Letters to the Editor

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Absolute Pitch: Prevalence, Ethnic Variation, and Estimation of the Genetic Component

To the Editor:

Absolute pitch (AP), also known as "perfect pitch," is a distinct cognitive ability possessed by a minority of musicians. The essential feature of this trait is the capacity to recognize and name the pitch of a musical note or ambient sound without the use of a reference pitch and with a minimum of deliberation. Elegant studies by Miyazaki (1988) have provided a method of measuring this ability, and Baharloo et al. (1998) recently reported on the characterization of a population of AP possessors, using a modification of this approach. These studies have emphasized that, although there is some variation in levels of accuracy in AP possessors, musicians with this ability are nevertheless distinct from those who do not possess AP. As such, AP ability is one of the few cognitive phenotypes that exhibit a clear qualitative difference between those who possess it and those who do not.

Although informal prevalence estimates for AP ($\leqslant$1:1,500 among amateur music students) have been suggested (Profita and Bidder 1988), the study by Baharloo et al. (1998) represents the only published data concerning the prevalence of the AP phenotype. In a survey of 612 highly accomplished musicians, Baharloo et al. (1998) observed a prevalence rate of 15%. We have now completed a survey of 2,707 music students at music conservatories as well as at university and college music programs in the United States. We surveyed student populations ranging in size from 20 to 390 students (mean [$\pm$ SD] 104 $\pm$ 78), using a two-page questionnaire asking about the presence of AP in the students and in their family members. We assumed AP ability to be present if students reported both the ability to perceive tones in an absolute manner and the ability to sing a note when given the letter name, but without a reference pitch. In our experience (see below), this correlates reasonably well with AP ability on objective testing, as has been observed by others (Takeuchi and Hulse 1991; Baharloo et al. 1998).

We observed large variations in AP prevalence among different student populations (range 0%–35%). There was a significant association ($P < .001$) between the type of institution or music program and the prevalence of AP in the students: conservatory (24.6% with AP), university-based school of music (7.3% with AP), or liberal arts/state university music program (4.7% with AP). We also noted a strong correlation between the prevalence of AP and the percentage of students in these schools who reported their ethnic background as "Asian or Pacific Islander" ($r = .81$, $P < .0001$, Spearman rank correlation coefficient). This raised the possibility that AP is more prevalent in Asian students in general.

Table 1

Prevalence of AP in Asian Music Students, Stratified by Type of Music Program

Type of Music Program (No. of Students Surveyed)	No. (%) of Students with AP
Conservatory (73)	36 (49.3)
University music program (152)	39 (25.7)
Liberal arts college (12)	1 (8.3)
All programs combined (237)	76 (32.1)

The prevalence data in table 1 (Asian students) and table 2 (non-Asian students) indicate that AP is significantly more prevalent in Asian students compared with all other ethnic groups (non-Asian) combined (32.1% vs. 7.0%, $P < .001$). Furthermore, the higher rate of AP in Asian students is observed in all types of educational institutions. Even among non-Asians, however, the rate of AP was significantly higher in students at major music conservatories (table 2). A multivariate logistic regression indicated that Asian ethnicity and attendance at a conservatory were independently associated with AP in the student populations. Asian ethnic background had a relative risk (RR) of 5.0 (95% CI 3.6–7.0), whereas attendance at a music conservatory (vs. other music programs) had RR = 3.5 (95% CI 2.6–4.8). There were insufficient numbers of Hispanic or African American students to perform a meaningful subgroup analysis within the non-Asian group. Most of the individuals in the non-Asian group were white, and there were no obvious trends among the other broad ethnic groupings.

As has been reported by others, we also observed a significant association between AP and the age at which an individual first began playing music. For the AP group as a whole, the mean age of starting musical activities

Table 2

Prevalence of AP in Non-Asian Music Students, Stratified by Type of Music Program

Type of Music Program (No. of Students Surveyed)	No. (%) of Students with AP
Conservatory (276)	50 (18.1)
University music program (1,844)	107 (5.8)
Liberal arts college (350)	16 (4.5)
All programs combined (2,470)	173 (7.0)

was 5.4 ± 2.8 years, whereas, for the non-AP group, the mean age was 7.9 ± 3.2 years ($P < .0001$). This same trend was observed for Asian students as well as non-Asian students.

The issue of familial aggregation of AP is important for assessing the genetic contribution to this phenotype. In a small study (Gregersen and Kumar 1996), we estimated λ_s (recurrence risk to sibs divided by population prevalence) at ~20, whereas the data of Baharloo et al. (1998) suggested a λ_s of ~7 (Gregersen 1998). Because our current survey populations have such highly variable rates of AP, we estimated λ_s using the recurrence risk in *sibs of unaffecteds* as the denominator in the RR calculation. For this survey population, the recurrence rate for AP in siblings was reported as 14.1% for probands with AP and 1.7% in the siblings of subjects who did not have AP, leading to an estimate of $\lambda_s = 8.3$. By this method, the λ_s estimate for Asians was 11.1. Of course, this approach to estimating λ_s will tend to underestimate its value, since the background prevalence of AP in the general population is undoubtedly much lower than it is in the sibs of unaffected musicians. Notably, the prevalence of AP in the parents of AP probands is also higher than in the parents of music students without AP (6.5% vs. 1.6%), similar to our previous report (Gregersen and Kumar 1996).

These data indicate that estimates of the prevalence of AP are highly dependent on the selection of the population under study. Although AP may occur in non-musicians, the method of ascertainment of the AP phenotype restricts prevalence surveys to musically educated populations. This fact makes it especially difficult to separate the environmental from the genetic factors that predispose to AP, since exposure to music is both required for ascertainment as well as implicated in the development of the phenotype. In addition, the presence of AP almost certainly increases the probability that musical education will be pursued, and it may well provoke educational activities at an earlier age, thus confounding the interpretation of the association between early-childhood musical activities and AP. Our data also suggest that more-professionally oriented music schools are especially likely to attract or admit individuals with AP, independent of the ethnic background of students in these schools.

There are several possible reasons for the markedly increased prevalence of AP in students of Asian background. The presence of AP in a child may provoke more-serious parental efforts at music education in certain cultural groups and may lead to preferential selection of this population into higher levels of music education. Alternatively, certain childhood educational systems (for example, the Yamaha method in Japan) may foster the development of AP. We do not currently have information on our study population concerning childhood exposure of the Asian students to these methods. Finally, the possibility that certain Asian populations may have a higher prevalence of AP susceptibility genes should be considered.

Because these data are derived from a survey, the results must be treated as preliminary. In our experience, self report for AP is a very good indicator of AP ability; >80% of 173 subjects who reported AP have passed a rigorous test of their pitch-naming ability (E. Kowalsky and P. K. Gregersen, unpublished data). However, the reliability of reporting on AP ability in sibs or parents is uncertain and needs to be validated. It would also be valuable to obtain data on early-childhood music exposure and education from sibs of AP probands, to better control for the influence of environment on the development of AP. Familial aggregation of AP appears to be common, yet the measurement of background prevalence is not possible in the general population. Thus, more-extensive contact with the family members of a large number of AP subjects will be required, to provide further epidemiological evidence for genetic predisposition to AP, independent of environment. On the other hand, many subjects report the spontaneous appearance of AP in very early childhood. It is likely that inheritance plays a significant role in AP, perhaps in the setting of environmental exposure to music during a "critical" period (Goodman and Schatz 1993).

PETER K. GREGERSEN,[1] ELENA KOWALSKY,[1] NINA KOHN,[2] AND ELIZABETH WEST MARVIN[3]
Division of [1]Biology and Human Genetics and [2]Biostatistics, North Shore University Hospital, Manhasset, NY; and [3]Department of Music Theory, Eastman School of Music, Rochester, NY

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Address for correspondence and reprints: Dr. Peter K. Gregersen, Division of Biology and Human Genetics, North Shore University Hospital, 350 Community Drive, Manhasset, NY 11030. E-mail: peterg@nshs.edu

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Extremely Skewed X-Chromosome Inactivation Is Increased in Women with Recurrent Spontaneous Abortion

To the Editor:

Recurrent spontaneous abortion (RSA), defined as three or more consecutive losses at ⩽20 wk gestation (Stirrat 1990), affects 1%–2% of couples trying to have a family (Stray-Pedersen and Lorentzen-Styr 1979; Roman 1984). Although spontaneous abortion occurs quite frequently in humans, affecting ~15% of all clinically recognized pregnancies (Warburton and Fraser 1964; Edmonds et al. 1982; Wilcox et al. 1988), the observed rate of RSA is much higher than the expected rate of 0.3% due to chance alone. This suggests the presence of factors that may predispose particular couples to multiple pregnancy losses. Nearly 60% of RSA cases can be potentially explained by identifiable autoimmune, endocrine, anatomical, or infectious factors or by structural chromosome rearrangements in one partner (Stephenson 1996). However, >40% of RSA is still unexplained. We suggest that a significant proportion of the unexplained cases of RSA may be caused by a genetic mutation or chromosomal abnormality that would not be discovered by routine investigation.

X-chromosome inactivation (XCI) is the process whereby one of the two X chromosomes present in each cell of female mammals is inactivated during early embryogenesis, to achieve dosage compensation with males (Lyon 1961). Generally, in a given cell type in humans, the maternal X chromosome is inactivated approximately equally as often as the paternal X chromosome (Belmont 1996). However, extremely skewed XCI, defined in this letter as >90% inactivation of one allele, is observed in ~2% of newborns and ~4.5% of 28–32-year-old women (Busque et al. 1996). This extremely skewed XCI pattern may be due to a number of possible causes: (1) chance; (2) a mutation in the XIST gene that is found on the X chromosome and is thought to be critical in the inactivation process (Plenge et al. 1997); (3) selection against cells with a growth disadvantage because of a deletion or mutation on one of the X chromosomes (Pegoraro et al. 1997) or to an X-autosome translocation (Gaal and Laszlo 1977); and (4) a reduction in the fetal precursor-cell pool size, as has been suggested to occur in twinning (Bamforth et al. 1996; Goodship et al. 1996). Trisomy mosaicism has also recently been shown to be associated with extremely skewed XCI (Lau et al. 1997). Extremely skewed XCI (>90% inactivation of one allele) was found in the majority (11 of 18) of prenatally detected mosaic cases when the trisomic cell line was of meiotic origin and absent from most fetal tissues (Lau et al. 1997; W.P. Robinson and M.S. Peñahererra, unpublished data). Skewing is hypothesized to result from a reduction in the number of embryonic precursor cells, because of selection against the trisomic cells shortly after XCI.

At least three causes of skewed XCI are expected to be associated with an increased risk of spontaneous abortion: (1) some deletions or mutations on the X chromosome may be lethal to male fetuses carrying the abnormal X chromosome (Pegoraro et al. 1997); (2) X-autosome translocations can lead to RSA, because some gametes may be deleted and/or duplicated for portions of each chromosome that are involved in the rearrangement (Byrne and Ward 1994); and (3) trisomy mosaicism may also be associated with RSA if the germline is affected, since recurrent aneuploidy may result (Kohn and Shohat 1987; Gersdorf et al. 1990; Satge et al. 1996). Although it is impossible to determine how often the germline is mosaic in individuals with a normal phenotype and blood karyotype, one case was reported in which trisomy 16 was found in placenta and oocytes but in no other fetal tissue (Stavropoulos et al. 1998). To evaluate the degree to which mosaicism or other genetic factors associated with extremely skewed XCI may contribute to RSA, we screened women with RSA in order to determine their XCI status and compared them with controls of similar age.

Patients were ascertained through the Recurrent Pregnancy Loss Clinic at British Columbia's Women's Hospital and Health Centre. Between September of 1997 and December of 1998, all new patients with a history of RSA who were seen by the one of the authors (M.D.S.) were offered participation in this study. RSA was defined as three or more consecutive pregnancy losses prior to 20 wk gestation, with each pregnancy documented by a positive result on serum or urinary hCG, ultrasound, or pathology. Ethics approval was obtained from the

University of British Columbia Clinical Research Ethics Board, and the consent form was thoroughly reviewed with each patient. A single tube of peripheral blood was collected from consenting women, and DNA was extracted by use of a standard protocol. Every effort was made to collect these blood samples when samples for other standard blood work were being collected, according to the RSA protocol of Stephenson (1996). Controls were mothers who had donated blood previously, each having had at least one full-term pregnancy.

The mean number of consecutive spontaneous abortions per patient was 4.1, with a range of 3–11. Patients' ages were 19–45 years, with a mean of 33.6 years. For the control group, the age at which the blood was drawn was available for 86 of the 111 individuals informative at the androgen-receptor (AR) locus; the controls' ages were 20–49 years, with a mean age of 35.0 years. Results of karyotype analysis using standard Giemsa banding at 550-band resolution, in patients with RSA, their reproductive partners, and prior spontaneous abortions were taken from patients' charts; a total of 49 aborted pregnancies from patients with RSA who were informative for XCI status underwent karyotype analysis.

The degree of skewed XCI was estimated by an assay based on a methylation-sensitive *Hpa*II restriction site located near the human AR gene. This site is known to be methylated on the inactive X chromosome and to be unmethylated on the active X chromosome (Allen et al. 1992). When *Hpa*II was used to digest the genomic DNA prior to PCR, the AR allele was amplified only from the inactive X chromosome, since the sequence to be amplified on the active X chromosome was cleaved by the restriction enzyme. A trinucleotide CAG-repeat polymorphism located within the amplified region was used to distinguish between the two X chromosomes. For each patient, two PCR reactions were performed—one with genomic DNA digested with *Hpa*II and one without *Hpa*II. The second reaction served as an internal control to establish a baseline level of amplification of each allele, specific to the individual. This measure corrected for any preferential amplification of one allele over the other, in a given patient. Genomic DNA from males was used as a digestion control, since their X chromosome is always active (unmethylated) and therefore should have been completely digested by *Hpa*II and should have yielded no amplification product. Products were separated by PAGE and were visualized by silver staining. Quantification of the resulting bands was performed as detailed elsewhere (Lau et al. 1997). The analysis was repeated for any patient in whom skewing was >70%, in order to verify the result. Thus, the degree of skewing reported in these cases was an average of two or three independent tests; the mean difference between two estimates of skewing for the same patient was four percentage points.

XCI status was informative in 76 of the 98 patients with RSA and in 111 of the 137 female controls. This frequency may be less than that in other reports because some heterozygous cases were considered to be uninformative if the two bands were too close to resolve adequately for densitometric analysis. Extremely skewed XCI, defined as >90% amplification of one allele, was found in 14 (18%) of the 76 informative females with RSA and in just 6 (5%) of the 111 controls ($P < .001$; χ^2 test) (see table 1). The mean rate of skewing in the control group was similar to the 4.5% rate of skewing observed by Busque et al. (1996) in women 28–32 years of age. This rate was not significantly altered if controls for whom no age data were available were excluded.

These results show that factors associated with extremely skewed XCI account for a significant proportion (i.e., as much as 18%) of couples with RSA. Similar results have been reported, by Lanasa et al. (1999), for women who have experienced two or more spontaneous abortions. What is the explanation for extreme skewing in these patients with RSA? A pattern of extremely skewed XCI is commonly seen in females who carry balanced translocations involving one X chromosome and an autosome. The normal X chromosome is usually preferentially inactivated, presumably to maintain a balanced chromosomal complement in each cell (Gaal and Laszlo 1977). Theoretically, X-chromosome rearrangements could also lead to recurrent pregnancy loss. However, in a study that reported karyotype results in 1,142 couples with recurrent abortion there was not a single rearrangement involving the X chromosome (Portnoi et al. 1988). Thus, this is too rare a finding to account for the significant number of patients with RSA whom we observed to have extremely skewed XCI. Furthermore, in our study, all 14 of the patients with RSA who had extreme skewing had normal results on karyotype analysis—that is, 46,XX (see table 2).

Extremely skewed XCI may also result from a mutation on one of the two X chromosomes because of selective advantage of those cells that have the normal X chromosome active. In such cases, any male conceptus inheriting the abnormal X chromosome would most likely be aborted, because of the presence of only the defective copy of the locus in question. One such family has been reported in which 100% of the females exhib-

Table 1

Proportion of Females with RSA Who Showed Extremely Skewed XCI Compared with Control Females

	No. (%) Observed With	
Group	<90% Skewing	>90% Skewing
Females with RSA	62 (82)	14 (18)
Control females	105 (95)	6 (5)

Note.—For comparison by χ^2, $P < .001$.

Table 2

Karyotype Data on Female Patients with RSA and on Their Male Reproductive Partners

Gender and Karyotype	No.
Females:	
46,XX[a]	86
46,XX,t(2;12)(q13;q24.31)	1
46,XX,t(8;12)(q22;q22)	1
46,XX,inv(2)(p11.2q13)	1
46,XX/45,X[b]	1
NA	8
Total	98
Males:	
46,XY	81
NA	17
Total	98

[a] All 14 patients with RSA who had extremely skewed XCI also had a 46,XX karyotype.

[b] A 45,X karyotype was found in 4/100 lymphocytes, and further examination showed a 46,XX karyotype in 30/30 skin fibroblast cells.

iting extremely skewed XCI showed the presence of an X chromosome with an inherited deletion (Pegoraro et al. 1997). The women in this family had a spontaneous-abortion rate more than twice that in their female relatives who did not have the deletion or skewing. They also had a greater proportion of live-born females than of live-born males. There is currently no efficient method that can screen for mutations on the X chromosome that affect viability. However, it would be expected that, if this were the cause of RSA in our group of patients with extremely skewed XCI, then a high rate of 46,XY karyotypes should be seen among their spontaneous abortions. Of the karyotyped spontaneous-abortion specimens that were euploid, 1 of the 2 abortuses from patients with skewing was male, compared with 14 males among the 25 normal abortuses from patients with RSA and without skewing (table 3).

It should be noted that, although the frequency of pregnancy loss would be higher in carriers of an X-chromosome mutation or deletion than in the general population, in theory only 25% of the conceptuses (i.e., one-half of the males) would be at risk of being aborted because of the mutation; this is because all of the females and half of the males should be protected from the mutation, by the presence of the normal X chromosome. If it is assumed that the population rate of abortion due to independent causes is 15%, then the joint probability of pregnancy loss would be ~36%. Thus, only a small number (i.e., ~5%) of women with such an X-chromosome mutation might be expected to have three or more consecutive spontaneous abortions and no live births. Thus, an X-chromosome mutation might be relatively more common among women with either only a few losses or several losses combined with some live births than it is among women who experience a larger number of losses and no successful full-term pregnancies. It is therefore interesting to note that the mean number of spontaneous abortions (4.1) in the 14 patients who had skewed XCI was equal to that of the entire group with RSA.

Finally, a factor that may be associated with both extremely skewed XCI and RSA is aneuploidy mosaicism. Although aneuploidy is the leading cause of random spontaneous abortion, accounting for ~50% of karyotyped losses (Boue et al. 1975; Hassold and Jacobs 1984), it is difficult to evaluate whether recurrence of the same aneuploidy occurs more often than would be expected by chance, since karyotype information on each loss is lacking in most RSA cases. Nonetheless, many cases of gonadal mosaicism for a trisomy have been reported, many of which are ascertained by the multiple recurrence of Down syndrome (Kohn and Shohat 1987; Nielsen et al. 1988; Gersdorf et al. 1990; Sachs et al. 1990; Pangalos et al. 1992; Tseng et al. 1994; Satge et al. 1996), and mosaicism is found in the lymphocytes of one of the two parents in 4% of families with Down syndrome (Uchida and Freeman 1985). However, even if germline mosaicism were a cause of RSA, we would not necessarily expect the same trisomy to recur, since it has been shown that the presence of an unpaired chromosome in mouse oocytes can cause both disruption of meiosis and missegregation of other chromosomes (Hunt et al. 1995). There is also evidence from rare cases of fertile 45,X women that the same effect occurs in humans (Warburton 1989). In our study, seven of nine spontaneous-abortion specimens from the group of women with extremely skewed XCI were found to be aneuploid, whereas, among the women with nonskewed XCI, just 15 (27%) of 40 spontaneous-abortion specimens were aneuploid ($P = .03$; see table 3). Although both skewed XCI and aneuploidy increase with maternal age (Hassold and Jacobs 1984; Busque et al. 1996), the mean age of the patients with extremely skewed XCI

Table 3

Karyotypes of Spontaneous Abortions among Female Patients with RSA Who Have Extremely Skewed XCI, versus Those Who Do Not Have Such Skewing

Type of Abortus Karyotyped	No. of Abortuses From Patients Who Have	
	<90% Skewing	>90% Skewing
46,XY	14	1
46,XX	11	1
Aneuploid	15[a]	7[a]
Total	40	9

[a] $P = .03$ (by Fisher's exact test comparing proportion of abnormal karyotypes in the two groups).

was lower (32.4 years) than that of the group without skewing (33.9 years). Thus, age cannot explain the higher rate of aneuploidy in the spontaneous-abortion specimens from patients with extremely skewed XCI.

It is estimated that 1%–2% of first-trimester pregnancies assessed by chorionic-villus sampling are mosaic (Vejerslev and Mikkelsen 1989). Although the abnormal cell line is often assumed to be confined to the placenta, this is difficult to prove. Even when mosaicism is found in amniotic fluid, the aneuploidy is frequently absent from fetal/newborn blood (Hsu et al. 1997), and trisomy mosaicism for most chromosomes is unlikely to be detected by routine blood karyotyping. Although analysis of skin fibroblasts may detect a greater proportion of mosaic cases, it is still possible for trisomic cells to be found in oocytes even when no other fetal tissues are affected (Stavropoulos et al. 1998). Alternatively, we can look at indirect indicators, such as skewed XCI, to provide clues as to whether an individual may be the product of a pregnancy associated with mosaicism.

Pregnancy loss is a devastating issue for many couples, and identification of an etiology is very important for counseling couples with RSA as to their treatment options. Clearly, genetic factors associated with extremely skewed XCI are important in at least some patients with RSA and, most likely, involve either an X-linked mutation or germline mosaicism. Further review of the medical histories and pedigrees may provide clues as to which etiology is involved in a particular case. A larger epidemiological study documenting the outcome of all pregnancies and including karyotype data from the spontaneous abortions is also necessary to clarify the mechanism involved and, subsequently, to improve counseling of patients with RSA, in regard to future pregnancy outcomes.

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KARAN K. SANGHA,[1,3] MARY D. STEPHENSON,[2,4] CAROLYN J. BROWN,[1] AND WENDY P. ROBINSON[1,3]
Departments of [1]Medical Genetics and [2]Obstetrics and Gynecology, University of British Columbia; [3]BC Research Institute for Children's & Women's Health; and [4]Children's & Women's Health Centre of British Columbia, Vancouver

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Address for correspondence and reprints: Dr. Wendy P. Robinson, BC Research Institute for Children's and Women's Health, 3086 – 950 West 28th Avenue, Vancouver, B.C., Canada V5Z 4H4. E-mail: wendyr@interchange.ubc.ca

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Demonstration of the Recurrence of Marfan-like Skeletal and Cardiovascular Manifestations Due to Germline Mosaicism for an *FBN1* Mutation

To the Editor:

Marfan syndrome (MFS [MIM 154700]) is a dominantly inherited disease of connective tissue. Cardinal manifestations involve the eye (lens dislocation and myopia), skeleton (dolichostenomelia, arachnodactyly, anterior chest deformity, spinal curvature, and joint laxity) and cardiovascular system (aortic root dilation and dissection, mitral valve prolapse, and mitral and aortic valve regurgitation). Striae distensae and inguinal hernia are frequent findings in the integument, and pneumothorax and dural ectasia occur in some patients (Pyeritz and McKusick 1979; First International Symposium on the Marfan Syndrome 1989). If untreated, the syndrome shortens life expectancy mainly because of cardiovascular complications. The disorder is characterized by considerable variation in the distribution and severity of organ system involvement between families, leading to the definition of diagnostic criteria listed first in the Berlin nosology (Beighton et al. 1988) and subsequently revised in the Ghent nosology (de Paepe et al. 1996). In 1986, Sakai and colleagues identified a 350-kD glycoprotein called "fibrillin," which represents the major structural component of connective tissue microfibrils. By using an anti-fibrillin antibody, Godfrey, Hollister, and their colleagues demonstrated a reduction of microfibrils in immunofluorescence studies of cultured dermal fibroblasts in patients with MFS (Godfrey et al. 1990; Hollister et al. 1990). Subsequent studies of fibrillin synthesis, secretion, and incorporation into the extracellular matrix showed abnormalities in most but not all MFS fibroblast strains (Milewicz et al 1992; Collod et al. 1994). Finally, mutations in the *FBN1* gene, encoding fibrillin, have been demonstrated to result in MFS or associated phenotypes (Dietz et al. 1991; Hayward et al. 1994; Kainulainen et al. 1994; Lonnqvist et al. 1994; Sood et al. 1996). The *FBN1* gene is ~200 kb in size, with a coding sequence fragmented into 65 exons (Corson et al. 1993; Pereira et al. 1993; Biery et al. 1999) located on chromosome 15q21.1 (Magenis et al. 1991).

It encodes a large glycoprotein composed of repeated modules, 47 of which are homologous to human epidermal growth factor (EGF) ("EGF-like modules") and are interspersed by seven modules displaying high homology to transforming growth factor β1–binding protein (TGFβ1-bp), ("8-cysteine modules") (Corson et al. 1993; Pereira et al. 1993). To date, >160 *FBN1* mutations in patients with MFS or associated phenotypes have been reported or submitted in the Marfan Database (Collod et al. 1996; Collod-Béroud et al. 1997, 1998). However, hardly any predictions of the resulting phenotype can yet be made on the basis of the nature of a specific mutation.

The prevalence of MFS has been estimated at 1/5,000, and $\geqslant$25% of patients represent sporadic cases. This high mutation rate should be associated with cases of germline mosaicism, as has been reported in other connective-tissue disorders or other genetic disorders with a high mutation rate. Therefore, it was surprising that, until recently (Montgomery et al. 1998; Rantamaki et al. 1999), no instance of somatic or germline mosaicism had been reported in MFS. Furthermore, since *FBN1* mutations are also associated with phenotypes overlapping MFS, mosaicism could also be identified in these subtypes. In this report, we demonstrate somatic mosaicism of a *FBN1* genomic mutation in the father of two siblings who presented with the typical skeletal and cardiovascular features observed in the Marfan syndrome.

The proband, MS48MA307, was identified at the Centre Hospitalier in Amiens (by M.M. and Y.M.), and his family was investigated at the Marfan Clinic at Ambroise Paré Hospital, Boulogne (by G.J.). The diagnostic criteria used were those reported by Beighton et al. (1988). The parents of MS48MA307 and his brother, MS48MA308, were unaffected. Patient MS48MA307, a 16-year-old boy, presented dilation of the ascending aorta (46 mm at the sinuses of Valsalva, 8 SD above the mean when standardized to age and body surface area), mitral valve prolapse with regurgitation, highly arched palate, arachnodactyly (positive wrist and thumb signs), tall stature (199 cm, +4 SD, 76 kg), and scoliosis. His 9-year-old brother (MS48MA308) displayed dilation of the ascending aorta (32 mm at the sinuses of Valsalva, 6 SD above the mean when standardized to age and body surface area), arachnodactyly (positive wrist and thumb signs), dolichostenomelia (arm span–to-height ratio 1.05), tall stature (144 cm, +3 SD, 31 kg), highly arched palate, and joint hypermobility. No other typical anomaly of MFS (including ectopia lentis) was found in either subject. Both parents were examined thoroughly, and the diagnosis of MFS was excluded for both. Blood samples were collected from the four family members and from 150 unrelated French subjects. DNA was extracted from white blood cells (Henry et al. 1984). Informed consent was obtained for all individuals. Sense and antisense primers designed from flanking intron sequences were used for PCR amplification of exons 1–65 of *FBN1* and were described, along with PCR amplification conditions, in Nijbroek et al. (1995). SSCP analysis of the *FBN1* gene from white blood cells revealed an abnormal pattern for the 419-bp fragment of exon 24 for patient MS48MA307. This abnormal pattern was also identified in his brother, MS48MA308, but was absent in the mother. However, the father presented a very slight abnormal pattern (fig. 1*a*). Paternity and maternity had been tested previously and indirectly by analysis of highly polymorphic markers on chromosomes 3, 5, and 15 (data not shown). The abnormal fragment from the SSCP gel was cut out of the gel, and DNA was eluted in water and reamplified by PCR. The PCR product was purified with the Promega Wizard Prep kit and was directly sequenced on both strands by means of a cycle-sequencing kit (Pharmacia). Sequencing revealed that the two boys carried the identical heterozygous 2954 G→A transition that results in a Gly→Glu change at codon 985 (G985E) (fig. 1*d*). There is a compelling body of evidence to suggest that G985E is indeed a disease-producing mutation. First, this alteration was not observed during screening of 306 chromosomes. Second, the mutation substitutes an uncharged for a negatively charged amino acid of much higher molecular weight. Finally, the mutational event occurs in the 8-cysteine module 3 at a position conserved in the bovine, murine, and porcine sequences.

Since MFS is characterized by a high mutation rate, the recurrence of the disease in the sibs could have been due to two unrelated de novo mutations. However, since mutations in the *FBN1* gene are essentially private, the presence of an identical mutation in the brothers suggested that the most likely hypothesis was that the mutation had been inherited from one of the parents. Since the mutation creates a new *Taq*I restriction site resulting in two fragments of 202 and 217 bp, it could easily be looked for in the family (fig. 1*b*). After transfer on Hybond N+ membrane (Amersham) and hybridization with the sense primer, the 217-bp fragment resulting from digestion was found in the father's white blood cell DNA (MS48MA305), at a very low level, but was not found in that from the mother (MS48MA306) or in three controls (fig. 1*c*). The finding of the alteration in the father's white blood cells and the recurrence of the disease in his children implied somatic and germline mosaicism in the father. Careful reassessment of clinical examination of the father (performed systematically before the identification of the mosaicism) revealed no skeletal or ocular sign but minor findings: discreet dilation of the ascending aorta (43 mm, +2 SD when standardized to age and body surface area [193 cm, 75 kg, at age 41 years]) and minimal aortic regurgitation. This

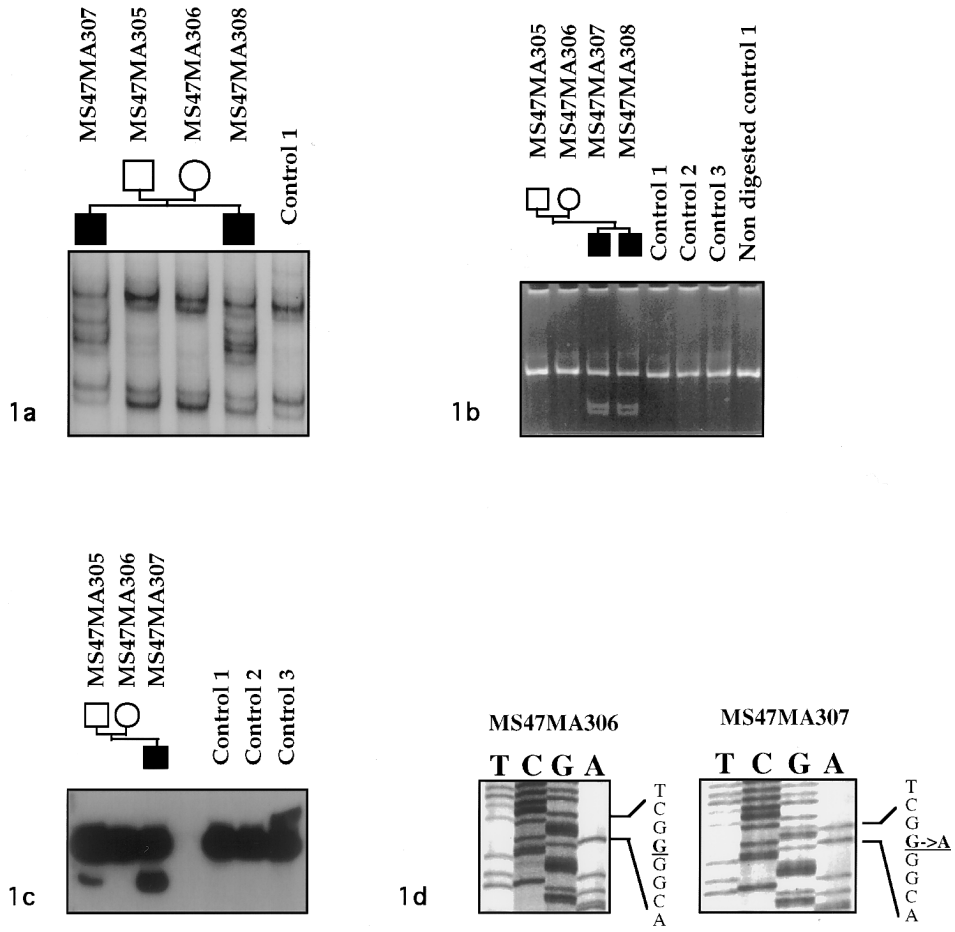

Figure 1 *a,* DNA single-strand analysis by nondenaturing PAGE (SSCP) of a 419-bp PCR product including exon 24. Aberrant migration of PCR product is found in subjects MS47MA307 and MS47MA308 compared with their parents and the normal control. *b,* The G→A transition creates a *Taq*I site within the 419-bp PCR product of exon 24, resulting in two fragments of 202 and 217 bp. *Taq*I digestion confirmed the G985E mutation in the two brothers MS47MA307 and MS47MA308. *c,* The digestion products were migrated and then were hybridized with the sense primer. Only the PCR product resistant to digestion can be found for normal controls 1, 2, and 3 and for the mother (MS47MA306). The heterozygous three-banded restriction enzyme pattern after *Taq*I digestion is present for MS47MA307 and the father MS47MA305, after overexposure. *d,* The normal (for MS47MA306) and abnormal (for MS47MA307) fragments from the SSCP gel (in *a,* above) were cut, eluted in water, and reamplified by PCR. Sequencing for MS47MA307 compared with normal sequence (MS47MA306) revealed a G→A transition at nucleotide position 2954, resulting in a Gly→Glu change at codon 985 (G985E).

mutation probably arose at an early mitotic stage in embryonic development, as reflected by distribution in somatic and germ cell tissues.

The MFS-like phenotype associated with the G985E mutation in exon 24 is not associated with ocular anomalies. Of interest, the Marfan Database (Collod-Béroud et al. 1998), when sorted for mutations in MFS patients who have no ocular anomaly, indicates that half (9/19) of these mutations are located in exons 23–29. This contrasts with mutations associated with the complete classic MFS, which are widely distributed throughout the gene. Furthermore, study of the distribution of mutations identified in 8-cysteine modules after their alignment by their consensus sequence indicates that the G985E mutation affects a residue close to three consecutive cysteines. This region harbors three other mutations (5137ins4 [Dietz et al. 1993], C1721Y [Collod-Béroud et al. 1998], and V984I [Collod-Béroud et al. 1998]) identified in probands that do not have ectopia lentis. The 8-cysteine modules are found only in fibrillins and latent TGFβ1-bp, and their function in fibrillins is still unclear. The absence of ectopia lentis and, therefore, the probable absence of major zonular alteration in subjects carrying mutations in this region of the 8-cysteine modules would tend to indicate the absence of a specific function of this module in the zonule.

Our observation shows that somatic and germline mosaicism are associated with MFS-like features and should be looked for in parents of sporadic cases presenting with these MFS-like features. In effect, if mild or isolated

features of the disease are found in one of the parents, genetic counseling should take into account the possible presence of the disease in another child. Somatic mosaicism could also explain the mild and incomplete features often seen in patients referred to MFS clinics for diagnosis. Again, caution is warranted in the follow-up of these patients and in evaluation of the risk of transmission.

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GWENAËLLE COLLOD-BÉROUD,[1] MARILYN LACKMY-PORT-LYS,[1] GUILLAUME JONDEAU,[2] MICHÈLE MATHIEU,[4] YVES MAINGOURD,[5] MONIQUE COULON,[1] MICHEL GUILLOTEL,[1] CLAUDINE JUNIEN,[1,3] AND CATHERINE BOILEAU[1,3]

[1]*INSERM U383, Hôpital Necker-Enfants Malades, Université René Descartes, Paris;* [2]*Service de Cardiologie et* [3]*Laboratoire Central de Biochimie, d'Hormonologie et de Génétique Moléculaire, Hôpital Ambroise Paré, Boulogne; and* [4]*Centre de Génétique Clinique and* [5]*Unité de Cardiologie Pédiatrique, CHU d'Amiens, Hôpital Nord, Amiens, France*

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Marfan Database, http://www.umd.necker.fr/ (for *FBN1* mutations)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for MFS [MIM 154700)

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Address for correspondence and reprints: Dr. C. Boileau, INSERM U383, Hôpital Necker-Enfants Malades, Clinique Maurice Lamy, 149-161, rue de Svres, 75743 Paris Cedex 15, France. E-mail: boileau@ceylan.necker.fr

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The Jewish Ashkenazi Founder Mutations in the BRCA1/BRCA2 Genes Are Not Found at an Increased Frequency in Ashkenazi Patients with Prostate Cancer

To the Editor:

BRCA1 and BRCA2, the predisposing genes for breast cancer (BC) and ovarian cancer (OC), have been suggested to increase the risk of prostate cancer (PrC) in male carriers (Ford et al. 1994; Thorlacius et al. 1996; Struewing et al. 1997); however, no direct evidence exists to confirm this hypothesis. A population with a high carrier frequency of BRCA1 and BRCA2 germinal mutations allows a direct approach to studying the role BRCA1 and BRCA2 play in the development of PrC; if germinal mutations in BRCA1 and BRCA2 increase the risk of PrC in carriers, it is to be expected that the carrier frequency in PrC patients will be higher than in the general population, as was demonstrated in female patients diagnosed with BC and OC (Ford et al. 1995; Claus et al. 1996, Abeliovich et al. 1997).

In the Ashkenazi Jewish population, three founder mutations, 185delAG and 5382insC in the BRCA1 gene and 6174delT in the BRCA2 gene, exist at a high frequency (2.5%) (Struewing et al. 1995; Oddoux et al. 1996; Roa et al. 1996; Fodor et al. 1998). To assess the contribution of the BRCA1/BRCA2 germinal mutations to PrC morbidity, we analyzed the Ashkenazi founder mutations in two groups (with the same age distribution) of Ashkenazi men, a group of unselected PrC patients, and a control group of men with no history of cancer. The study was designed around the fact that, in families known to segregate BRCA1/BRCA2 mutations, men with PrC were noted sporadically. It was thus assumed that, if BRCA1 and BRCA2 play a role in the development of PrC, they do so as risk modifiers rather than as major dominant genes, and therefore will not be confined to familial cases.

Patients diagnosed with adenocarcinoma of the prostate (n = 87) were recruited from the oncology outpatient clinic at Sharett Institute, Hadassah Hebrew University Hospital, with no preselection. The patients signed an informed-consent form approved by the hospital's ethics committee. Each patient was interviewed regarding his family history. Clinical and pathological records were the sources of the clinical data.

The control group included 87 healthy men with no history of cancer. These men were approached in Jerusalem-area homes for the elderly and were asked to participate in the study; if they agreed, they signed an informed-consent form. Their blood samples were kept anonymous, labeled only with the patients' ages and origins (table 1). The median age was 71 years at the time of diagnosis for the patients with PrC and 72 years at the time of blood sampling for the control group (table 2). The mutations were analyzed as described elsewhere (Abeliovich et al. 1997).

The risk of developing PrC is age-dependent and is determined by differential exposure to environmental factors. In addition, positive family history is a major risk factor for developing PrC at an early age (Steinberg et al. 1990; Spitz et al. 1991; Whittemore et al. 1995). It is assumed that ~10% of all cases of PrC and half of the cases diagnosed at an early age (≤60 years) are dominantly inherited. Linkage analyses in families with multiple cases of PrC pointed to a PrC-susceptibility gene (or group of genes) on chromosome 1 (Smith et al. 1996; Grönberg et al. 1997*a*; Berthon et al. 1998; Schaid et al. 1998), and, recently, an X-linked gene was suggested (Xu et al. 1998). It can be argued that BRCA1 and BRCA2 markedly reduce the age at onset of PrC and that therefore the effect of BRCA1/BRCA2 will be shown only in patients diagnosed with PrC at age <60 years, whereas in our study only five patients were ascertained in this age group. However, since 2.5% of Ashkenazi males are BRCA1/BRCA2 carriers, it would be expected that an excess of Ashkenazi men will develop PrC at age <60 years. The stratification of the ages

Table 1

Study Group of Ashkenazi Patients with PrC and Control Group: Age at Diagnosis, Cancer History, and the Ashkenazi BRCA1/BRCA2 Founder Mutations

Group	No. of Subjects Diagnosed or Tested at Age (in Years)						Group Total
	<50	50–59	60–69	70–79	>80	Unknown	
All subjects[a]	1	9	72	142	29	…	253
PrC study group	1	4	36	38	3	5	87
Carriers	…	1[b]	1[c]	1[d]	…	…	3
Carriers with second primary tumor[e]	…	1	1	…	…	…	2
Carriers with cancer in family	…	…	1	…	…	…	1
Noncarriers with second primary tumor[f]	…	…	4	4	…	1	9
Noncarriers with cancer in family[g]	…	2	10	11	1	2	26
Control group	…	3	27	31	26	3	87
Carriers	…	2[h]	…	1[i]	…	…	…

[a] Total number of Ashkenazi patients treated in Sharett Oncology Institute from January 1991 to July 1997.
[b] Patient A, carrying mutation 185delAG.
[c] Patient B, carrying mutation 6174delT.
[d] Patient C, carrying mutation 185delAG.
[e] Patient A had chronic lymphocytic leukemia; patient B had BC at age 59 years.
[f] Second primary tumors included melanoma ($n = 3$) and tumors of the bladder ($n = 2$), lung ($n = 1$), rectum ($n = 2$), and kidney and colon in the same patient.
[g] Six patients had first-degree relatives with PrC; eight patients had first-degree relatives with BC, including one male relative.
[h] Carriers of mutation 185delAG.
[i] Carrier of mutation 6174delT.

at diagnosis in the study group was similar to that of PrC patients in Israel, and only rarely are patients diagnosed before age 50 years (Israel Cancer Registry, 1994). The data of Struewing et al. (1997) also support the view that there is no excess of Ashkenazi patients with PrC diagnosed at age <50 years. They estimated the risk of cancer among relatives of Ashkenazi carriers of BRCA1 and BRCA2, which for PrC was 16% (95% confidence interval [CI] 4%–30%) by age 70 years; by age 80 years the risk increased to 39%. Interestingly, in the same study (Struewing et al. 1997), the risk of OC was 16% by age 70 years (95% CI 6%–28%), similar to that of PrC. It should be emphasized that, although BRCA1/BRCA2 are major dominant genes in BC and in OC and although carriers tend to develop those cancers at a young age, 18% of the female patients diagnosed at age ⩾50 years with BC or OC were carriers of BRCA1 or BRCA2—8% of the BC patients and 66% of the OC patients (Abeliovich et al. 1997).

Three patients in the study group were identified as mutation carriers: patients A and C with 185delAG (BRCA1) and patient B with 6174delT (BRCA2). In the control group, three individuals were identified as carriers, two with 185delAG and one with 6174delT (table 1).

Two of the three carrier patients had second primary tumors: CLL (chronic lymphocytic leukemia) in patient A, and breast cancer in patient B. Among the noncarrier patients, 9 (11%) of 84 had a second primary tumor (including one patient with two second primary tumors).

A history of cancer in first-degree relatives was noted in patient B: his sister had BC, his father had PrC, and his son had testicular cancer. The two carrier patients, A and C, did not have positive family histories (the close relatives of patient A died in the Holocaust). Cancer history in first-degree relatives was noted in 26 (31%) of the 84 patients (table 1); in 5 of these patients the cancer was PrC, and the mothers of 4 had BC (both the mother and the daughter of one of these 4 had BC). The father of 1 patient had BC; the sisters of 2 others had BC; and the mother of 1 other had OC.

The clinicopathological data of the carrier and noncarrier patients is given in table 2. The carrier patients

Table 2

Clinicopathological Characteristics of Patients

Stage (No. of Patients)	PSA[a] at Diagnosis (No. of Patients)	Gleason Score (No. of Patients)
Noncarrier patients		
A (5)	5.9 (3)	5.4 (5)
B (35)	13.6 (28)	5.9 (31)
C (27)	32.8 (21)	6.2 (24)
D (12)	37.4 (10)	7.6 (5)
Carrier patients		
B (patient A[b])	60	>8
B (patient B[c])	47	8
B (patient C[b])	60	7

[a] In mg/ml.
[b] Carrier of mutation 185delAG.
[c] Carrier of mutation 6174delT.

were diagnosed at ages 57, 62, and 73 years (average 64 years). The average level of prostate serum antigen (PSA) in the carrier patients was 55.8 mg/ml, higher than the average (23.6) in noncarrier patients at all stages; the difference in the PSA level was highly significant ($\chi^2 > 30$). The three carrier patients were diagnosed at stage B with Gleason scores of 7, 8, and >8, higher than the average (5.9) for the noncarrier patients at stage B and similar to the average at stage D. The clinicopathological records of the patients indicated that the tumors in the three carriers were highly proliferative. This may suggest that mutations in BRCA1 and BRCA2 may have some role in the progression of the disease. A similar observation was made of PrC in patients who belong to HPC1-linked families (Grönberg et al. 1997*b*) and in BRCA1-associated breast cancers (Eisinger et al. 1996; Marcus et al. 1996; Blackwood and Weber 1998; Robson et al. 1998). However, this conclusion is based on three patients and should be confirmed in a larger number of patients.

The frequency of carriers in the study group of PrC patients and in the group of healthy men was 3.4% (95% CI 1.48%–5.4%), which is within the range of the population frequency (2.5%) (Fodor et al. 1998). In order to detect a minor difference between the two groups, a much larger sample was needed. Instead, we chose a different approach in which we calculated the expected percentage of carriers of BRCA1/BRCA2 founder mutations among the PrC patients, on the basis of the existent risk figures: 16% by age 70 years and 39% by age 80 years (Struewing et al. 1997). Assuming that we follow Ashkenazi men from age 50 years through age 80 years, we further assumed that the rate of carriers is 2.5% and that among the carriers the average risk of developing PrC prior to age 80 years is ~20%. We would then expect that every year 33 of 100,000 new Ashkenazi patients with PrC would be carriers of any of the BRCA1/BRCA2 founder mutations. Israeli data show that the number of new cases among Ashkenazi men at this age (50–80 years), is ~260 in 100,000 (Israel Cancer Registry, 1994); hence the carriers would be ~13% of the patients (33/260). We had 87 patients, and therefore expected 11 carriers in our study group, but observed 3. The difference between the expected and observed result is highly significant ($P < .0005$ in the exact-binomial test). The size of the sample enables a power of $\geq$80% for detecting a difference in carriers of 2.5% in the control group and at least 12.5% in the patients group. It is interesting to note that the strong association found among Israeli females between ethnic origin and breast cancer is not evident for prostate cancer. The age-standardized rate of breast cancer among Jewish women born in Europe or America (i.e., having an Ashkenazi origin) is 1.57 times that of Jewish women born in North Africa (non-Ashkenazi origin), whereas the respective rate for men having prostate cancer is 0.9 (Israel Cancer Registry, 1994). The age-adjusted rate of PrC (per 100,000) in Israeli Jewish men by place of birth is 32.2 for those born in Europe and North America (Ashkenazi Jews), 32.5 for men born in Africa and Asia, and 43.5 for men born in Israel (Israel Cancer Registry, 1994). We therefore suggest that the contribution of BRCA1/BRCA2 germinal mutations to PrC morbidity is negligible. Our conclusion is in agreement with other studies in which PrC patients were tested directly (Langston et al. 1996; Johannesdottir et al. 1996; Wilkens et al. 1999) and with some of the epidemiological studies (Isaacs et al. 1995; McCahy et al. 1996). However, our conclusion contradicts other epidemiological studies (Arason et al. 1993; Ford et al. 1994; Thorlacius et al. 1996; Struewing et al. 1997), in which the data were based on information received about first-degree relatives of carriers, while the PrC patients themselves were not analyzed. It would be interesting to explore the possibility of other sources of variation, such as environmental factors that affect BRCA1/BRCA2 carriers to a greater extent than noncarriers and to which men in Israel are not exposed.

Ayala Hubert,[1] Tamar Peretz,[1] Orly Manor,[2] Luna Kaduri,[1] Naomi Wienberg,[3] Israela Lerer,[3] Michal Sagi,[3] and Dvorah Abeliovich[3]
[1]*Sharett Institute of Oncology,* [2]*School of Public Health and Community Medicine, and* [3]*Department of Human Genetics, Hadassah Hebrew University Hospital, Hadassah Hebrew University Medical School, Jerusalem*

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Address for correspondence and reprints: Dr. Dvorah Abeliovich, Department of Human Genetics, Hadassah Hospital, P.O. Box 12 000, Ein Kerem, Jerusalem 91120, Israel. E-mail: dvoraha@cc.huji.ac.il

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An HFE Intronic Variant Promotes Misdiagnosis of Hereditary Hemochromatosis

To the Editor:

Hereditary hemochromatosis (HH; MIM 235200), an autosomal recessive disorder of iron metabolism, can result in numerous clinical complications and is estimated to affect ~1/300 individuals of northern European origin (Merryweather-Clarke et al. 1997). Two mutations—C282Y and H63D—that contribute to HH have been identified (Feder et al. 1996), and screening for the C282Y mutation, in particular, is routinely done to identify carriers and affected individuals. Biochemical markers indicate a relatively clear distinction between these two groups, with minimal clinical consequences for heterozygotes (Bulaj et al. 1996). We initiated screening for the C282Y mutation, using the primer sequences provided by Feder et al. (1996) and subsequent restriction digestion of PCR products (Jazwinska et al. 1996). Re-

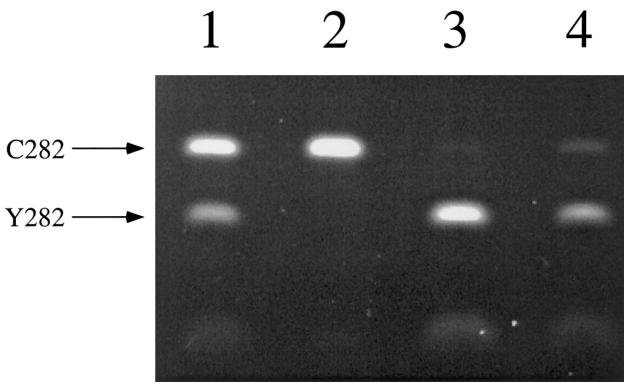

Figure 1 PCR amplification and *Sna*BI digestion of DNA (Jazwinska et al. 1996) from individuals referred for HH testing. Lane 1, C282Y carrier. Lane 2, Normal homozygote. Lane 3, C282Y homozygote. Lane 4, Individual with an anomalous pattern with trace amounts of undigested PCR product. All 25-μl PCR reactions were performed in parallel, with use of 150 ng DNA template and 1.25 U PLATINUM®*Taq* (GIBCO-BRL), in 20 mM Tris-HCl (pH 8.4), 50 mM KCl, and 1.5 mM $MgCl_2$. PCR conditions were as follows: 94°C for 2 min; then 30 cycles at 94°C for 30 s, 65°C for 30 s, and 72°C for 30 s; and a final extension at 72°C for 10 min, by means of a GeneAmp PCR System 9600 (Perkin-Elmer). PCR products were digested with *Sna*BI at 37°C for 3 h and were resolved by use of 1.5% agarose gel.

cently, we have identified anomalous results in some individuals while screening for the presence of the C282Y mutation. We initially identified eight individuals, seven of whom were unrelated, who appeared to be C282Y homozygotes with trace amounts of undigested DNA (fig. 1). It was assumed that these individuals were homozygotes with some form of sample contamination. Clinical histories of these individuals did not include previous blood transfusion or tissue transplantation. Increased amounts of restriction enzyme and incubation time, as well as resampling of these individuals, did not resolve the anomalous results. An increase in the stringency of the PCR conditions, achieved either by increasing the annealing temperature or by decreasing the amount of genomic template, reduced the amount of amplified normal product to generate a C282Y homozygote pattern (results not shown). Biochemical data on serum iron levels, serum ferritin levels, and transferrin saturation were available for two of these individuals—a 35-year-old man and a 71-year-old woman—and values were below the affected range. In one particular family, two sibs showed this pattern, yet, when their parents were tested, only the mother was found to be a C282Y carrier. In this instance, a combination of two independent incidents of nonpaternity and sample contamination would be required to explain the results.

We reanalyzed these cases, using two different approaches: (1) the Baty et al. (1998) amplification refractory mutation system (ARMS), which includes an alternative reverse primer with the Feder et al. (1996) forward primer, and (2) a modification of the previous protocol (Jazwinska et al. 1996) to incorporate two alternative primers that flank the Feder et al. primer sites (fig. 2). With both techniques, we found that all eight samples gave a clear carrier pattern for C282Y (results not shown). In all cases, sequencing across the Feder et al. primer sites revealed a G→A substitution at nucleotide position 5569 (GenBank accession number Z92910) on the non-C282Y allele. This sequence variant is located in intron 4, 5 bases from the 3′ terminus of the reverse-primer site identified by Feder et al., and is not predicted to disrupt normal hemochromatosis gene (HFE) splicing (fig. 2). In all eight samples, this mutation was seen on a non-C282Y, non-H63D chromosome. PCR using this reverse primer can result in dramatically reduced amplification of the polymorphic allele, such that a C282Y carrier can appear to be a C282Y homozygote. The relative intensity of the non-C282Y PCR product is inversely proportional to the stringency of the PCR conditions. Sequencing of both alleles in the eight individuals in the present study allowed clear assignment of carrier, rather than affected, status.

The 5569 G→A substitution introduces an *Mse*I restriction site. We used this enzyme, in conjunction with the Feder et al. (1996) forward primer and the HCS:

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ccttcctccaacctatagaaggaagtgaaagttccagtcttcctggcaagggt
aaacagatccctctcctcatccttcctctttcctgtcaagTGCCTCCTTTGG
TGAAGGTGACACATCATGTGACCTCTTCAGTGACCACTCTACGGTGTCGGGCC
TTGAACTACTACCCCCAGAACATCACCATGAAGTGGCTGAAGGATAAGCAGCC
AATGGATGCCAAGGAGTTCGAACCTAAAGACGTATTGCCCAATGGGGATGGGA
CCTACCAGGGCTGGATAACCTTGGCTGTACCCCCTGGGGAAGAGCAGAGATAT
ACGTACCAGGTGGAGCACCCAGGCCTGGATCAGCCCCTCATTGTGATCTGGGg
tatgtgactgatgagagccaggagctgagaaaatctattgggggttaagagga
gtgcctgaggaggtaattatggcagtgagatgaggatctgctctttgttaggg
gatgggctg
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Figure 2 DNA sequence of HFE exon 4, with flanking introns. The relative locations of the C282Y mutation (G→A) in exon 4 and the 5569 G→A (892+48 G→A) polymorphism in intron 4 are shown. Sequences and locations are highlighted for Feder et al. (1996) forward and reverse primers and for the two alternative flanking primers (HCS: F and HCS:R) that were used to amplify this region. Conditions for PCR with HCS:F and HCS:R followed those outlined for the Feder et al. primer set (fig. 1), with an annealing temperature of 55°C. The 486-bp HCS PCR product was cleaved into 320- and 166-bp fragments by *Sna*BI in the presence of the C282Y mutation. The intronic polymorphism was confirmed, by sequencing and by *Mse*I digestion of HCS PCR products, in all samples that showed an anomalous *Sna*BI digestion pattern by use of Feder et al. (1996) PCR products.

R primer, to screen 48 individuals (45 of whom were unrelated) whom we had previously identified as C282Y homozygotes. In this group, we identified one additional unrelated individual with the C282Y mutation and the 5569 G→A polymorphism. Closer examination of the assay on the basis of which the previous diagnosis was made in this individual revealed an extremely faint normal band that had been interpreted to result from partial digestion. In total, therefore, the polymorphism has been found in 8 of 202 unrelated individuals who were referred for HH testing. An estimate of the allele frequency can be made on the basis of the C282Y carrier frequency. We found these 8 polymorphism carriers among a total of 43 unrelated C282Y (non-H63D) carriers. Our estimated population frequency of this allele is, therefore, 8/43 (=.186). Consequently, in our population, this polymorphism had the potential to result in ~19% of C282Y heterozygotes being misidentified as homozygotes.

We identified the 8 polymorphism carriers, in addition to 44 unrelated C282Y homozygotes, from our total sample of 202 unrelated individuals referred for testing. If the assumption of homozygosity, along with access to parental genotypes, had been made in all individuals with the polymorphism, as well as in those with the homozygosity, this would have led to an estimate of ~8/52 (=.154) for nonparentage, of which half of these cases, or 8%, would have been assumed to result from nonpaternity. The frequency of this polymorphism is high enough to warrant concern that the interpretation of homozygosity in these cases will result in an overestimate of the C282Y-allele frequency, a misdiagnosis of this condition, and an incorrect assumption of nonpaternity in some families. In our hands, the polymorphism promoted misinterpretation of a restriction-digestion–based assay, but any form of analysis (including allele-specific oligonucleotide hybridization, ARMS, or direct sequencing) that incorporates the Feder et al. (1996) reverse primer is equally prone to misdiagnosis. It is recommended that all laboratories using the Feder et al. reverse primer to test for the C282Y mutation confirm C282Y-homozygote results by using a flanking primer set and *Mse*I digestion.

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MARTIN J. SOMERVILLE, KATHLEEN A. SPRYSAK, MARK HICKS, BASIL G. ELYAS, AND LEANNE VICEN-WYHONY
Department of Medical Genetics
University of Alberta
Edmonton

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Address for correspondence and reprints: Dr. Martin J. Somerville, 8-26 Medical Sciences Building, Department of Medical Genetics, University of Alberta, Edmonton, Alberta, Canada T6G 2H7. E-mail: martin.somerville@ualberta.ca

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No Mutations in the Coding Region of the *PRKCG* Gene in Three Families with Retinitis Pigmentosa Linked to the *RP11* Locus on Chromosome 19q

To the Editor:

Retinitis pigmentosa (RP) and allied degenerations of the retina are genetically heterogeneous, with well over 50 loci implicated so far through gene identifications or linkage-based chromosomal assignments. Among these genes, the dominantly inherited *RP11* locus (MIM 600138) on chromosome 19q is noteworthy because some carriers develop RP that is symptomatic at age <20 years, whereas others are asymptomatic and show no funduscopic or electroretinographic signs of disease even at age >70 years (Berson et al. 1969; Berson and Simonoff 1979; Evans et al. 1995; Nakazawa et al. 1996; McGee et al. 1997). On the basis of its chromosomal

assignment, the *PRKCG* gene is a candidate for *RP11*. This gene encodes a form of protein kinase C that is expressed in the retina. Last year, Al-Maghtheh et al. (1998) described two families with *RP11*-linked dominant RP in which a missense change (Arg659Ser) in *PRKCG* cosegregated with disease. Only one of these two families (RP1907) clearly exhibited asymptomatic, obligate carriers who transmitted the disease to offspring. The authors failed to discover a mutation in *PRKCG* in three other families with reduced penetrance showing linkage to this region. Nevertheless, the authors speculated that *PRKCG* could be the *RP11* gene.

In response to that report, we have undertaken an analysis of the *PRKCG* gene in three additional families with dominant RP with reduced penetrance. All three families have unaffected, obligate carriers, and we previously reported linkage data pointing to *RP11* as the cause of RP in these families (McGee et al. 1997). An affected individual from each family was chosen for the current study (the patients were individuals III-2 from family 1295, IV-8 from family 2474, and IV-34 from family 1562) (McGee et al. 1997), as well as DNA from an unrelated control individual without RP and without a family history of retinal degeneration. We amplified each of the 18 exons of the *PRKCG* gene individually, using PCR from leukocyte DNA obtained from these individuals. Primer pairs were the same as those reported elsewhere (Al-Maghtheh et al. 1998) except for exons 5, 10, and 11, for which we used primers (sense/antisense) as follows: exon 5, 5′ portion, TGAGGTGCTACCCGCAGCTT / CAGTTACGTGGATCTCATCT; exon 5, 3′ portion, AGGCTGCGAGATGAACGTGC / AGGCGAGGGGGCGGGGCCTC; exon 10, GGCTGTGTAAGGTCTAAGTG / CACAGGAGCCCAGTCTCTTC; exon 11, CTGGGTTCCCAACATGGACT / CTTGCCTCTCCCTAAACTCA. The amplified fragments were sequenced directly by means of standard methods.

None of the patients had a defect in codon 659. Furthermore, none of the patients had an abnormality in the coding region or the flanking-intron splice-acceptor or -donor sites, except for one patient who heterozygously carried a silent change in codon 24 (Ala24Ala, GCT→GCC). In all eight gene copies carried by the three patients and the control individual, the sequence of codons Phe19 and Ser148 in exons 1 and 5, respectively, was different from that published elsewhere (TTT instead of TTC, and TCC instead of TCT, respectively), suggesting that the previously reported sequence (GenBank accession number M13977) is an allelic variant, a sequencing artifact, or an error. The previously reported (Al-Maghtheh et al. 1998) silent polymorphism at codon Asn189 was encountered, with two of the three patients being heterozygotes (carrying the sequences AAT and AAC for that codon) and the third patient being a homozygote for the sequence AAT. We previously documented a recombination event in the vicinity of *RP11* in one branch of family 1562 (McGee et al. 1997); however, the intragenic polymorphisms were not informative in this branch of that family and did not allow us to determine on which side of the crossover the *PRKCG* locus lies.

Our analysis provides no evidence that *PRKCG* is the *RP11* gene. Although our data cannot exclude the pathogenicity of the Arg659Ser missense change, the possibility that it is a rare, nonpathogenic variant remains plausible, since none of the three families analyzed here and only two of the five families analyzed elsewhere (Al-Maghtheh et al. 1998) carry an anomaly that would change the sequence of the encoded protein. Another formal possibility is that there are two RP genes in this region, but it would be necessary that both RP loci exhibit reduced penetrance. In either case, it appears that an RP gene in this region remains to be identified.

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THADDEUS P. DRYJA,[1,2] JENNIFER MCEVOY,[1] TERRI L. MCGEE,[1] AND ELIOT L. BERSON[2]
[1]*Ocular Molecular Genetics Institute and* [2]*Berman-Gund Laboratory for the Study of Retinal Degenerations, Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston*

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Address for correspondence and reprints: Dr. Thaddeus P. Dryja, Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, MA 02114. E-mail: dryja@helix.mgh.harvard.edu

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