possibility but emphasize that, in evaluating the possible effect of stress on mutation rates, one must account for any differences in population dynamics in stressful and nonstressful environments. One of the important lessons of the directed mutation controversy is just how difficult it is to rigorously quantify mutation rates, which cannot be measured directly but must be inferred indirectly from the population dynamics of mutants and their progenitors (*4*).

Watson hypothesizes that excision allows the conjugative Tn*916* to escape from an ill-fated genome. We agree that it is important to consider the consequences of transposon-induced mutations to the fitness of a transposon as well as its host, and we have similarly suggested that *Mu* excisions may arise as that transmissible element attempts to leave a starving host (*5*).

Hurst and Grafen describe the interesting phenomenon of ripping in fungi. Ripping appears to have evolved to eliminate parasitic genetic elements, such as transposons, that spread by making copies of themselves and to prevent nonhomologous pairing during meiosis (*6*). The question raised by Hurst and Grafen is whether ripping qualifies as directed mutation. According to our definition of directed mutation (*4*, p. 188), "comparable increases in rate do not occur . . . for the same mutation in similar environments where it is not advantageous." With respect to ripping, Hurst and Grafen define similar environments on the basis of whether or not a gene duplication has occurred, and they conclude that ripping mutations occur only when they are advantageous. However, an occasional gene duplication that had fortuitously beneficial effects would also presumably be subject to ripping (in which case the ripping mutations would be misdirected). Thus, we regard ripping as a mutational process that is advantageous on average, rather than a process that occurs at a higher rate specifically when the mutation is advantageous.

Our difference with Hurst and Grafen, therefore, seems to be a matter of semantics. Our restrictive definition for directed mutation was intended to (i) emphasize the need for proper controls in experimental tests of the directed mutation hypothesis; (ii) distinguish that hypothesis from others concerning possible advantages of elevated rates of random mutation in certain genes or under certain circumstances; and (iii) highlight the issue of whether an organism can somehow test the value of a novel phenotype before introducing the corresponding change into its genome.

Ripping is similar in certain respects to other processes that repair damaged DNA and protect against invasion by foreign DNA. For example, methyl-directed repair preferentially resolves DNA mismatches in favor of the unchanged allele (*7*), while restriction-modification immunity tends to exclude the incorporation of foreign DNA into the genome (*8*). Like ripping, these processes appear to be adaptations to restore the status quo to a genome, rather than adaptations to generate variation that might allow an organism to track a changing environment. Thus, they provide an interesting contrast to the mechanisms discussed by Rainey and Moxon. None of these mechanisms, however, involves the reverse flow of information from phenotype to genotype, which might allow an organism to test the value of a novel phenotype before introducing the corresponding change into its genome, as Cairns *et al.* (*9*) so provocatively proposed.

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Evidence of Genetic Heterogeneity in the Long QT Syndrome

The long QT syndrome (LQT) is a familial predisposition to sudden death from cardiac arrhythmias (*1*, *2*). M. Keating *et al.* performed linkage analysis in a large Utah family and found that the disease was closely linked to the Harvey *ras*-1 (H-*ras*-1) locus on chromosome 11 (*3*). With the use of the probe pTBB-2 at the H-*ras*-1 oncogene, a logarithm of the likelihood ratio for linkage (lod score) of +16.44 was obtained by Keating *et al.* In a subsequent study (*4*), tight linkage of LQT to the H-*ras*-1 locus was found in six other small LQT families. The combined lod score from these two studies was +21.65 at a recombination fraction of 0. This tight linkage suggests that mutations at the H-*ras*-1 locus or at a closely linked locus resulted in LQT in the families studied.

In view of the clinical heterogeneity and possible genetic heterogeneity in this syndrome, we analyzed a large Jewish family with a history of LQT. This family, whose origin is the island of Jerba near Tunis and whose members reside in Israel, is probably the largest family with LQT outside the United States. It comprises 131 individuals, of whom 28 have been affected (Fig. 1).

Clinical and electrocardiographic data collected over 7 years were available for 92 family members and blood samples for

Table 1. LQT status by age and gender. The QT interval was measured in standard lead II and corrected for heart rate with the use of the Bazett's formula (*8*). Multiple electrocardiograms recorded over several years were available for most living family members. To account for the possible variability over time in QT_c per individual, QT_c phenotypic cutoffs were applied to the mean QT_c of all measured QT_c intervals per individual.

Status	Demographic subset [mean QT_c (in seconds)]		
	Children	Adult males	Adult females
Affected	>0.46	>0.45	>0.47
Unaffected	<0.44	<0.43	<0.45
Equivocal	0.44 to 0.46	0.43 to 0.45	0.45 to 0.47

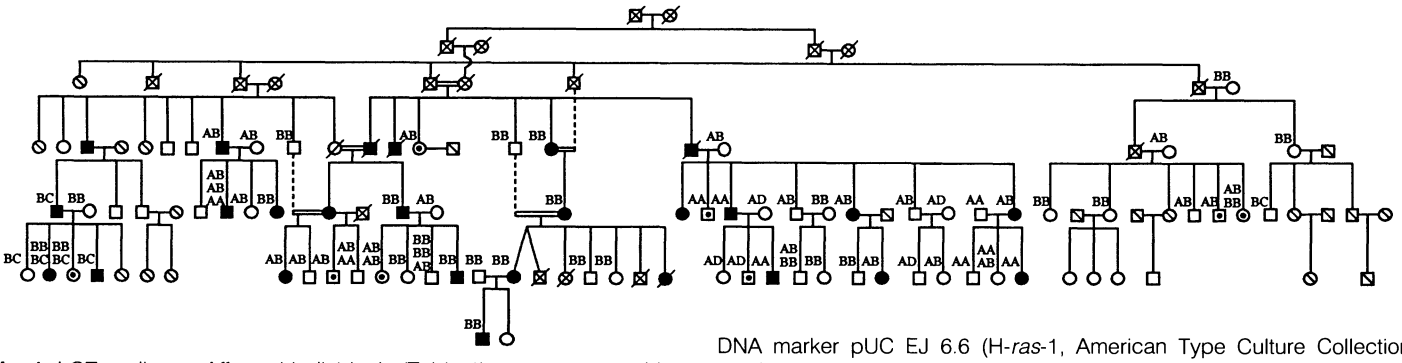

Fig. 1. LQT pedigree. Affected individuals (Table 1) are represented by solid circles (females) or solid squares (males); unaffected individuals by open circles and squares; individuals with equivocal LQT status by a central dot; those with unknown status by a short diagonal line from top left to bottom right; deceased individuals by a long diagonal line from top right to bottom left; and consanguineous marriages by two horizontal lines. Above and to the left of each genotyped individual, alleles of the DNA marker pUC EJ 6.6 (H-*ras*-1, American Type Culture Collection number 41028) are listed. At this locus, the restriction enzyme Bam HI revealed four distinct alleles: (A) 7.8 kb, (B) 6.5 kb, (C) 7.1 kb, and (D) 6.8 kb. Individuals with identical status and gender within the same nuclear family are represented by a single pedigree symbol, and their genotypes are presented separately above each symbol. The pedigree structure has been altered to protect confidentiality. Informed consent was obtained from all family members.

genetic analysis were available for 74. Strict electrocardiographic criteria for QT interval prolongation, derived from a normal electrocardiographic database (5), were used to characterize the phenotype. Although the conventional upper limit for the corrected QT interval (QT_c) is 0.44 s, this criterion has been shown to be inaccurate in separating normal individuals from those with LQT (6). To accommodate uncertainty in phenotypic classification at intermediate values of QT_c, we characterized all family members in our study according to three phenotypic subsets: affected, unaffected, and equivocal (Table 1). To account for age and gender differences (5), we generated separate phenotypic definitions for three demographic subsets: children (<16 years old), adult (>16 years old) males, and adult females. Cytogenetic analysis of selected affected family members revealed no chromosomal aberrations.

We carried out genetic analyses using two methods. First, we used a complex segregation analysis that resulted in evidence for a single, completely dominant allele (7). We used this major locus and the H-*ras*-1 marker in a combined linkage and segregation analysis. This analysis (Fig. 2, bottom curve) shows that the major LQT locus is not tightly linked to H-*ras*-1 for the family in our study. The best estimate of the recombination fraction is 0.494, which is close to the value of 0.50 that corresponds to no linkage. Linkage can be excluded for $\theta < 0.13$ by conventional exclusion criteria (lod score of -2.0, which corresponds to a P value of 0.0024) or for $\theta < 0.23$ by chi-square with $P = 0.05$.

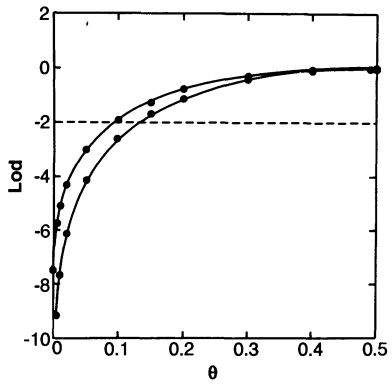

Fig. 2. Profile lod functions for linkage of H-*ras*-1 to LQT. Bottom curve shows lod scores from combined linkage and segregation analysis ($\theta = 0.494$). Top curve shows lod scores from conventional linkage analysis ($\theta = 0.500$).

Second, for comparison with the results of Keating *et al.* (3), we performed conventional linkage analysis using their assumptions (dominant disease allele with a frequency of 0.001 and 0.9 penetrance) and their phenotypic definition. The use of their phenotypic definition, which is slightly different from the definition used by us (Table 1), resulted in a change in the status of 18 individuals from our studied pedigree, 17 from unaffected to equivocal, and one from affected to equivocal. Conventional linkage analysis (Fig. 2, top curve) also excludes linkage to H-*ras*-1 for $\theta < 0.095$ according to the lod score criterion and yields an estimated recombination fraction of 0.500.

Our pedigree differs in several ways from the large Utah pedigree analyzed by Keating *et al.* (3). One difference is ethnicity; because different Jewish ethnic groups tended to live as relatively isolated populations until recent times, specific genetic defects might be expected in their gene pool that are different from those in other populations. Second, in the Israeli pedigree there are several consanguineous marriages. Although inbreeding creates a computational burden for linkage analysis, we were able to retain four of the five inbreeding loops in the pedigree. Only one inbreeding loop, in the third generation, has been broken. The major consequence of not accounting for inbreeding is overestimation of the frequency of the LQT allele; thus, inferences concerning linkage should not be affected.

This analysis, together with that of Keating *et al.*, provides evidence for genetic heterogeneity in the determination of the LQT.

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Response: In our 1991 report (*1*), my colleagues and I described tight linkage between LQT and a polymorphism within the H-*ras*-1 gene on chromosome 11p15.5. Because the family used in our study was large and because the polymorphic marker was informative, the statistical support for linkage was strong, even though we used conservative phenotyping (*2*, *3*). The LOD score for linkage between LQT and the H-*ras*-1 gene was more than 16, which indicated that the odds in favor of linkage were greater than 10^{16}:1. The maximum score of 16.44 was identified at a recombination fraction of 0, which indicated that the gene for LQT was likely to be close to H-*ras*-1. This discovery meant that genetic markers on chromosome 11p15.5 could be used for presymptomatic diagnosis of LQT in this family. We characterized six other families with autosomal dominant LQT (*4*) and found the LQT gene linked to markers on chromosome 11p15.5, which indicated that presymptomatic diagnosis was possible.

Evidence that a second locus might be involved in the pathogenesis of LQT has been presented by J. A. Tobin (*5*). His preliminary data, which used markers at the H-*ras*-1 locus, suggested that the disease phenotype in one large family in Iowa was not linked to chromosome 11p15.5. In their comment, Benhorin *et al.* present another example of locus heterogeneity for LQT. In a study that used a carefully characterized, large Israeli family, they found that the LQT phenotype was not linked to the H-*ras*-1 gene. Again, because of the large size of this family and because of the highly informative nature of the marker, the statistical support for this negative finding was strong.

Locus heterogeneity has been described for many inherited disorders including the myotonias, Charcot-Marie-Tooth, and familial hypertrophic cardiomyopathy. That LQT in the Israeli pedigree is not caused by a gene on chromosome 11p15.5 suggests that what we currently refer to as LQT consists of at least two distinct disorders.

It is not yet clear what percentage of familial LQT will be caused by mutations in a gene on chromosome 11p15.5, nor is the chromosomal location of a second LQT locus known. We recently found two families with LQT in which the phenotype was clearly not linked to chromosome 11p15.5 (*6*). A great deal of work needs to be done before we fully understand the molecular basis of these potentially deadly disorders. Locus heterogeneity has, in the short run, complicated genetic testing for LQT and disappointed many members of families with LQT. In the long run, however, the identification and characterization of two or more genetic mechanisms for this phenotype will teach us much about cardiac repolarization and arrhythmias.

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