there are some examples where such testing could be desirable to protect public safety, and we have discussed this issue in detail in the accompanying letter (Natowicz et al. 1992*b*).

Dr. Lowden maintains that discrimination on the basis of genetic testing is in the public's interest, with respect to the successful operation of the health and life insurance industries. He further argues that the availability of genetic testing, in the absence of insurance companys' access to this personal information, will result in behaviors by insurance applicants and policyholders that will pose serious financial risks for insurers (adverse selection). Despite considerable rhetoric in support of this claim, we are unaware of substantiating data and request that Dr. Lowden and his colleagues in the insurance industry provide documentation of the phenomenon of adverse selection.

It is Dr. Lowden's belief that "underwriting is inherently fair because it spreads risk among a large cohort but requires those who are expected to make early or excessive claims to bear some of the financial responsibility" (actuarial fairness). Actuarial fairness is not moral fairness. The question of whether actuarial fairness may indeed be fair is a moral and political one. Our health insurance system as currently constructed and implemented serves primarily to provide for the economic health and well-being of insurance companies. Dr. Lowden's statement is particularly disingenuous since "some of the responsibility" often means that those who are in need of health insurance as a result of, for example, a chronic medical condition not infrequently find themselves penalized as a result of a medical circumstance over which they have no control. They must often pay exorbitant premiums or suffer universal denial of health insurance coverage. As Light has recently stated, "actuarial fairness is morally unfair because it reduces access to life opportunities and increases suffering for those disadvantaged by risk, pain, and illness" (Light 1992, p. 2507).

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Perils of Gene Mapping with Microsatellite Markers

To the Editor:

The discovery of microsatellite polymorphisms has revitalized the genetic mapping of the human genome and promises to have a dramatic effect on human disease gene mapping. The high polymorphicity, relative abundance, and amenability of these markers to assay by PCR amplification gives them a significant advantage over previous markers, which explains their general acceptance and widespread use (Litt and Luty 1989; Weber and May 1989). Preliminary chromosome maps have been constructed using microsatellites exclusively (Weber et al. 1991; Hazen et al. 1992; Kwiatkowski et al. 1992), and disease loci have been mapped by linkage to these markers (Wijmenga et al. 1991). The markers provide new optimism for the mapping of disease genes, particularly for the mapping of complex genetic disorders. We present evidence that the very qualities that render these markers so efficient for chromosome mapping in large reference pedigrees can lead to dramatic lod score bias when applied to the typical pedigrees used to study genetic

disorders, particularly when the disorder under study is complex.

We have recently genotyped over 160 microsatellite markers against seven families who segregate a complex genetic illness, panic disorder. In the course of this study we encountered two problems in the analysis of disease pedigrees by microsatellite markers. First, we have found that the practice of assigning equal marker-allele frequencies for polymorphic alleles leads to systematic and pronounced bias in lod scores. Second, we have discovered complications in assigning alleles for microsatellite markers. This second problem complicates attempts to remedy the first. These problems are not peculiar to microsatellite markers; indeed, VNTR alleles are often impossible to resolve with current technology, and the estimation of allele frequencies is exceedingly complex. However, only a fraction of VNTR markers show continuous allelic variation, and it is probable that disease gene mapping will in the future become dominated by the more efficient and more abundant PCR-based microsatellite markers. The majority of microsatellites are multiallelic, and the typical skew in the allele distributions (often two or three alleles will be quite common, and the rest will be quite rare) makes assignment of equal allele frequencies particularly problematic. In this letter, we describe the problems we have encountered and offer suggestions for researchers who use and generate microsatellite markers.

We analyzed the panic-disorder pedigrees, assuming a dominant mode of inheritance, and calculated lod scores with the LINKAGE program (Lathrop et al. 1985). For each microsatellite marker, allele frequencies were initially assigned as $1/n$ for all alleles, where n is the total number of alleles reported for the marker. When a marker yielded a lod score in excess of 1, the analyses were repeated using the published allele frequencies.

A total of five markers initially yielded lod scores of 1 or more, when allele frequencies were specified as $1/n$. Table 1 shows the initial lod scores for these markers, as well as the lod scores obtained on reanalysis using published allele frequencies. The lod scores for four of the five markers decreased on reanalysis. The magnitude of these changes, which are due to the presence of untyped (missing) individuals at key positions in some of the pedigrees, was somewhat surprising.

For instance, in one case (marker 3 of table 1), the marker initially yielded a lod score of 2.14 at a recombination fraction of 0 for the seven families, but when published allele frequencies (Weber and May 1989) were used, the lod score dropped to 0.34. Pedigree 5009, which accounted for most of the change in this lod score, is shown in figure 1. The inflation of the lod score when the allele frequencies were assigned as $1/n$ occurred because of the number of untyped individuals in the first two generations of the pedigree and because affected individuals tended to share a particular common allele. When this allele is assigned frequency $1/n$ (11% for a nine-allele marker), the likelihood of sharing of the allele that is identical by descent (IBD) in the affected individuals is inflated, and this is reflected in the inflated lod score at small recombination fractions. However, when the observed frequency (from a random sample) of 38% is assigned, the likelihood that the allele is shared IBD is significantly lower, and its occurrence in the affected individuals contributes much less evidence for linkage. Note that while the average effect of assuming equal allele frequencies is an inflation of the lod score, it is possible, in any given case, for this practice either to deflate or to have little effect on the lod score. For instance, in the (less likely) event that there is linkage of the marker to the disease gene but that the marker allele that happens to be segregating with the disease gene in the family (or, less likely still, in several families) happens to be one of the very rare ones, evidence for linkage could be greatly underestimated. This is, however, the less likely scenario. Using simulation, Ott (1992) illustrates that the practice of using equal allele frequencies with the presence of untyped individuals in the family tends to inflate lod scores and that this inflation can be substantial.

In theory, observed marker-allele frequencies can be easily ascertained from the published literature, by

Table 1

Changes in Lod Scores Due to Marker-Allele Frequencies

	Maximum Lod Scores of	
Marker	Marker-allele Frequencies for $1/n$	Published Marker-Allele Frequencies
1	1.53	.06
2	3.08	1.25
3	2.14	.34
4	1.34	1.02
5	1.44	1.52

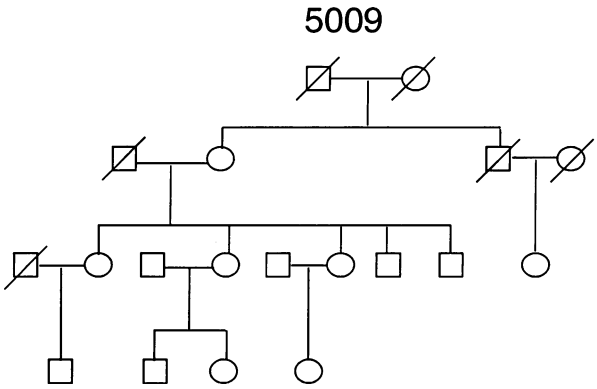

Figure 1 Pedigree of the panic disorder family. Affected status is not shown, to keep laboratory personnel blind to diagnosis. All deceased members of the pedigree (indicated by a slash [/] through the symbol) were not typed.

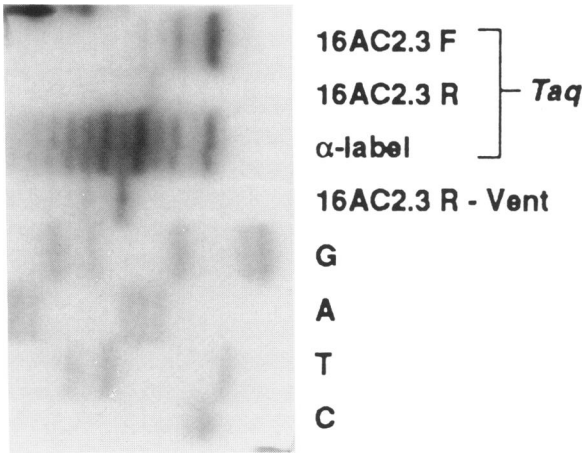

Figure 2 Oligonucleotide primers (16AC2.3F and 16AC2.3R) were 5′ end-labeled according to a method described elsewhere (Maniatis et al. 1982) and were used without further purification in 14-μl PCR reactions containing 10 mM Tris pH 8.3 at 25°C, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM each dNTP, 19 ng of each primer, 56 ng of genomic DNA, 0.5 units of *Taq* polymerase (Boehringer-Mannheim). Alternatively, 0.3 units of Vent polymerase (New England Biolabs) was used with its supplied buffer. PCR products labeled with alpha-dCTP were synthesized according to a method described by Weber and May (1989). PCR products were resolved on 0.4 mm 6% acrylamide, 7 M urea sequencing gels by using 96-well combs, and allele sizes were estimated by comparison with pUC18 DNA sequencing ladder made with a dsDNA Cycle Sequencing System (BRL).

comparison of the observed allele size with the published sizes (in base pairs). For technical reasons, however, this is not at all straightforward. The size of the PCR product can vary depending on the method of labeling (5′ vs. internal), the type of thermostable polymerase, and which member of the primer pair is kinased. As shown in figure 2, an apparent size difference of 4–5 bp is observed, depending on which of the oligonucleotides is kinased (compare lanes 1 and 2 of fig. 2). This effect has been observed for 24 (89%) of 27 primer pairs we have tested, and the observed migration difference has a range of 1–6 bp. The differences may be attributable to asymmetric addition of 3′ nucleotides to the two strands by the *Taq* polymerase (Clark 1988). Vent polymerase synthesizes a product that is 1 bp shorter (lane 4 of fig. 2), presumably because it has a 3′-to-5′ exonuclease activity giving flush ends (Lohff and Cease 1992).

In our experience, it is generally possible to correlate observed:published allele sizes by identifying the most common alleles from the married-in individuals in our pedigrees and by comparing these to the published allele frequencies. A solution practiced by Weber et al. (1991) is to type two reference members of the Centre d'Etude du Polymorphisme Humaine (CEPH) pedigree against each new microsatellite marker and to report these genotypes (in base pairs) as part of the marker characterization. This would allow other laboratories to include these individuals on their gels and hence be able to make accurate allele assignments for the disease pedigree under study.

Assignment of accurate allele numbers and hence frequencies is further complicated when, inevitably, new alleles are identified by typing disease pedigrees. In our study of 160 markers, 29 (18%) have yielded alleles that were not in the initial marker report. While this is not a problem for an individual laboratory, the ability of different laboratories to share genotypings will be hindered, as new alleles found in different laboratories will be assigned different allele numbers. Again, complex genetic traits are likely to suffer most because of the highly collaborative effort required to attain a critical mass of pedigrees. Often the new alleles could have been predicted to exist, as they occurred between two reported alleles. Since many markers with allele frequencies based on only 20–40 individuals are reported, it is to be expected that, in the course of a genetic search, new alleles may be found. These new alleles often occur within the published size set, which disturbs the numbering of the alleles. Even when allele numbers are correctly assigned, there will be ambiguity regarding the relevance of allele frequencies from a random but unrelated population to the

families being studied. In some cases it may be helpful to calculate allele frequencies from the married-in members of the families in the linkage study or from individuals in the same sampling population.

Unlike the CEPH reference pedigrees used for marker mapping, pedigrees used for linkage studies of diseases often have members of the first or second generation who are unavailable for genotyping. The reconstruction of these "missing individuals" for lod score analysis is sensitive to marker-allele frequency. In consideration of the large collaborative effort that will be required to find linkage to the complex genetic disorders, there is a need to standardize the way in which alleles are numbered and reported. The ideal method would be for all genotypes to be recorded and reported by size in base pairs, not by allele number, but this is impractical with respect to the present linkage-analysis programs. We therefore suggest the following.

1. Inclusion of the genotypes and allele sizes of two CEPH individuals (1331-01 and 1331-02) for each marker, as done by Weber et al. (1991), to eliminate the variability of both labeling and enzyme effects observed here.
2. Improved reporting of the methods used to generate the allele frequency data. This includes the enzyme type and manufacturer, the labeling method, and the type of DNA sequencing ladder used. For end-labeled PCR products, the kinased member of the oligonucleotide pair should be noted.
3. Generation of allele frequencies from as large a sample as practical, to reduce the chances of new alleles being found. We are able to generate genotypes of 88 unrelated individuals on one sequencing gel (using a 96-well comb). This number of individuals has the power to detect alleles present at a frequency of .017 95% of the time and at a frequency of .026 99% of the time. Also, a brief description of the sample, e.g., North American Caucasian, etc., should be included.
4. Assignment of allele numbers, with 1 being the largest observed allele, and assignment of numbers to all alleles whose existence is postulated.

These changes will necessitate modifications of the existing data bases in use by the human-gene-mapping community. The CEPH data base currently accepts only 15 alleles at a given locus. The inclusion of alleles that were predicted but not observed will increase the number of alleles to greater than 15, for many markers. A dinucleotide-repeat marker with 25 *observed* alleles has been generated in this laboratory (K. Petrukhin, personal communication), and the CEPH data base is unable, at present, to accommodate this marker. The Genome Data Base will need new fields for the genotypes of the CEPH reference individuals, as well as fields for the methods used to generate the allele-size data and optimum PCR amplification conditions. Furthermore, the greater number of alleles for these markers and the need to assign actual allele frequencies for the computation of lod scores will require accommodations, by linkage programs, of markers with as many as 30–40 alleles.

There is a need to standardize the sizing and numbering of microsatellite alleles, as well as the subsequent estimation of allele frequencies, so that the disease-mapping community can derive the maximum benefit from these new highly polymorphic markers. It is unfortunate that the solutions proposed will not be easily implemented. Marker development is primarily driven by efforts to map the human genome. Accurate mapping in CEPH families, and even calculation of heterozygosity values, do not demand accurate documentation of individual allele sizes, which the disease mapper will need. In the past, impetus for careful characterization of marker alleles came from publication in peer-reviewed journals in which authorship is attributed. The current trend toward documentation of new markers in public data bases could, ironically, exacerbate the situation. It is clear that some problems, such as the relevance of population frequencies to a particular disease study, cannot be avoided when highly polymorphic markers are analyzed for linkage in incomplete pedigrees. In spite of these impediments, the mapping of complex disorders will require attention to these problems.

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Abuse of Genetics in Nazi Germany

To the Editor:

Dr. Harper (1992) discusses the abuse of genetics in the first decades of this century in the United States and particularly in Germany, using Huntington disease as an example. Although there are some early reports (e.g., Platen-Hallermund 1948), it is true that after 1945 it took many years until the involvement of scientists and health officials in compulsory sterilization and killing of psychiatric and other patients in Nazi Germany was analyzed in detail. Harper, however, gives the impression that the denial of the contribution of scientists in the Nazi crimes continued until recently in Germany. This impression deserves some commentary.

During the past decades an immense German literature came out in which many aspects of this outrageous part of German history were analyzed. Some major publications should be mentioned in this context. The historians Zmarzlik (1963) and Wehler (1974) thoroughly examined the aims of social Darwinism in the first decades of this century in Germany, as well as its influence on later history. An early and detailed analysis of eugenics was published by Conrad-Martius (1955). The late professor of history of medicine at the University of Mainz, Mann (1973) initiated a long-standing project on the biological ideas in the 19th and early 20th centuries, a prehistory necessary for the understanding of the later development. Under his guidance a whole series of doctoral theses on biographies of men who became key figures for Nazi ideology and on other central topics were published. The psychoanalyst Mitscherlich and the young physician Mielke gave a detailed report of the trials against the medical doctors who were involved in experimentation in humans, originally written in 1948 for the West German physicians' association (Mitscherlich and Mielke 1960). The psychiatrist Schmidt (1965) gave a frightening account on the "selection" in a psychiatric hospital, and the psychiatrist Dörner (1967) wrote an early review on the killing of patients in Nazi Germany. Nowak (1980), an East German theologist, gave a detailed analysis of euthanasia and sterilization in the Third Reich, focusing on the role of the Protestant and Catholic Churches. Klee (1983), Schmuhl (1987), and Weingart et al. (1988) published excellent books on the history of "racial hygiene" (the German term for eugenics) and "euthanasia" in Nazi Germany, including the history before 1933. In my own textbook on psychiatric genetics I devoted 34 of 368 pages to historical aspects (Propping 1989). This became possible for me only because a vast literature existed already on this topic.

There are still gaps to fill in the history of the abuse of genetics during the Nazi period. On the basis of biographies of the involved doctors and scientists, their intentions, their hopes, and their fears, we have to understand why and how they became guilty. Nevertheless, some general conclusions appear already warranted: The combination of a poor understanding of population genetics; the old and widespread fear of degeneration, in view of the loss of millions of young