maternal age. Subsequent investigation of the family revealed that the inversion is present in the father. The couple had previously had two normal children and one spontaneous abortion at 11 wk gestation (chromosomes were not studied). The study was approved by the university ethics committee, and the sperm donor provided informed consent. The sperm donor had a normal sperm profile, with a volume of 3 ml, concentration of 138×10^6/ml, 80% motility, and forward progression of 8/10. A partial karyotype of the normal and inverted chromosomes 14 in the 41-year-old father is presented in figure 1. Sperm chromosome complements were obtained by fusion of golden hamster oocytes with human sperm and analysis of the Q-banded

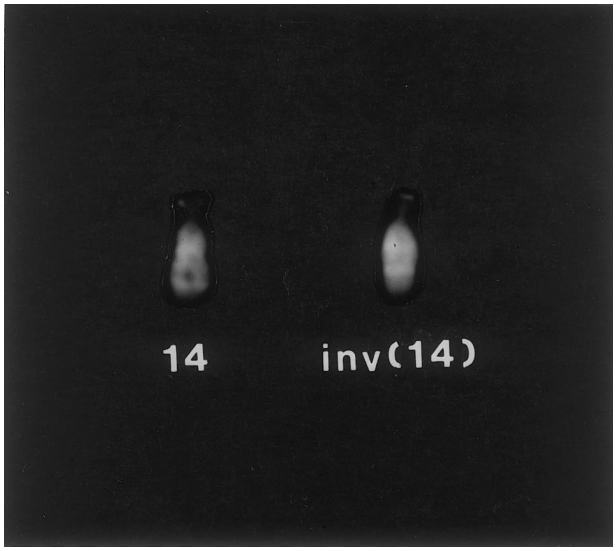

Figure 1 Q-banded human sperm chromosomes demonstrating normal and inverted chromosomes 14.

pronuclear chromosomes. This technique has been described in detail elsewhere (Martin et al. 1994*b*).

A total of 120 sperm chromosome complements were obtained. The results are summarized in the Appendix. Fifty complements (41.7%) contained a normal chromosome 14, whereas 70 (58.3%) had the inverted chromosome 14. This segregation was not significantly different from the expected 1:1 ratio ($\chi^2 = 3.3$, $P > .05$). An example of a sperm chromosome complement with an inverted chromosome 14 is shown in figure 2. The number of X- and Y-bearing sperm was 55 and 65, respectively, which was not significantly different from the expected (60 X- and 60 Y-bearing) number.

None of the spreads contained a recombinant chromosome 14. There were no dicentric, acentric, or duplicated/deficient chromosomes, as would be expected if a crossover had occurred within the inverted segment. Abnormal sperm chromosome complements were present in 10% of the spreads. Three complements had a numeric abnormality, six had a structural abnormality, and three had both. Details of these abnormalities are provided in the Appendix. This frequency of abnormalities is similar to my results from 84 chromosomally normal control donors, who had a mean frequency of 12.8% abnormal sperm (Martin 1995).

In a paracentric-inversion heterozygote, pairing of homologues during meiosis is maximized by the formation of an inversion loop. If an unequal number of crossovers occur within this loop, dicentric and acentric chromosomes are formed. The acentric chromosomes are generally lost in subsequent cell divisions. Dicentric chromosomes tend to break, since the two centromeres are pulled to opposite poles of the cell. Thus, the resulting gametes can have a variety of duplications or deficiencies.

Pettanati et al. (1995) have reviewed 446 cases of paracentric inversions, with 17 cases of recombinant chromosomes reported. The majority of the cases were monocentric chromosomes with duplications or deficiencies. Two maternally inherited cases had offspring with dicentric chromosomes: one case was an inversion of chromosome 9 (q22.1q34.3) (Worsham et al. 1989), and the other was an inversion of chromosome 14 (q24.2q32.3), with breakpoints very similar to those seen in the present case (Mules and Stamberg 1984). The paracentric inversion of chromosome 14 (q24q32) appears to be relatively common in humans (Pettenati et al. 1995), with six index cases reported, including this report. Two of the six cases were paternally inherited. The only case with recombinant chromosomes reported for this inversion is the maternally inherited dicentric case cited above. Because this inversion is ~30% of the length of long arm of chromosome 14, and because chro-

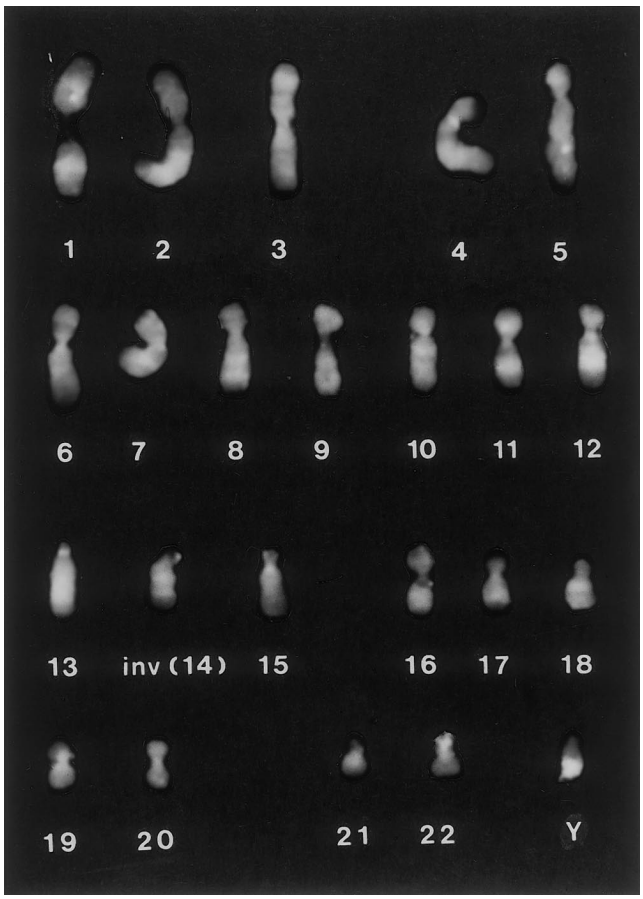

Figure 2 Q-banded karyotype of human sperm chromosomes, 23,Y,inv(14)(q24q32).

mosome 14 has an average of 1.8 chiasmata (Chandley 1975), one would expect that a crossover within the inverted segment would occur ~50% of the time. Because one half of the chromatids are involved in the crossover, ~25% of the gametes should be chromosomally unbalanced. However, my results from sperm chromosome analysis did not detect a single recombinant chromosome in 120 spermatozoa. The lack of any recombinant chromosomes in sperm suggests either that the chromosomes 14 did not pair by an inversion loop or that crossing-over was suppressed within the loop.

Very few meiotic studies of inversion carriers have shed light on these possibilities. The only meiotic analysis of a paracentric inversion was performed in mice, by Poorman et al. (1981). They found that 100% of spermatocytes at early pachytene contained a fully synapsed loop. There have been no cytogenetic meiotic studies of paracentric inversions in humans, and the studies of pericentric inversions have produced conflicting results: some have shown homosynapsis whereas others have demonstrated heterosynapsis and asynapsis (Gabriel-Robez and Rumpler 1994). A recent study reported the analysis of meiotic recombination, by sperm typing in a man carrying a 9q32q34 inversion (Brown et al. 1998). The authors concluded that there was a reduced frequency of recombination within the inversion, suggesting that an inversion loop had not been formed.

Sperm karyotyping has been performed on a total of nine inversion heterozygotes: two with paracentric inversions (Martin 1986; present study) and seven with pericentric inversions. The other paracentric-inversion carrier (7q11q22) also did not have any recombinant sperm, despite expectation that the estimated frequency of recombinant sperm would be 25% (Martin 1986). Of the seven pericentric-inversion carriers, four had no recombinant sperm (Balkan et al. 1983; Jenderny et al. 1992; Martin et al. 1994*a;* Colls et al. 1997), whereas three demonstrated frequencies of 11%, 18%, and 31% recombinant sperm (Martin 1991, 1993; Navarro et al. 1993). The inversions that produced recombinant chromosomes in sperm were all large inversions encompassing more than half the chromosome length. The inversions that did not produce recombinant chromosomes were, in general, smaller, being less than one-third the length of the chromosome. The exception to this was the pericentric inversion of chromosome 20 studied by Jenderny et al. (1992), which failed to produce recombinant sperm despite a relatively large size. However, this may simply be a matter of sample size, because only 26 sperm were analyzed. This dependence on size of the inversion to produce recombinant chromosomes is reminiscent of empirical studies that have suggested that, for a pericentric-inversion heterozygote to produce live-born children with a recombinant chromosome, the inverted segment must involve one-third of the chromosome length (Trunca and Opitz 1977). It is possible that, in small inversions, crossing-over is suppressed within the loop or that pairing is accomplished by heterosynapsis.

Ashley (1988) has formulated a hypothesis that predicts that, if G-light bands are aligned with G-light bands, lack of homology will be recognized and an inversion loop will be formed; in contrast, if the arrangement aligned two G-dark bands or a G-light band with a G-dark band, then lack of homology would not be recognized and heterosynapsis would proceed. de Perdigo et al. (1989) and Gabriel-Robez and Rumpler (1994) have reviewed synaptic data and localization of chromosomal breakpoints in human pericentric inversions and have determined that Ashley's hypothesis is consistent with the data: loops are formed when both breaks occur in G-light bands, whereas heterosynapsis without loop formation or asynapsis occurs when one of the breaks is in a G-dark band. The results of sperm chromosome–complement analysis of pericentric-inversion carriers are also generally consistent with this hypothesis. All three inversions with recombinant chromosomes in sperm had breakpoints in G-light areas (allowing homosynapsis, loop formation, chiasmata, and recombinant chromosomes). Of the four pericentric inversions with no recombinant chromosomes in sperm, all except the paracentric inversion of chromosome 20 (p13q11.2) studied by Jenderney et al. (1992) had at least one breakpoint in a G-dark region. However, as discussed above, 26 sperm complements may have been an insufficient sample size for detection of recombinant chromosomes. Of the two paracentric inversions studied by sperm karyotyping, both had breakpoints in G-light areas, yet neither had recombinant chromosomes in sperm. Thus, data from sperm karyotyping of the two paracentric inversions studied to date do not agree with Ashley's hypothesis. It may be that, for paracentric inversions, small size is a major detriment to homologous pairing and crossing-over.

The possibility of an interchromosomal effect for inversion heterozygotes has been raised, because children with unrelated chromosomal abnormalities have been born to inversion carriers. Canki and Dutrillaux (1979) described two cases of familial paracentric inversions associated with sex-chromosomal aneuploidy, and Fryns and van den Berghe (1980) reported a child with trisomy 21 who was born to a father heterozygous for a paracentric inversion. However, my data do not support such an effect, because the frequency of chromosomal abnormalities was not increased in either the case that was heterozygous for a paracentric inversion of chromosome 14 or the case that was heterozygous for a paracentric inversion of chromosome 7 (Martin 1986). Similarly, previous studies of sperm chromosomes in men with pericentric inversions have not demonstrated any increased frequency of abnormalities in other chromo-

somes (Balkan et al. 1983; Martin 1991, 1993; Navarro et al. 1993; Colls et al. 1997). Colls et al. (1997) specifically searched for an interchromosomal effect involving chromosome 21, by performing FISH analysis of >10,000 sperm nuclei from a case that was heterozygous for a paracentric inversion of chromosome 9, as well as by analyzing >300 sperm complements. Even these large sample sizes did not uncover an interchromosomal effect.

Further studies of sperm chromosomes in paracentric-inversion carriers are required, because only two have been reported. Information from these studies will provide estimates of the frequency of chromosomally unbalanced gametes. These studies will also help us to elucidate some of the factors that influence the production of recombinant chromosomes at meiosis.

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I would like to thank Drs. I. Lange and N. Spence for referring the family, Dr. J. Chernos for lymphocyte chromosome analysis, Evelyn Ko and Leona Barclay for expert technical assistance, Debbie Bell for preparing the manuscript, and the family for their cooperation and interest in the study. The support of the Medical Research Council of Canada (grant MA-7961), the Alberta Heritage Fund for Medical Research, and the Alberta Children's Hospital Research Foundation is gratefully acknowledged.

R. H. MARTIN

Department of Medical Genetics, University of Calgary, and Department of Genetics, Alberta Children's Hospital, Calgary

Appendix

Sperm Chromosome Complements in a Male Heterozygous for a Paracentric Inversion of Chromosome 14 (q24q32)

Normal sperm (n = 108):
- Normal chromosome 14, n = 44
- Inverted chromosome 14, n = 64

Abnormal sperm (n = 12):
- Normal chromosome 14, n = 6
- Inverted chromosome 14, n = 6

Details of the 12 abnormal sperm complements:
- Numerical:
  - 22,Y,−6
  - 22,X,−13
  - 22,X,−18
- Structural:
  - 23,X,chrb(12)(q14or15)
  - 23,X,chte(19;20)(q13;1or13.2;q13.3)(tr, incomplete)
  - 23,X,chrb(12)(q12or13),[−14,+inv(14)]
  - 21,Y,chte(1;3;17)(cx),[14,+inv(14)]
  - 22,X,dic(7;18)(pter→cen::p11.3→qter), [−14,+inv(14)]
  - —,Y,MB+R,[−14,+inv(14)]
- Both numerical and structural:
  - 24,XiY,+ace
  - 21,X,−22,dic(5;7)(p15;q35or36)+ace, [−14,+inv(14)]
  - 22,Y,−17,chrg(7)(q21,chtg(9)(p21), [−14,+inv(14)]

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Address for correspondence and reprints: Dr. R. H. Martin, Genetics Department, Alberta Children's Hospital, 1820 Richmond Road SW, Calgary, Alberta, Canada. T2T 5C7. E-mail: renee@ach.ucalgary.caThis letter is dedicated to R. Brian Lowry, clinic geneticist, on the occasion of his retirement.

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A Program for the Monte Carlo Evaluation of Significance of the Extended Transmission/ Disequilibrium Test

To the Editor:

The extended transmission/disequilibrium test (ETDT) package (Sham and Curtis 1995*a*) calculates three likelihood ratio transmission/disequilibrium test (TDT) statistics for a multiallelic marker: an allelewise statistic that assumes an allele-specific effect on transmission distortion from heterozygous parents, a genotypewise statistic that allows an independent effect for each parental genotype, and a goodness-of-fit statistic that assesses the adequacy of the allelewise model compared with the genotypewise model. Since a marker with m alleles will have $m(m-1)/2$ possible heterozygous genotypes, there may be very few observations of certain genotypes when m is large, so that P values based on asymptotic χ^2 distributions may be inaccurate, especially for the latter two tests. The χ^2 approximation is more likely to be adequate for the allelewise test unless there are very few observations or very many alleles. Since we have proposed the allelewise test to be the most useful test in most circumstances, we did not at first implement a Monte Carlo approach (e.g., Sham and Curtis 1995*b;* Cleves et al. 1997; Kaplan et al. 1997*a;* Miller 1997) in the ETDT program to obtain empirical P values. We now believe that it is useful to have such an option, and we have therefore extended the ETDT package to include a program called "MCETDT," which carries out such a procedure to obtain empirical P values for all three tests.

Table 1

Comparison of Different TDT Statistics Applied to Schizophrenia and D22S278

Type	χ^2	df	Asymptotic P Value	Empirical P Value	SE (P)
ETDT:					
Allelewise	22.025	10	.0151	.0142	.0012
Genotypewise	66.228	30	.0002	.0009	.0003
Goodness-of-fit	44.203	20	.0015	.0085	.0009
MATLAB:					
Spielman-Ewens	22.358	10	.0134	.0115	.0011
Stuart	18.976	10	.0406	.0202	.0014

MCETDT works by taking input to the LRTDT program of the ETDT package and then generating transmissions at random according to the null hypothesis of equal transmission, conditional on the observed parental genotypes. The three statistics are then calculated by LRTDT in the usual fashion for multiple replicates of simulated data, and the proportion of times each statistic is equal to or greater than the corresponding one from the real data provides an empirical *P* value for that test. If *n* replicates are used to estimate this *P* value, then the standard error (SE) of the estimate is $\sqrt{P(1-P)/n}$.

Example application. In a large collaborative linkage study of schizophrenia, data on transmissions of alleles at D22S278 to subjects affected with schizophrenia were available in 574 families (Vallada et al. 1998). Asymptotic and empirical results based on 10,000 replicates were calculated by use of MCETDT and, for comparison, the Spielman-Ewens (Spielman and Ewens 1996) and Stuart (1955) statistics were also calculated by use of MATLAB.

The results are shown in table 1; the asymptotic *P* values for the genotypewise and goodness-of-fit tests are anticonservative. Nevertheless, the empirical *P* value for the genotypewise test of ETDT is more highly significant than the results of any other method of analysis and hence provides the strongest support for transmission distortion.

There have been extensive discussions regarding the analysis of multiallelic TDT data (e.g., Kaplan et al. 1997*a*, 1997*b*; Miller 1997; Sham 1997; Lazzeroni and Lange 1998). Monte Carlo methods provide a useful way to obtain empirical *P* values for tests conducted on sparse data sets. (The new program is provided as part of the ETDT package, which is freely available at http://www.gene.ucl.ac.uk/users/dcurtis/software.html).

Acknowledgments

The authors are grateful to Dr. Mike Miller for helpful discussions in his analysis of GAW10 data during development of the program and for kindly providing his MATLAB macros for calculating empirical *P* values for the Spielman-Ewens and Stuart χ^2 statistics. We thank Richard Spielman and an anonymous referee for helpful comments. Jing Hua Zhao is supported by Wellcome Trust grants 043279 and 055379.

JING HUA ZHAO,[1] PAK CHUNG SHAM,[1] AND DAVID CURTIS[2]

[1]*Department of Psychological Medicine, Institute of Psychiatry, De Crespigny Park, and* [2]*Academic Department of Psychological Medicine, St. Bartholomew's and Royal London School of Medicine and Dentistry, Royal London Hospital, Whitechapel, London*

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Address for correspondence and reprints: Dr. Jing Hua Zhao, Department of Psychological Medicine, Institute of Psychiatry, De Crespigny Park, London SE5 8AF, United Kingdom. E-mail: j.zhao@iop.bpmf.ac.uk

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Genomewide Transmission/Disequilibrium Testing: A Correction

To the Editor:

This response is to address comments made by several investigators regarding the sample sizes required for genomewide transmission/disequilibrium testing (TDT) in my earlier article (Camp 1997). There are two main comments: first, the issue of independence of parental transmissions and, second, the issue of the definition for the random variable (RV) B_i (for detailed explanations, see Risch and Merikangas 1996; Camp 1997).

Table 1

Sample Sizes Necessary to Gain 80% Power in a Genomewide TDT, for the C and R&M Definitions of RV B_i and with Consideration of Dependence between Parental Transmissions

	Sample Size Required[a]											
	Multiplicative			Additive			Recessive			Dominant		
		R&M			R&M			R&M			R&M	
γ and p	C; No	No	Yes	C; No	No	Yes	C; No	No	Yes	C; No	No	Yes
4.0:												
.01	523	1,097	1,097	549	1,137	1,135	4.3×10^6	4.3×10^6	4.3×10^6	562	1,158	1,155
.10	86	150	150	123	200	197	5,056	5,559	5,643	153	239	234
.50	103	103	103	222	222	220	205	205	208	712	712	698
.80	291	222	222	663	560	558	337	263	264	9,873	9,476	9,388
2.0:												
.01	4,154	5,817	5,817	4,154	5,817	5,817	3.8×10^7	3.9×10^7	3.9×10^7	4,317	6,014	6,008
.10	533	695	695	533	695	695	43,331	44,808	45,065	766	960	954
.50	340	340	340	340	340	340	949	949	959	1,861	1,861	1,840
.80	750	640	640	750	640	640	976	851	855	22,728	22,126	21,995
1.5:												
.01	16,008	19,300	19,300	15,550	18,794	18,801	1.5×10^8	1.5×10^8	1.5×10^8	16,487	19,829	19,821
.10	1,908	2,216	2,216	1,485	1,757	1,762	1.7×10^5	1.7×10^5	1.7×10^5	2,554	2,911	2,901
.50	949	949	949	464	464	466	3,078	3,078	3,099	4,599	4,599	4,568
.80	1,833	1,662	1,662	816	701	703	2,553	2,351	2,359	51,914	51,003	50808

[a] "No" denotes that dependence of parental transmissions were not accounted for; "Yes" denotes that dependence of parental transmissions were accounted for.

Fengzhu Sun and Rong Cheng have kindly pointed out that the assumption that I made (Camp 1997) about the independence of parental transmissions is incorrect. This assumption of independence is valid only under the multiplicative mode of inheritance (MOI) (Knapp et al. 1993). Sun and Cheng have shown that, for the number of samples that are required in order to perform a genomewide TDT using singletons, the correct version of the formula given in Camp (1997) is

$$N \geq \frac{\left[z_{1-\alpha}\sqrt{2pq} - z_{\beta}\sqrt{S - 2h_s^2(2\tau_s - 1)^2}\right]^2}{2h_s^2(2\tau_s - 1)^2},$$

where $S = pq[(1-q^2)f_2 + (1-2pq)f_1 + (1-p^2)f_0]/K$, p is the frequency of the putative disease allele ($q = 1 - p$), h_s is the probability that a parent will be affected, given that she or he has a single affected offspring (SAO), τ_s is the conditional probability that the disease allele is transmitted, given that the parent is heterozygous and has an SAO, and K is the population prevalence of the disease. Correct formulas for h_s, τ_s, and K can be found in Camp (1997).

Both I and Sun and Cheng have recalculated, using the revised formula given above, and have found that the new sample sizes are extremely similar to those shown in table 3 in Camp (1997). The average discrepancy, although not always in the same direction, was ~0.65%. I agree with Sun and Cheng that it is important that the correct formula be available in the literature (hence it is included it here); however, I think that it is equally important and interesting to note that the incorrect assumption of independence of parental transmissions that I made (Camp 1997) has little impact on the sample size calculated (see table 1). A similar conclusion is also true in the case of genomewide TDT with affected sib pairs (not shown).

A second issue—and one with greater impact on sample size—regards the choice of definition for the RV B_i. Recall that in Risch and Merikangas (1996) the RV B_i takes the values $+(h_s)^{-\frac{1}{2}}$, $-(h_s)^{-\frac{1}{2}}$, and 0 for the cases in which the parent is heterozygous and transmits the disease allele, is heterozygous and transmits the normal allele, or is homozygous, respectively; Camp (1997) alternatively used $+(2pq)^{-\frac{1}{2}}$, $-(2pq)^{-\frac{1}{2}}$, and 0. These two definitions for the RV B_i result in two different TDT statistics (in which the subscripts "R&M" and "C" denote "Risch and Merikangas" and "Camp," respectively): $T_{\text{R\&M}} = \{(b-c)^2/[h_s(2N)]\}$ and $T_{\text{C}} = \{(b-c)^2/[2pq(2N)]\}$, where b and c are the standard symbols used, in the TDT statistic, for the number of times that the disease and the normal alleles, respectively, are transmitted from heterozygous parents and where N is the total number of trios collected for study.

The denominator in the true TDT statistic is $(b + c)$—that is, the total number of heterozygous parents (M, say) within the $2N$ total possible parents in the N trios collected. Thus, the two different RVs for B_i are effectively using two different estimates for M: $h_s(2N)$ and $2pq(2N)$. Now, it is true that $E(M) = h_2(2N)$, as

used by Risch and Merikangas. However, the calculation of h_s includes information on the genotypic relative risks (GRRs), or γ, which are unknown. Hence the RV specified by Risch and Merikangas (1996) could never be used to actually perform the test, since the values assigned to the RV B_i assume knowledge of the values for the GRRs. This was the rationale for changing the RV B_i in Camp (1997) to one that was not dependent on unknown parameters. Under the null hypothesis ($\gamma = 1$), $h_s = 2pq$; hence, the false-positive rates under both definitions will be as expected under the true TDT statistic. For power, however, the definition as given by Risch and Merikangas (1996) estimates power more accurately (M. M. Iles, personal communication). It is also worth noting that, when $p = q = .5$, it is also the case that $h_s = 2pq$, and so discrepancies between the two methods are small when p and q are near equifrequent or when γ is near 1.0, and discrepancies are larger for those cases in which p and q are more divergent and when γ is large. I believe that the RV B_i as used by Risch and Merikangas (1996) leads to the correct sample sizes for a given power but that their B_i is inappropriately parameterized for use in a test statistic. The RV given by Camp (1997) was an attempt to gain both a tool for power and a valid RV for use in a TDT test statistic. Table 1 illustrates the sample sizes necessary to gain 80% power in a genomewide TDT using the two different types of RV B_i defined above and for various values for γ and p. For each MOI, the third column illustrates (using B_i as defined by Risch and Merikangas [1996]) the sample sizes when the dependence of parental transmissions are considered, as discussed above. The formula for this column is as follows:

$$N \geqslant \frac{\left[z_{1-\alpha} - z_{\beta}\sqrt{S' - 2h_s(2\tau_s - 1)^2}\right]^2}{2h_s(2\tau_s - 1)^2},$$

where $S' = S/h_2$.

Qualitatively, the results from all the alternatives discussed above are concordant—that is, they all indicate that genomewide TDT analysis could be useful as an alternative to classical affected-sib-pair linkage analysis for localization of genes of small effect in complex disease. Quantitatively, with respect to the RV B_i, results differ for large γ and extreme values for p. It is now left to the reader to determine whether either of these issues is worthy of further investigation.

NICOLA J. CAMP
Genetic Research, Intermountain Health Care, and Genetic Epidemiology, Department of Medical Informatics, University of Utah, Salt Lake City

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Address for correspondence and reprints: Dr. Nicola J. Camp, Genetic Research, 391 Chipeta Way, Suite C, Salt Lake City, UT 84108. E-mail: nicki@gr.ihc.com

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