Background
Because linkage analysis observes the co-segregation of marker alleles and phenotype, there is a concern that errors in pedigrees or genotypes could result in false-negative or false-positive results. The use of Genetic Analysis Workshop (GAW) data to examine the presence and nature of pedigree errors is not new [1-3]. In this exercise we detect and describe the pedigree and genotyping errors in the GAW13 Framingham Heart Study data.

Methods
Pedigree error detection and correction
The program PREST [4] was used to detect pedigree errors. PREST estimates the probabilities, $p_0$, $p_1$, and $p_2$ of two individuals sharing 0, 1, and 2 alleles identically by descent (IBD), respectively. We calculated this over all of the relationship pairs known to PREST (parent-offspring, full-sibs, half-sibs, avuncular, first-cousins, grandparent-grandchild, half-avuncular, half-first cousin, half-sib plus first-cousin, monozygotic twins, and unrelated) within and between pedigrees. Pedigree errors were first screened with PREST’s analytical tests: conditional estimated identity by descent (EIBD), adjusted identity by state (AIBS), and IBS, in that order and where applicable, at $\alpha = 0.0001$, to focus on the more significant problems. This index pair and their relatives were then examined more thoroughly using PREST’s accompanying program ALTERTEST that can test two individuals for each of the 11 relationship classes.

PREST comes with an R script written by Dan Weeks to plot the IBD estimation of a single relative pair on a relationship triangle [5]. We modified this program to provide a scatter diagram of IBDs on the triangle. The result is an informative graphical summary of the pedigree errors in the sample. Pedigrees were drawn with PEDIGREE/DRAW [6].

Genotyping error detection and correction
Genotyping errors are detected using SIMWALK [7,8]. SIMWALK2 applies a Markov-chain Monte Carlo method to data from the pedigree, population allele frequencies, and a genetic map to assign probabilities of mistyping for each genotype. Because this is a computationally intensive exercise, we examined genotyping errors only on chromosome 7.

We ran SIMWALK2 in two phases. In the first phase, Mendelian errors were detected and corrected independently for each marker. Marker genotypes were blanked.
(changed to a missing value) for all probabilities of mistyping above a given threshold. The threshold was chosen conservatively, i.e., to blank no more genotypes for a marker than necessary to calculate a likelihood for that marker. The mistyping probability was decremented from 1.0 until a calculable likelihood was reached.

In the second phase, genotypes that suggest improbable double recombination events were blanked. Mistyping probabilities were assigned using the genetic maps supplied with the GAW13 data. In this phase, the proportion of genotypes potentially blanked at a series of thresholds is plotted to provide a visual guide for choosing a threshold.

Following the corrections of the pedigree and genotype data, we recalculated for comparison, the chromosome 7 genetic map using MULTIMAP/CRIMAP [9,10].

**Results**

**Pedigree errors**

Scatter diagrams of the estimated IBD probabilities $p_0$, $p_1$, and $p_2$ on relationship triangles are shown in Figure 1. These reveal almost no full-sibling errors. A few avuncular and first-cousin relationships are likely to be unrelated, and a great many unrelated individuals are likely in fact to be related, some being parent-offspring pairs.

Consistent observations among relatives were required before changes were made to the pedigrees. For example, the four unrelated pairs that are undoubtedly parent-offspring display the required parent-offspring relationships with a female in a disconnected pedigree, as determined by ALTERTEST and illustrated in Figure 2. All four were unrelated to the originally designated mother, now the stepmother. None of the other alternative tests suggested any other arrangement.

In another example shown in Figure 3, two pedigrees were joined through two ungenotyped half-sibs, detected through consistent half-avuncular and half-first cousin relationships.

These simple examples belie the difficulties of correcting pedigree errors. In the Framingham data we were able to resolve the errors in 16 of 21 pedigrees, out of 329 pedigrees in total. The five unresolved pedigrees displayed patterns of errors that contradicted all testable alternative hypotheses, and were left unchanged.

Figure 1

**Scatter diagrams of estimated IBDs on the relationship triangles for six relationship categories**

Scatter diagrams of the IBD probabilities estimated by PREST on a relationship triangle reveal the extent of pedigree errors within a given relationship category. By far, the largest number of errors involves "unrelated" pairs that in fact appear to be related.
Five Mendelian errors were revealed following the pedigree changes. The distribution of mistyping probabilities for the double-recombinant detection is shown in Figure 4. We chose a threshold probability of 0.25 to define erroneous genotypes, so as to blank without a too great loss of data. With this threshold, 142 genotypes were blanked.

Following the blanking, a new genetic map for the 21 markers on chromosome 7 was estimated using MULTI-MAP/CRIMAP [9,10]. The new map was 167 cM in length, or 24 cM shorter than the map provided with the data. In comparison, the length of the corresponding Marshfield map is 175 cM [11].

**Discussion**

The Framingham data were relatively free of pedigree errors, particularly those involving close relatives. The most frequent type of error involved individuals in the upper generations, detected through their descendants due to the lack of genotype data for the ancestors. These errors were frequently corrected through the joining of disconnected pedigrees. It is difficult to generalize these findings to other populations in which the types and distribution of pedigree errors may be quite different. Presumably in Framingham, kinship terms are usually given a biological interpretation. In other populations where this is not strictly the case, serious errors would seem possible.
The small number of Mendelian genotyping errors in the Framingham data that had not been detected previously became evident only following the pedigree corrections. Genotype errors that imply unlikely double recombination events were more common, the exact number depending on a definition of probable mistyping.
Figure 4
Distribution of double-recombinant genotyping errors The distribution of mistyping probabilities for the markers in chromosome 7 is shown on the left, and the proportion of genotypes blanked on the right, as determined by the mistyping probability thresholds on the x-axis. We chose to blank genotypes with mistyping probabilities greater or equal to 0.25.
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References
1. Broman KW: Cleaning genotype data. Genet Epidemiol 1999, 17(suppl 1):S79-S83.
2. Sun L, Abney M, McPeek MS: Detection of mis-specified relationships in inbred and outbred pedigrees. Genet Epidemiol 2001, 21(suppl 1):S36-S41.
3. Cherny SS, Abecasis GR, Cookson WOC, Sham PC, Cardon LR: The effect of genotype and pedigree error on linkage analysis: analysis of three asthma genome scans. Genet Epidemiol 2001, 21(suppl 1):S117-S122.
4. Sun L, Wilder K, McPeek MS: Enhanced pedigree error detection. Hum Hered 2002, 54:99-110.
5. Ihaka R, Gentleman R: A language for data analysis and graphics. J Comp Graph Stat 1996, 5:299-314.
6. Mamalka PM, Dyke B, MacCluer JW: Pedigree/Draw for the Apple Macintosh, Technical Report No. 1. San Antonio, TX, Population Genetics Laboratory, Southwest Foundation for Biomedical Research 1989.
7. Sobel E, Lange K: Descent graphs in pedigree analysis: applications to haplotyping, location scores, and marker-sharing statistics. Am J Hum Genet 1996, 58:1323-1337.
8. Sobel E, Papp JC, Lange K: Detection and integration of genotyping errors in statistical genetics. Am J Hum Genet 2002, 70:496-508.
9. Matise TC, Perlin M, Chakravarti A: Automated construction of genetic linkage maps using an expert system (MultiMap): a human genome linkage map. Nat Genet 1994, 6:384-390.
10. Green P, Falls K, Crooks S: CRI-MAP Documentation. St. Louis, MO, Department of Genetics, Washington University in St. Louis 1990.
11. Broman KW, Murray JC, Sheffield VC, White RL, Weber JL: Comprehensive human genetic maps: individual and sex-specific variation in recombination. Am J Hum Genet 1998, 63:681-689.
12. Dyke B: PEDSYS, a Pedigree Data Management System User's Manual, Technical Report No. 2. San Antonio, TX, Population Genetics Laboratory, Southwest Foundation for Biomedical Research 1994.