and novel ~835-bp fragments from normal and abnormal PrP alleles are shown, as are the two larger heteroduplex fragments. The ~835-bp fragments represent homoduplex PCR amplification products from the PrP alleles containing ~20–30 bp interstitial deletions, while the two larger heteroduplex fragments represent hybrid DNA molecules formed by the annealing of corresponding coding and anticoding segments derived from the normal (860-bp) and partially deleted (~835-bp) PrP alleles. The migration of the heteroduplex molecules is slowed by confirmational changes imposed by the presence of unpaired bases from the normal allele, which correspond to the deleted bases in the abnormal allele. No samples analyzed were homozygous for the deletion.

Further, prior to DNA sequence analysis, we used DASH to rapidly determine whether the deleted alleles in controls were identical to that of the proband; equal amounts of PrP PCR products from each heterozygous control were mixed with those of the proband, were denatured at 95°C for 10 min, were reannealed at room temperature for 30 min, and were subjected to gel electrophoresis (fig. 2). The formation of only two heteroduplex bands (fig. 2, lanes 1–4 and 6) suggests that the DNA of both individuals may have the same deletion. In contrast, the formation of four heteroduplex bands (fig. 2, lane 5) formed by the four possible combinations of coding and anticoding strands indicates that there are allelic differences between the deletions occurring in the PCR products of the heterozygous proband and those in the control.

In summary, our findings of PrP interstitial gene deletions suggest that the 24-bp deletion, identified in our FCJD patient as well as in the recently cloned PrP gene and, most likely, in the uncharacterized ~20-bp deletion reported in a Moroccan family with no history of neurologic disorders (Laplanche et al. 1990), represent polymorphisms, which as described here, appear to be heterogeneous. Further, DASH—the screening method we used to detect allele-specific heteroduplexes—is a rapid technique to distinguish allelic differences resulting from small deletions or small insertions and can be used to prioritize samples for DNA sequence analysis.

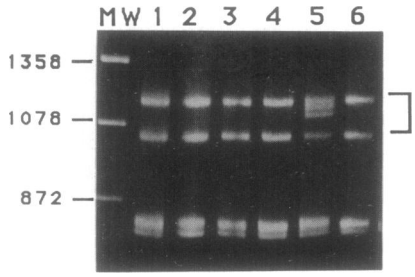

Figure 2 Ethidium bromide–staining patterns showing results of DASH. Equal amounts of PCR products from controls (lanes 1–6, as in fig. 1) and from a FCJD patient were mixed. Bracket indicates differences in heteroduplex patterns.

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Mitochondrial ND-1 Mutation in Leber Hereditary Optic Neuropathy

To the Editor:

Leber hereditary optic neuropathy (LHON) is a maternally inherited form of acute visual loss which occurs predominantly in young men (Johns 1990). Discovery of a point mutation at position 11778 in the ND-4 gene of mtDNA provided the first documentation of

a human disease caused by an inherited mtDNA mutation (Wallace et al. 1988). The 11778 mutation accounts for about one-half of the cases of LHON (Huoponen et al. 1991), and two other causative mutations have recently been described in the ND-1 mtDNA gene (Howell et al. 1991; Huoponen et al. 1991). A G-to-A transition at position 3460 in the ND-1 mtDNA gene was recently discovered in 3 (14%) of 21 LHON families in Finland and in none of 60 controls. The 3460 mutation, which was homoplasmic in all 3 families, changes conserved alanine 52 to threonine and could be readily detected by the loss of an *Aha*II restriction site (GPuCGPyC) (Huoponen et al. 1991).

In order to verify the occurrence of the 3460 mutation in a different population of LHON patients, we screened American individuals with acute visual loss for this mutation and also determined its frequency in a larger group of disease and normal controls. A region of the ND-1 gene bracketing the mutation was amplified by PCR with a forward primer at position 3346–3363 and a reverse primer at position 3636–3650. Digestion of the resultant PCR product with the restriction enzyme *Bsa*HI (an isoschizomer of *Aha*II) resulted in either two wild-type bands of 190 nucleotides and 114 nucleotides or a single 3460 mutant band of 304 nucleotides (because of the loss of the single *Bsa*HI site). The presence of the 3460 mutation in those patients who lost the *Bsa*HI site was verified by direct sequencing of the PCR product (Johns et al. 1989), since any alteration in the six nucleotides that constitute the *Bsa*HI site (including two in the third position of a codon) could cause the abnormal restriction-fragment pattern noted.

Nine independent probands harbored the 3460 mutation, and no false positives were detected among 200 disease and normal controls. The 3460 mutation was rarer in our population: the ratio of 3460-positive probands to 11778-positive probands was 9:68 (.13), versus 3:11 (.27) in the Finnish LHON population. Seven of nine individuals were homoplasmic, and direct sequencing verified that all contained the G-to-A transition. All probands were Caucasian Americans, and none were of known Finnish extraction. Four probands carried a simultaneous mutation in another complex I mtDNA gene, at positions 4917 (ND-2) (one proband) and 13708 (ND-5) (three probands). The 4917 and 13708 mutations have previously been demonstrated in multiple families with LHON (Johns and Berman 1991) and most likely represent synergistic or indicator mutations that are in linkage dysequilibrium with the primary pathogenetic mutation. None of the nine probands harbored the 11778 mutation.

We therefore verified the international occurrence of the 3460 mtDNA mutation in patients with LHON and noted that it had a two- to three-fold lower prevalence in our study population. Simultaneous mtDNA mutations in other complex I genes were noted in four (44%) of nine families, which is consistent with the hypothesis that such alternative mtDNA mutations occur frequently in LHON (Johns and Berman 1991). These data confirm that the genetic heterogeneity underlying LHON is due to locus heterogeneity (mutations in multiple different mtDNA complex I genes), as opposed to allelic heterogeneity (mutations at multiple sites in the ND-4 gene) (Howell and McCullough 1990; Huoponen et al. 1990; Johns 1991*b*). The 3460 mutation occurs in one of the two regions of greatest sequence conservation in the ND-1 gene identified by evolutionary sequence comparison (Burger and Werner 1985) and therefore is likely to be pathogenetically significant.

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The 4% Solution (Approximately)

To the Editor:

Medical researchers spend a great deal of energy in measuring the frequencies of various disorders, signs, symptoms, and events. Yet, when they make statements about these frequencies, it sometimes appears that they are not thinking about how best to do it. A precise way to state a frequency is as a mean and SD or, less formally, as a mean with a verbal indication of its degree of imprecision, such as "about," "around," or "roughly." Instead, many authors seem reluctant to state a specific figure but prefer to state a range. For example, "2–3% of all couples are at high and recurrent risk of having a child with an inherited disease." (This and subsequent quotations are all taken from the published literature, but references are not given, for diplomacy's sake.) Or, again, "the frequency of congenital malformations is 3–5%." What does this mean? According to Humpty Dumpty it means whatever the writer means it to mean, nothing more and nothing less. But the writer does not say what she or he means it to mean.

Does it mean that the frequency is not lower than 3% and not greater than 5%? Probably not. Or that estimates of the frequency range from 3% to 5%? From what one knows of such estimates, this meaning also seems unlikely. Or that 3% and 5% are the confidence limits of the estimate of the mean? Again, probably not. What it really means, I fear, is that the writer can't make up his or her mind. It would be more charitable, perhaps, to say that the writer is unwilling to give a single figure because that would give a false impression of precision and is also reluctant to use an adjective implying imprecision, such as "about," "around," or, to sound more scientific, "approximately."

Some authors state frequencies as ranges to imply that the estimates vary widely. Fair enough. "The prevalence of sleepwalking is 1–6% of different patient populations" or, more striking, "hypopigmented patches occur in 15% to 85% of cases of tuberous sclerosis." "Depressive symptoms have been reported in 35–70% of post-viral fatigue patients." "Mild forms [of hyperstimulation syndrome] may be seen in 3–83% of women undergoing treatment." Not very helpful to the reader—or to the practitioner—but at least one gets the impression either that frequencies are vary variable or that estimates are very imprecise, or both. When the stated range is small, however, this cannot be the interpretation. "Klackenburg gives a prevalence rate of 5–6%." Or consider "a significant mortality rate of 0.5–1%." Surely these statements are not meant to imply a range but simply reflect a reluctance to state a single figure.

In contrast to those who feel impelled to state a range, others give a false sense of precision by stating a figure to an unrealistic number of decimal points. "Estimates [of the prevalence of sleepwalking] include 2.2% of young healthy adults, 2.5% of the general population, the highest being at age 11–12 (16.7%)." Do the figures after the decimal point mean anything?

Similarly for "a frequency of 1 in 42221 births," a figure presumably derived from letting the calculator complete the division, but do we really need that final 221 births? Does it not give a false sense of precision?

Some authors combine the two approaches. For example, "8.2% of CVS cases go on to amniocentesis. The risk of malformation is 2–3%." Or "the frequency [of x] is 3.5 to 15–20%." "The recurrence risk for autism is 8.6% with confidence limits of 5.8 to 12.2%" (what precision!); and the figure given for use in counseling is "8 to 9%"! Certain procedures for egg retrieval result in "an *average* of *about* 4–6 eggs per cycle" (emphasis mine). And some just don't seem to be paying attention. What does "at least 100 to 1000 cells" mean? Or "a frequency of approximately greater than 1%"? Or "in nearly 55.1% of . . . "? I could go on but hope I have made the point. Perhaps I am being finicky and pedantic, but when stating a frequency