Letters to the Editor

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A Gene for Premature Ovarian Failure Associated with Eyelid Malformation Maps to Chromosome 3q22-q23

To the Editor:

Premature ovarian failure and XX gonadal dysgenesis leading to female infertility have been reported in association with an autosomal dominantly inherited malformation of the eyelids: blepharophimosis-ptosis-epicanthus inversus syndrome (BPES; MIM 110100 [McKusick 1992]). This association distinguishes BPES type I from BPES type II, in which affected females are fertile and the transmission occurs through both sexes (Zlotogora et al. 1983). Recently, a gene responsible for BPES type II has been mapped to chromosome 3q22-q23 (Amati et al. 1995; Small et al. 1995), and the critical region for the gene location has been reduced to the interval between loci D3S1615 and D3S1316 (Lawson et al. 1995). Hitherto, however, no information regarding the localization of the gene for BPES type I, in which female ovarian failure is associated with eyelid malformation, has been available. We have studied two independent families (fig. 1) affected with BPES type I, including a total of 12 affected individuals (6 infertile women) and 6 healthy relatives. The diagnostic criteria for the ophthalmological anomaly included (i) reduced horizontal diameter of palpebral fissures, (ii) drooping of the upper eyelids, and (iii) an abnormal skinfold running from the lower lids. Telecanthus and a flat nasal bridge were present in most cases. In both families the disease was transmitted only by the male, and no affected woman of childbearing age was fertile.

Family 1 (nine affected individuals and five healthy relatives) originated from southern Italy and was ascertained at the Medical Genetics Department of San Giovanni Rotondo. Patient IV-4 was referred, at the age of 25 years, for a history of oligomenorrhea (menstrual periods of 45 d). Menarche occurred at the age of 13 years, and menstruation became irregular beginning at the age of 23 years. Apart from eyelid malformation, she had a normal appearance, with normal secondary sex characteristics (height 154 cm, weight 58 kg). Gynecological examination was normal, and pelvic ultrasonography revealed a normal uterus (81 × 42 mm) and ovaries. Several gonadotropin measurements showed high levels of both follicle-stimulating hormone (FSH) (mean = 35 mIU/ml; normal = 3–12 mIU/ml) and luteinizing hormone (LH) (mean = 15 mIU/ml; normal = 0.5–1 mIU/ml), suggesting ovarian failure. Her karyotype, performed on lymphocytes, was normal (46,XX). Female patients III-4 (56 years old), IV-1 (37 years old), and IV-2 (35 years old) were all affected with BPES. They all had normal menarche and, thereafter, a history of increasing oligomenorrhea with primary infertility. Subject IV-5 (17 years old) had menarche at the age of 14 years but subsequently had irregular menstrual periods. In this family, two adult females (III-1 and III-5) with normal eyelids are normally fertile, without menstrual dysregulation.

Family 2 was of Palestinian Arab origin and was ascertained at the Human Genetics Clinic of the Hadassah-Hebrew Medical Center, Jerusalem. Patient III-1 was referred because of secondary amenorrhea. She had menarche at the age of 16 years, but menses stopped 4 years later. At the age of 26 years she was obese (height 160 cm, weight 90 kg) and had typical features of BPES, without additional clinical signs. Hormone tests revealed a high level of LH and FSH, with normal levels of testosterone, suggesting ovarian failure. Her lymphocyte karyotype was normal, 46,XX. The father's eldest sister (II-3; fig. 1) was married and sterile. Two additional paternal aunts were reported to have "abnormal menses"; all these females had similar dysmorphic eyelids. The other members of the family were not available for examination, and DNA samples were obtained only from three affected individuals and one healthy individuals (II-2, III-1, III-4, and III-3, respectively; fig. 1).

We conducted a linkage study to map this disease and focused first on the long arm of chromosome 3, in which

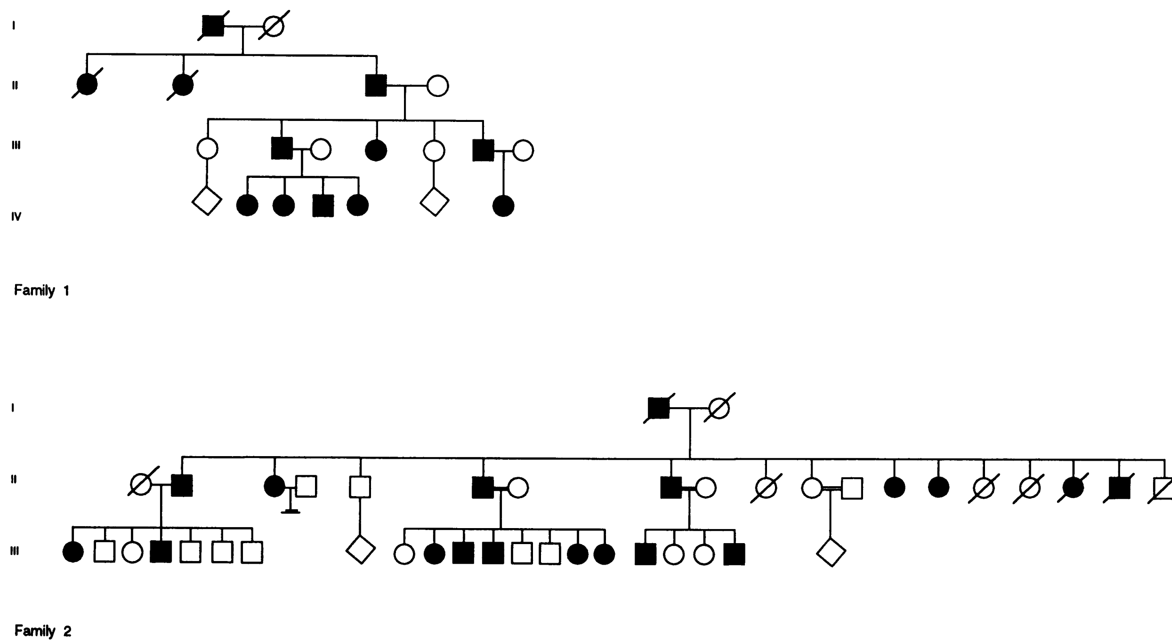

Figure 1 Pedigrees of the two families affected with premature ovarian failure and BPES

a locus for blepharophimosis alone without sterility (BPES type II) has recently been described. Four microsatellites (D3S1292, D3S1549, D3S1316, and D3S1607) were chosen from the Généthon linkage map, on the basis of their informativity and their localization in the 3q22-q23 region. Linkage data shows a maximum pairwise LOD score (Z_{max}) for marker AFM 268vc9 at the D3S1316 locus (Z_{max} = 3.31 at recombination fraction [θ] 0; table 1). The maximum-likelihood estimate for the BPES type I gene location is in the interval defined by loci D3S1292 and D3S1607 (location score in log base 10 = 3.31; fig. 2). Haplotype analysis supplements the multipoint analysis by providing direct evidence of two recombinant events, at loci D3S1292 and D3S1607, respectively, in two affected females from family 1. This result indicates that BPES type I, which involves sterility in affected females, is localized on chromosome 3q22-q23 at the locus where BPES type II previously has been

Table 1

Two-Point LOD Scores for Linkage of BPES Type I to Four Polymorphic DNA Markers of Chromosome 3q

Locus (Probe) and Family	LOD Score at θ =						Z_{max}	Maximum θ
	0	.01	.05	.10	.20	.30		
D3S1292 (AFM199xd6):								
1	−∞	−.51	.12	.32	.42	.36		
2	.55	.54	.48	.42	.28	.15		
Total	−∞	.03	.60	.74	.70	.51	.75	.15
D3S1549 (AFM182yc5):								
1	1.50	1.47	1.37	1.23	.93	.58		
2	.30	.30	.25	.21	.13	.07		
Total	1.80	1.77	1.62	1.44	1.06	.65	1.80	0
D3S1316 (AFM268vc9):								
1	2.70	2.66	2.48	2.25	1.74	1.17		
2	.61	.60	.54	.46	.32	.17		
Total	3.31	3.26	3.02	2.71	2.06	1.34	3.31	0
D3S1607 (AFM319yb1):								
1	−∞	.67	1.21	1.30	1.14	.80		
2	.60	.59	.53	.46	.32	.17		
Total	−∞	1.26	1.74	1.76	1.46	.97	1.76	.1

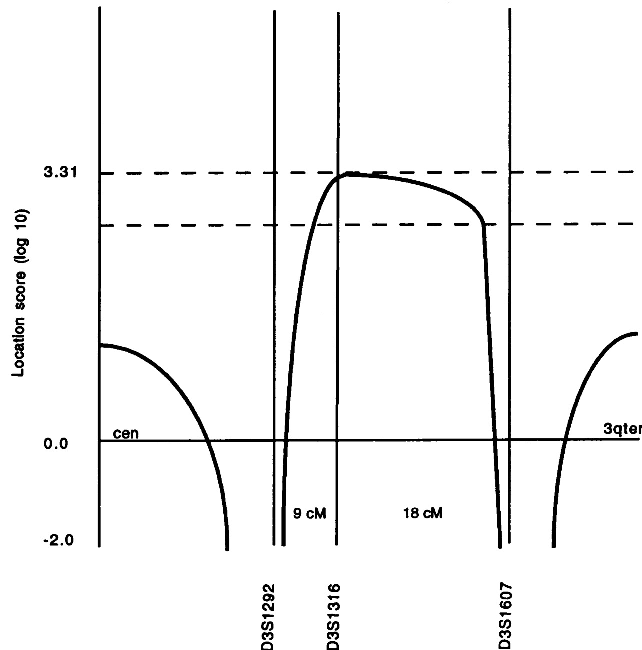

Figure 2 Support for location of the BPES type I gene, with respect to three chromosome 3q markers. Likelihood estimates are given in log base 10. Distances between marker loci are shown, in centimorgans, along the abscissa. The maximum location score for the disease-causing gene is between D3S1292 and D3S1607, over the locus D3S1316.

mapped. In BPES type I, the cause of female infertility is either premature ovarian failure or XX gonadal dysgenesis. In the reports by Zlotogora et al. (1983), Jones and Collins (1984), and Smith et al. (1989), as in both families reported here, there was secondary ovarian failure: affected females have normal menarche, normal secondary sex characteristics, and, subsequently, premature (before the age of 40 years) oligo-amenorrhea with high levels of gonadotropins. However, primary female hypogonadism has also been reported in BPES I (Townes and Muechler 1978; Nicolino et al. 1995). Affected females have poorly developed secondary sex characteristics, and laparoscopy reveals small atrophic ovaries and a small uterus. Histological examination of the ovary, performed in one case, showed fibrous tissue without any primordial, developing, or atretic follicles (Nicolino et al. 1995). All affected females have high levels of gonadotropins and a normal 46,XX karyotype. These findings are typical of XX gonadal dysgenesis (Aittomäki 1994). The clinical variation of ovarian failure observed in BPES type I indicates that an autosomal gene, localized on chromosome 3q22-q23, is necessary for both development and maintenance of ovarian function that was, until now, thought to be under the control of genes localized on the X chromosome.

To explain how gene(s) responsible for both eyelid and ovarian failure are localized at a single locus, it can be hypothesized either that the two types of BPES are allelic disorders or that BPES type I is a contiguous-gene syndrome. In that case, one or several genes implicated in female reproduction should be closely linked, at 3q22-23, to the gene responsible for the eyelid malformations. How a gene or a cluster of genes can be involved in both eyelid and ovarian development is unclear, but it has been suggested that the critical event could take place during the third month of intrauterine life, when both ocular and female genital formation occur (Oley and Baraitser 1988). Recently, Vassilli et al. (1994) have reported that mice homozygous for a targeted mutation in the activin/inhibin βB subunit gene have a defect in both eyelid development and female reproduction. These mutants suffer from failure of eyelid fusion, and females have a normal ovarian function but a profoundly impaired reproductive ability, resulting from the early mortality of their offspring. Although this animal model does not completely correspond to BPES type I, it does, however, demonstrate that a single-gene mutation can be responsible for both eyelid malformation and reproductive failure. Activin and inhibins are dimeric growth factors of the transforming growth factor b superfamily. None of these genes is localized to the chromosome 3q and can therefore be regarded as a candidate gene for BPES. Further characterization of the gene(s) involved in the two types of BPES at 3q22-q23 will, it is hoped, give new clues to the understanding both eyelid and ovary development.

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Limits on Fine Mapping of Complex Traits

To the Editor:

We recently published a paper in the *Journal* on high-resolution genetic mapping of complex traits (Kruglyak and Lander 1995). In that paper, we considered the confidence region for the position of a gene localized by genetic mapping. We showed that the size of this confidence region increases rapidly as the trait becomes more complex (that is, as the relative risk or the proportion of alleles shared by affected relatives decreases). We concluded that using affected-relative-pair analysis to localize a gene conferring a modest increased risk to a region suitable for positional cloning (e.g., 1 cM) requires a large number of relative pairs (or, more generally, meioses).

Since the paper appeared, we have discovered an intuitive way to understand the difficulty of fine mapping of susceptibility genes for complex traits. The insight was motivated by a colleague who inquired why one could not simply confine the search for a susceptibility gene to the region of maximum allele sharing in a sib-pair (or other relative pair) data set.

The key question is thus: what is the chance that a susceptibility gene will *not* lie in the region of maximum allele sharing? The answer is easily obtained by using the methods described in our previous paper (Kruglyak and Lander 1995).

Proposition

Consider a susceptibility locus at which affected sibs share a proportion of alleles $z > 1/2$. (This proportion is given by $z = (z_1 + 2z_2)/2$, where z_1 and z_2 are the proportions of affected sib pairs sharing 1 and 2 alleles identical by descent at the susceptibility locus.) The probability that the gene will not lie in the region of maximum allele sharing in an affected-sib-pair study is $(1 - z)(3z - 1)/z^2$.

Proof

In fact, one can easily show a stronger result. Consider an affected-relative-pair study involving relative pairs with allele-sharing proportion α at random loci and allele-sharing proportion z at a susceptibility locus. The chance that the number of pairs sharing alleles at the true susceptibility locus is lower by at least Δ than the maximum observed number is $q^{\Delta}(2 - q^{\Delta})$, where the quantity $q = \alpha(1 - z)/[z(1 - \alpha)]$. The proof follows from appendices D and E of Kruglyak and Lander (1995). The special case above corresponds to $\alpha = 1/2$ and $\Delta = 1$. The proof implicitly assumes that a large number of relative pairs has been studied; this is a realistic assumption in the context of fine mapping.

Consider the consequences of this relation for positional cloning based on sib-pair data. Let λ_O, λ_S, and λ_M denote the relative risk ratios for an offspring, a sibling, and a monozygotic twin of an affected individual, respectively. Then, $z = (\lambda_O/\lambda_S + \lambda_M/\lambda_S)/4$ for a single-locus trait and $z = (3\lambda_S - 1)/4\lambda_S$ in the special case of an additive single-locus trait for which $\lambda_O = \lambda_S$ and $\lambda_M = \lambda_S - 1$ (Risch 1990*a*, 1990*b*). Thus, for an additive trait with $\lambda_S = 40, 6, 3, 2$, and 1.5, the sharing proportion $z = 0.74, 0.71, 0.67, 0.63$, and 0.58, respectively. The corresponding chance that the gene lies outside the region of maximal sharing is 0.57, 0.65, 0.74, 0.84, and 0.92. By looking only in the region of maximum sharing, one will thus miss a gene conferring sixfold increased risk ~2/3 of the time, a gene conferring threefold increased risk ~3/4 of the time, and a gene conferring