Africa food supply may potentially limit hyena litter sizes; in the nearby Serengeti whole litters sometimes starve (*1*).

Competition for nutrition does not, however, explain the marked difference in survival between same- and mixed-sex litters. This phenomenon may be related to differential dispersal; that is, males disperse after puberty, whereas females remain in the maternal clan (*5*). Thus, mixed-sex twins are not competitors as adults, and the benefits gained from disposal of a sibling may not outweigh the costs of prolonged combat and the loss of inclusive fitness through siblicide. Competition within same-sex pairs may occur for different reasons. Rank affects reproductive success in both sexes (17). For females, elimination of a sister results in the removal of a close-ranking competitor, because females acquire the mother's rank in the social hierarchy (*5*). For males, confronting the difficult task of integration into a new clan, large size may be a particular advantage, and elimination of a brother does result in a more rapid weight gain during the first year of life (*15*).

Nonlethal intra-litter aggression occurs in neonatal domestic pigs and at a later age in several canids (*18*); this is the first report of habitual siblicide in a mammal. Siblicide appears to kill nearly 25% of spotted hyena offspring: at birth, 50% of litters are same sex, and half of those individuals succumb. Why does the mother apparently permit her offspring to reduce her own fitness? The fact that aggression was not fatal in captivity, where the mother had constant access to the neonates, suggests that the denning habit of spotted hyenas, whereby the infant pair is safe from predators, but out of reach of the female when not nursing, may permit aggression to proceed unchecked. The fatal outcome of fighting may thus be in part a result of ecological factors limiting potential parental influence on infant behavior, resolving parent-offspring conflict in favor of the offspring. Once aggression appeared, even if as a by-product of selection for some other complex of traits mediated by prenatal androgenization, immense advantage would accrue to the survivor, and there would be powerful selection for adaptations permitting "victory" in intra-litter combat, for example, precocial tooth eruption and general physical maturation (*19*).

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9. Assays were performed on ether extracted plasma as described by P. Licht, I. Zucker, G. Hubbard, and M. Boshes [*Biol. Reprod.* **21**, 411 (1982)] using a testosterone antiserum (from G. Niswender, Colorado State University, Fort Collins) that was cospecific for testosterone and 5α-dihydrotestosterone, and a specific antiserum against androstenedione (from S. Monroe, University of California, San Francisco); labeled testosterone and androstenedione were from New England Nuclear.
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Linkage of a Cardiac Arrhythmia, the Long QT Syndrome, and the Harvey *ras*-1 Gene

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Genetic factors contribute to heart disease. In this study, linkage analyses have been performed in a family that is predisposed to sudden death from cardiac arrhythmias, the long QT syndrome (LQT). A DNA marker at the Harvey *ras*-1 locus (H-*ras*-1) was linked to LQT with a logarithm of the likelihood ratio for linkage (lod score) of 16.44 at θ = 0, which confirms the genetic basis of this trait and localizes this gene to the short arm of chromosome 11. As no recombination was observed between LQT and H-*ras*-1, and there is a physiological rationale for its involvement in this disease, *ras* becomes a candidate for the disease locus.

CARDIOVASCULAR DISEASE IS A MAJOR cause of morbidity and mortality in the industrialized world. Over the last 10 years it has become increasingly apparent that inherited traits are involved in the pathogenesis of most cardiovascular disorders. Much attention has been and continues to be focused on the genes that regulate lipid metabolism and their role in atherogenesis. In this study, we have begun to investigate the genetic basis of a type of cardiovascular disease that is not directly linked to lipid abnormalities, that of cardiac arrhythmias.

Ventricular arrhythmias are a common cause of cardiac arrest and death (*1*). The pathogenesis of these arrhythmias is poorly understood, but predisposing factors include myocardial ischemia and infarction, metabolic abnormalities, and genetic factors. In this study we have examined a large

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family with a syndrome that predisposes affected members to recurrent fainting and sudden death at an early age from ventricular tachycardia and fibrillation. Because affected individuals have prolongation of the QT interval on electrocardiograms, this disorder is called the long QT syndrome (LQT) (*2*). The onset of ventricular arrhythmias in LQT appears to be related to heightened autonomic tone, and symptoms typically occur during periods of exercise, excitement, or anxiety (*3*). For example, affected individuals have frequently died after diving into a pool following exercise on a hot summer day.

The incidence of LQT is not known. However, more than 300 families have been entered in an international registry (*4*) and the extended family used in the present study is estimated to contain more than 400 affected members (*5*). As many affected individuals have only a prolonged QT interval and are otherwise asymptomatic, this trait may be more common than was previously believed.

The chromosomal location of the LQT locus was unknown (*6*). We undertook genetic linkage studies in a branch of a large multi-generation family that includes 40 affected individuals (Fig. 1). Diagnosis of LQT was made on the basis of a corrected electrocardiographic QT interval (QT_c) of 0.45 s (7) or greater in individuals with symptoms, a QT_c of 0.47 s or greater in the absence of symptoms and the exclusion of other causes of ventricular arrhythmias. Phenotypic criteria were identical for females and males. As the electrocardiographic findings of LQT are generally manifest in infancy and throughout life, the age of each individual was not considered during phenotypic evaluation. No individuals under the age of five were included in this study because of limitations in drawing blood samples. Because accurate phenotypic characterization is critical for determination of linkage, asymptomatic individuals who had an intermediate QT interval (QT_c of 0.42 to 0.46 s) were considered of unknown phenotype during linkage analysis.

We tested 245 markers in this family before a linked marker was found and excluded more than 60% of the human genome, including the HLA locus (*8*). Linkage analysis was performed with the program LINKAGE (*9*). The segregation pattern of the LQT phenotype has indicated an autosomal dominant pattern of inheritance with high penetrance (*10*). We assumed autosomal dominant inheritance of a LQT gene with a penetrance of 0.90. This conservative assumption of high but incomplete penetrance was based on a segregation ratio estimated from this pedigree of 45%. A

Table 1. Lod scores (pairwise) between LQT and a DNA marker (pTBB-2) in a five-generation pedigree. Lod scores have been calculated assuming the penetrance is 0.90. When penetrance was varied from 0.60 to 0.95, maximum lod scores at $\theta = 0$ ranged from 15.09 to 16.52. The one-lod confidence interval varied from 3.9 to 4.4% recombination on either side of H-*ras*-1 over this range of penetrances; the one-lod confidence interval was 4% at penetrance of 0.90. Allele frequencies were estimated from the CEPH database (*24*) and from calculations based on gene frequencies in 18 unrelated individuals. Since no estimates of LQT gene frequency exist, for purposes of this study the frequency of this rare disease gene was assumed to be 0.001.

Lod scores at recombination fraction of:					
0.00	0.05	0.10	0.20	0.30	0.40
16.44	15.18	13.81	10.79	7.42	3.76

DNA marker defining a restriction site polymorphism at the H-*ras*-1 locus, pTBB-2 (*11*), was found to be tightly linked to the LQT locus, with a maximum lod score of 16.44 at a recombination fraction of zero. This score corresponds to odds of greater than 10^{16} to 1 in favor of linkage (Table 1). A second DNA probe at H-*ras*-1 (pUC EJ 6.6) (*12*) gave identical results (*8*). This was not unexpected because this marker is in complete disequilibrium with pTBB-2 in family members (*8*).

The H-*ras*-1 locus had been mapped to chromosome 11p by linkage with other known loci and by physical mapping with somatic cell hybrids (*13*). In situ hybridization confirmed this chromosomal assignment and provided independent subregional localization of the H-*ras*-1 locus to 11p15.5 (*14*).

H-*ras*-1 is an intermediary in many different signal transduction pathways. Studies of *ras* have demonstrated its ability to transform mammalian cells (*15*) as well as to promote yeast cell survival (*16*) and mating (*17*). *Ras* proteins reside on the inner surface of cell membranes (*18*) and are similar to G proteins (*19*). As G proteins can regulate myocardial (*20*) and cardiac pacemaker ion channels (*21*), *ras* proteins may similarly regulate myocardial excitability. Physiological data show that p21 *ras* protein and GAP (guanosine triphosphatase–activating protein) regulate cardiac potassium channels (*22*). Therefore, it is reasonable to hypothesize that mutations in the H-*ras*-1 gene cause LQT. As the lod score indicating linkage of LQT to H-*ras*-1 was high, and no recombination was observed between the two loci, the H-*ras*-1 proto-oncogene remains a candidate for the LQT disease gene.

Available clinical evidence suggests that one mutant gene may be responsible for LQT in other families. Prolongation of the QT interval and cardiac arrhythmias are also present in the Jervell and Lange-Nielsen syndrome. However, this syndrome also af-

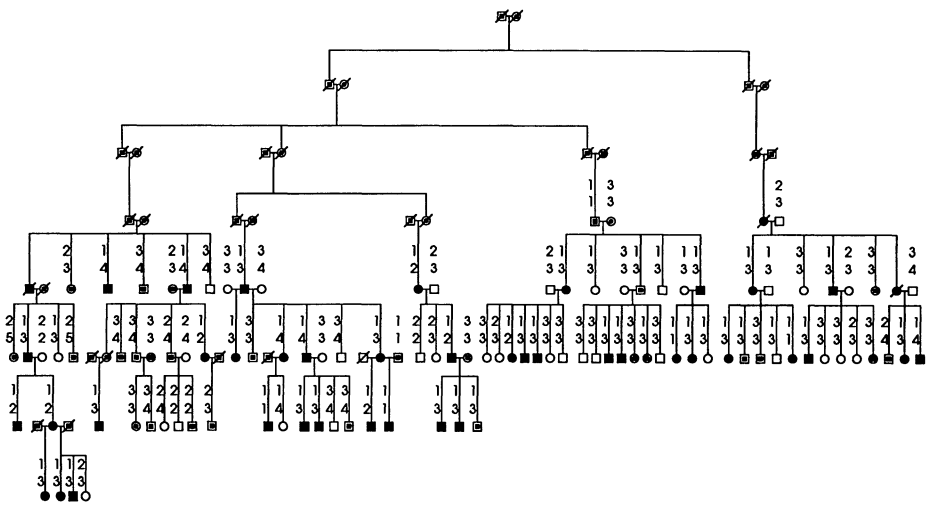

Fig. 1. LQT pedigree. Individuals having the characteristic symptoms of fainting or sudden death due to ventricular arrhythmias, with prolongation of the QT interval on electrocardiogram, are represented by filled circles (females) or filled squares (males). Unaffected individuals are represented as empty circles and squares; individuals with an equivocal or unknown phenotype are represented by a central dot. Above each genotyped individual, alleles are listed for the marker pTBB-2 (H-*ras*-1). At this marker locus, the restriction enzyme *Taq* I revealed five distinct alleles ranging in size from 2.4 to 4.0 kb (*11*). The disease gene cosegregates with the 4.0-kb *ras* allele (allele 1), which has a frequency of 11% in the general population (*24*). The pedigree structure has been altered to protect confidentiality. Informed consent was obtained from each family member before inclusion in the study.

fects hearing and is inherited as an autosomal recessive trait (*23*). The issue of genetic heterogeneity can be resolved by testing additional families with LQT and Jervell and Lange-Nielsen syndrome with the markers described here.

We conclude that a mutation at a single genetic locus on the short arm of chromosome 11 predisposes individuals in this family to ventricular arrhythmias and sudden death. Presymptomatic diagnosis of LQT has previously been difficult because of the normal variability of the QT interval. The existence of genetic markers will greatly improve our ability to identify families and family members who are at risk for sudden death. Attention can then be directed to a search for the gene that causes LQT and to an explanation for the distinctive pattern of ventricular arrhythmias seen in this syndrome.

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Identification of p53 Gene Mutations in Bladder Cancers and Urine Samples

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Although bladder cancers are very common, little is known about their molecular pathogenesis. In this study, invasive bladder cancers were evaluated for the presence of gene mutations in the p53 suppressor gene. Of 18 tumors evaluated, 11 (61 percent) were found to have genetic alterations of p53. The alterations included ten point mutations resulting in single amino acid substitutions, and one 24–base pair deletion. In all but one case, the mutations were associated with chromosome 17p allelic deletions, leaving the cells with only mutant forms of the p53 gene product. Through the use of the polymerase chain reaction and oligomer-specific hybridization, p53 mutations were identified in 1 to 7 percent of the cells within the urine sediment of each of three patients tested. The p53 mutations are the first genetic alterations demonstrated to occur in a high proportion of primary invasive bladder cancers. Detection of such mutations ex vivo has clinical implications for monitoring individuals whose tumor cells are shed extracorporeally.

CARCINOMA OF THE BLADDER IS THE fifth most common cancer in the United States, with an annual incidence of approximately 18 cases per 100,000. The disease prevalence peaks in the seventh decade of life, and over 45,000 cases were detected last year (*1*). Patients with superficial tumors often have recurrences and require careful observation and clinical follow-ups. Those patients with invasive tumors are at risk of dying from their disease, despite radical surgery, radiation, and chemotherapy.

Although genetic alterations are thought to underlie the development of malignancies, no such alterations have yet been identified in a high proportion of invasive bladder carcinomas. The first example of a point mutation in a human tumor (a *ras* oncogene mutation) was identified in a bladder carcinoma cell line (*2*), but similar mutations have rarely been found in the primary bladder tumors subsequently examined (*3*). Differences in the expression of other oncogenes have also been observed in bladder tumors (*4*), but the expression patterns are not necessarily specific for the neoplastic state and have not been associated with mutations.

Cytogenetically, several specific chromosomal losses have been reported to commonly occur in bladder cancers (*5*). The cytogenetic findings have been confirmed and extended by restriction fragment length polymorphism analysis; this analysis revealed frequent losses of chromosomal arms 9q, 11p, and 17p in tumors of this type (*6*). Such chromosomal losses are thought to indicate the presence of putative tumor suppressor genes within the deleted areas (*7*). These suppressor genes presumably exert a negative regulatory effect on neoplastic cell growth. When such suppressor genes are inactivated (by mutation or deletion), the cell acquires a competitive growth advantage compared to other cells, and the neoplastic process progresses.

We suspected that the chromosome 17p deletions in bladder cancers reflected underlying mutations in the p53 suppressor gene; p53 gene mutations have been observed in other human tumor types with chromosome 17p deletions (*8*). To test this idea, we obtained cystectomy or biopsy specimens from 18 patients who had invasive bladder carcinomas. DNA was purified from these specimens (*9*), and exons 5 to 9 were amplified from tumor DNA by means of the polymerase chain reaction (PCR) (*10*). The PCR products were then isolated and subcloned into phage, and pooled clones were sequenced together to evaluate p53 mutations (*11*) (a representative example is in Fig. 1). Eleven of the 18 tumor specimens (61%) contained p53 gene mutations (Table 1). When present, the mutations appeared to be clonal, that is, they were present in the majority of the tumor cells, as

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