On the probability of identity states in permutable populations: reply to Cannings

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Letters to the Editor

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A *BRCA1* Nonsense Mutation Causes Exon Skipping

To the Editor:

Nonsense mutations can induce the skipping of constitutive exons, as has been shown in a few disease-causing genes (Dietz et al. 1993; Gibson et al. 1993; Naylor et al. 1993; Hull et al. 1994; Santisteban et al. 1995; Pié et al. 1997). Shortened translational reading frames are also sometimes associated with a reduction in mRNA abundance (Maquat 1996). However, few of the premature-termination codons involved in genetic diseases have been comprehensively assayed for induced transcript defects.

The *BRCA1* gene, located on chromosome band 17q21, has a coding sequence of 5,592 nucleotides scattered on 22 exons (Miki et al. 1994). Germ-line mutations in this gene lead to the predisposition to breast and ovarian cancer. More than 1,250 different mutations of the *BRCA1* gene have been reported worldwide, spread over the entire length of the gene (Breast Cancer Information Core 1997 [http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/]); most of them lead to premature-termination codons, as a result of frameshift, nonsense, or splice-site mutations (Couch et al. 1996) or large rearrangements (Puget et al. 1997; Swensen et al. 1997). Most mutation-screening studies are conducted on genomic DNA only, and, as yet, little effort has been made to check for possible induced transcript defects. By contrast, evaluation of the phenotypic risk associated with mutations has received a great deal of attention because it is very important in genetic counseling. A correlation between the location of the mutation in the *BRCA1* gene and the ratio of the incidence of breast to ovarian cancer has been shown (Gayther et al. 1995; Shattuck-Eidens et al. 1995). However, such a trend has not been found systematically. The reason may be that taking into account the location of the mutation rather than its consequences at the level of the transcript could be misleading in such studies, because it is impossible to predict which premature stop codon will lead to a reduction in mRNA abundance or to exon skipping.

We describe here, for the first time, nonsense-mediated exon skipping in the *BRCA1* gene, in one breast and ovarian cancer family (number 3497), recruited by Dr. H. Lynch at Creighton University, Omaha, that contains four breast and four ovarian cancer cases (among which are one case of bilateral breast cancer and one case of bilateral breast cancer and ovarian cancer). A fragment of *BRCA1* cDNA covering exons 16–22 was shown to produce, after amplification by PCR and migration on an agarose gel, two bands of equal intensity in affected family members, one of the expected size (596 bp) and one slightly smaller. Simultaneous direct sequencing of both templates demonstrated a 78-bp in-frame deletion in the smaller fragment corresponding to the entire exon 18. Alternative splicing removing exon 18 does not occur naturally (Xu et al. 1997; authors' unpublished data). In order to identify the basis of exon skipping, we amplified, by PCR, a 352-bp fragment from genomic DNA encompassing 78 bp of intron 17, all of exon 18, and 196 bp of intron 19. We observed no mutation in all the *cis*-acting consensus elements known to be involved in RNA splicing (i.e., the 3′ and 5′ splice sites and the predicted branchpoint flanking exon 18: TTCTAAT at positions −31 to −25). However, a G→T substitution was found in exon 18 at nucleotide 5199 (codon 1694), which changes a glutamic acid to a stop codon (Glu1694ter). To find out whether any transcript with exon 18 containing the stop codon was expressed, we specifically amplified from cDNA a fragment with primers in exons 16 and 18 (thereby preventing amplification from the transcript without exon 18), which was then sequenced. Only a wild-type exon 18 sequence was found in all carriers, which implies that the mutant allele produces only mRNA in which exon 18 had been skipped.

Although the Glu1694ter mutation would be expected to lead to the truncation of 169 amino acids from the BRCA1 protein, the skipping of the in-frame exon 18 removes 26 amino acids (Asp1692–Phe1717). Nevertheless, it leads to the disruption of the first BRCT domain, since it removes part of block D, the most highly conserved motif within this domain (Callebaut and Mornon 1997). The block D motif is organized around a conserved aromatic residue. In the case of *BRCA1*, this residue is Trp, which is changed to Gly because of the junction of exon 17 to exon 19. It is therefore unlikely that the mutant BRCA1 protein would remain totally functional.

In at least two cases of nonsense-mediated exon skipping (Santisteban et al. 1995; Pié et al. 1997), the mutation was found to be located in a purine-rich sequence that could function as an exonic splicing enhancer (ESE), thereby impairing its function. These ESE sequences have been shown to affect the selection of the splice sites (Cooper and Mattox 1997). In the case of Glu1694ter in *BRCA1* exon 18, exon skipping is probably not the result of the inactivation of an ESE, since the G→T substitution does not take place in a purine-rich sequence. It has also been proposed that point mutations in an exon could lead to disruption of secondary structure, with consequent aberrations in RNA splicing. We cannot exclude a structural effect of Glu1694ter on the splicing of *BRCA1* exon 18, the mutation occurring only six nucleotides downstream of the intron 17 splice acceptor. By contrast, experimental data suggest that maintenance of an open reading frame can serve as an additional level of scrutiny during exon definition; nonsense codons could thus directly alter splice-site selection (Dietz and Kendzior 1994). Additional work is needed to better identify the relevant mechanism and machinery that evaluate the coding potential of nuclear pre-mRNAs.

Glu1694ter has already been reported four times (Breast Cancer Information Core 1997 [http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/]), but on genomic DNA, so the skipping of exon 18 resulting from this mutation has probably been missed. Clearly, it would be interesting to investigate the consequence of each *BRCA1* mutation, found using genomic DNA, at the cDNA level. In addition to the fundamental aspect of finding the mutation, these findings could have implications to draw a genotype-phenotype correlation.

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Mutations of the *RET-GDNF* Signaling Pathway in Ondine's Curse

To the Editor:

The congenital central hypoventilation syndrome (CCHS, or Ondine's curse; MIM 209880; McKusick 1997) is a hitherto unexplained disorder of the ventilatory response to hypoxia and hypercapnia, leading to life-threatening hypoxic episodes starting immediatly after birth (Mellins et al. 1970). The alveolar hypoventilation observed during sleep is regarded as the consequence of a failure of the autonomic control of ventilation, located in the ventral medulla of the brain stem. Although the majority of CCHS cases are sporadic, several familial forms have been reported, and segregation analyses have suggested that the multifactorial and the major-locus models are almost equally likely in CCHS (Weese-Mayer et al. 1993). Interestingly, the CCHS-Hirschsprung disease association has frequently been reported (Haddad syndrome; MIM 209880 [McKusick 1997]; Haddad et al. 1978; Nakahara et al. 1995), and all CCHS families reported, to date, have included at least one sibling with this association.

On the other hand, Hirschsprung disease (HSCR; aganglionic megacolon; MIM 249200; McKusick 1997) is a frequent congenital malformation (1/5,000 live births) that is characterized by the absence of parasympathetic intrinsic ganglion cells of the hindgut and is regarded as a neurocristopathy (Bolande 1973). *RET* mutations account for 50% and 15%–20% of isolated familial and sporadic HSCR, respectively (Attié et al. 1995*a*). Mutations in the endothelin signaling pathway, including the endothelin B receptor gene (*EDNRB*) and the endothelin 3 gene (*EDN3*), account for no more than 5% of HSCR cases (Puffenberger et al. 1994; Attié et al. 1995*b;* Amiel et al. 1996; Edéry et al. 1996). Since CCHS is also regarded as the consequence of an abnormal migration of the neural crest cells toward the central autonomic respiratory system, genes involved in HSCR were considered as candidate genes in CCHS as well, including the *RET* proto-oncogene and its ligand, the glial cell line–derived neurotrophic factor (*GDNF*; Durbec et al. 1996; Salomon et al. 1996), *EDNRB,* and *EDN3*.

Although a heterozygous *EDN3* frameshift mutation has been found in one CCHS patient (Bolk et al. 1996*b*), we failed to detect *EDNRB* or *EDN3* mutations in our series. By contrast, screening the coding sequence of the *RET* and *GDNF* genes in five unrelated cases of isolated CCHS and in two cases of CCHS-HSCR association, we found mutations of the *RET* and the *GDNF* genes in children with isolated CCHS (1/7) and the CCHS-HSCR association (1/7), respectively.

All patients fulfilled the inclusion criteria for diagnosis of CCHS, namely (*i*) hypoventilation, hypoxemia, and hypercapnia during quiet sleep on polygraphic respiratory recording, with (*ii*) no cardiac, pulmonary, neuromuscular, electroencephalographic or cerebral magnetic resonance imaging anomaly. Histopathological criteria for HSCR were (*i*) the absence of enteric plexuses with histological evaluation of the aganglionic tract and (*ii*) increased acetylcholinesterase histochemical staining in nerve fibers. We screened *RET* and *GDNF* genes by SSCP analysis (Attié et al. 1995*a;* Salomon et al. 1996). The PCR products were heated for 10 min at 95°C, loaded onto a Hydrolink mutation detection enhancement gel (Bioprobe), and electrophoresed at 4 W. The gel was then dried and autoradiographed for 48 h. When

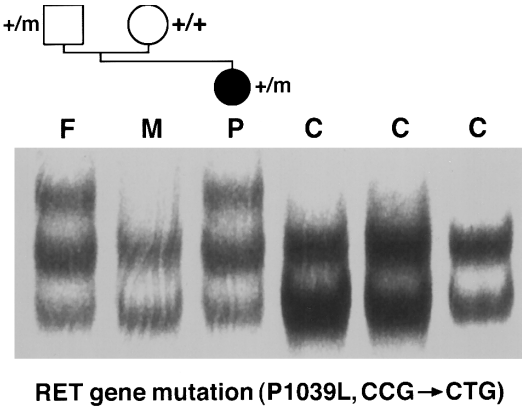

Figure 1 SSCP analysis of *RET* exon 19 in case 1. F = father; M = mother; P = proband; and C = control.

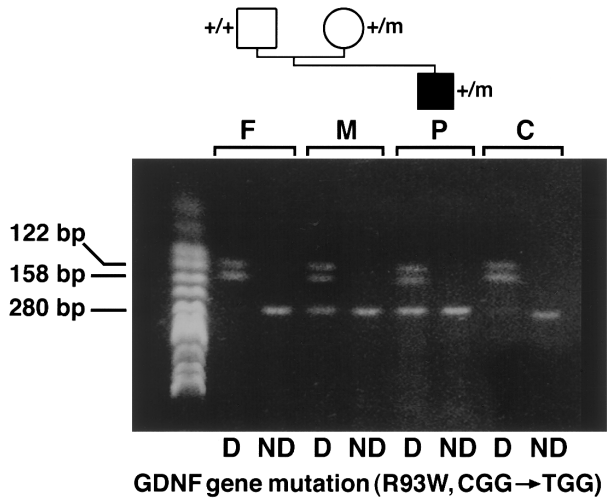

Figure 2 Screening for the R93W mutation by *Hin*fI restriction analysis. The nucleotide change abolishes the *Hin*fI restriction site. Partial digestion is found in the proband (case 2) and his mother (M), showing heterozygosity for the R93W mutation, whereas complete digestion is found in the father (F) and the control (C). D = digested DNA; ND = nondigested DNA.

an abnormal SSCP pattern was observed, direct DNA sequencing was performed by the fluorometric method (DyeDeoxyTerminator cycle sequencing kit, Applied Biosystems).

In a female patient with CCHS and total colonic aganglionosis, we found a hitherto unreported C→T transition at the second nucleotide of codon 1039 in exon 19 of the *RET* gene (C<u>C</u>G→C<u>T</u>G), changing a proline into a leucine in the protein (P1039L; fig. 1). This mutation is located in the terminal end of the intracellular domain of *RET* and is predicted to alter both the long and short carboxy-terminal isoforms of the *RET* protein. The mutation was inherited from the healthy father, and no other deleterious variation in *RET* coding sequence was detected (the A432A and G691S polymorphisms were noted in exons 7 and 11, respectively). In a male patient with CCHS and growth hormone deficiency, we found a C→T transition at the first nucleotide of codon 93 of the *GDNF* gene (<u>C</u>GG→<u>T</u>GG), changing a highly conserved arginine into a tryptophan in the protein (R93W; fig. 2). This mutation was inherited from the healthy mother. No other deleterious variation in the *GDNF* coding sequence was detected. The two mutations were absent in 90 normal controls (180 chromosomes). The R93W mutation in the *GDNF* gene has already been reported in sporadic and familial HSCR (Angrist et al. 1996; Salomon et al. 1996). In the latter case, since a *RET* mutation was associated to the *GDNF* mutation, the R93W mutation was regarded as neither necessary nor sufficient to cause isolated HSCR. The lack of penetrance of both *RET* and *GDNF* mutations reported here suggests that a major secondary event, yet to be defined, or the involvement of modifier loci are required for the expression of the CCHS phenotype.

The identification of mutations in the RET-GDNF pathway and the endothelin pathway in Ondine's curse sheds light on the genetic bases of this life-threatening condition and further suggests that CCHS is a neural crest cell disorder. Nevertheless, mutations have been reported in a minority of patients tested thus far (Bolk et al. 1996*a*). Finally, the involvement of at least three genes belonging to distinct signaling pathways, the incomplete penetrance of the mutation in carrier parents, and the variable expression of the respiratory control defect observed in the Ret −/− homozygous mice exposed to hypercapnia (Burton et al. 1997) support the view that an interactive polygenic inheritance is involved in Ondine's curse.

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De Novo mtDNA nt 8993 (T→G) Mutation Resulting in Leigh Syndrome

To the Editor:

Recently, Blok et al. (1997) analyzed mtDNA in oocytes from an asymptomatic mother of three children exhibiting heteroplasmic expression of the mtDNA nt 8993 (T→G) (T8993G) mutation associated with Leigh syndrome (MIM 516060). The mother had 50% mutant mtDNA in her blood. It was striking that one of the seven oocytes analyzed showed no evidence of the mutation, while the remaining six had a mutant load of >95%. Blok et al. suggested that this observation reflected preferential amplification of the mtDNA variant during oogenesis. During formation of the zygote, mtDNA is derived exclusively from the oocyte (Giles et al. 1980). Thus, it is possible that a de novo mutation may arise during oogenesis. A first carrier of a de novo mutation may be a mother who exhibits mosaicism for the mutation restricted to oocytes. However, it has been shown by previous investigators that the mothers of patients with Leigh syndrome associated with the mutation usually have substantial levels of the mutant mtDNA (Tatuch et al. 1992; Santorelli et al. 1993; Tulinius et al. 1995). The proportion of the mutant mtDNA in the lymphocytes from such mothers has been reported to be 38%–76%. Here we report the case of a 1-year-old boy with Leigh syndrome associated with the T8993G mutation, whose mother did not have the mutant mtDNA in her blood or urine sediment cells. It was shown that a de novo T8993G mutation in mtDNA may occur spontaneously at a high level in oocytes, thereby causing Leigh syndrome in the second generation.

The present patient was a Japanese boy born at term after an uncomplicated pregnancy. He was the second child of a 25-year-old mother and a 23-year-old father, who were healthy and unrelated. A 3-year-old sister was also healthy. His birth weight was 2,842 g, and the occipito-frontal circumference (OFC) was 33.2 cm (50th percentile). Generalized hypotonia was noted at birth. He developed apnea attacks and altered consciousness, after upper respiratory infections at the ages of 2 and 4 mo. From the age of 7 mo, he showed symptoms of brain-stem dysfunction, such as irregular respiration and swallowing difficulty. At the age of 9 mo, growth retardation (height 72.0 cm; body weight 6.84 kg, −2.3 kg SD) and microcephaly (OFC 43.0 cm, <10th percentile) were obvious. Although he could follow objects with his eyes and could respond to auditory stimuli, his head control was poor, because of severe generalized hypotonia.

Laboratory examination revealed increased lactate

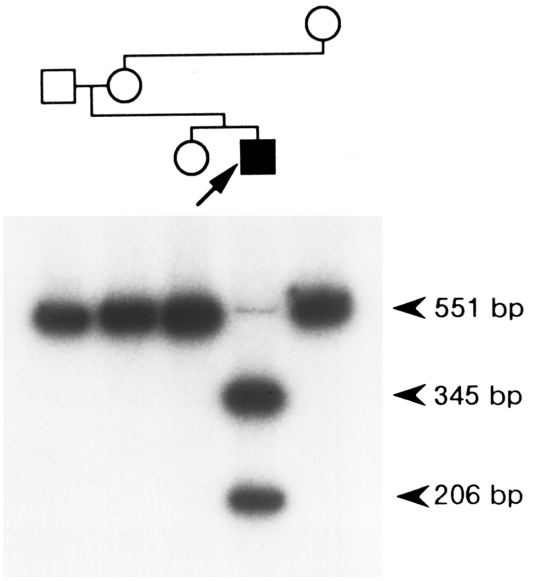

Figure 1 Pedigree of the family and autoradiograph of *Ava*I-digested ^{32}P-labeled PCR products of mtDNA encompassing the ATPase 6 gene. Template DNA was prepared from lymphocytes of family members. In the proband, the 551-bp PCR product harboring the mtDNA nt 8993 (T→G) mutation was cleaved into 345-bp and 206-bp fragments. No mutant mtDNA was detected for the proband's parents, sister, or maternal grandmother.

and pyruvate levels in blood (lactate 28 mg/dl; pyruvate 2.0 mg/dl) and cerebrospinal fluid (lactate 50 mg/dl; pyruvate 2.7 mg/dl) with high lactate/pyruvate and β-hydroxybutyrate/acetoacetate (2.9; normal range 1.84 ± 0.96) ratios. Plasma amino acid analysis revealed an increased level of alanine (62.9 μmol/dl; normal range 16.9–48.3 μmol/dl). On arterial blood gas analysis, metabolic acidosis was noted with respiratory compensation, i.e., pH 7.37; carbon dioxide tension 26.8 mmHg; partial pressure of oxygen 113.3 mmHg; bicarbonate 15.4 mEq/liter; and base excess −8.0 mEq/liter. Brain magnetic-resonance images revealed symmetrical necrotic foci in the striatum and periaqueductal gray matter, which are characteristic of Leigh syndrome. Histological examination of biopsied quadriceps femoris muscle showed variation in fiber size, but ragged-red fibers were not seen. Electron microscopy, however, showed a marked increase in the number of variably sized mitochondria with aberrant cristae. The activities of mitochondrial respiratory chain enzymes in the biopsied muscle were normal (NADH cytochrome c reductase 162.9 nmol/min/mg mitochondrial protein, control 27.3 ± 11.6; succinate cytochrome c reductase 139.8, control 76.6 ± 17.7; cytochrome c oxidase 63.9, control 33.0 ± 16.1).

After informed consent had been granted, blood and urine samples for DNA extraction were obtained from the patient and from his parents, sister, and maternal grandmother. In addition, the patient's DNA was also extracted from muscle and skin fibroblasts. Because the ratio of mutant to nonmutant mtDNA found in mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes associated with pathogenic point mutations is not constant in lymphocytes and muscle (Poulton and Morten 1993), we extracted mtDNA from at least two different somatic cells. DNA in lymphocytes, muscle, and skin fibroblasts was extracted by the phenol/chloroform method, although the procedure involving proteinase K digestion/boiling treatment was required for urine sediment cells. mtDNA encompassing the ATPase 6 gene was amplified by PCR, by use of a pair of primers, 5′-ccg act aat cac cac cca ac-3′ (nt 8648–8665) and 5′-tgt cgt gca ggt aga ggc tt-3′ (nt 9180–9199). The PCR conditions were as follows: 30 cycles, each consisting of denaturation for 0.5 min at 95°C, annealing for 0.5 min at 55°C, and extension for 0.5 min at 72°C. One extra cycle was then performed, after addition of 10 μCi of α-[^{32}P]dATP (3,000 Ci/mmol), 10 pmol of each primer, and 1.0 unit of *Taq* polymerase. Condition for this last cycle were 5 min at 95°C, 0.5 min at 55°C, and 5 min at 72°C. Adding radioactive dATP in only the last PCR cycle avoids the formation of heteroduplex DNA molecules, which would cause underestimation of the mutant mtDNA levels after restriction-enzyme digestion (Schoffner et al. 1990). Five microliters of the PCR product were digested in a final volume of 20 μl for 1 h at 37°C with 10 units of *Ava*I (Toyobo). The digestion products were electrophoresed through a 10% nondenaturing polyacrylamide gel. The gel was dried and autoradiographed at room temperature for 1 h by use of Kodak x-ray film. PCR restriction-digestion analysis of mtDNA from the patient revealed a T→G change at nt 8993, creating a new *Ava*I restriction site (fig. 1). The proportion of the mutant mtDNA was calculated as the ratio between the intensity of the 551-bp band and that of the 345-bp + 206-bp bands, by use of a scanning densitometer. The sensitivity of the PCR assay for the mutation was 0.2%, which was determined by analyzing serial dilutions of mutant mtDNA. The patient had >95% mutant mtDNA in all examined samples, including lymphocytes, urine sediment cells, skeletal muscle, and skin fibroblasts, but no mutant mtDNA was detected in lymphocytes or urine sediment cells from the mother, sister, or maternal grandmother.

Cases have been reported in which novel mtDNA mutations in sporadic patients with mitochondrial encephalomyopathy appeared to be confined only to skeletal muscle (Fu et al. 1996; Weber et al. 1997). It is speculated that this phenomenon may reflect loss of the mutation by random genetic drift in mitotic tissues and proliferation of mitochondria containing the mutant mtDNA in postmitotic cells. Although muscle for DNA

analysis was obtained only for the patient, it has been reported that the proportion of the mutant mtDNA was fairly constant in muscle and lymphocytes in patients carrying the T8993G mutation (Mäkelä-Bengs et al. 1995). Furthermore, the de novo occurrence of the T8993G mutation has previously been described, but in these families the amount of mutant mtDNA gradually increased during several generations, before reaching levels sufficiently high to cause Leigh syndrome (Santorelli et al. 1993; Tulinius et al. 1995). The finding that the mutation was present at high percentages in all samples from the patient but not in the mother or sister may be interpreted as follows: the mutation occurred spontaneously in some, but not all, oocytes, and consequently the mother's ovary may have had a mosaic status for the mutation, although it is not clear when the mutation arose. The germ-line mosaicism for the T8993G mutation identified by Blok et al. (1997) supports our interpretation. The recurrence risk cannot be estimated correctly, whereas the germ-line mosaicism may lead to familial clustering of affected individuals. We believe that this observation is useful for genetic counseling for families with affected patients.

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A Kerato-Epithelin (βig-h3) Mutation in Lattice Corneal Dystrophy Type IIIA

To the Editor:

The lattice corneal dystrophies (LCDs) are a class of inherited stromal amyloidoses characterized by pathognomonic, branching "pipestem" lattice figures in the cornea (Klintworth 1967). Four different LCD subtypes have been described. Type I (MIM 122200), the autosomal dominant form not associated with systemic amyloidosis (Gorevic et al. 1984), has its onset early in childhood and possesses a delicate network of interdigitating filaments in the cornea (fig. 1*A*). Type II (MIM 105120), the Finnish type (Meretoja 1972), on the other hand, is a condition associated with systemic amyloidosis. LCD type III has a presumed recessive inheritance pattern, is characterized by thicker lattice lines, and is not associated with systemic amyloidosis (Hida et al. 1987). Type IIIA resembles type III clinically but differs in that type IIIA has an onset age of 70–90 years and an autosomal dominant inheritance pattern (Stock et al. 1991). To the best of our knowledge, only two families with LCDIIIA have been reported (Stock et al. 1991), and, unlike

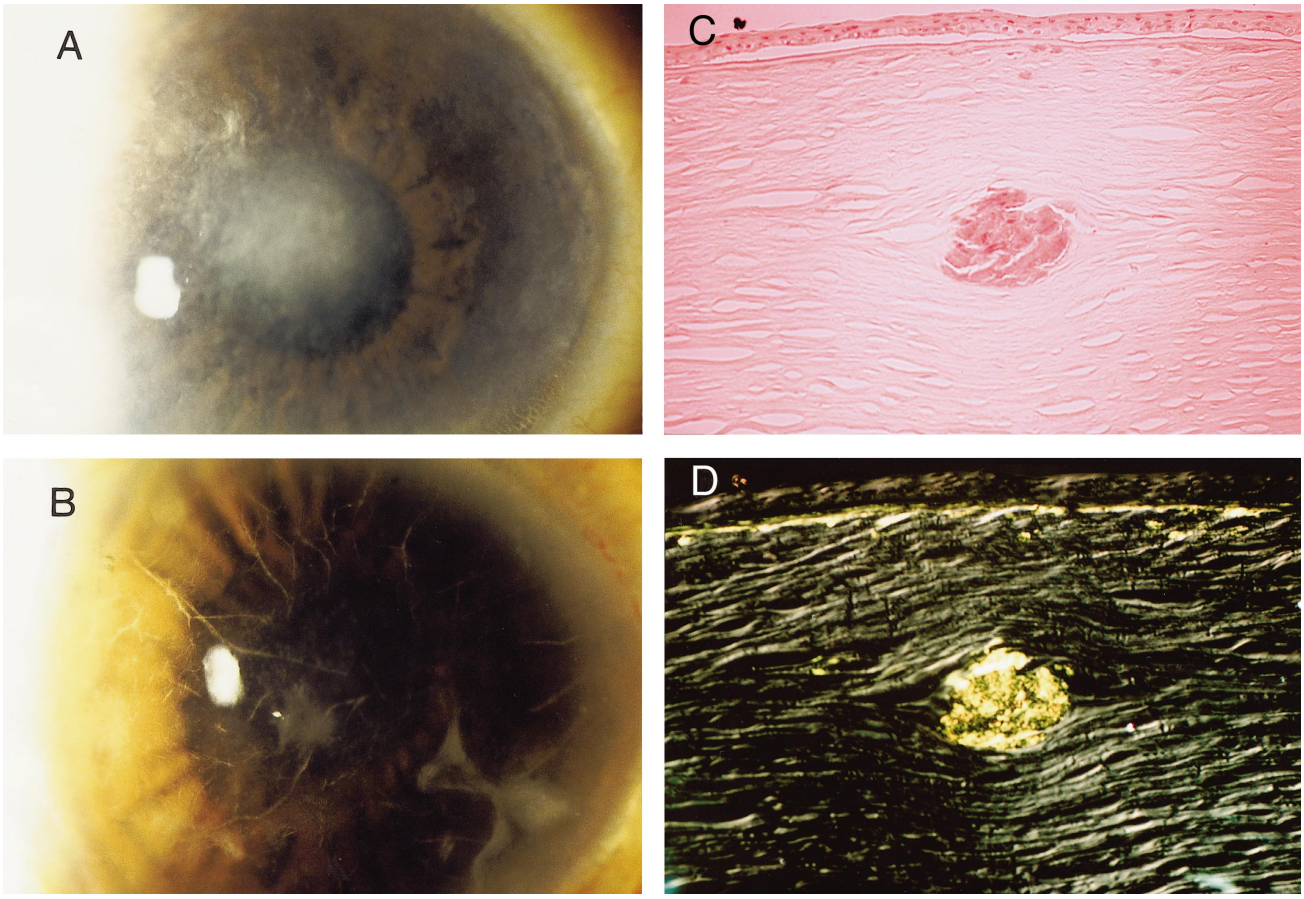

Figure 1 Clinical and histological appearance of LCD corneas. *A,* LCD type I, showing central opacities. Fine lattice lines are present in this patient, but they are difficult to reproduce photographically. In this case, we identified a heterozygous missense mutation, R124C, that was identical to that of a previous report (Munier et al. 1997) (original magnification × 5). *B,* LCD type IIIA, showing thick, ropy branching lattice lines throughout the cornea on sclerotic scatter (patient A-3, in family A) (original magnification × 5). *C* and *D,* Histology of the stroma from one of our four sporadic LCD type IIIA cases stained with congo red (*D* is viewed under polarized light), showing an amyloid deposit (original magnification × 100).

LCD1, which along with three other 5q31-linked (Stone et al. 1994) autosomal dominant corneal dystrophies is the result of a mutation in the βig-h3 gene (Munier et al. 1997), the molecular defect in LCDIIIA has not been identified.

We encountered three Japanese families with LCDIIIA, and, in a molecular analysis of nine affected patients from these families, we detected a novel missense mutation in the βig-h3 gene. The same mutation was also detected in four additional sporadic LCDIIIA patients from whom no family history was available. βig-h3 encodes an extracellular adhesion protein inducible by transforming growth factor-β (TGF-β), first isolated by Skonier et al. (1992) and recently termed "kerato-epithelin" (Munier et al. 1997).

All affected individuals had late-developing thick, ropy lattice lines in the corneal stroma typical of LCDIIIA (fig. 1*B*). In each family, the disease showed an autosomal dominant inheritance pattern (fig. 2). No corresponding systemic abnormalities were seen in any of the patients. Histopathological examination (two cases) revealed characteristic accumulations of amyloid deposits in the stroma (fig. 1*C, D*). Furthermore, of 13 LCDIIIA patients (9 members of three families and 4 sporadic cases), 8 had a history of recurrent corneal erosions like those described by Stock et al. (1991).

After obtaining informed consent, we analyzed genomic DNA isolated from leukocytes of the LCDIIIA patients and their family members, using standard methods. The 13 exons of the βig-h3 gene (Munier et al. 1997) were amplified using the PCR with oligonucleotide primers. The PCR products were then subjected to SSCP analysis (Orita et al. 1989). In LCDIIIA patients, we identified an abnormal conformer of exon 11. Sequencing analysis demonstrated that one of the alleles of every patient had a C→A transition (CCA→ACA) at

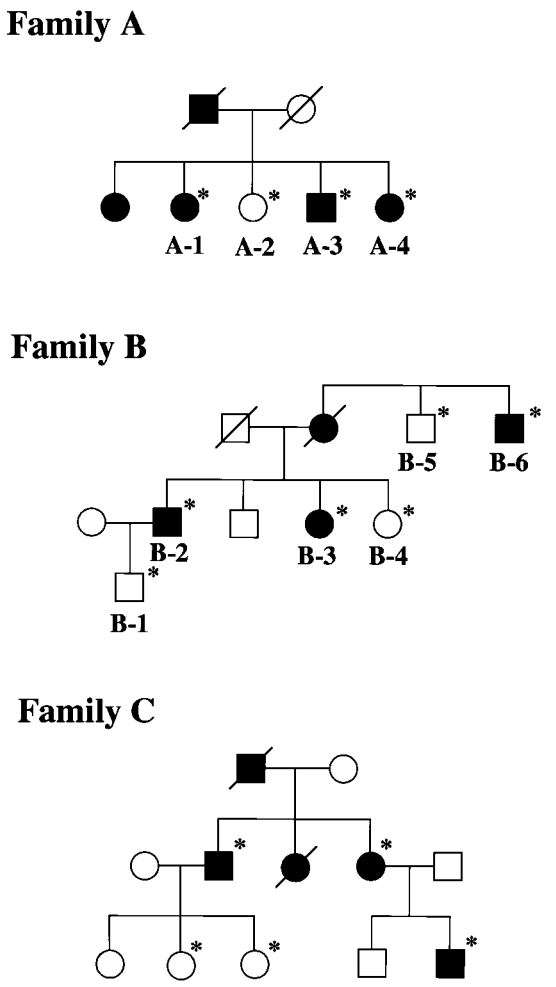

Figure 2 Pedigrees of families with LCD type IIIA. Asterisks (*) denote individuals whose leukocyte DNA was analyzed.

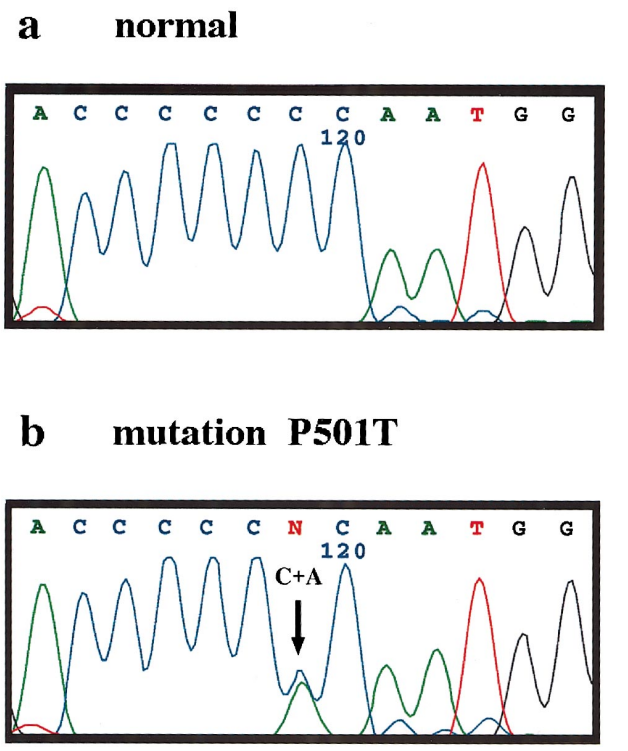

Figure 3 Direct sequencing of exon 11 of the βig-h3 gene. *a*, Unaffected control. *b*, Patient B-2, the proband of family B (fig. 2). Sequencing of the normal and mutant alleles of the patient identified the C→A transition (CCA→ACA) at position 1501, resulting in a proline-to-threonine substitution (Pro501Thr) in the protein. Other affected members had the same mutation. The nucleotide N indicates the presence of both C and A.

position 1501 (fig. 3) that caused a proline-to-threonine substitution (Pro501Thr). To specifically rule out mutations in codons 124 and 555, where mutations have been found in other corneal dystrophies including LCDI (Munier et al. 1997), we sequenced exons 4 and 12 of the βig-h3 gene. No mutations were found, and codons 124 and 555 were intact.

The LCDIIIA families were analyzed using a mutation-specific primer we synthesized that generates a *Dra*III site. Under standard PCR conditions (94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, with a final extension step at 72°C for 10 min), the following forward and reverse PCR primers were used; BIGexon11F (Munier et al. 1997) (5′-CTC GTG GGA GTA TAA CCA GT-3′) and BIG11RLCDIII (5′-GAC ATC CAT GAC AGT CCA CAT-3′). We observed the mutation-specific *Dra*III digestion pattern in all LCDIIIA-affected individuals but not in unaffected family members (fig. 4), indicating that the missense change Pro501Thr perfectly cosegregated with the disease. In addition, this mutation was not found among 41 patients with granular corneal dystrophy type I (MIM 121900), Reis-Bücklers corneal dystrophy, or Avellino corneal dystrophy, nor was it found in 106 normal individuals (data not shown). On the basis of this evidence, we conclude that the Pro501Thr mutation is the cause of LCDIIIA.

The βig-h3 gene encodes an adhesion molecule characterized by four internal homologous domains, which can be folded into a potential bivalent structure and may act as a bridge between cells expressing the appropriate ligand (Skonier et al. 1992, 1994). Pro501 is located in the third internal repeat and is conserved in humans, mice, chicks, and pigs. The mutation (Pro501Thr) we detected in LCDIIIA changes a nonpolar residue to a polar residue. Although the mechanism by which the Pro501Thr mutation leads to LCDIIIA is still unknown, proline is important in producing bends in a peptide chain. Therefore, it is possible that the tertiary structure of the mutant kerato-epithelin is deranged in LCDIIIA, leading to the formation of amyloidogenic intermediates.

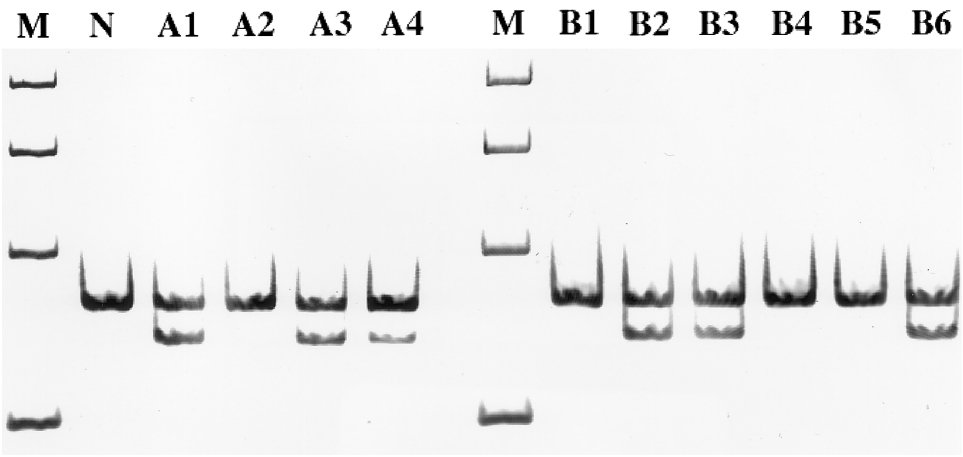

Figure 4 Cosegregation study by restriction-digestion analysis of exon 11. N = normal individual, for control; M = 100-bp ladder marker. The upper band (165 bp) represents the wild type, and the lower digested band (146 bp) represents the mutation Pro501Thr. All affected patients carry the mutation-specific lower band.

Munier et al. (1997) identified four missense mutations at codons 124 and 555 of the βig-h3 gene, in four corneal dystrophies, and all four mutations occurred in a CpG dinucleotide of arginine codons. They postulated that the mutation R124 resulted in amyloidogenic intermediates. Our cases suggest that P501-mutated kerato-epithelin may also form amyloidogenic intermediates that precipitate in the cornea.

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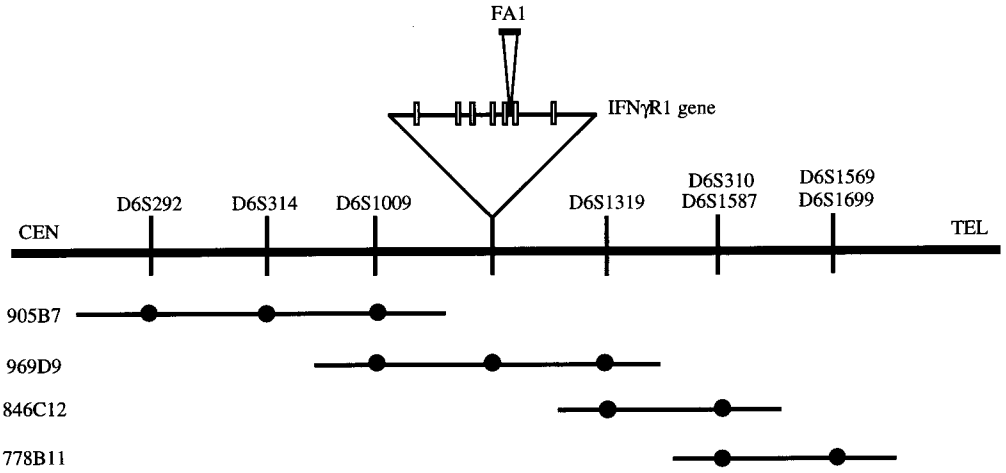

Figure 1 Genetic and physical map of the *IFNgR1* gene. Restriction digests of cosmids COSTCFP-2.8 and COSTCFP-7.13, carrying the *IFNgR1* gene (Merlin et al. 1997), were hybridized with a peroxydase-labeled (CA)30 probe. A specific fragment was found to contain a repeat consisting of 22 CAs located in the sixth intron (FA1; Genbank accession number U84721). When tested on 80 unrelated individuals, this marker was found to be highly informative, with 12 alleles and >90% heterozygosity. Linkage analyses were performed on three-generation CEPH families genotyped with polymorphic markers (Dausset et al. 1990). The FA1 marker was positioned on a framework consisting of the map of 5,264 markers (Dib et al. 1996), by use of the GMS algorithm (Lathrop and Lalouel 1988). On the basis of the best-supported order for the framework map, recombination fractions between adjacent markers were estimated using the LINKAGE program (Lathrop et al. 1984). The map was reevaluated until no further double recombination event could be eliminated. The FA1 microsatellite was thus genetically mapped between microsatellites D6S292 and D6S1699 (<4 cM). The CEPH YAC WC6.15 contig was previously reported with a series of polymorphic and nonpolymorphic markers (Dausset et al. 1992). Selected YACs of this contig were confirmed by PCR, to define a smaller contig encompassing the microsatellite FA1 and its flanking markers defined by the genetic mapping. The physical map of the region was then enriched by testing an additional panel of dinucleotide (Dib et al. 1996) and tetranucleotide (Sheffield et al. 1995) repeats on the same subset of YACs. The YAC contig consisting of 905B7, 969D9, 846C12, and 778B11 spans <4 Mb. All markers positioned on this contig are polymorphic CA repeats, except D6S1009 and D6S1003 (polymorphic tetranucleotide repeats) and D6S1319 (nonpolymorphic marker). The relative positions of D6S310 and D6S1587 and of D6S1569 and D6S1699 are not known.

Am. J. Hum. Genet. 62:423–426, 1998

A Causative Relationship between Mutant *IFNgR1* Alleles and Impaired Cellular Response to IFNγ in a Compound Heterozygous Child

To the Editor:

Mutations in the *IFNgR1* gene have recently been identified in two consanguinous kindreds from Malta and Tunisia (Jouanguy et al. 1996; Newport et al. 1996). Affected children were found to be homozygous for two different alleles. No IFNγR1 protein molecules could be detected at the surface of peripheral blood cells, accounting for impaired IFNγ-mediated immunity. Patients had disseminated infections due to *Mycobacterium bovis*, bacille Calmette Guérin (BCG) vaccinal strain, or environmental nontuberculous mycobacteria (NTM), such as *M. avium, M. fortuitum,* and *M. chelonei* (MIM 209950). No other opportunistic infections were documented, with the possible exception of salmonellosis in one child, and four of five affected children died of mycobacterial infection. Thus, complete IFNγR1 deficiency appears to be an autosomal recessive immune disorder associated with severe and selective susceptibility to poorly pathogenic mycobacteria.

To better characterize this condition, it is important to demonstrate that there is a genuine causal relationship between mutant *IFNgR1* alleles and impaired cellular responses to IFNγ. It is also important to determine whether compound heterozygous mutations of the *IFNgR1* gene may be responsible for BCG or NTM infections in other kindreds (Casanova et al. 1995, 1996; Levin et al. 1995; Emile et al. 1997). We have therefore investigated an Italian child, born to nonconsanguinous parents, who presented at 3 years of age with disseminated infection due to *M. smegmatis* (Pierre-Audigier et al. 1997). The child was not vaccinated with BCG and died at 8 years of age of a progressive mycobacterial disease, despite intensive antimycobacterial therapy. Four older siblings had received the BCG vaccine in infancy with no adverse effect and were healthy.

To facilitate the genetic analysis of IFNγR1 deficiency, we first identified an intragenic polymorphic CA-repeat, designated "FA1" (fig. 1). The *IFNgR1* gene has been previously mapped to the broad chromosome region 6q16–6q22, by use of somatic cell hybrids (Pfizenmaier

et al. 1988), and to 6q24.1–6q24.2, by in situ hybridization (Le Coniat et al. 1989; Pappanicolaou et al. 1997). The segregation of FA1 alleles within CEPH reference families provided a genetic mapping of FA1 between D6S292 (centromeric) and D6S1699 (telomeric) (4 cM). Physical mapping of the gene and surrounding markers on a YAC contig was then achieved. Intrafamilial genotyping showed that the affected child had a unique pattern for FA1 and the associated D6S1009, D6S310, and D6S1587 microsatellites, when compared with her four healthy siblings (not shown). This segregation was compatible with the diagnosis of autosomal recessive IFNγR1 deficiency.

Amplification of the seven *IFNgR1* exons and associated intronic consensus splice sites was performed for each member of the family. Sequencing revealed a 4-bp insertion, designated "107ins4," within *IFNgR1* exon 2 at one locus in the child and in the father (not shown). The four inserted nucleotides (TTAC) were found to duplicate flanking nucleotides 104–107, as has occasionally been observed with insertions causing other genetic diseases (Cooper and Krawczak 1991). The frameshift is expected to cause premature termination of translation before the transmembrane segment, because of a stop codon at nucleotides 115–117. A substitution of the first base of the consensus splice-donor site of *IFNgR1* intron 3 was found at the other locus in the child and in the mother, designated "200+1G→A" (not shown). This type of *5′* splice-site mutation is frequent in other genetic diseases and is expected to cause exon 2 skipping and/or cryptic splice-site usage (Krawczak et al. 1992). The affected child was the only member of the family carrying the two mutant alleles, further suggesting that he had IFNγR1 deficiency.

Analysis of *IFNgR1* mRNA in the patient's fibroblasts, by northern blot and amplification of the full-length *IFNgR1* cDNA coding region, failed to detect any alternative splicing product, when compared with healthy individuals (not shown). Likewise, amplification of the patient's cDNA, with primers specific for exon 1 (sense) and exon 3 (antisense), failed to detect exon 2 skipping and cryptic site usage. Even though the patient's *IFNgR1* mRNA appeared to be of normal molecular weight and to be expressed at a normal level by northern blot, the patient's *IFNgR1* cDNA-PCR, however, was found to be 4 bp longer than the wild type. Moreover, direct sequencing of the amplicon confirmed that the only detectable *IFNgR1* mRNA species in this patient was encoded by the 107ins4 mutant allele. Together, these results suggest that the 107ins4 allele transcript is expressed at a normal level and that none of the 200+1G→A allele potential transcripts, including full-length, exon 2 skipping, and cryptic splice-site transcripts, is detectable.

We then analyzed cell surface expression of the receptor by flow cytometry with two IFNγR1-specific antibodies as reported elsewhere (Jouanguy et al. 1996). Whereas IFNγR1 molecules were present on the surface of peripheral blood mononuclear cells from healthy individuals, there were no detectable IFNγR1 molecules on the patient's cells (not shown). These data are consistent with the analysis of *IFNgR1* mRNA expression and strongly suggest that both the 107ins4 and 200+1G→A mutations preclude transcription of full-length wild-type mRNA and expression of detectable IFNγR1 molecules at the cell surface.

To document a functional defect in this patient, we took advantage of a previously reported method for the investigation of human leukocyte antigen (HLA)–class II deficiency (Lisowska-Grospierre et al. 1994) and analyzed the IFNγ-mediated induction of HLA-DR in fibroblasts. Concentrations of IFNγ, witin the range of 10–100,000 UI/ml, were tested for the induction of HLA-DR after 48 h and 72 h of incubation. In all conditions, there was no induction of HLA-DR on the patient's cells, as detected by flow cytometry, in contrast to the dramatic induction obtained with control cells after only 48 h in response to $\geqslant$10 UI of IFNγ/ml (fig. 2*A*). Intracellular HLA-DR could also be detected by microscopic immunofluorescence in control cells but not in the patient's cells (not shown). These results suggested that the patient's fibroblasts were not responsive to IFNγ because of a lack of functional surface IFNγR1 molecules.

Finally, we attempted to demonstrate a causal relationship between the two mutant *IFNgR1* alleles identified and impaired cellular response to IFNγ. We thus transiently transfected the patient's fibroblasts with wild-type *IFNgR1* allele and assessed the induction of intracellular HLA-DR in response to IFNγ, by microscopic immunofluorescence. In response to even low IFNγ concentrations (10 UI/ml) and after only a brief incubation (48 h), a number of cells intensely fluoresced, attesting that transfection of the *IFNgR1* gene conferred normal IFNγ-dependent induction of HLA-DR in the cytoplasm (fig. 2*B*). By contrast, transfection of the control vector was ineffective in inducing intracellular HLA-DR. Similar results were obtained with high concentrations of IFNγ (100,000 UI/ml) and 72-h incubation (not shown). Analysis of HLA-DR surface expression by flow cytometry was also consistent with these results (not shown).

We have thus demonstrated a cause-and-effect relationship between mutant *IFNgR1* alleles and impaired cellular response to IFNγ in this kindred. These data corroborate the results previously obtained for two kindreds and strengthen the association between inherited IFNγR1 deficiency and susceptibility to mycobacteria in affected children. This kindred provides further important information concerning IFNγR1 deficiency. The af-

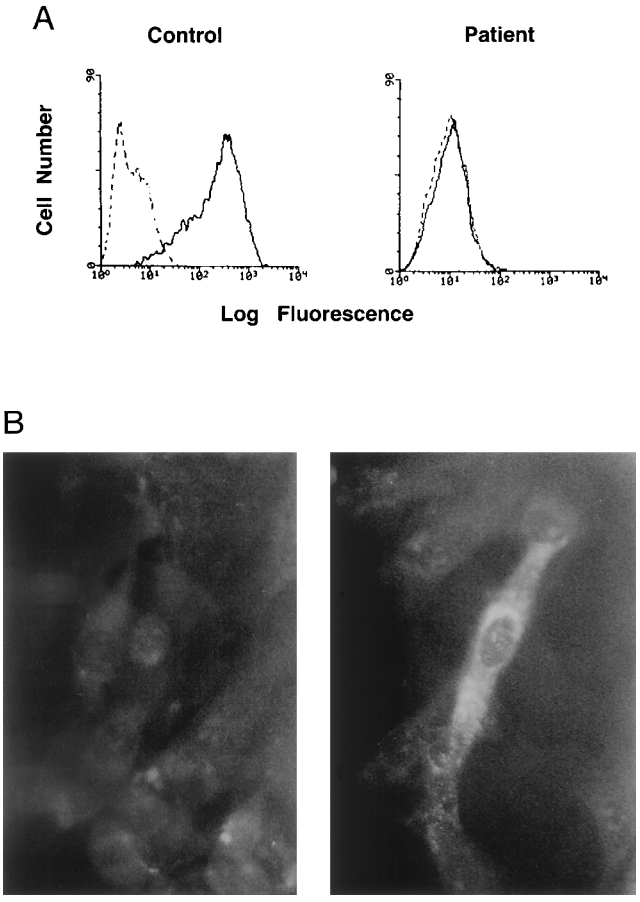

Figure 2 Impaired IFNγ-mediated induction of HLA-DR on the patient's fibroblasts and complementation with wild-type *IFNgR1* gene. *A,* Flow cytometry analysis of HLA-II on fibroblasts before (*dashed lines*) and after (*solid lines*) addition of IFNγ in a control (*left*) and the patient (*right*). An adherent fibroblastic cell line was established from a skin biopsy of the patient, grown in RPMI 1640 (Gibco BRL) supplemented with 10% FCS (Gibco BRL) (complete medium) and transformed with SV40T as described elsewhere (Lisowska-Grospierre et al. 1994). One million fibroblastic cells were treated with 200 IU recombinant human IFNγ/ml (Genex, Biogenex Laboratories) for 48 h, and the induction of HLA-DR was documented following trypsinization by flow cytometry with a fluorescein isothiocyanate–conjugated monoclonal antibody anti–HLA-DR (Becton Dickinson). *B,* Microscopic fluorescence analysis of IFNγ-induced HLA-II expression in the patient's fibroblasts transiently transfected with control plasmid (*left*) and with IFNγR1 expression vector (*right*). The 4×10^5 fibroblasts were transferred to sterile microscopic slides for in situ electrophoration in 100 μl of 10 mM sodium phosphate, pH 7.2, 1 mM $MgCl_2$, and 250 mM sucrose with 4 μg plasmid DNA (either an IFNγR1 expression vector referred to as "pSFFVhgR" or the vector with no insert, referred to as "pSFFVneo") (Farrar et al. 1991). Six pulses were delivered (5 ms, 320 V, 1 Hz, E = 0.8 kV/cm) with a JOUAN GHT 128/A electropulser, and 5 min later the slides were placed in complete medium. After 3 h of culture, recombinant IFNγ was added at 10 IU/ml, and after 24 h the medium with IFNγ at the same concentration was replaced. Antibody binding (Bu27) on ethanol-fixed fibroblasts at 48 h following transfection was revealed ,as described elsewhere (Lisowska-Grospierre et al. 1994). Immunofluorescence analysis was carried out with a Leitz Orthoplan optical microscope.

fected child was found to be the first compound heterozygous patient. Worthy of note, the two mutations found in this child were different mutations from the two identified in previous kindreds. The four mutations identified, to date, include a nonsense mutation, a deletion, an insertion, and a splice mutation. Thus, these data not only show that the disease is not limited to consanguinous families but also suggest that there is probably a relatively high allelic heterogeneity underlying inherited IFNγR1 deficiency.

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On the Probability of Identity States in Permutable Populations: Reply to Cannings

To the Editor:

To extend the affected-sib-pair method to consanguineous populations, we derived the probabilities of the nine condensed identity states as a function of the probability, α, that two genes, drawn at random from the population, will be identical by descent (IBD) (Génin and Clerget-Darpoux 1996). Cannings (1998 [in this issue]) criticizes our derivations and argues that they are correct only in certain very restricted and uninteresting models of populations. He proposes another treatment, which requires three coefficients, α_2, α_3, and α_4. These coefficients represent the probability that two, three, and four genes, respectively, drawn at random from the population, will be IBD (table 1). He compares this model to ours. It should be noted that his comparison was performed with incorrect formulas that we have since corrected in a letter (Génin and Clerget-Darpoux 1998 [in this issue]); unfortunately, our letter had not been published at the time when Cannings (1998) wrote his article. The corrections explain the inconsistencies he notes

Table 1

Probabilities of the Nine Condensed Identity States in Cannings's Model and in Our Model

State	Cannings's Model	Our Model
S1 (1,1,1,1)	α_4	α^3
S2 (1,1,2,2)	$\alpha_2^2 - \alpha_4$	$\alpha^2(1-\alpha)$
S3 (1,1,1,2)	$2(\alpha_3 - \alpha_4)$	$2\alpha^2(1-\alpha)$
S4 (1,1,2,3)	$\alpha_2(1-\alpha_2) - 2(\alpha_3 - \alpha_4)$	$\alpha(1-\alpha)(1-2\alpha)$
S5 (1,2,2,2)	$2(\alpha_3 - \alpha_4)$	$2\alpha^2(1-\alpha)$
S6 (1,2,3,3)	$\alpha_2(1-\alpha_2) - 2(\alpha_3 - \alpha_4)$	$\alpha(1-\alpha)(1-2\alpha)$
S7 (1,2,1,2)	$2(\alpha_2^2 - \alpha_4)$	$2\alpha^2(1-\alpha)$
S8 (1,2,1,3)	$4[\alpha_2(1-\alpha_2) - 2(\alpha_3 - \alpha_4)]$	$4\alpha(1-\alpha)(1-2\alpha)$
S9 (1,2,3,4)	$1 - 6\alpha_4 + 8\alpha_3 - 6\alpha_2 + 3\alpha_2^2$	$(1-\alpha)(1-2\alpha)(1-3\alpha)$

in our derivations; these inconsistencies are no longer present in the corrected formulas (table 1).

First of all, we would like to emphasize that our derivations were approximations. We agree that Cannings's (1998) derivations of the probabilities of the condensed identity states are more correct, in the sense that they are not approximations. Cannings's coefficient α_2 is equivalent to the kinship coefficient, α, that we have used, but Cannings's other two coefficients, α_3 and α_4, were approximated in our model by α^2 and α^3, respectively. Of course, if either the coefficients α_3 and α_4 or genealogies are available, it is better to use them. However, in most situations, accurate estimates of these two coefficients are either unavailable or very difficult to obtain, and this is also true of genealogies. Weir (1994) reported this difficulty elsewhere and suggested the use of approximations that depend only on the probability α that two genes will be IBD (θ, in his article); these are, in fact, the same approximations that we used. Hence, he proposed to approximate α_3 by α^2, assuming that three genes, a, b, and c, are IBD if a and b are IBD, if b and c are IBD, and if these two events are independent. He further showed that, for the purpose of forensic calculations, these approximations are fairly accurate and that they have the advantage of being analytically simpler. The problem now is to determine whether, for our purpose, the approximations were or were not correct.

To answer this question, we must further describe the population model that we used in our paper (Génin and Clerget-Darpoux 1996); we admit that the model was not discussed in sufficient detail. We considered a population in which mating was random but that derived from a few founders. Consequently, even if the population was in Hardy-Weinberg equilibrium, there was some random inbreeding (Allen 1982). This is exactly the same population model considered by Cannings (1998). The only further assumption in our model was that the F initial founders were assumed to be unrelated and heterozygous for different alleles at the locus under consideration (which means that, in the initial population, α was assumed to be zero). Therefore, a total of $2F$ distinct alleles were present in the population, both at the beginning and across generations. The probability α that two alleles, taken at random in the population, are IBD was thus $\frac{1}{2F}$ (after the first generation). Under these conditions, α can take on only discrete values—for example, $\frac{1}{2}$, $\frac{1}{4}$, or $\frac{1}{6}$, for one, two, or three founders, respectively (the identity-state probabilities are then always greater than or equal to zero). The model assumes that all of the alleles present in the founders are maintained in the population, across generations; this is not true for a small population, because of genetic drift, but it is expected for an infinite population, as in the first model considered by Cannings (1998). The assumption that alleles are unique in the first generation is the only way, in our view, to ensure that two alleles observed to be identical are in fact IBD—and is therefore not a major assumption of the model. Under these conditions, each allele is expected to have the same frequency, and, therefore, as shown by Cannings (1998) himself, α_3 exactly equals α^2, and α_4 equals α^3, which makes our approximations correct. If the population is of finite size and the number of generations is not too large, then allele frequencies will not differ significantly from the original frequencies in the founder population (i.e., alleles are also expected to have approximately the same frequencies, and approximations would also hold). This can be shown by simulations: if we assume that there are 10 founder genes and a population size of 100 genes at each generation, after 10 generations of random mating, the expected distribution of identity states for the 100 genes, computed with our approximations, is significantly different, from the one computed with Cannings's model, in only 6/1,000 replicates (when the mutation rate is zero) and in 2/1,000 replicates (when the mutation rate is 10^{-5}). Of course, if the population has diverged for only 10 generations, the stability of the inbreeding coefficient α and of the kinship coefficient ϕ is not reached; but, in most situations, the variation of these coefficients from one generation to the next is expected to be small, so that $\alpha \approx \phi$. In conclusion, we think that our approximations are correct in most situations that involve a population that is diverging from a few unrelated founders and is expanding rapidly.

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Consanguinity and Relative-Pair Methods for Linkage Analysis

To the Editor:

The recent paper by Génin and Clerget-Darpoux (1996) makes an important and timely point: that linkage analyses using nonparametric statistics in an affected-sib-pair design can be made more powerful by assaying identity-by-descent sharing within inbred siblings. However, we believe some of the technical details of their mathematical model and derivations may be in error.

In Appendix A of their paper, Génin and Clerget-Darpoux derive "IBW-state probabilities" (or "condensed coefficients of identity" [Jacquard 1974]) for siblings from a population with a mean inbreeding coefficient α, which is assumed to be constant over time and to be equal to the mean kinship coefficient. These are perhaps unrealistic assumptions in terms of many inbred human populations, as mean kinship coefficients change over time because of changing demographic factors (Khoury et al. 1987), and mean inbreeding coefficients are often much higher than mean kinship coefficients (De Braekeleer et al. 1993, 1996). Even so, under the modeling assumptions made by Génin and Clerget-Darpoux, the probabilities that they present fail to satisfy the following two consistency checks:

1. Let Δ_i represent the probability of being in condensed identity state S_i, where the range of i is 1–9, and assume that the two sibs are numbered "3" and "4" and that the parents are numbered "1" and "2." Then, according to a formula presented by Jacquard (1974), the kinship coefficient between the siblings, Φ_{34}, should be

$$\Delta_1 + \frac{1}{2}(\Delta_3 + \Delta_5 + \Delta_7) + \frac{1}{4}\Delta_8 \ . \qquad (1)$$

We can derive what the kinship coefficient should be by classical recursion methods:

$$\begin{aligned}\Phi_{34} &= \frac{1}{2}(\Phi_{31} + \Phi_{32}) \\ &= \frac{1}{4}(\Phi_{11} + \Phi_{21} + \Phi_{12} + \Phi_{22}) \\ &= \frac{1}{4}\left[\frac{1}{2}(1+\alpha) + \alpha + \alpha + \frac{1}{2}(1+\alpha)\right] \\ &= \frac{1}{4}(1+3\alpha) \ , \end{aligned} \qquad (2)$$

because, under Génin and Clerget-Darpoux's assumptions, the inbreeding coefficient of each parent is α and the kinship between the parents is also α. However, when we apply equation (1) to Génin and Clerget-Darpoux's Δ's, we get $\Phi_{34} = \frac{1}{4} + \frac{\alpha}{2} + \frac{\alpha^3}{4}$, which is clearly incorrect (except when $\alpha = 0$ or when $\alpha = 1$).

2. Karigl (1981, eq. [7]) presents a matrix that permits one to derive a vector of several different kinship coefficients from the vector of Δ's. However, when this matrix is applied to Génin and Clerget-Darpoux's Δ's, the correct kinship coefficients are not recovered.

In passing, it is important to point out that the derivation, in Génin and Clerget-Darpoux's Appendix B, of condensed identity coefficients for two sibs from a first-cousin marriage is also incorrect. For example, they appear to compute Φ_{224} (sampling twice from one individual—i.e., person 2—and once from an unrelated person) as α^2. However, kinship sampling is done with replacement, so that the chance that the same gene is sampled twice from person 2 is $\frac{1}{2}$, and then it is identical by descent (IBD) with the gene from person 4 with probability α. Likewise, the chance that different genes are sampled from person 2 is $\frac{1}{2}$, and then, since the three different genes are IBD with probability α^2, $\Phi_{224} = \frac{1}{2}\alpha + \frac{1}{2}\alpha^2$. There appear to be similar mistakes throughout the derivation (e.g., for three unrelated people—2, 4, and 6—Φ_{2426} is not α^3). Génin and Clerget-Darpoux's Δ's in their Appendix B do pass the first consistency check above, but they fail the second consistency check.

So why do Génin and Clerget-Darpoux's results fail to satisfy these checks? They derive their Δ's for the sibs by first deriving the parental Δ's and then multiplying these by a transition matrix. Since the transition matrix appears to be correct to us, we believe that the problem lies in the specification of the parental Δ's. This can be illustrated by the following scenario: Suppose that we have a population in which $\frac{1}{4}$ of the individuals, C, are offspring (siblings) of the same first-cousin marriage and in which the remaining $\frac{3}{4}$ of the individuals, U, are noninbred unrelated individuals. This population has a mean inbreeding coefficient, α, of $\frac{1}{64}$. Then, if we randomly sample two individuals to form the parents of our sib pair, we see, from the data in table 1, that we have a *zero* chance of getting a parental pair in state S_2, whereas Génin and Clerget-Darpoux's Appendix A indicates that the parents should be in state S_2 with a *nonzero* probability $\alpha^2(1-\alpha)$. If we judge on the basis of this slightly artificial example, it seems that one cannot specify correctly the condensed identity coefficients for the parents in terms of α alone; rather, one must take the *specific type and frequency* of consanguineous matings into account. (Similarly, one cannot recover condensed identity coefficients for a pair of individuals on the basis of knowledge of their kinship coefficient alone, since a parent-offspring pair has the same kinship co-

Table 1

Probabilities of Ordered Pairs, Kinship Coefficients, and Condensed Identity Coefficients for Two People Drawn at Random from a Population Consisting of 25% Sibs from the Same First-Cousin Marriage (C) and 75% Noninbred Unrelated Individuals (U)

			Condensed Identity Coefficient								
Pair	Probability	Kinship	Δ_1	Δ_2	Δ_3	Δ_4	Δ_5	Δ_6	Δ_7	Δ_8	Δ_9
(C,U)	$\frac{3}{16}$	0	0	0	0	$\frac{1}{16}$	0	0	0	0	$\frac{15}{16}$
(U,C)	$\frac{3}{16}$	0	0	0	0	0	0	$\frac{1}{16}$	0	0	$\frac{15}{16}$
(C,C)	$\frac{1}{16}$	$\frac{9}{32}$	$\frac{1}{64}$	0	$\frac{1}{32}$	$\frac{1}{64}$	$\frac{1}{32}$	$\frac{1}{64}$	$\frac{15}{64}$	$\frac{15}{32}$	$\frac{3}{16}$
(U,U)	$\frac{9}{16}$	0	0	0	0	0	0	0	0	0	1

efficient as does a pair of siblings.) At least, one should use any available information about population sizes over time: Jacquard (1974, pp. 167–171) discusses how to adjust kinship coefficients properly for the background level of inbreeding due to finite population size (and, in fact, derives eq. [2] under slightly different modeling assumptions).

Finally, at the end of Génin and Clerget-Darpoux's paper, they state that the affected-pedigree-member (APM) method of linkage analysis (Weeks and Lange 1988) fails to take "full advantage of IBW states, since they only use a part of the information that concerns IBD between individuals" (Génin and Clerget-Darpoux 1996, p. 1158). Although this is true, we would like to point out that one of us (D.E.W.) has explored, in his dissertation, assaying for increased marker similarity *within* inbred individuals in the context of the APM method (Weeks 1988). The Appendix presented here contains a relevant (and slightly edited) extract regarding the theoretical development of this procedure. Note that to take full advantage of this extension of the APM method requires that the relationships of the affected individuals be known and specified. As our critique of Génin and Clerget-Darpoux's paper suggests, it may be difficult to properly analyze pedigrees from an inbred population, unless one devotes much effort to determining, as best as possible, the precise structure of each family.

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Appendix

Usually "a sample of unrelated individuals can give no information about linkage" (Smith 1953, p. 159), but this principle does not hold in the case of inbred individuals. For example, consider an affected offspring of a first-cousin marriage. If the disease is very rare and recessive, then the affected person is almost certainly homozygous by descent at the disease locus and so would also show increased homozygosity at any marker loci closely linked to the disease locus. If this increased homozygosity can be observed, then, as Smith (1953) first observed, a sample of inbred individuals may contain information about linkage. Lander and Botstein (1987) later elaborated this approach, naming it "homozygosity mapping."

The original APM statistic involves comparisons of marker identity-by-state (IBS) status *between* affected individuals (Weeks and Lange 1988). In order to apply this statistic in the context of homozygosity mapping, it must be modified to include comparisons *within* individuals. The APM statistic was constructed in terms of a random variable, Z_{ij}, which measures the IBS marker similarity between two affected individuals, i and j. Let i have maternal marker allele G_{ix} and paternal marker allele G_{iy}. Likewise, let j have maternal marker allele G_{jx} and paternal marker allele G_{jy}. The original definition of Z_{ij}, for $i \neq j$, was

$$Z_{ij} = \frac{1}{4}\delta(G_{ix}, G_{jx})f(p_{G_{ix}}) + \frac{1}{4}\delta(G_{ix}, G_{jy})f(p_{G_{ix}}) + \frac{1}{4}\delta(G_{iy}, G_{jx})f(p_{G_{iy}}) + \frac{1}{4}\delta(G_{iy}, G_{jy})f(p_{G_{iy}}) ,$$

where the Kronecker delta is defined as

$$\delta(G, G') = \begin{cases} 1 & G \text{ and } G' \text{ match in state} \\ 0 & G \text{ and } G' \text{ do not match in state} \end{cases} .$$

Each match is weighted by the function $f(p)$ of the

frequency of the allele involved. Z_{ij} can be interpreted as a conditional expectation. In the usual notation for conditional expectations,

$$Z_{ij} = \mathrm{E}[\delta(G_i, G_j) f(p_{G_i}) | \text{obs. marker genotypes of } i \text{ and } j] \ ,$$

where G_i and G_j are randomly selected marker genes from i and j, respectively. If we permit i to equal j, then this definition gives

$$Z_{ii} = \frac{1}{4} f(p_{G_{ix}}) + \frac{1}{2} \delta(G_{ix}, G_{iy}) f(p_{G_{ix}}) + \frac{1}{4} f(p_{G_{iy}}) \ .$$

To construct an analytically computable statistic, we need to find formulas for the mean and variance of Z_{ii}. The mean of Z_{ii} is easy to calculate by exploiting the logic of Weeks and Lange (1988):

$$\mathrm{E}(Z_{ii}) = \Phi_{ii} \sum_{k=1}^{n} p_k f(p_k) + (1 - \Phi_{ii}) \sum_{k=1}^{n} p_k^2 f(p_k) \ .$$

In order to simplify this expression, let Φ_{FM} be the probability that the paternal and maternal genes of person i are IBD. Recall that $\Phi_{ii} = \frac{1}{2}(1 + \Phi_{\mathrm{FM}})$. Then,

$$\begin{aligned} \mathrm{E}(Z_{ii}) = {} & \frac{1}{2} \sum_{k=1}^{n} p_k f(p_k) \\ & + \frac{1}{2} \sum_{k=1}^{n} [\Phi_{\mathrm{FM}} p_k f(p_k) + (1 - \Phi_{\mathrm{FM}}) p_k^2 f(p_k)] \ . \end{aligned}$$

From the intimidating formula (6) presented by Weeks and Lange (1988) for $\mathrm{E}[Z_{ij} Z_{kl}]$, we find that, when $i = j = k = l$,

$$\begin{aligned} \mathrm{E}(Z_{ii} Z_{ii}) = {} & \left[\sum p_m f(p_m)^2\right] \Phi\{(G_i, G_i, G_i, G_i)\} \\ & + \left[\sum p_m f(p_m)\right]^2 \Phi\{(G_i, G_i)(G_i, G_i)\} \\ & + \left[\sum p_m^2 f(p_m)^2\right] \left\{ \begin{array}{l} 2\Phi\{(G_i, G_i)(G_i, G_i)\} \\ +4\Phi\{(G_i, G_i, G_i)(G_i)\} \end{array} \right\} \ , \end{aligned}$$

where the $\Phi()$'s are generalized kinship coefficients as described by Weeks and Lange (1988). Now,

$$\begin{aligned} & \Phi\{(G_i, G_i, G_i, G_i)\} \\ & = P[(G_i, G_i, G_i, G_i) \mid \text{picked the same allele four times}] \\ & \quad \times P_{[\text{picked the same allele four times}]} \\ & \quad + P[(G_i, G_i, G_i, G_i) \mid \text{picked both alleles}] \\ & \quad \times P_{[\text{picked both alleles}]} = (1)\left(\frac{1}{8}\right) + \Phi_{\mathrm{FM}}\left(\frac{7}{8}\right) \ . \end{aligned}$$

Similar reasoning leads to $\Phi\{(G_i, G_i)(G_i, G_i)\} = \frac{1}{8}(1 - \Phi_{\mathrm{FM}})$ and $\Phi\{(G_i, G_i, G_i), (G_i)\} = \frac{1}{8}(1 - \Phi_{\mathrm{FM}})$. Thus,

$$\begin{aligned} \mathrm{E}(Z_{ii} Z_{ii}) = {} & \left[\sum p_m f(p_m)^2\right]\left[\frac{1}{8} + \frac{7}{8}\Phi_{\mathrm{FM}}\right] \\ & + \left[\sum p_m f(p_m)\right]^2 \left[\frac{1}{8}(1 - \Phi_{\mathrm{FM}})\right] \\ & + \left[\sum p_m^2 f(p_m)^2\right]\left[\frac{6}{8}(1 - \Phi_{\mathrm{FM}})\right] \ . \end{aligned}$$

Note that, in order to find $\mathrm{E}(Z^2)$, we also need to calculate terms such as $\mathrm{E}(Z_{ii} Z_{ij})$. However, in the computer program, it is easier to use the general formula (6) presented by Weeks and Lange (1988) than to use specific expressions for each special case.

For a pedigree, from the various statistics Z_{ij}, we form the overall statistic $Z = \Sigma_{i \leqslant j} Z_{ij}$. The mean and variance of Z may be computed by the following equations:

$$\mathrm{E}(Z) = \sum_{i \leqslant j} \mathrm{E}(Z_{ij})$$

and

$$\mathrm{E}(Z^2) = \sum_{\substack{i \leqslant j \\ k \leqslant l}} \mathrm{E}(Z_{ij} Z_{kl}) \ .$$

When combining the Z statistics from different pedigrees into the test statistic T (see Weeks and Lange 1988, eq. [7]), we cannot use the weights w_m in equation (8) of Weeks and Lange (1988), since we may now have pedigrees with only one inbred affected individual. If r_m is the number of affected and typed individuals in the mth pedigree and if Z_m is the Z statistic for this pedigree, then we choose to use $w_m = \sqrt{r_m} / \sqrt{\mathrm{Var}(Z_m)}$. There is no rigorous justification for this choice of weights. However, intuitively it seems better than giving all pedigrees equal weight.

Weeks (1988) investigated some sample applications of this extended APM statistic, which is based on measurement of IBS marker similarity *within* affected

individuals as well as *between* pairs of affected relatives. Note that the computation of this statistic requires that the pedigree structure linking the affected relatives be known.

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Reply to Weeks and Sinsheimer

To the Editor:

In their letter, Weeks and Sinsheimer (1998 [in this issue]) point out some mistakes in the mathematical derivations in our article published in an earlier issue of the *Journal* (Génin and Clerget-Darpoux 1996). Although the main results of our earlier article are not invalidated, there were undoubtedly some errors in the formulas that it presented in Appendixes A and B.

First, contrary to what is believed by Weeks and Sinsheimer, it is possible to derive the IBW-state probabilities (or condensed identity coefficients) for two individuals in a population as a function of the mean inbreeding coefficient α of this population. The argument of Weeks and Sinsheimer is indeed based on an example that did not follow our basic assumption of a mean inbreeding coefficient α constant over time and equal to the mean kinship coefficient. Hence, Weeks and Sinsheimer give the example of a population in which $\frac{1}{4}$ of the individuals, C, are offspring from the same first-cousin marriage and in which the remaining $\frac{3}{4}$ of the individuals, U, are noninbred unrelated individuals. We agree that the mean inbreeding coefficient of the population is thus $\frac{1}{64}$, but, in this case, the mean kinship coefficient is different from the mean inbreeding coefficient.

However, we agree that the IBW-state probabilities of Appendix A of our earlier article were incorrect, and we have corrected them in Appendix A below. It should be noted that, with these corrected IBW-state probabilities, Δ_i ($i = 1$–9), the two consistency checks noted by Weeks and Sinsheimer are satisfied:

1. The kinship coefficient between the siblings, ϕ_{34}, is

$$\phi_{34} = \Delta_1 + \frac{1}{2}(\Delta_3 + \Delta_5 + \Delta_7) + \frac{1}{4}\Delta_8$$

$$= \frac{1}{4}(1 + 3\alpha) \ ;$$

that is consistent with the result obtained, by means of classical recursion methods, by Weeks and Sinsheimer.

2. The second check is the use of matrix **K** of Karigl (1981) to derive the vector $\mathbf{V}_{ij}$ of kinship coefficients from the vector $\mathbf{I}_{ij}$ of IBW-state probabilities between two individuals i and j. If we apply matrix **K** to $\mathbf{I}_{34}$, the vector of IBW-state probabilities of the two siblings, we obtain the correct vector $\mathbf{V}_{34}$ (see Appendix A below).

With these IBW-state probabilities, it is possible to derive the probability that the sib pair shares zero, one, or two alleles identical by descent (the IB-state probabilities). The correct IB-state probabilities are also reported below in Appendix A. Using these formulas, we have redone the study of the robustness of the three tests (the t_1 test, the t_2 test, and the IB test) considered in our earlier article (Génin and Clerget-Darpoux 1996). For small values of the mean inbreeding coefficient α, the type 1 error of the three tests is not changed; for greater values of α, the type 1 error increases slightly, as shown in table 1, in a manner dependent on the type of test.

In Appendix B of our earlier article, we did not, as is noted by Weeks and Sinsheimer, consider that kinship

Table 1

Corrected (and Original [Génin and Clerget-Darpoux 1996]) Type 1 Errors for Three Tests of Linkage, for Samples of 100 Affected Sib Pairs in a Population with Mean Inbreeding Coefficient α

	Corrected (Original) Type 1 Error		
α	t_1	t_2	IB
.000	.050 (.050)	.050 (.050)	.050 (.050)
.001	.051 (.051)	.052 (.052)	.050 (.050)
.005	.057 (.057)	.061 (.060)	.051 (.051)
.010	.064 (.064)	.075 (.070)	.054 (.053)
.020	.082 (.082)	.108 (.097)	.066 (.060)
.030	.103 (.103)	.150 (.129)	.086 (.073)
.040	.128 (.127)	.202 (.169)	.115 (.090)
.050	.156 (.155)	.263 (.215)	.152 (.114)
.060	.189 (.187)	.332 (.268)	.198 (.143)
.070	.226 (.223)	.407 (.326)	.252 (.179)
.080	.266 (.262)	.486 (.390)	.312 (.220)
.090	.311 (.305)	.565 (.456)	.377 (.267)
.010	.358 (.350)	.641 (.523)	.446 (.318)

Table A1

K Matrix and I and V Vectors

K									$\mathbf{I}_{12}$	$\mathbf{V}_{12}$
1	1	1	1	1	1	1	1	1	Δ_1	1
2	2	2	2	1	1	1	1	1	Δ_2	$2\phi_{11}$
2	2	1	1	2	2	1	1	1	Δ_3	$2\phi_{22}$
4	0	2	0	2	0	2	1	0	Δ_4	$4\phi_{12}$
8	0	4	0	2	0	2	1	0	Δ_5	$8\phi_{112}$
8	0	2	0	4	0	2	1	0	Δ_6	$8\phi_{122}$
16	0	4	0	4	0	2	1	0	Δ_7	$16\phi_{1122}$
4	4	2	2	2	2	1	1	1	Δ_8	$4\phi_{11,22}$
16	0	4	0	4	0	4	1	0	Δ_9	$16\phi\Delta_{12,12}$

sampling is done with replacement. This leads to small differences in the IBW-state probabilities, Δ_i, which have been corrected in Appendix B below. For $\alpha = 0$, there is no difference, and, for $\alpha < .05$, the difference is negligible. The power results are thus almost not changed, and the figures given in our earlier article are still valid.

Although there were some regrettable errors in our earlier article (Génin and Clerget-Darpoux 1996), which Weeks and Sinsheimer detected and which have been independently noted by Cannings (1998 [in this issue]), we have shown that it is possible to correct them and that they do not invalidate the robustness and power results.

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Appendix A

IBW-State Probabilities for Two Sibs in a Consanguineous Population

Let us consider that the population from which sib pairs are sampled has a mean inbreeding coefficient α equal to the mean kinship coefficient in a same generation. Let individuals "1" and "2" be the parents of the sib pair, "3" and "4." Let Δ_k denote the probability for IBW state S_k (k = 1–9). Let $\mathbf{I}_{ij}$ be the vector of Δ_k (k = 1–9) for the two individuals i and j. It is possible to compute $\mathbf{I}_{12}$ as a function of α, by use of Karigl's (1981) extended-kinship coefficient. Eight extended-kinship coefficients should be computed:

$$\phi_{11} = \phi_{22} = \frac{1}{2}(1 + \alpha) \ ;$$

$$\phi_{12} = \alpha \ ;$$

$$\phi_{112} = \phi_{122} = \frac{1}{2}\alpha(1 + \alpha) \ ;$$

$$\phi_{1122} = \frac{1}{4}\alpha(1 + \alpha)^2 \ ;$$

$$\phi_{11,22} = \frac{1}{4}(1 + \alpha)^2 \ ;$$

$$\phi_{12,12} = \frac{1}{4}\alpha(1 + 3\alpha) \ .$$

Karigl (1981) showed that the matrix $\mathbf{K}$ multiplied by $\mathbf{I}_{12}$ equals $\mathbf{V}_{12}$, where the matrix $\mathbf{K}$ and the vectors $\mathbf{I}_{12}$ and $\mathbf{V}_{12}$ are as reported in table A1; by use of that relation, it is thus possible to derive the vector $\mathbf{I}_{12}$ of IBW-state probabilities:

$$S_1\ (1111) = \alpha^3 \ ;$$

$$S_2\ (1122) = \alpha^2(1 - \alpha) \ ;$$

$$S_3\ (1112) = 2\alpha^2(1 - \alpha) \ ;$$

$$S_4\ (1123) = \alpha(1 - \alpha)(1 - 2\alpha) \ ;$$

$$S_5\ (1222) = 2\alpha^2(1 - \alpha) \ ;$$

$$S_6\ (1233) = \alpha(1 - \alpha)(1 - 2\alpha) \ ;$$

$$S_7\ (1212) = 2\alpha^2(1 - \alpha) \ ;$$

$$S_8\ (1213) = 4\alpha(1 - \alpha)(1 - 2\alpha) \ ;$$

$$S_9\ (1234) = (1 - \alpha)(1 - 2\alpha)(1 - 3\alpha) \ .$$

Once the IBW-state probabilities of parents are known, the IBW-state probabilities for the sib pair can

Table A2

IBW States of Parents and Sibs

IBW State of Sib	IBW State of Parents								
	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9
S_1	1	0	$\frac{1}{4}$	0	$\frac{1}{4}$	0	$\frac{1}{8}$	$\frac{1}{16}$	0
S_2	0	0	0	0	0	0	$\frac{1}{8}$	0	0
S_3	0	0	$\frac{1}{4}$	0	$\frac{1}{4}$	0	$\frac{1}{4}$	$\frac{1}{8}$	0
S_4	0	0	0	0	0	0	0	$\frac{1}{16}$	0
S_5	0	0	$\frac{1}{4}$	0	$\frac{1}{4}$	0	$\frac{1}{4}$	$\frac{1}{8}$	0
S_6	0	0	0	0	0	0	0	$\frac{1}{16}$	0
S_7	0	1	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{3}{16}$	$\frac{1}{4}$
S_8	0	0	0	$\frac{1}{2}$	0	$\frac{1}{2}$	0	$\frac{3}{8}$	$\frac{1}{2}$
S_9	0	0	0	0	0	0	0	0	$\frac{1}{4}$

be obtained by use of matrix $\mathbf{M}_{ps}$, shown in table A2. The IBW-state probabilities for the sib pair, individuals 3 and 4, are thus the product $\mathbf{M}_{ps}\mathbf{I}_{12}$:

$$\Delta_1 = \frac{1}{4}\alpha^3 + \frac{1}{2}\alpha^2 + \frac{1}{4}\alpha \ ;$$

$$\Delta_2 = -\frac{1}{4}\alpha^3 + \frac{1}{4}\alpha^2 \ ;$$

$$\Delta_3 = -\frac{1}{2}\alpha^3 + \frac{1}{2}\alpha \ ;$$

$$\Delta_4 = \frac{1}{2}\alpha^3 - \frac{3}{4}\alpha^2 + \frac{1}{4}\alpha \ ;$$

$$\Delta_5 = -\frac{1}{2}\alpha^3 + \frac{1}{2}\alpha \ ;$$

$$\Delta_6 = \frac{1}{2}\alpha^3 - \frac{3}{4}\alpha^2 + \frac{1}{4}\alpha \ ;$$

$$\Delta_7 = -\frac{1}{2}\alpha^3 + \frac{1}{4}\alpha + \frac{1}{4} \ ;$$

$$\Delta_8 = 2\alpha^3 - 2\alpha^2 - \frac{1}{2}\alpha + \frac{1}{2} \ ;$$

$$\Delta_9 = -\frac{3}{2}\alpha^3 + \frac{11}{4}\alpha^2 - \frac{3}{2}\alpha + \frac{1}{4} \ .$$

These IBW-state probabilities verify the two consistency checks discussed by Weeks and Sinsheimer:

1. The kinship coefficient between the siblings, ϕ_{34}, is

$$\phi_{34} = \Delta_1 + \frac{1}{2}(\Delta_3 + \Delta_5 + \Delta_7) + \frac{1}{4}\Delta_8$$

$$= \frac{1}{4}(1 + 3\alpha) \ .$$

2. By multiplying this vector, $\mathbf{I}_{34}$, by the matrix $\mathbf{K}$, we obtained

$$\mathbf{V}_{34} = \begin{pmatrix} 1 \\ 1+\alpha \\ 1+\alpha \\ 1+3\alpha \\ 1+5\alpha+2\alpha^2 \\ 1+5\alpha+2\alpha^2 \\ 1+8\alpha+6\alpha^2+\alpha^3 \\ 1+\frac{9}{4}\alpha+\frac{3}{4}\alpha^2 \\ \frac{3}{2}+\frac{17}{2}\alpha+6\alpha^2 \end{pmatrix} ,$$

which is the correct vector of kinship coefficient for two sibs in a population with mean inbreeding coefficient α. The IB-state probabilities for the sib pair are then

$$P(\mathrm{IB}=0) = \Delta_2 + \Delta_4 + \Delta_6 + \Delta_9$$

$$= \frac{1}{4} - \alpha + \frac{3}{2}\alpha^2 - \frac{3}{4}\alpha^3 \ ;$$

$$P(\mathrm{IB}=1) = \Delta_3 + \Delta_5 + \Delta_8 = \frac{1}{2} + \frac{1}{2}\alpha - 2\alpha^2 + \alpha^3 \ ;$$

$$P(\mathrm{IB}=2) = \Delta_1 + \Delta_7 = \frac{1}{4} + \frac{1}{2}\alpha + \frac{1}{2}\alpha^2 - \frac{1}{4}\alpha^3 \ .$$

Appendix B

Corrections of Appendix B in Our Earlier Article (Génin and Clerget-Darpoux 1996)

In Appendix B of our earlier article, we showed how to account for the remote consanguinity in the computation of IBW-state probabilities for two sibs from first-cousin matings, using the algorithm of Karigl (1981). The pedigree in which extended-kinship coefficients have been computed is shown in figure B1.

As is pointed out by Weeks and Sinsheimer, there was an error in the computation of the extended-kinship coefficients, because we did not consider that kinship sampling is done with replacement. This leads to some differences in the kinship coefficients ϕ_{778}, ϕ_{7788}, and $\phi_{78,78}$:

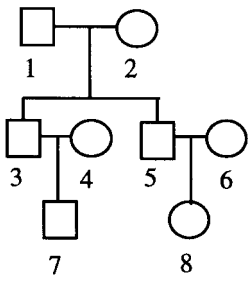

Figure B1 Pedigree in which individuals 7 and 8 are first cousins

$$\phi_{778} = \phi_{887} = \frac{1}{32} + \frac{17}{32}\alpha + \frac{7}{16}\alpha^2 \ ;$$

$$\phi_{7788} = \frac{1}{64} + \frac{19}{64}\alpha + \frac{1}{2}\alpha^2 + \frac{3}{16}\alpha^3 \ ;$$

$$\phi_{78,78} = \frac{1}{64} + \frac{21}{64}\alpha + \frac{21}{32}\alpha^2 \ .$$

The kinship coefficients ϕ_{77}, ϕ_{78}, and $\phi_{77,88}$ are not changed:

$$\phi_{77} = \phi_{88} = \frac{1}{2}(1 + \alpha) \ ;$$

$$\phi_{78} = \frac{1}{16}(1 + 15\alpha) \ ;$$

$$\phi_{77,88} = \frac{1}{4}(1 + \alpha)^2 \ .$$

The IBW-state probabilities for individuals 7 and 8, obtained by use of the inverse of matrix **K**, as explained in Appendix A above, are thus

$$P(S_1) = \frac{1}{4}\alpha^2(1 + 3\alpha) \ ;$$

$$P(S_2) = \frac{3}{4}\alpha^2(1 - \alpha) \ ;$$

$$P(S_3) = \frac{1}{4}\alpha(1 + 5\alpha - 6\alpha^2) \ ;$$

$$P(S_4) = \frac{3}{4}\alpha(1 - 3\alpha + 6\alpha^2) \ ;$$

$$P(S_5) = \frac{1}{4}\alpha(1 + 5\alpha - 6\alpha^2) \ ;$$

$$P(S_6) = \frac{3}{4}\alpha(1 - 3\alpha + 6\alpha^2) \ ;$$

$$P(S_7) = \frac{1}{4}\alpha(1 + 5\alpha - 6\alpha^2) \ ;$$

$$P(S_8) = \frac{1}{4}(1 + 9\alpha - 34\alpha^2 + 24\alpha^3) \ ;$$

$$P(S_9) = \frac{1}{4}(3 - 18\alpha + 33\alpha^2 - 18\alpha^3) \ .$$

The use of matrix $\mathbf{M}_{ps}$, defined in Appendix A above, allows derivation of the IBW-state probabilities for sib pairs from first-cousin marriages in this population:

$$P(S_1) = \frac{3}{16}\alpha^3 + \frac{1}{2}\alpha^2 + \frac{19}{64}\alpha + \frac{1}{64} \ ;$$

$$P(S_2) = -\frac{3}{16}\alpha^3 + \frac{5}{32}\alpha^2 + \frac{1}{32}\alpha \ ;$$

$$P(S_3) = -\frac{3}{8}\alpha^3 - \frac{1}{8}\alpha^2 + \frac{15}{32}\alpha + \frac{1}{32} \ ;$$

$$P(S_4) = \frac{3}{8}\alpha^3 - \frac{17}{32}\alpha^2 + \frac{9}{64}\alpha + \frac{1}{64} \ ;$$

$$P(S_5) = -\frac{3}{8}\alpha^3 - \frac{1}{8}\alpha^2 + \frac{15}{32}\alpha + \frac{1}{32} \ ;$$

$$P(S_6) = \frac{3}{8}\alpha^3 - \frac{17}{32}\alpha^2 + \frac{9}{64}\alpha + \frac{1}{64} \ ;$$

$$P(S_7) = -\frac{3}{8}\alpha^3 - \frac{3}{32}\alpha^2 + \frac{15}{64}\alpha + \frac{15}{64} \ ;$$

$$P(S_8) = \frac{3}{2}\alpha^3 - \frac{21}{16}\alpha^2 - \frac{21}{32}\alpha + \frac{15}{32} \ ;$$

$$P(S_9) = -\frac{9}{8}\alpha^3 + \frac{33}{16}\alpha^2 - \frac{9}{8}\alpha + \frac{3}{16} \ .$$

These IBW-state probabilities verify the two consistency checks discussed by Weeks and Sinsheimer:

1. The kinship coefficient of the two sibs is, as expected, $\frac{9}{32} + \frac{23}{32}\alpha$. If $\alpha = 0$, then we obtain $\frac{9}{32}$; that is the correct kinship coefficient for two siblings whose parents are first cousins.

2. Using matrix **K** of Karigl (1981), we obtained the correct vector **I** of extended-kinship coefficients:

$$\mathbf{I} = \begin{pmatrix} 1 \\ \frac{17}{16} + \frac{15}{16}\alpha \\ \frac{17}{16} + \frac{15}{16}\alpha \\ \frac{9}{8} + \frac{23}{8}\alpha \\ \frac{5}{4} + 5\alpha + \frac{7}{4}\alpha^2 \\ \frac{5}{4} + 5\alpha + \frac{7}{4}\alpha^2 \\ \frac{23}{16} + \frac{133}{16}\alpha + \frac{11}{2}\alpha^2 + \frac{3}{4}\alpha^3 \\ \frac{73}{64} + \frac{141}{64}\alpha + \frac{21}{32}\alpha^2 \\ \frac{61}{32} + \frac{281}{32}\alpha + \frac{85}{16}\alpha^2 \end{pmatrix}.$$

We can verify our formulas, since, for $\alpha = 0$, the extended-kinship coefficients for the two sibs, individuals 9 and 10, are

$$\phi_{99} = \phi_{1010} = \frac{1}{2}\left(1 + \frac{1}{16}\right) = \frac{17}{32}$$ (and the second entry and the third entry $[2\phi_{99}]$ of **I** are correct) ;

$$\phi_{910} = \frac{9}{32}$$ (and the fourth entry $[4\phi_{910}]$ of **I** is correct) ;

$$\phi_{9910} = \phi_{10109} = \frac{1}{2}(\phi_{910} + \phi_{7810})$$

$$= \frac{1}{2}\left\{\frac{9}{32} + \left[\frac{1}{16}\left(\frac{1}{2}\right)\right]\right\} = \frac{5}{32}$$

(and the fifth entry and the sixth entry $[8\phi_{9910}]$ of **I** are correct) ;

$$\phi_{991010} = \frac{1}{2}(\phi_{91010} + \phi_{781010}) = \frac{5}{64} + \frac{1}{256} + \left[\frac{1}{4}\left(\frac{1}{32}\right)\right]$$

$$= \frac{23}{256}$$ (and the seventh entry $[16\phi_{91010}]$ is correct) ;

$$\phi_{99,1010} = \frac{1}{2}(\phi_{1010} + \phi_{78,1010}) = \frac{73}{256}$$ (and the eighth entry $[4\phi_{991010}]$ is correct) ;

$$\phi_{910,910} = \frac{1}{2}(\phi_{91010} + \phi_{710,810}) = \frac{5}{64} + \frac{1}{8}$$

$$(2\phi_{7810} + \phi_{77,88} + \phi_{78,78}) = \frac{61}{512}$$

(and the last entry $[16\phi_{910910}]$ is correct) .

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Health, Life, and Disability Insurance and Hereditary Nonpolyposis Colorectal Cancer

To the Editor:

With the discovery of germ-line mutations in the mismatch-repair genes, genetic testing for hereditary nonpolyposis colorectal cancer (HNPCC) has become a reality. However, concern about potential genetic discrimination, with regard to insurance and employment, has surfaced among affected individuals and health-care providers. Owing to the lack of information regarding insurance providers' attitudes toward both HNPCC individuals diagnosed with cancer and asymptomatic gene carriers, we decided to assess the insurance-industry attitude regarding the offering of health, life, and disability insurance to HNPCC gene carriers and at-risk family members.

An anonymous survey with 14 questions, which included a self-addressed, stamped envelope, was mailed to 1,000 health, life, and disability insurance company presidents. An insurance company database consisting of 5,178 companies was purchased from NAIC Database products. Twenty companies, from each state, that were reported to sell health, life, and disability insurance were chosen randomly from the purchased database. An introductory letter defining HNPCC and the anonymous nature of the study accompanied each mailed questionnaire. Only one mailing was sent. A gene carrier was defined as someone with a germ-line mutation in one of the genes responsible for HNPCC. An at-risk family member was defined as someone who had a 50% chance of carrying the mutation for the defective gene.

Of the 1,000 surveys mailed, 4 were not delivered and 79 were returned. Two of the returned surveys were excluded, since they were mailed to different locations of the same company. There were 77 (7.7%) responses to the mailing. Five surveys were returned unanswered, and 6 were returned labeled "not applicable." Even though we did not know the size of the companies that responded to our survey, we estimated, on the basis of our response rate, that our respondents probably issue <5% of the insurance policies sold in the United States.

The survey consisted of three sections, with six questions on health insurance, four questions on life insurance, and four questions on disability insurance. A total of 66 usable questionnaires were returned, but not all were completed for all three types of insurance: Responses were as follows: 49/66 responded as health insurance providers, 46/66 as life insurance providers, and 30/66 as disability insurance providers. These 66 questionnaires served as the basis for this report.

With regard to health insurance, 48 (98%) insurance providers responded that they would insure at-risk family members, whereas 1 (2%) insurer would not. Three of the 48 insurers would provide the insurance, with the following conditions: a higher premium (1); if the insured "does not carry a bad gene" (1); and depending on a guarantee (1). Forty-four (90%) of the insurers would provide insurance to a gene carrier. Those who would not provide the insurance had the following reasons: no explanation (1); not unless state mandated (1); cost of surveillance and treatment too high (1); would offer colorectal cancer rider (1); and depends on guarantee (1).

Thirty-seven (76%) providers would not increase the premium for an at-risk member. However, 9 (18%) providers would increase the premium for an at-risk member, whereas 2 providers were not sure whether the premium would be increased. One company answered that it would not increase the premium but would consider inserting an exclusion rider. With regard to health insurance for gene carriers, 38 (78%) providers would not increase the premium, 10 (20%) providers would, and 1 (2%) provider was not sure.

Tables 1 and 2 indicate the responses to the questions regarding (1) payment for colonoscopy at more-frequent intervals for HNPCC at-risk individuals than for the general population and (2) payment for prophylactic abdominal colectomy and ileorectal anastomosis for an HNPCC gene carrier. It can be seen that the majority (61%) of the health insurance providers would pay for more-frequent colonoscopies and that 35% of the in-

Table 1

Health Insurance Providers' Willingness to Pay for Colonoscopies More Frequently for HNPCC At-Risk Individuals than for the General Population

	No. (%) of Providers
Yes	30 (61)[a]
No	16 (33)
Not applicable	3 (6)

[a] Four providers responded that payment was subject to the contract, one that payment would be ⩽$100/year, and one that payment would be made if the patient was already covered and if there was no rider.

Table 2

Health Insurance Providers' Willingness to Pay for Prophylactic Surgery for an HNPCC Gene Carrier

	No. (%) of Providers
Yes	17 (35)[a]
No	22 (45)[b]

[a] Three providers responded that payment would be made if medical necessity had been established, one that payment would be made if there was no rider, and one that payment would be made if medical necessity had been established and if the procedure was not experimental.

[b] One provider responded that payment would not be made unless the procedure was standard care, one responded that payment was subject to review of each case, and one responded that payment normally is not made for preventive surgery.

surers would pay for prophylactic surgery for a gene carrier.

With regard to life insurance, 32 (70%) companies would sell life insurance to at-risk individuals, 2 (4%) would not, and 12 (26%) would sell it at a higher premium. Thirty-two (70%) of the respondents would sell life insurance to gene carriers, whereas 2 (4%) would not, 10 (22%) would sell it at a higher premium, 1 (2%) was not sure, and 1 (2%) did not answer the question. Forty-three (93%) of the life insurance providers did not require genetic testing prior to insuring a member of an HNPCC kindred. One company did require genetic testing. Two companies would require genetic testing prior to selling life insurance if the coverage was >$50,000–$100,000 or >$350,000, respectively.

With regard to disability insurance, 24 (80%) companies would sell disability insurance to at-risk individuals, 1 (3%) would not, and 5 (17%) would sell it at a higher premium. Gene carriers would be insured by 23 (77%) companies. Three (10%) companies would not sell disability insurance to gene carriers, whereas 4 (13%) would charge a higher premium.

Twenty nine (97%) of the disability insurance providers did not require genetic testing prior to insuring a member of an HNPCC kindred. One company did require genetic testing. As shown in table 3, the majority of the life and disability insurance providers would be allowed access to DNA test results.

In the interpretation of our results, there are several factors that should be kept in mind. Even though our survey was completely anonymous, only 79 (7.9%) of the surveys were returned. This is a limitation of our data set. This could have been improved if we had sent additional mailings. However, because of the anonymity of the survey, the logistics of remailing 1,000 questionnaires was insurmountable, and, hence, the decision was

Table 3

Access to DNA Test Results, by Type of Insurance Provider

Access to DNA Test Results	No. (%) of Life Insurance Providers	No. (%) of Disability Insurance Providers
Yes	26 (57)[a]	20 (67)
No	14 (30)	8 (30)
Not applicable	2 (4)	1 (3)
Do not know	1 (2)	1 (3)
Maybe	1 (2)	…
No answer	2 (4)	…

[a] One provider stated that they would have access to test results but would decline to insure for economic reasons.

made to mail the survey only once. There were several potential reasons for the poor response rate. Among them was that the survey may have been mailed to an inappropriate insurance company executive, which could have led to fear of identification and of adverse public relations for the company or fear that the company could be held accountable, in the future, for any answers provided. Importantly, we do not claim that the results reflect the whole insurance industry. However, they at least provide some information about insurance-provider attitudes toward HNPCC gene carriers and at-risk individuals.

Even though we had a limited response (7.7%) to the questionnaires and the survey respondents probably issue <5% of the insurance policies sold in the United States, our results indicate that the majority of health, life, and disability insurance providers with an opinion would be willing to sell insurance to both HNPCC gene carriers and at-risk individuals. Nevertheless, there were some that would not insure these individuals. The interpretation of our results indicates that there is probably a minority of insurance providers that potentially will discriminate against HNPCC gene carriers and at-risk individuals. It is the latter insurance providers who need to be educated about the condition and the benefits of surveillance and early detection.

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Likelihood Calculation Conditional on Observed Pedigree Structure

To the Editor:

The article by Vieland and Hodge (1995) has addressed the ascertainment problem from a theoretical point of view and has commendably explained the difference between the true and the observed likelihoods for a set of data. After pointing out the difference in the two likelihood expressions, they give an example, using a universe of sibships of size 3, to illustrate this difference. Their figure 1 shows a tree diagram for calculating $L_{\text{TRUE}}(\theta)$, and their figure 2 shows a tree diagram for calculating $L_{\text{OBS}}(\theta)$. We should like to clarify that the presence of figure 2 might mislead the reader, because it is irrelevant under the stated conditions.

The true likelihood is conditional on being ascertained and the true pedigree structure, whereas the observed likelihood is conditional on being ascertained and the observed pedigree structure. When the observed likelihood $L_{\text{OBS}}(\theta)$ is computed, the sampling rule for the relatives of the proband(s) is not taken into account and, hence, is irrelevant. We therefore find the tree diagram shown in Vieland and Hodge's figure 2, meant to aid the reader in the computation of $L_{\text{OBS}}(\theta)$, confusing. However, Vieland and Hodge correctly indicate, below the diagram, that the step from their level (ii) to their level (iii) in the tree diagram is not governed by the sampling rule and that the location of the probands within the sibships is irrelevant. Since the investigator constructs the likelihood conditional on the observed pedigree structure (i.e., on $s = 2$ or $s = 3$, where s is the observed sibship size in the example) with no allowance given for the sampling rule, the second column of Vieland and Hodge's table 1, which gives the probabilities for $L_{\text{OBS}}(\theta)$, is more simply obtained as follows, without referring to their figure 2.

First, there are three possibilities for a given subject: (1) not affected (probability $= 1 - \theta$); (2) affected proband (probability $= \theta\pi$); and (3) affected nonproband (probability $= \theta(1 - \pi)$).

When two sibs are observed, the first two entries of the second column of Vieland and Hodge's table 1 are, where $D_2 = 1 - (1 - \theta\pi)^2$,

$$\begin{aligned} &P(1 \text{ affected} \mid \text{ascertained}) \\ &\quad = P(1 \text{ proband and } 1 \text{ unaffected})/P(\text{ascertained}) \\ &\quad = 2\theta\pi(1 - \theta)/D_2, \end{aligned}$$

and

$$\begin{aligned} &P(2 \text{ affected} \mid \text{ascertained}) \\ &\quad = P(2 \text{ probands, or } 1 \text{ proband and} \\ &\qquad 1 \text{ affected nonproband})/P(\text{ascertained}) \\ &\quad = [(\theta\pi)^2 + 2\theta\pi\theta(1 - \pi)]/D_2 \\ &\quad = \theta^2[1 - (1 - \pi)^2]/D_2. \end{aligned}$$

Similarly, when three sibs are observed, the last three entries of the second column of Vieland and Hodge's table 1 are, where $D_3 = 1 - (1 - \theta\pi)^3$,

$$\begin{aligned} &P(1 \text{ affected} \mid \text{ascertained}) \\ &\quad = P(1 \text{ proband and } 2 \text{ unaffected})/P(\text{ascertained}) \\ &\quad = 3\theta\pi(1 - \theta)^2/D_3 , \end{aligned}$$

$$\begin{aligned} &P(2 \text{ affected} \mid \text{ascertained}) \\ &\quad = P(1 \text{ proband, } 1 \text{ affected nonproband and } 1 \\ &\qquad \text{unaffected, or } 2 \text{ probands and } 1 \text{ unaffected})/ \\ &\qquad P(\text{ascertained}) \\ &\quad = \{6\theta\pi[\theta(1 - \pi)](1 - \theta) + 3(\theta\pi)^2(1 - \theta)\}/D_3 \\ &\quad = 3\theta^2(1 - \theta)[1 - (1 - \pi)^2]/D_3 , \end{aligned}$$

and

$$\begin{aligned} &P(3 \text{ affected} \mid \text{ascertained}) \\ &\quad = P(1 \text{ proband and } 2 \text{ affected nonprobands}, \\ &\qquad 2 \text{ probands and } 1 \text{ affected nonproband}, \\ &\qquad \text{or } 3 \text{ probands})/P(\text{ascertained}) \\ &\quad = \{3\theta\pi[\theta(1 - \pi)]^2 + 3(\theta\pi)^2\theta(1 - \pi) + (\theta\pi)^3\}/D_3 \\ &\quad = \theta^3[1 - (1 - \pi)^2]/D_3 . \end{aligned}$$

We stress that the purpose of this letter is merely to make the example presented by Vieland and Hodge (1995) easier to understand and that it in no way detracts from the main point of their paper—namely, that the observed likelihood is correct for ascertained data only in very special situations. As genetic epidemiology moves—whether by segregation or linkage analysis or a combination of both—away from the mere detection of trait genes, toward the precise estimation of their effects (e.g., in terms of relative risks or attributable risks), it will be necessary, in order to obtain good parameter estimates, to design studies for which the observed likelihood (which is what we can calculate) is as close as possible to the intractable true likelihood. By under-

standing how these two likelihoods may differ when we ascertain large pedigrees, we can be guided toward sampling and analytical techniques that minimize their difference (Elston 1995). Furthermore, current attempts to locate, by model-based linkage analysis, genes underlying complex traits can be made more powerful by the use of a realistic model for the genetic mechanism underlying the trait phenotype. Clerget-Darpoux et al. (1986) showed how the LOD-score profile may be affected by misspecification of various genetic parameters, leading to biased estimates of the recombination fraction. In order to estimate the most appropriate trait models for linkage analysis, it is necessary both to allow for familial correlations due to causes other than the locus to be linked (Demenais and Lathrop 1993) and to take proper account of ascertainment considerations. Linkage analysis of multigenerational data allowing for residual correlations is implemented in the program package S.A.G.E. (1997), but only careful planning at the time when data are collected (Elston 1995; Zhao et al. 1997) will ensure that relevant likelihoods can be formulated.

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Reply to Karunaratne and Elston

To the editor:

We thank Karunaratne and Elston for their careful and appreciative remarks on our article (Vieland and Hodge 1995). As they say, in that article we included figure 2 in order to illustrate the calculation of the entries in the second column of table 1. But their derivation of the entries in the table is of course equally reasonable, and if there are readers who find their derivation easier to follow than the original figure, then we are grateful for the additional illumination.

It is also gratifying to read that they agree with our central point—namely, that the observed likelihood is correct for ascertained data "only in very special situations" (notably, ascertainment independent of phenotype and single ascertainment; see Hodge and Vieland 1996) and that calculation of the true likelihood in all other situations is "intractable."

We agree as well with their remarks on the importance of study design for complex segregation analysis. However, we would like to add one minor clarification to their remarks on linkage analysis. Karunaratne and Elston might appear to be citing Clerget-Darpoux et al. (1986) in support of the view that use of a realistic model for the genetic mechanism can increase the power to detect linkage by use of model-based analyses (LOD scores). What Clerget-Darpoux et al. (1986, p. 398) actually concluded, however, was that, when certain genetic parameters (penetrances or gene frequencies) are misspecified, "the underestimation of the maximum lod score is negligible in most cases." Again, we thank Karunaratne and Elston for their thoughtful comments.

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