Analysis of Meiotic Segregation using Single-Sperm Typing: Meiotic Drive at the Myotonic Dystrophy Locus

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Running Title: Segregation at Myotonic Dystrophy Locus

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Summary

Meiotic drive at the myotonic dystrophy (DM) locus has recently been suggested as being responsible for maintaining the frequency, in the human population, of DM chromosomes capable of expansion to the disease state. In order to test this hypothesis, we have studied samples of single sperm from three individuals heterozygous at the DM locus, each with one allele larger and one allele smaller than 19 CTG repeats. To guard against the possible problem of differential PCR amplification rates based on the lengths of the alleles, the sperm were also typed at another closely-linked marker whose allele size was unrelated to the allele size at the DM locus. Using statistical models specifically designed to study single-sperm segregation data, we find no evidence of meiotic segregation distortion. The upper limit of the two-sided 95% confidence interval for the estimate of the common segregation probability for the three donors is at or below 0.515 for all models considered, and no statistically significant difference from 0.5 is detected in any of the models. This suggests that any greater amount of segregation distortion at the myotonic dystrophy locus must result from events following sperm ejaculation. The mathematical models developed make it possible to study segregation distortion with high resolution using sperm typing data from any locus.

Introduction

The transmission of genetic traits from parent to offspring is expected to conform to Mendel's laws. When the transmission ratios among offspring are altered from those expected, a number of explanations are possible, including alterations in chromosomal segregation patterns and differential effects on viability. In a few experimental organisms, altered chromosome segregation has been shown to be the basis for the distortion of the expected transmission ratios (reviewed in Lyttle 1993; Silver 1993).

Recently, Carey et al. (1994) proposed that a specific class of normal alleles at the myotonic dystrophy (DM) locus are transmitted preferentially. DM is the most common form of muscular dystrophy in adults and results from a trinucleotide repeat (CTG/CAG) expansion (Brook et al. 1992; Fu et al. 1992; Mahadevan et al. 1992). It has previously been proposed, based on linkage disequilibrium studies, that all disease-causing alleles are ultimately descended from a single founder chromosome (Imbert et al. 1993; Neville et al. 1994). This hypothetical founder allele is believed to have given rise to a class of normal DM alleles with greater than 19 repeats which are especially susceptible to further expansion into the disease causing range (> 50 repeats).

Noting that the significant loss of reproductive fitness that accompanies severe DM is difficult to reconcile with the high incidence (around 1 in 8000) of this disease, Carey et al. (1994) studied segregation of normal alleles in families with no known history of DM. In heterozygous normal males with one allele having ≥ 19 CTG repeats and the other allele having < 19 repeats, they found segregation distortion in favor of the larger allele. This effect appeared to them to be more pronounced in men than in women. They suggested that a meiotic drive process may be responsible for maintaining the frequency of DM chromosomes capable of expansion to the disease state in the human population. Similar results were obtained by Gennarelli et al. (1994). However, both data sets were reanalyzed by Hurst et al. (1995) who concluded that, on the contrary, there was no evidence for male-specific meiotic drive of large normal DM alleles. Shaw et al. (1995) also reported segregation distortion in favor of larger DM alleles and suggested meiotic drive as an explanation. However, they reported female-specific meiotic drive rather than male-specific.

Indirect evidence for equal meiotic segregation of large and small DM alleles has come from small pool PCR analysis of DNA isolated from total sperm compared to blood (Monckton et al. 1995). However it was reported that small alleles may have a PCR amplification advantage over large alleles. Such an advantage could mask an excess of larger alleles. We have used sperm typing (Li et al. 1988) to directly examine the specific question of whether a deviation from expected transmission ratios is a result of a prefertilization process, including altered chromosomal segregation patterns during meiosis or differential viability during or immediately following spermiogenesis. The analysis of sperm-typing data can, however, be confounded by experimental error. Thus a number of mathematical models have been developed for the analysis of genetic recombination using sperm typing (Cui et al. 1989; Goradia et al. 1991; Lazzeroni et al. 1994; Schmitt et al. 1994). Because Mendelian segregation of alleles is assumed in these linkage models, they cannot be used to address questions about the segregation of DM alleles using sperm-typing data. Williams et al. (1993) applied sperm typing to the analysis of meiotic segregation patterns of the wild type and ΔF508 alleles at the cystic fibrosis (CF) locus.

Data from a sample of 539 sperm typed only for the CF locus suggested distorted segregation. However, when the segregation frequencies of CF and a co-amplified non-linked marker were compared in 177 sperm, no differences were detected. These studies did not take into account any of the PCR errors that are considered in the mathematical models described above, such as differential amplification efficiency or contamination. We have studied the segregation patterns of large and small alleles at the DM locus as well as at tightly linked dinucleotide repeat markers to eliminate possible bias in amplification efficiency due to allele size. We have also developed specific mathematical models that do not assume Mendelian allele segregation ratios, but do take experimental errors into consideration. Analysis of three sperm donors, each carrying one DM alleles with > 19 repeats and one with < 19 repeats, showed no evidence for altered chromosomal segregation patterns or pre-ejaculatory sperm viability.

Materials and Methods

Samples

All three semen donors were caucasian and judged to have normal sperm by an andrologist. Donor 1 (5048) was 43 years old. Donor 2 (5041) was 44 and Donor 3 (5012) was 38 years of age. Donors 1, 2 and 3 each had a 5 repeat DM allele and a 37, 27 or 20 repeat allele respectively (see Leeflang and Arnheim, 1995 for a description of the unusual 37-repeat allele).

PCR

Multiplex PCR was performed as described in Leeflang and Arnheim (1995), including primers for the linked markers D19S112 (Jansen et al. 1992) or D19S207 (Jansen et al. 1993).

First round PCR included 0.2 μM of each linked marker primer set. For D19S112, MDQH 128 (5′ GCCAGCCATTCAGTCATTTGA) (Imbert et al. 1993) and MDQH 131 (5′ CTGAAAGACACGTCACACTGGT) (Jansen et al. 1992) were used. For D19S207, MDDRP1 (5′ TGCGGTGTTTGAACCCTCGCTG) and MDDRP2 (5′ ACTGCACTGCAGCCTGAGTGAC) (Jansen et al. 1993) were used.

Second round PCR was performed using 2 μL of first round PCR product and using the same reagent concentrations as the first round, except for the use of 1.0 μM of each heminested set of primers MDQH 128 and MDQH 129 (5′ ATGCACGTGTGCGCTCATACA) (Imbert et al. 1993) or MDDRP1 and MDDRP3 (CAAAGCGACGAGACTCTG).

The thermal cycling program for D19S112 was 5 minutes, 95°C, 30 cycles of 1 minute, 95°C, 1 minute 48°C and 1 minute 72°C, followed by 5 minutes, 72°C. The thermal cycling program for D19S207 was similar, except using a 52°C annealing temperature and 15 rounds of PCR.

Linked Markers

Allele sizes at the linked marker loci do not bear any particular relation to those at the DM locus (see Table 1). Thus, the marker should segregate with DM, but should not be affected by the same pattern of differential amplification. If there is differential amplification based on allele size, it should be unmasked by the difference between observed segregation at the DM locus and observed segregation at the marker locus. For donors 1 and 3, the marker used was D19S207, while for donor 2, the marker used was D19S112 (see Table 1).

PCR products were analyzed on 8% non-denaturing polyacrylamide gels, post-stained with ethidium bromide.

Mathematical Models

Table 2 summarizes the main models of interest. The simplest model considered is the general multinomial (Model 1) in which each data event has a corresponding probability that varies from individual to individual. The only constraints are that the probabilities are nonnegative and sum to 1 for each individual. The estimated parameters are simply the observed proportions of each data event. This model could be potentially useful for assessing the goodness of fit of the sperm-typing models below.

Our sperm-typing models are based on the model of Cui et al. (1989), which incorporates explicit error parameters for the PCR process. In our most fully parametrized sperm-typing model (Model 2), we have 11 parameters for each individual: (i) the segregation probability s for that individual's larger DM allele; (ii) the Fluorescent Activated Cell Sorting (FACS) deposit probabilities γ_i of i sperm present in a tube, $i = 0, 1, 2$, where $\gamma_0 + \gamma_1 + \gamma_2 = 1$; (iii) the probabilities that particular polymorphic regions will be amplified to a detectable level, α_A, α_a, α_B, and α_b, where A represents the larger DM allele, B is the marker allele linked with the larger DM allele, a is the smaller DM allele, and b is its linked marker allele; and (iv) the probabilities of contamination events, β_A, β_a, β_B, and β_b. Note that the larger DM allele A varies in size for the three individuals, while the marker alleles B and b not only vary in size, but actually refer to a different marker for donor 2 than for donors 1 and 3. Only a refers to the same allele for all three donors (see Table 1). There is assumed to be no recombination between the DM locus and the associated marker locus. In this model, all eleven of the parameters are allowed to vary from individual to individual. We give expressions for the probabilities of the possible observed events in terms of these parameters in the Appendix.

In order to properly analyze segregation, we want to be certain that we have adequately modeled the data. Model 2 is an overparametrization of the data, as discussed below. There are clearly some sub-models of the full sperm-typing model that are of interest, for instance, the model in which the segregation parameters for the three donors are assumed equal, $s_1 = s_2 = s_3$ (Model 3), or the one in which all these parameters are assumed equal to 0.5 (Model 4). The latter model is of interest for hypothesis testing. Other possible models would include setting some or all of the contamination parameters equal, and similarly for the amplification and deposit parameters.

From the many possible models, we have selected certain parsimonious ones using a backwards stepwise method. That is, starting from the full sperm-typing model (Model 2), at each step, a number of sub-models with one fewer parameter than the current model are compared, and the one with the highest log-likelihood is chosen as the current model for the next step (all logarithms are base e). This creates a sequence of nested models. Two criteria are considered for choosing the best model from among these: the Akaike information criterion (AIC) (Akaike 1972) and the Bayesian information criterion (BIC) (Schwartz 1978), where $AIC = -2L + 2k$, and an equivalent formulation of BIC would be $BIC = -2L + k \log n$, L is the log-likelihood, k is the number of model parameters, and n is the number of observations. For a given criterion, the model in the nested sequence of models that minimizes that criterion would be selected. Ordinarily, there will be several different models of the same size that all have approximately the same value of the criterion as the selected model. Thus, the procedure should not be viewed as selecting a unique best model, but ordinarily several roughly equivalent models should be reported.

AIC can be thought of as imposing a penalty of 2 against $-2L$ for each parameter in the model, while the more stringent penalty imposed by BIC is $\log n$ per parameter. Thus, the AIC criterion will tend to choose a model with a larger number of parameters. Asymptotic comparisons of prediction error in the context of normal regression suggest that AIC is the more effective criterion when there are a large number of parameters of roughly equal contribution, while BIC is more effective when there are some very important parameters and some unimportant ones (Speed and Yu 1993). The latter situation seems more applicable here, as for instance, the separate contamination parameters do not seem to contribute much, while the deposit and amplification parameters seem to have more effect. Thus, although we are not in the same setting described by Speed and Yu (1993), we might speculate that the BIC criterion would be more appropriate.

Results

The data are presented in Tables 3 and 4. Some of donor 3's sperm were typed only at the DM locus (see Table 4). These data have been properly incorporated into the analysis via the EM algorithm (Dempster et al. 1977) for likelihood maximization with incomplete data. Thirty-one anomalous observations, in which obvious external contamination was detected in addition to the alleles under study, are excluded from the data presented here. The statistical analysis described was performed both including and discarding these anomalous data points, and no qualitative difference resulted.

Table 5 displays the results of fitting the full sperm-typing model (Model 2) to the data by maximizing the likelihood using the EM algorithm. Estimated parameter values are given along with estimated asymptotic standard deviations obtained by inverting the observed Fisher information matrix.

A higher amplification rate for smaller alleles does not appear to be a great problem overall in these data. For donor 2, under Model 2, the smaller DM allele did have a significantly higher estimated amplification probability than the larger one, 0.88 vs. 0.80, $p = 0.036$, but this was not the case for other allele pairs, nor did it hold for the three donors combined. In general, the variability in amplification rates for different loci proved to be greater than the variability among alleles for a given locus (see Table 5). The average amplification rate for DM was 0.82, that for D19S207 was 0.91, and that for D19S112 was 0.75.

A comparison of Models 2 and 3 based on their maximized log-likelihoods (Table 6) suggests that the estimated segregation probabilities do not vary significantly among the three donors (p=0.53). A similar comparison of Models 2 and 4 based on their maximized log-likelihoods (Table 6) suggests that none of the segregation parameters differ significantly from 0.5 (p=0.55). These two hypothesis tests are based on the result that for nested models, twice the difference in log-likelihoods should be approximately χ^2-distributed with degrees of freedom equal to the difference in the number of parameters, under the null hypothesis that the more parsimonious model holds. We note that in each of Models 2, 3, and 4, four parameters are estimated to be 0, which is on the boundary of the parameter space, in violation of the assumptions for the χ^2 test. Since the same four parameters are estimated as 0 in all three models, the χ^2 approximation might nonetheless be expected to hold. However, to be more secure in our conclusions about homogeneity of segregation probabilities across donors and lack of deviation from 0.5, we perform model selection below and retest these hypotheses in a context in which the assumptions for the χ^2 approximation are met.

In order to be confident in our analysis of the data and our evaluation of the segregation probability, we want to be certain that we have adequately modeled the data. Model 2 has 33 parameters, while 16 possible events could be observed for each person, giving a total of 48 possible events (45 freely varying counts). However, among the 48 possible events, 21 have observed counts of only 0 or 1 (see Table 3), so that their corresponding frequencies are not well-estimated. Thus, fitting a model with 33 parameters is an overparametrization. A symptom of this is that four of the (nonnegative) parameters of Model 2 are estimated on the boundary of the parameter space, i.e. they are estimated as 0, while six other nonnegative parameters are estimated to be within one standard deviation of 0 (see Table 5). It is worth considering whether the estimation of the segregation parameter and its standard deviation are affected by applying a more parsimonious model. Furthermore, the χ^2 tests for heterogeneity across donors and for deviation from Mendelian segregation will be applicable in the more parsimonious models, whereas their applicability was questionable in the overparametrized models.

The parsimonious model selected by the AIC criterion is Model 7, while that selected by BIC is Model 10, both described in Table 2. Table 6 gives the values of AIC and BIC for these and several other models. Of course, many more models were actually considered. Alternatives to Models 7 and 10 could be obtained by setting different combinations of the amplification and contamination parameters to be equal, while keeping the same number of parameters. For example, Model 7 could be modified to have locus-specific amplification parameters and to have all contamination parameters equal, and Model 10 could be similarly modified, but with the additional constraint that the amplification parameter for

DM be equal to the amplification parameter for D19S112.

A valid χ^2 test for inhomogeneity of the segregation parameters across donors is performed by comparing the log-likelihoods of Models 5 and 6, or alternatively, by comparing the log-likelihoods of Models 8 and 9 (see Tables 2 and 6). In neither test is inhomogeneity detected (p-values are both 0.49). Similarly, comparison of Models 6 and 7 shows that the segregation parameter does not differ significantly from 0.5, as does comparison of Models 9 and 10 (p-values are both 0.46).

It is interesting to note that even in Model 2, the most fully parametrized model, the FACS deposit parameters for donor 3 could not be set equal to those for donors 1 and 2 without creating significant model misfit (p= 5.3×10^{-5}). This p-value again depends on a questionable χ^2 approximation, but here, too, we can avoid this problem by testing for inhomogeneity of the FACS deposit parameters across donors in the more parsimonious models where the assumptions of the approximation hold. For instance, in Model 5, the p-value for the test is 5.1×10^{-9}, supporting the above conclusion.

Ideally, any model under consideration could be compared to Model 1 to assess model misfit using a χ^2 test. However, Model 1 has 14 parameters estimated on the boundary, i.e. 14 of the observed counts are equal to 0 (see Table 3), so the χ^2 test does not apply. If the χ^2 test is blindly applied without regard to this difficulty, then no significant model misfit is detected in any of the models described in Table 2 (all p-values > 0.5). One way to alleviate the problem of having parameters estimated on the boundary would be to consolidate some of the possible events. For each individual, the events representing those PCR errors in which genetic material from at least two different chromosomes was detected (–Bb, -aB-, -aBb, A–b, A-Bb, Aa–, Aa-b, AaB-, and AaBb, where, for example, AaB- refers to the event that A, a, and B are detected, but not b), could be consolidated into a single event. Then there would be 8 different types of events recorded for each person, each occurring at least four times in the data. Models 1, 2, 3, and 4 could not be fit to these data, because they have more than 21 parameters. However, some models containing fewer parameters could be fit, and a better assessment of model misfit would be possible for these. This avenue was not pursued here, as it would almost certainly not make any difference in the results.

An approximate 95% confidence interval for the segregation parameter estimated under Model 3 ranges from 0.46 to 0.51. Note that this interval excludes the segregation proportions observed by Carey et al. (1994): 0.56 overall and 0.60 for males, Gennarelli et al. (1994): 0.58 overall and 0.60 for males, and Shaw et al. (1995): 0.55 overall and 0.52 for males. This clearly indicates that segregation distortion at the level observed in those studies is not present in ejaculated sperm. Table 7 gives estimates of and confidence intervals for the segregation probability s under several of the more parsimonious submodels in which s is allowed to vary. All of the estimates are slightly below, and not significantly different from, 0.5, and the confidence intervals are in very close agreement with one another.

Finally, Table 8 gives the estimates for the segregation parameters obtained by simply ignoring some of the observed events resulting from apparent PCR errors and taking simple proportions. For instance, the estimate of the segregation proportion for the larger DM allele for donor 1 in Table 8 was calculated by taking $(n_{A---} + n_{A-B-})/(n_{A---} + n_{A-B-} + n_{-a--} + n_{-a-b})$, where, e.g., n_{A---} represents the number of events of type A—. An approximate 95% confidence interval for the combined estimate of segregation probability of the larger DM allele obtained by this naive method ranges from 0.447 to 0.501, and an approximate 95% confidence interval for the combined estimate of the segregation probability of the larger associated marker obtained by the naive method ranges from 0.461 to 0.515. The latter is in close agreement with the confidence intervals obtained from the more sophisticated analysis using the sperm-typing models, while the former gives slightly lower values.

Discussion

Carey et al. (1994) have suggested that a meiotic drive process may be responsible for maintaining the frequency of DM chromosomes capable of expansion to the disease state in the human population. In their study, described above, the proportion of larger alleles segregating was 0.56 (150/266) with approximate 95% confidence interval shown in Figure 1. Males appeared to have a slightly higher rate of segregation of the larger allele than did females, but the difference was not statistically significant: 0.60 (74/124) for males and 0.55 (65/118) for females, with approximate 95% confidence intervals shown in Figure 1, and with p-value for the difference close to 0.5.

Gennarelli et al. (1994) studied segregation of DM alleles in 251 Italian and Spanish DM pedigrees. Among the children of heterozygous affected parents, they found significant segregation distortion in favor of the mutant (DM-causing) allele. The proportion of mutant alleles segregating was 0.58 (521/897) overall, with 0.60 (314/525) for affected fathers and 0.56 (207/372) for affected mothers (approximate 95% confidence intervals shown in Figure 1). Although the rate for fathers appears to be slightly higher than that for mothers, the difference is not statistically significant ($p = 0.21$). Separate segregation rates for the mutant allele were calculated for father-to-son transmission (0.61), father-to-daughter transmission (0.58), mother-to-son transmission (0.61), and mother-to-daughter transmission (0.50). However, the null hypothesis that the four underlying rates are all the same cannot be rejected ($p = 0.43$). Their results also led them to cautious speculation about meiotic drive.

Hurst et al. (1995) reanalyzed the data of Carey et al. (1994) and Gennarelli et al. (1994). Hurst et al. (1995) identified errors in the p-values reported by Carey et al. (1994), and they pointed out the lack of a significant sex difference in transmission in either dataset. Furthermore, Hurst et al. (1995) proposed that all the rejection levels for the hypothesis tests in Carey et al. (1994) be Bonferroni-corrected, rendering the observed segregation distortion statistically insignificant. We note that the use of multiple hypothesis tests does not automatically require use of the Bonferroni correction. Use of the correction is warranted in cases in which a number of hypothesis tests are performed and only the most significant ones are selected for consideration. This might occur when a series of hypothesis tests is used as a screening procedure, as in the case of the t-test for outliers in regression (see e.g. Weisberg 1985). The arguments for Bonferroni correction apparently do not apply, for instance, to the overall test for non-sex-specific segregation distortion in the data of Carey et al. (1994). That result would be considered significant with p-value 0.036. Of course, replicability of such a borderline-significant result is much more important and interesting than the exact p-value assigned to it.

Finally, Shaw et al. (1995) studied 495 transmissions from heterozygous parents with both DM alleles within the normal range which they took to be 5-30 repeats. They simply looked at the rate of transmission of the larger allele from each pair, regardless of whether it was above or below 19 repeats. The overall rate of transmission of the larger allele in their data is 0.55 (272/495), with paternal rate 0.52 (126/242) and maternal rate is 0.58 (146/253) (approximate 95% confidence intervals shown in Figure 1). They remark that a higher transmission rate among mothers than among fathers is in contradiction with the previous results. However, we note that, as in the previous two studies, the sex difference here is not statistically significant ($p = 0.21$), although the overall finding of segregation distortion is significant. Combining the three studies also does not give a statistically significant sex difference (0.58 for males, 0.56 for females, $p = 0.56$).

We have studied the segregation of DM alleles in 1818 sperm from three males using single-sperm typing. Analysis of a large sample of sperm from each donor was an important consideration since we wanted to demonstrate the power of sperm typing to detect even small deviations from normal Mendelian segregation within individuals. Each male was heterozygous for a normal allele of < 19 repeats and one of > 19 repeats. To avoid the problem of differential PCR amplification based on allele length, each sperm was coamplified for a polymorphic microsatellite within 90 kb distal of the DM repeat, close enough to preclude any significant crossing over between them. Using specifically designed mathematical models that do not assume normal allele segregation ratios, our analysis showed no evidence for altered chromosomal segregation patterns or allele-specific differences in pre-ejaculatory sperm viability. Our data on three donors rule out the generalization that normal DM alleles with > 19 repeats exhibit meiotic drive. Studies on many more donors will be needed however to rule out the possibility that distorted segregation of alleles with > 19 repeats occurs in a small proportion of males. If the family studies suggesting segregation distortion are eventually confirmed, the possibility that sperm carrying DM alleles with > 19 repeats have a selective advantage in fertilization could also be considered.

Sperm typing is capable of providing the large sample sizes needed to detect altered chromosomal segregation patterns during meiosis or differential viability during or immediately following spermiogenesis in sperm from humans and other animals. Analysis of the data using the mathematical models we have derived in this paper gave us an upper limit of no more than 0.515 on the two-sided 95% confidence interval for the segregation probability for the larger DM alleles, which is quite a strong statement in light of the previous (post-fertilization) estimates of 0.56 (Carey et al. 1994), 0.58 (Gennarelli et al. 1994), and 0.55 (Shaw et al. 1995). Our results show that this approach has the power to detect even relatively small differences between alleles in these factors which, at least potentially, could contribute to altered transmission ratios among offspring.

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Appendix

Let x_1 be an indicator for the event that allele A is detected in a sample, x_2 for B, x_3 for a, and x_4 for b. Furthermore, define $\alpha_1 = \alpha_A, \alpha_2 = \alpha_B, \alpha_3 = \alpha_a$ and $\alpha_4 = \alpha_b$ (amplification parameters), similarly for β (contamination parameters). Recall that γ_i is the chance that i sperm are deposited $i = 0, 1, 2$, $\gamma_0 = 1 - \gamma_1 - \gamma_2$, and s is the chance that a randomly chosen sperm is of type AB, $1 - s$ the chance that it is of type ab. For $i = 1, 2, 3, 4$, let $q_i = 1 - (1 - \alpha_i)(1 - \beta_i)$, the chance of observing allele i conditional on deposit of one sperm containing that allele, and $r_i = 1 - (1 - \alpha_i)^2(1 - \beta_i)$, the chance of observing allele i conditional on deposit of two sperm containing that allele. Note that the chance of observing allele i conditional on deposit of zero sperm containing that allele is β_i. Then the probability of the joint event (x_1, x_2, x_3, x_4) is $P(x_1, x_2, x_3, x_4) = (1 - \gamma_1 - \gamma_2) \prod_{i=1}^{4} \beta_i^{x_i}(1 - \beta_i)^{1-x_i} + \gamma_1[s \prod_{i=1}^{2} q_i^{x_i}(1 - q_i)^{1-x_i} \prod_{j=3}^{4} \beta_j^{x_j}(1 - \beta_j)^{1-x_j} + (1-s) \prod_{i=1}^{2} \beta_i^{x_i}(1-\beta_i)^{1-x_i} \prod_{j=3}^{4} q_j^{x_j}(1-q_j)^{1-x_j}] + \gamma_2[s^2 \prod_{i=1}^{2} r_i^{x_i}(1-r_i)^{1-x_i} \prod_{i=3}^{4} \beta_j^{x_j}(1-\beta_j)^{1-x_j} + 2s(1 - s) \prod_{i=1}^{4} q_i^{x_i}(1 - q_i)^{1-x_i} + (1 - s)^2 \prod_{i=1}^{2} \beta_i^{x_i}(1 - \beta)^{1-x_i} \prod_{j=3}^{4} r_j^{x_j}(1 - r_j)^{1-x_j}]$, where the first term is the chance of observing (x_1, x_2, x_3, x_4) when zero sperm are deposited, the second term is the chance of observing it when one sperm is deposited, subdivided into two terms corresponding to which of the two types of sperm is deposited, and the third term is the chance when two sperm are sperm deposited, subdivided into three terms corresponding to which types of sperm were deposited.

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Table 1 Donor alleles, where A and B cosegregate as do a and b

Designation	Donor 1	Donor 2	Donor 3
A	DM 182 bp (37 repeats)	DM 152 bp (27 repeats)	DM 131 bp (20 repeats)
a	DM 86 bp (5 repeats)	DM 86 bp (5 repeats)	DM 86 bp (5 repeats)
B	D19S207 105 bp	D19S112 104 bp	D19S207 114 bp
b	D19S207 124 bp	D19S112 92 bp	D19S207 128 bp

Table 2 Descriptions of models

Model	Description
1. General multinomial	ith observed event for jth donor has prob. $p_{ij} \geq 0$ where $\sum_i p_{ij} = 1$ for each j
2. Full sperm-typing	For each donor j, separate segreg. params. (s_j) and deposit params. $(\gamma_{0j}, \gamma_{1j}, \gamma_{2j} = 1 - \gamma_{0j} - \gamma_{1j})$, while amplif. params. $(\alpha_{Aj}, \alpha_{Bj}, \alpha_{aj}, \alpha_{bj})$ and contam. params. $(\beta_{Aj}, \beta_{Bj}, \beta_{aj}, \beta_{bj})$ vary by donor and by allele
3. $s_1 = s_2 = s_3$	Same as Model 2, but segreg. params. assumed equal across donors
4. $s_1 = s_2 = s_3 = 0.5$	Same as Model 3, but with $s = 0.5$
5. Parsimonious model I with $s_1 \neq s_2 \neq s_3$	Same as Model 2, but donors 1 and 2 have same deposit params., different from donor 3: $\gamma_{i1} = \gamma_{i2}$, $i = 0, 1, 2$; one contam. param.: $\beta_a = \beta_b$ and $\beta_A = \beta_B = 0$, where, e.g., β_a assumed equal for all donors; 3 amplif. params.: $\alpha_{A1} = \alpha_{A2} = \alpha_{A3}$ $= \alpha_{a1} = \alpha_{a3}$, $\alpha_{B1} = \alpha_{B3} = \alpha_{b1} = \alpha_{b3} = \alpha_{a2}$, and $\alpha_{B2} = \alpha_{b2}$, where, e.g., α_{A1} is α_A for donor 1

6. Parsimonious model I with single s	Same as Model 5, but with $s_1 = s_2 = s_3$
7. Parsimonious model I with $s = 0.5$	Same as Model 6, but with $s = 0.5$ for all donors
8. Parsimonious model II with $s_1 \neq s_2 \neq s_3$	Same as Model 5, but 2 amplif. params.: $\alpha_{A1} = \alpha_{A2} = \alpha_{A3} = \alpha_{a1} = \alpha_{a3} = \alpha_{B2} = \alpha_{b2}$, $\alpha_{B1} = \alpha_{B3} = \alpha_{b1} = \alpha_{b3} = \alpha_{a2}$
9. Parsimonious model II with single s	Same as Model 8, but with $s_1 = s_2 = s_3$
10. Parsimonious model II with $s = 0.5$	Same as Model 9, but with $s = 0.5$ for all donors

Table 3 Counts of observed events

Observed events[a]	Donor 1	Donor 2	Donor 3
—-	51	103	27
—b	37	25	50
–B-	38	41	54
–Bb	1	0	0
-a–	20	64	24
-a-b	160	185	227
-aB-	0	2	0
-aBb	1	1	0
A—	13	51	21
A–b	0	0	0
A-B-	171	162	186
A-Bb	4	1	2
Aa–	0	0	0
Aa-b	0	0	0
AaB-	2	1	1
AaBb	2	4	1
Total	500	640	593

[a] The letters are indicators for the corresponding alleles. For example, AaB- refers to the event that A, a, and B are detected, but not b.

Table 4 Additional data for donor 3, typed only at DM locus

Observed events	Counts
–	14
-a	33
A-	38
Aa	0
Total	85

Table 5

Maximum likelihood estimates of the parameters in the full sperm-typing model (Model 2) with estimated standard deviations in parentheses

Parameter	Donor 1	Donor 2	Donor 3
s	0.51 (0.02)	0.48 (0.02)	0.48 (0.02)
γ_0	0.09 (0.02)	0.13 (0.03)	0.03 (0.01)
γ_1	0.90 (0.02)	0.85 (0.02)	0.97 (0.01)
γ_2	0.01 (0.01)	0.02 (0.01)	0.004 (0.004)
α_A	0.82 (0.03)	0.80 (0.03)	0.79 (0.02)
α_a	0.81 (0.03)	0.88 (0.02)	0.82 (0.02)
α_B	0.93 (0.03)	0.76 (0.03)	0.90 (0.02)
α_b	0.89 (0.02)	0.74 (0.03)	0.90 (0.02)
β_A	0.0	0.0	0.0
β_a	0.008 (0.006)	0.001 (0.007)	0.003 (0.003)
β_B	0.003 (0.006)	0.007 (0.007)	0.0
β_b	0.02 (0.01)	0.002 (0.004)	0.007 (0.005)

Table 6

Model selection

Model	# Params.	# Params. Est. on Boundary	Log_e-Likelihood	AIC	BIC
1	45	14	-3034.0	6158.1	6405.8
2	33	4	-3039.5	6144.9	6326.6
3	31	4	-3040.1	6142.2	6312.9
4	30	4	-3040.5	6141.0	6306.2
5	11	0	-3047.1	6116.2	6176.8
6	9	0	-3047.8	6113.6	6163.2
7	8	0	-3048.1	6112.2	6156.2
8	10	0	-3050.1	6120.3	6175.3
9	8	0	-3050.8	6117.7	6161.7
10	7	0	-3051.1	6116.2	6154.8

Table 7

Estimates of the segregation probability under various sub-models of the full sperm-typing model, with estimated 95% confidence intervals

Model 3	Model 6	Model 9
0.49 (0.464,0.514)	0.49 (0.466,0.515)	0.49 (0.466,0.515)

Table 8

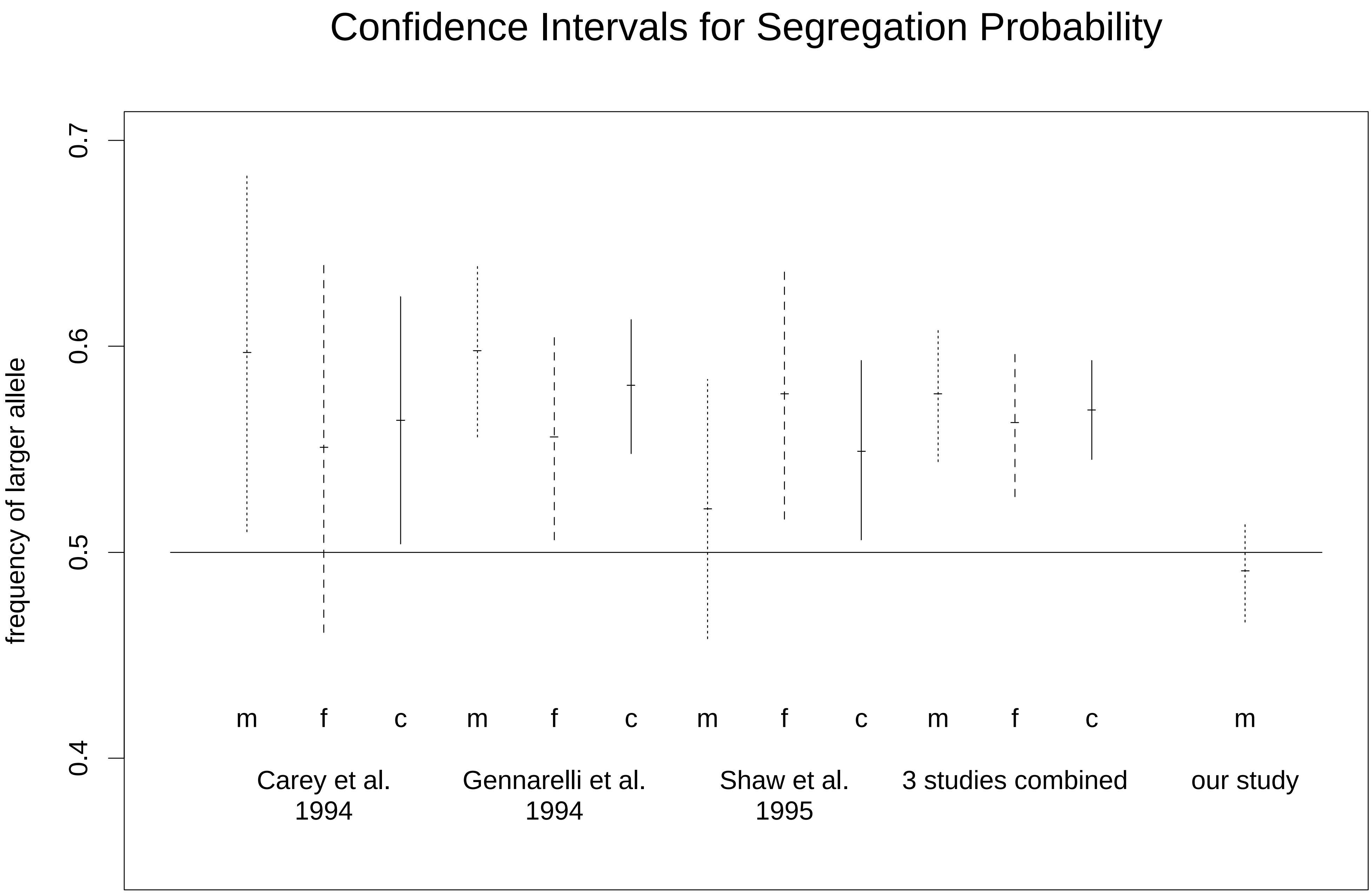
Naive estimates of segregation probability with estimated standard deviations

	Donor 1	Donor 2	Donor 3	Combined
larger DM allele	0.51 (0.03)	0.46 (0.02)	0.46 (0.02)	0.47 (0.01)
associated marker	0.52 (0.03)	0.49 (0.03)	0.46 (0.02)	0.49 (0.01)

Figure Legend

Confidence intervals for the segregation probability of the larger DM allele in various studies

Here “m” denotes male, “f” female, and “c” male and female combined.


Confidence Intervals for Segregation Probability
frequency of larger allele
0.4
0.5
0.6
0.7
m f c m f c m f c m f c m
Carey et al. 1994
Gennarelli et al. 1994
Shaw et al. 1995
3 studies combined
our study