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Individual Variation in Recombination among Human Males

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Summary

Studies of recombination between the markers D6S291 and D6S109 in individuals by sperm typing provide direct evidence for significant variation in recombination among humans. A statistically significant difference in the recombination fraction (range 5.1%–11.2%) was detected among five donors. This variation could reflect polymorphisms in genes affecting recombination or in chromosome structure. Ignoring this variability in studies designed to examine the relationship between physical and genetic distances could lead to incorrect inferences. Individual variation in recombination makes it difficult to predict the recombination fraction for an interval in any particular individual. This could be important in certain genetic counseling situations.

Introduction

Studies of a variety of nonhuman species have revealed genetic variation for recombination within and among both natural and laboratory populations (Brooks 1988). Although differences in recombination between the sexes in humans and mice are well documented (Donis-Keller et al. 1987; Roderick and Hillyard 1990), relatively little is known of the extent to which recombination may vary among individuals independent of the influence of sex. In mice, sizable differences in the recombination fraction (θ) across the same interval have been observed in different interspecific and intersubspecific crosses (Reeves et al. 1990). In humans, cytogenetic studies of bivalent chiasma frequencies have suggested individual variation among men (Laurie and Hulten 1985). Individual variation has also been inferred from limited human family data supporting linkage heterogeneity, on the basis of allele-specific effects on recombination. The LOD scores for recombination between the immunoglobulin marker Gm and the linked marker Pi (alpha-1 anti-trypsin) appear to vary depending on whether the parents were segregating for the MZ allele at the Pi locus (Gedde-Dahl et al. 1972; Weitkamp et al. 1978; Babron et al. 1990). Studies on the telomeric region of chromosome 4p, using the Venezuelan Huntington disease (HD) pedigrees, have suggested that recombination may be suppressed in individuals carrying the HD mutation, when compared with non-HD individuals (MacDonald et al. 1989; Buetow et al. 1991).

No parent, of course, can have enough offspring for meaningful comparisons among individuals to be made, and the way in which genetic recombination is usually studied in human families makes it difficult to examine the question of linkage heterogeneity. The required pooling of data from different families obscures individual differences, yet subdividing the available families into groups by some criteria to look for variation necessarily reduces the sample size of available meioses and limits the precision of the comparison. Finally, families exhibiting elevated recombination between markers, as compared with the average θ, are usually considered suspect and are subjected to special error-checking procedures.

Sperm typing (Li et al. 1988) allows the direct detection of individual differences in male recombination. Large sample sizes of meiotic products can be obtained from individuals, permitting direct comparisons. Sperm-typing data are likely to reflect true differences in crossing-over among males, resulting, possibly, from population variation in chromosome structure or polymorphisms in genes involved in recombination.

We measured the θ between the dinucleotide repeat markers D6S291 and D6S109 on the short arm of human chromosome 6 in five individual sperm donors. Statistically significant differences in recombination were detected. Our results raise a number of questions about the reliability of estimates of human θs and the consequences of these individual differences.

Subjects and Methods

Sperm Donors

All sperm samples were derived from Caucasian men who were being evaluated for fertility in a diagnostic

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laboratory. All subjects were found to have normal sperm counts and sperm motility. The ages of the subjects were 46 years (donor 5001), 38 years (donor 5012), 31 years (donor 5033), 43 years (donor 5043), and 43 years (donor 5048).

Sperm Typing

Single-sperm typing was first carried out according to methods described elsewhere, by use of primer extension preamplification (PEP) followed by PCR (Leeflang et al. 1994). After PEP, 2 μl were used for typing. The PCR reaction conditions were 10 mM Tris-HCl, 50 mM KCl, 2.5 mM $MgCl_2$, 50 μM of each dNTP, 0.2 μM each primer, and 1 unit *Taq*. PCR began with a 94°C, 4-min denaturation and ended with incubation at 72°C for 5 min. PCR products were sized on 8% polyacrylamide gels.

Amplification was carried out by use of two rounds of PCR—a first round with a pair of outside primers and a second round with two inside primers (D6S291) or one outside primer and one inside primer (D6S109 and D6S265). Sequence data provided by Dr. Harry Orr were used to design three D6S109 primers. Primers 109a (5′-CAACCTGGGCAATAAGAGCG) and 109c (5′-CAAGCCAACAGAATATATATGTG) were used in the first round, 109c and 109d (5′-AGACTCCATCTCACACAAAA) in the second round. For D6S265, primers 265a (5′-ACGTTCGTACCCATTAACCT) and 265b (5′-ATCGAGGTAAACAGCAGAAA) were used in the first round, 265a and 265c (5′-TAGTATCCAGAGGCTGGGAA) in the second round. Sequence data provided by Dr. Jean Weissenbach were used to design the primers for D6S265 and the four primers for D6S291. Primers 291a (5′-CCATCCGGCATTCAGG) and 291b (5′-GGATGACGAATTATTCACTAAC) were used in the first round, 291c (5′TTGTGGTGATGGTTTCACAG) and 291d (5′-CTCACAGTTTGGTAAGTGACTC) in the second round. The PCR cycling conditions were the same for both D6S291 and D6S109. For the first round, a 94°C, 4-min denaturation of 2 μl PEP product was followed by 11 cycles of 94°C for 45 s and 60°C for 3 min and 14 cycles of 94°C for 45 s and 60°C for 2 min. For the second round, 2 μl of first-round product was used as template in 25 cycles of 94°C for 45 s and 60°C for 1 min.

For amplification of D6S265, the cycling conditions differed from the other two loci. The first round consisted of 11 cycles of 94°C for 45 s and 60°C for 4 min, followed by 14 cycles of 94°C for 45 s and 60°C for 3 min. The second round consisted of 23 cycles of 94°C for 45 s and 60°C for 1 min.

Additional experiments were carried out without PEP and involved coamplification of D6S291 and D6S109 in single sperm. The primer pairs described above were used in the coamplification experiments. The primer concentrations were 0.3 μM each (D6S291) and 0.1 μM each (D6S109). Other reagents were identical to those used after PEP, except for the presence of 10% glycerol and a higher dNTP concentration (100 μM each). The first-round cycling conditions were 11 cycles of 94°C for 45 s and 58°C for 3 min and 16 cycles of 94°C for 45 s and 60°C for 2 min. The second-round cycling conditions, for D6S291, were 29 cycles of 94°C for 45 s and 60°C for 1 min and, for D6S109, 27 cycles of 94°C for 45 s and 63°C for 1 min. Sperm typing the chromosome 19 markers followed methods described elsewhere (Hubert et al. 1992).

Calculation of θ

Maximum-likelihood methods were used (1) to test whether individual donors had different θs for the D6S109 and D6S291 (or Mfd232 and Mfd11) intervals and (2) to obtain estimates and confidence intervals for these θs. The appropriate form of the likelihood depends on whether the sperm cells were directly typed or amplified according to the PEP protocol prior to typing. Thus, the PEP data set, containing typing data from all five donors, and the direct data set, containing separate typings from donors 5001 and 5043, were analyzed separately. See the study by Lazzeroni et al. (1994) for details of the likelihood formulation and computation for sperm typing, including the method used when multiple typings for a locus were carried out on a PEP sample.

Variability in the sperm-typing process is apparent in observations in which either zero or two alleles have been detected at a locus. Such data arise from imprecision in the cell-sorting process, inefficiency in allele amplification and detection, and contamination by exogenous DNA. We considered several statistical models of this process, choosing separately among the following alternatives to model the efficiency and contamination rates. The possibilities included distinct values for each allele of each donor, for each locus of each donor, for each locus only, for each donor only, or a single common value shared by all donors at all loci. Note that, in these models, donor variability can also reflect variability in the processing of donor samples. For these data, the occurrence rate of tubes containing two sperm was estimated as zero, under several models, which conforms to a large body of sperm-typing data (Cui et al. 1989; Goradia et al. 1991; Schmitt et al. 1994). Thus, the results are reported under models in which tubes can only contain either one or no sperm. In addition, we addressed the possibility of variation in PCR errors among the 96-well plates typed for a given individual, by fitting models that contained distinct efficiency and contamination rates for each individual plate. We found no evidence of variability, in PCR errors, among plates for any of the donors.

Within each data set, we tested homogeneity of the

recombination rate, using likelihood-ratio tests. Models containing distinct θs for each donor were compared with otherwise identical models containing a single recombination rate common to all donors. Using software developed for multipoint sperm-typing data (Lazzeroni et al. 1994), we allowed for distinct θs by treating loci from each donor as different from the same loci in all other donors.

Results under several models were compared to ensure that tests and estimates did not depend on model selection. The largest model contained distinct efficiency and contamination rates for each allele of each donor; the smallest model contained one overall efficiency rate and one overall contamination rate. We again used likelihood-ratio criteria to find a parsimonious model, accounting for most of the variability in the data, under which to make final inferences.

When the PEP and direct data provided independent estimates of a single θ, these estimates were combined according to the following weighted-least-squares procedure. Let $\hat{\theta}^p$ and $\hat{\theta}^d$ be the maximum-likelihood estimates of θ from the PEP and direct-data sets, respectively, with estimated SEs $\widehat{SE}(\hat{\theta}^p)$ and $\widehat{SE}(\hat{\theta}^d)$. Let $\hat{v}_p = [\widehat{SE}(\hat{\theta}^p)]^2$ and let $\hat{v}_d = [\widehat{SE}(\hat{\theta}^d)]^2$. Then, the combined estimate of θ is $\hat{\theta} = (\hat{v}_d\hat{\theta}^p + \hat{v}_p\hat{\theta}^d)/(\hat{v}_p + \hat{v}_d)$, and its SE is estimated by $[\hat{v}_p\hat{v}_d/(\hat{v}_p + \hat{v}_d)]^{1/2}$. Simultaneous confidence intervals were constructed by use of a Bonferroni-type adjustment (see Neter et al. [1996] or other statistics texts, for a description of weighted least squares and Bonferroni corrections).

Consequences of Linkage Heterogeneity on the Precision of Estimates of θ

In the discussion, we attempt to describe some consequences of individual variability in θs in terms of the amount of variability actually detected in our analysis. To do this, we treat our sperm donors as if they formed a random sample representative of the general population. The consequences of the observed variability also depend on the way in which θs are distributed in the population. One possible model would be to suppose that the θs of individuals in the population are independent and follow a normal distribution, with overall mean θ and variance σ_p^2. In this model, the true fraction θ_i of each sperm donor i in the sample is an unobserved random effect. Given θ_i, the observed estimate $\hat{\theta}_i$ for that donor is approximately normal, with conditional mean θ_i and conditional variance σ_i^2. The estimated correlation matrix for the estimates $\hat{\theta}_i$ shows that they can be reasonably treated as independent normal variables. When they are not conditioned on the unobserved θ_i, it follows that the estimates $\hat{\theta}_i$ are independent and approximately normally distributed, with mean θ and variance $\sigma_p^2 + \sigma_i^2$.

After we substitute $[\widehat{SE}(\hat{\theta}_i)]^2$, obtained above as an estimate of the conditional variance σ_i^2, the likelihood of the five observed estimates for the D6S291–D6S109 interval can be written as

$$\prod_{i=1}^{5} [\sigma_p^2 + \widehat{SE}(\hat{\theta}_i)^2]^{-1/2} \exp\left[- \frac{1}{2(\sigma_p^2 + \widehat{SE}(\hat{\theta}_i)^2} (\hat{\theta}_i - \theta)^2 \right].$$

The maximum-likelihood estimates of the population-wide average θ and its variance are $\hat{\theta} = .079$ and $\hat{\sigma}_p^2 = .021$. The estimated SE of $\hat{\theta}$ is $\widehat{SE}(\hat{\theta}) = .012$. This result contrasts with that based on the assumption of homogeneity in θs in which the combined estimate of θ is .076 and has an estimated SE equal to .005, or less than half the value under the assumption of individual variability.

With the above model, a conservative prediction interval for recombination in an unexamined individual, in the D6S29–D6S109 interval, is

$$\hat{\theta} \pm 1.96\sqrt{[\widehat{SE}(\hat{\theta}_i)]^2 + \hat{\sigma}_p^2} = (.031, .127) .$$

This is considerably wider than the confidence interval for the population average under the homogeneity model, because of the contribution of $\hat{\sigma}_p^2$. Larger sample sizes would reduce the value of $\widehat{SE}(\hat{\theta})$ but not $\hat{\sigma}_p$.

Results

Recombination between D6S291 and D6S109

Sperm typing was carried out on 3,180 sperm from five donors whose age range was 31–46 years. Statistical analysis of the data took into consideration errors in single-sperm isolation, PCR efficiencies, contamination and experiment-to-experiment variation (Cui et al. 1989; Lazzeroni et al. 1994). We also evaluated the effect of using two different modes of sperm typing: (1) direct locus-specific PCR or (2) whole-genome amplification (PEP; Zhang et al. 1992) followed by locus-specific PCR on aliquots from the PEP reactions. Estimates and confidence intervals for the θ_is were obtained by use of the statistical approaches described in the Subjects and Methods section.

The hypothesis of identical θs, for all five donors, in the PEP data was strongly rejected by the likelihood-ratio test under the final model. Table 1 shows results under the final model and the largest and smallest models described in the Subjects and Methods section. In the final model, the estimates of θ_i vary from a low of .051, for donor 5043, to a high of .112, for donor 5001, a greater-than-twofold difference. Several other intermediate models were analyzed and yielded similar results (not shown). The likelihood-ratio test statistic is 26.63 with 4 df and has a highly significant P-value of $<.0001$. Figure 1 shows simultaneous 95% confidence intervals for all five donors. Note that the intervals for 5001 (46

Table 1

Likelihood-Ratio Test Statistics for PEP and Direct PCR, and Estimates of θ_i and SEs between D6S291 and D6S109, for the Five Sperm Donors

	Final Model	Smallest Model	Largest Model
Source (n)	Likelihood-Ratio Test Statistic (P)		
PEP (2,396)	26.63 (<.0001)	29.50 (<.0001)	31.44[a]
Direct PCR (784)	3.45 (.0632)	4.01 (.0452)	2.95[a]
	Estimate (SE) of θ_i		
Donor 5001 (743)	.112 (.012)	.114 (.012)	.114 (.012)
Donor 5012 (190)	.109 (.024)	.111 (.024)	.114 (.023)
Donor 5033 (1,312)	.066 (.007)	.066 (.007)	.062 (.007)
Donor 5043 (808)	.051 (.008)	.049 (.008)	.052 (.008)
Donor 5048 (127)	.071 (.024)	.073 (.024)	.071 (.025)

Note.—The final model allowed efficiency of the amplification process to depend on the donor (and, thus, the experiment) but not on the locus. Contamination rates were allowed to depend on the locus but not on the allele donor. However, the results given above were remarkably insensitive to model choices regarding efficiency and contamination rates.

[a] The large-sample approximation is not valid, because estimates of some contamination rates were 0. *P*-values are not provided.

years old) and 5043 (43 years old) do not overlap. For these two donors the difference in θs is estimated as .061 with a SE of .014. The 95% confidence interval for this difference is (.025, .098) when a Bonferroni correction is used to adjust for the fact that we are looking, after the fact, at the largest of 10 possible pairwise comparisons of the individual θ_is.

The smaller data set, based only on direct locus-specific PCR, by itself provides insufficient evidence to determine conclusively whether donors 5001 and 5043 have different θs. With 1 df, the likelihood-ratio test statistic is 3.45 with a *P*-value of .0632. The corresponding 95% confidence interval for the difference between the θs of donors 5001 and 5043 is (−.0003, .0710), which is consistent with both equal and distinctly unequal θs in the two donors.

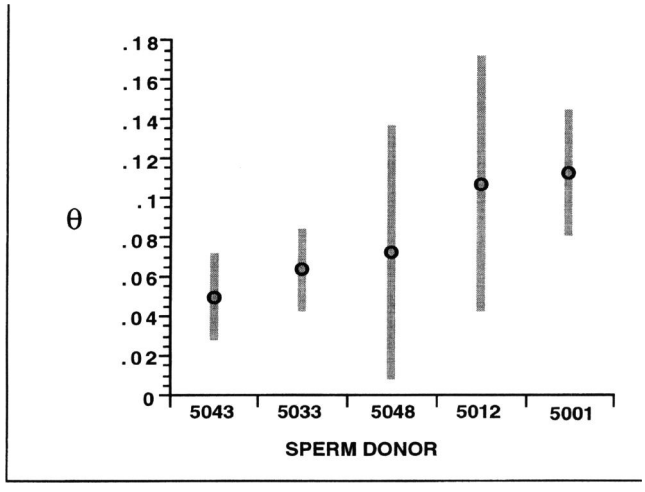

Figure 1 Estimated θs (*circles*) and simultaneous 95% confidence intervals (*bars*) for the D6S291–D6S109 interval, for five sperm donors.

PEP aliquots from 36 recombinants from 5001 and from 19 recombinants from 5043 were typed for a third marker (D6S265) that lies within the HLA class I region and between D6S291 and D6S109. In the D6S291–D6S265 interval there were 3.5 times as many recombinants in 5001 (14) as in 5043 (4), whereas in the D6S265–D6S109 interval there were only 1.5 times as many recombinants in 5001 (22) as in 5043 (15). However, a χ^2 test of homogeneity is not significant. Thus, we cannot exclude the possibility that increased recombination in 5001 relative to 5043 occurs over a chromosomal domain that is larger than the D6S291–D6S109 interval itself.

Recombination between Mfd232 and Mfd11

Recombination between two chromosome 19 markers (Mfd232 and Mfd11) was studied in 336 sperm by use of PEP aliquots from donors 5001 and 5043. The estimates of θ are .202 and .175, respectively (table 2). The difference in θ between the individuals was estimated to be .027 (95% confidence interval; −.087, .141). Since this interval overlaps zero, it is consistent with equal θs in the two donors. On the basis of CEPH family studies, the male-specific θ between these loci has been estimated

Table 2

Likelihood-Ratio Test Statistic (1 df), and Estimates of θ_i and SEs between Mfd232 and Mfd11, in Donors 5001 and 5043

	Final Model	Smallest Model	Largest Model
Source	Likelihood-Ratio Test Statistic (P)		
336 Samples	.06 (.8065)	.54 (.4635)	.58 (.4456)
	Estimate (SE) of θ_i		
Donor 5001	.202 (.045)	.204 (.046)	.205 (.046)
Donor 5043	.175 (.036)	.175 (.036)	.172 (.037)

NOTE.—The final model for this data set allowed both efficiencies and contamination rates to depend on both the donor and the locus; however, the results are insensitive to changes in choice of model.

to be .14, with a support interval of .09–.2 (J. Weber, personal communication).

Discussion

The D6S291–D6S109 genetic interval is estimated to include 20–25 Mb of DNA (A. Zeigler and A. Volz, personal communication). On the basis of studies of eight CEPH families (a maximum of 186 meioses) and including data on 40 microsatellite markers, the average male-specific θ between D6S291 and D6S109 is .10 (B. Cottingham, personal communication). Although the sperm-typing studies revealed statistically significant individual variation in θ with a range of .051–.112, the results are generally compatible with the family studies, allowing for statistical variation.

The θ between D6S291 and D6S109 for donors 5001 and 5043 differed by more than twofold (5.1% vs. 11.2%). When recombination between two markers on chromosome 19 was studied in these two individuals, no statistically significant difference was detected (17.5% and 20.2%, respectively). It should be noted that the chromosome 6 sperm sample size was far greater than that for chromosome 19 (1,551 and 336, respectively). However, these estimates suggest that there is less difference between the θs of these two donors on chromosome 19 than on chromosome 6. Therefore, we think that it is likely that the variation in recombination is not genomewide. It may be restricted to chromosome 6, some region of chromosome 6, or the specific interval itself.

The human major histocompatibility complex (MHC) is found within the D6S291–D6S109 interval. In mice, there is evidence that clusters of allelic differences at the MHC (H-2 haplotype) may affect recombination (Yoshino et al. 1995). In cattle, sperm- typing experiments on two different bulls have shown a difference in recombination over an interval that also includes the bovine MHC (Park et al. 1995). Preliminary data from family studies have suggested that recombination in the human MHC (human leukocyte antigen [HLA]) region may also vary according to haplotype (Thomsen et al. 1994). Our data are inconclusive about whether the difference in recombination between individuals 5001 and 5043 is localized to the HLA region.

The existence of sizable linkage heterogeneity implies two important consequences for geneticists. First, ignoring this variability when estimating θs will lead to the appearance of greater precision in the resulting estimates than is actually justified. In other words, SEs and confidence intervals will be too small. For example, if these donors were representative of the general population, maximum-likelihood analysis under the assumption of linkage homogeneity would yield a 95% confidence interval of (.066, .086) for the D6S291–D6S109 interval. However, under one possible model of linkage heterogeneity described in the Subjects and Methods section, an appropriate 95% confidence interval for the average θ would be distinctly wider (.056, .101).

The increasingly detailed genetic and physical maps that are being generated (Guyer et al. 1995) open up the possibility of analyzing the details of genetic recombination in humans in a way not previously feasible. However, the currently available genetic maps, while dense in markers, are relatively low in resolution; the individual genetic distances between closely linked markers have wide confidence intervals. This, in turn, will compromise the precision of estimates of the relationship between genetic and physical distance over regions less than a few megabases. In addition, since the current maps provide an estimate of the θ averaged across all the families studied, chromosomal regions that exhibit unusual recombination properties only in some individuals may not be identified. Because of sample size considerations and the ability to study recombination in individuals, sperm typing is an especially useful alternative approach.

Another consequence of individual variation in recombination is that it will be impossible to predict exactly the θ for an interval in a new individual who was not part of the sample even when the average for the population is known exactly. This could be important in certain genetic-counseling situations when the reliability of a diagnosis depends on estimates of linkage between a marker and the disease locus. If there were no individual variability, a new individual's θ would be the same as that of the population average, and the 95% prediction interval for the individual's θ would be the same as the 95% confidence interval for the population average. However, if individual variability exists, the prediction interval for any individual must allow not

only for uncertainty in the estimate of the population average but also for individual variability about that average. For example, on the basis of the model that acknowledges individual variation (see the Subjects and Methods section), the prediction interval for the D6S29–D6S109 region in an unexamined individual is (.031, .127). This is considerably wider than the confidence interval for the population average (.066, .086), when linkage homogeneity is assumed. Regardless of the sample size used to estimate the population average, some uncertainty about any particular unexamined individual will always remain.

Of course, the random-effects model is only one of a variety of models that might conceivably describe the distribution of θs in that population. An alternative, consistent with these data, is to suppose that there are two groups of males in the population—one group with θs centered at ~.06 and another group with θs centered at ~.11. This type of bimodal variability would have similar consequences for estimates and predictions of the θ. However, a quantitative statement of these consequences would depend on the precise form of the bimodal distribution.

Our sperm-typing results offer direct experimental evidence for significant variation in recombination over a specific chromosomal interval among human males. It is likely that similar variation occurs among females, but for obvious reasons, this is a much more difficult question to study. Individual variation in recombination could reflect either polymorphisms in genes affecting recombination or chromosome-structure polymorphisms. Because sperm typing is capable of measuring, at high resolution, θs in individuals, genomewide screens for variation could easily be made. Variation in recombination may be a useful phenotype to identify. It could lead to the identification of either (*a*) chromosomal variation, in the human population, that may not be detectable by cytogenetic methods or (*b*) modifier genes linked or unlinked to the interval under study. Such modifiers may be involved in the recombination process itself and may affect the whole genome or only parts of it. Consideration of individual variation in recombination may lead to new insights into the factors that influence genetic events in particular families. The detection of males with altered recombination might also lead to the identification of fathers susceptible to chromosomal nondisjunction. XY nondisjunction in males leading to XXY sons, for example, has been shown to be associated with lower pseudoautosomal recombination (Hassold et al. 1991).

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