Letters to the Editor

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Corrections to "Linkage Strategies for Genetically Complex Traits. III. The Effect of Marker Polymorphism on Analysis of Affected Relative Pairs" (Am. J. Hum. Genet. 46:242–253, 1990)

To the Editor:

A number of errors in the above-named paper have been brought to my attention. Martin Farrall has pointed out three errors to me: (1) in table 2, for arrangement 5 in the lod-score column, the z's in the denominator should be α's; (2) in table 4, for mating type 4, the equals sign (=) should be a plus (+) sign; and (3) the maximum-likelihood estimates of z_2, z_1, and z_0 for the data in table 1, given in the text underneath the table, are incorrect; the correct values are $\hat{z}_2 = .47$; $\hat{z}_1 = .43$; $\hat{z}_0 = .10$, and MLS = 2.79. Formula (4) for the E-M algorithm is correct but was not carried fully to completion to get the maximum.

Peter Holmans and David Clayton have pointed out a more serious error. The curves given in figures 3–7, corresponding to scheme 1, understate the actual expected lod scores and power; this was due to a programming error. The figures for scheme 2 are correct. The corrected relationship between percent maximum possible EMLS and PIC is given in table 1 (corresponding to the original fig. 3). Similarly, corrected values for EMLS and power as a function of PIC for 100 relative pairs and $\lambda_0 = 3$ are given in table 2 (corresponding to the original figs. 4 and 6), and corrected values for EMLS and power as a function of PIC for 40 relative pairs and $\lambda_0 = 10$ are given in table 3 (corresponding to the original figs. 5 and 7).

Furthermore, it also needs to be clarified that for the simulations described for sib pairs, with results given in figures 2–7 (and the new tables 1–3 described above), the MLS procedure, as described on page 243 was not precisely followed. The lod-score test was actually based on the assumption that $z_1 = \frac{1}{2}$, so that only z_0 (and, equivalently, $z_2 = \frac{1}{2} - z_0$) was estimated; in other words, a single parameter (z_0), rather than two parameters (z_1 and z_0), was estimated. This can be achieved using a formula similar to equation (4) on page 243 but focusing only on the expected number of pairs sharing two or zero marker alleles, excluding those sharing one (assuming $z_1 = \frac{1}{2}$); the probabilities of sharing two versus zero alleles are estimated from these expectations in the E-M algorithm. These probabilities are then divided by 2 to obtain estimates of z_0 and z_2. For the example in table 1 on page 243, the estimates for z_2 and z_0, constraining $z_1 = \frac{1}{2}$, are .423 and .077, respectively, giving an

Table 1

Percent of Maximum Possible EMLS as a Function of PIC for Sibs, and Second-Degree and Third-Degree Relatives, under Scheme I

	Percent of Maximum EMLS for		
PIC	Sibs	Second-Degree Relatives	Third-Degree Relatives
.38.....	.26	.13	.09
.59.....	.41	.21	.15
.70.....	.50	.28	.21
.77.....	.57	.35	.26
.86.....	.70	.49	.37
.89.....	.75	.56	.44
.95.....	.86	.73	.63

Table 2

EMLS and Power (1 − β) as a Function of PIC for 100 Pairs of Sibs and Second-Degree and Third-Degree Relatives, for $\lambda_0 = 3$, using Scheme 1

	SIBS		SECOND-DEGREE RELATIVES		THIRD-DEGREE RELATIVES	
PIC	EMLS	1 − β	EMLS	1 − β	EMLS	1 − β
.38.....	1.47	.09	.75	.01	.61	.01
.59.....	2.25	.25	1.20	.05	.94	.03
.70.....	2.80	.39	1.61	.10	1.25	.06
.77.....	3.15	.50	1.99	.18	1.53	.11
.86.....	3.90	.67	2.86	.42	2.27	.22
.89.....	4.13	.72	3.25	.52	2.63	.34
.95.....	4.75	.83	4.23	.73	3.84	.65
.99.....	5.47	.92	5.90	.93	6.29	.94

MLS of 2.62. The assumption of $z_1 = ½$ is reasonable because deviation from this value is caused only by significant dominance variance in the genetic model; evidence for such dominance variance (as provided by greater sibling than offspring risk) is lacking for most common familial traits being analyzed. The single-parameter (z_0) test increases the power compared with a two-parameter test.

As can be seen from the tables, expected lod scores and power are substantially greater for scheme 1 (typing relative pairs only) than originally reported, especially at lower values of PIC. In fact, in theory, typing pairs only may be more efficient than typing additional relatives, even at small PIC values. For example, the expected lod score is decreased only by, at most, ⅓ for sib pairs when scheme 1 is used, whereas only half the number of individuals is typed. For second-degree relatives, the expected lod score is typically decreased by less than 60% but requires typing only 40% of the number of relatives. For third-degree relatives, the lod score is decreased by less than 70% but requires typing only 25% of the number of relatives.

However, practical considerations also influence the decision about typing additional relatives. The new highly polymorphic molecular markers (e.g., minisatellites and microsatellites) are the most useful for linkage studies but can also lead to ambiguities in determining whether two individuals have matching bands (alleles); usually, it is necessary to study additional family members (such as parents) to confirm that two relatives (e.g., sibs) actually share a common allele or alleles. Furthermore, identity-by-state methods, that is, those that use only affected pairs without other relatives (such as scheme 1, described above)

Table 3

EMLS and Power (1 − β) as a Function of PIC for 40 Pairs of Sibs, Second-Degree Relatives, and Third-Degree Relatives, for $\lambda_0 = 10$, using Scheme 1

	SIBS		SECOND-DEGREE RELATIVES		THIRD-DEGREE RELATIVES	
PIC	EMLS	1 − β	EMLS	1 − β	EMLS	1 − β
.38.....	1.12	.01	.89	.00	1.01	.01
.59.....	1.77	.10	1.49	.02	1.68	.12
.70.....	2.17	.24	2.01	.22	2.25	.24
.77.....	2.54	.34	2.49	.28	2.80	.40
.86.....	3.13	.53	3.53	.64	4.13	.74
.89.....	3.40	.61	3.99	.78	4.81	.86
.95.....	3.89	.73	5.17	.92	6.80	.98
.99.....	4.54	.84	6.94	.99	10.45	1.00

may be sensitive to incorrect specification of marker-allele frequencies, as well as to population stratification with regard to those allele frequencies (author's unpublished data). Therefore, scheme 1 does not provide the robustness (i.e., false-positive results have a higher probability) that scheme 2 provides, because scheme 2 is based entirely on identity by descent and is uninfluenced by allele frequencies.

Perhaps the most efficient overall strategy would be a two-stage process involving both scheme 1 and scheme 2. First, only the affected relative pairs would be typed at an array of polymorphic markers and analyzed for linkage (i.e., scheme 1). Then, for only those loci that show at least suggestive evidence of linkage would the other relatives be typed and entire families be analyzed (i.e., scheme 2).

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Screening Duchenne and Becker Muscular Dystrophy Patients for Deletions in 30 Exons of the Dystrophin Gene by Three-Multiplex PCR

To the Editor:

Deletion mutations of the dystrophin gene may cause either the severe Duchenne muscular dystrophy (DMD) or the milder, allelic Becker muscular dystrophy (BMD) and are clustered in two high-frequency-deletion regions (HFDRs) located, respectively, 500 kb and 1,200 kb downstream from the 5′ end of the gene. The screening of deletions for postnatal and prenatal diagnosis of DMD/BMD is carried out by using Southern blotting with cDNA probes or PCR. Primers for the latter procedure have been devised by Chamberlain et al. (1989) and by Beggs et al. (1990) for two different DNA amplifications, each including nine exons, located in the two HFDRs of the dystrophin gene. Five additional exons can be amplified for the same purpose (Kunkel et al. 1991). We have now modified these three reactions, using primers that we designed—i.e., 20F, 5′ GTGTTAATGCAGATAGCATCAAAC 3′; 20R, 5′ ACAAATTTTTAACTGACTTTTAATTG 3′; 22F, 5′ TTGACACTTTGCCACCAATGCGCTATC 3′; and 22R, 5′ CAATTCCCCGAGTCTCTGCTCCATG 3′—in addition to the primers reported elsewhere by other groups (Beggs et al. 1991; Meng et al. 1991).

The Chamberlain reaction using primers for the original nine exons plus primers for exons 20, 22, and 29 yielded the results shown in figure 1*a*, under the following conditions: 94°C for 40 s, 51°C for 40 s, 65°C for 5 min, for 25 cycles. We also added primers for the amplification of exon 21 to the Beggs reaction, using the following conditions: 94°C for 30 s, 54°C for 30 s, 65°C for 4 min, for 25 cycles. In the third reaction, by combining primers already reported and by modifying the annealing temperature to 53°C (fig. 1*b*) we amplified simultaneously the following eight exons: brain promoter region (i.e., Pb) and exons 49, 16, 41, 32, 42, 34, and 46. These exons partially fill the gap between the two HFDRs of the dystrophin gene and therefore allow the identification of deletions in this region of the gene, without using Southern blotting and cDNA probes. In all cases, the PCR products were analyzed on a 3% regular agarose + 1% NuSieve agarose gel (FMC).

The three PCR reactions just described allowed the analysis of a total of 30 exons and led, in our laboratory, to the identification of three additional deletions involving the following exons: (*a*) 42 only, (*b*) 28–42, and (*c*) 16 only, none of which were detected with the two original multiplex reactions (Chamberlain et al. 1989; Beggs et al. 1990). Therefore, the three modified multiplexes detected 95 of the 96 deletions we identified among the 152 patients we studied so far by using Southern analysis and cDNA probes. The only deletion that remained undetected with this system involves exons 22–25 and generates the junction fragment described elsewhere (Covone et al. 1991).

The percentage of deletion mutations among our DMD/BMD patients amounts to 63%, which is in agreement with similar estimates from other laboratories. When field-inversion gel electrophoresis is coupled to Southern analysis, the detection rate of deletion and duplication mutations reaches 65% (Den