Biochemical and genetic characterisation shows that the BRCA1 IVS20 insertion is a polymorphism

EDITOR—Two breast cancer susceptibility genes, BRCA1 and BRCA2, have been identified. Combined, these large and complex genes have over 800 reported genetic variants. More than 50 variants occurring within the introns of these genes are known. The clinical significance of these intronic variants, which could potentially impact RNA splicing, is largely undetermined.

A variant within intron 20 of BRCA1 occurs through the duplication of 12 base pairs (bp) (GTATTCCACTCC) 48 bp from the donor junction. This variant has been observed in patients from cancer families. Furthermore, biochemical analysis showed the loss of contribution by one chromosome to the normal RNA in a breast cancer patient with IVS20ins12. This was shown by sequencing cDNA which showed the loss of a heterozygous base at the silent polymorphism at codon 1436 (serine TCT or TCC). These authors concluded that IVS20ins12 could represent a clinically significant regulatory mutation, although this variant was also present in a control specimen. More recently, IVS20ins12 was reported in four controls (with varying family histories of cancer, but otherwise healthy) and one early onset breast/ovarian cancer patient in a Polish BRCA1 study. No aberrant splicing products were detected in these samples, but mRNA abundance and possible loss of transcript have not been assayed. These authors concluded that the issue merits a more extensive study.

Here, a patient diagnosed with breast cancer at the age of 40 and with cancer reported on the maternal side of her family is described. Clinical full sequence analysis of BRCA1 and BRCA2 (BRACAnalysis, Myriad Genetic Laboratories, Salt Lake City, UT) identified IVS20ins12, as well as common single nucleotide polymorphisms (SNPs), particularly S1613G (AGT for serine and GGT for glycine). The patient was counselled regarding the uncertain clinical significance of this variant and elected to participate in this research analysis.

cDNA was synthesised from RNA isolated from peripheral blood mononuclear cells from both the patient and a control sample that also contained S1613G. PCR reactions generated fragments spanning S1613G to exon 21. These products were separated by agarose gel electrophoresis and fragments corresponding in size to the normal transcript were isolated. No notable bands that might represent alternative/aberrant splice products were observed in either sample. The isolated fragments served as targets in subsequent PCR reactions designed to evaluate the sequence at either the polymorphism or the exon junctions. DNA sequencing clearly shows a heterozygous base at codon 1613 (fig 1A) and normal RNA splice junctions for exons 18-21 (fig 1B), indicating that both chromosomes contribute to normally spliced transcripts.

In seeming contrast to our analysis, Robledo et al. observed loss of transcript in an IVS20ins12 carrier. In patient RNA, only the sequence TCC was detected at...
IVS20ins12. A single nucleotide polymorphism haplotype lost. The question remained whether the lost allele came from the chromosome carrying the insertion. It appears that the loss of transcript resulted from a hitherto unidentified BRCA1 mutation on the other chromosome.

These data and analyses strongly suggest that BRCA1 IVS20ins12 is a polymorphism with no obvious clinical significance. Biochemical evaluation of RNA encoding BRCA1 from a patient carrying the variant shows that both chromosomes contribute to the normally spliced transcript. Although these transcripts were isolated from leucocytes, it is highly unlikely that tissue specific splicing events would create mutant transcripts. This conclusion is augmented by the clarification of a seemingly conflicting result. Here, genetic analysis showed the presence of IVS20ins12 on the only functional copy of the BRCA1 gene in a previously described carrier of a loss of transcription.

Figure 2

Informative polymorphisms and haplotype pair assignment among the set of IVS20ins12 carriers. (A) SNP haplotype designations. The presence of a non-consensus allele in common haplotypes is depicted schematically with solid squares. (B) Polymorphisms among IVS20ins12 carriers. The figure contains numbers of non-consensus alleles in each informative locus (0, 1, or 2), as well as the pair of common haplotypes carried by each individual. Filled squares indicate the haplotype pair assignment. The database contained ethnic ancestry identifiers, whenever available. Note that the probands were not ascertained using a single set of criteria, and phenotype/family history information has been stripped on anonymisation. Therefore, essentially no genotype-phenotype correlation could be made solely on the basis of the information present in the database. The following frequent BRCA1 SNP loci were used for haplotype pair assignment: exon 4-49C/T, Q356R, D693N, S694S, L771L, P871L, E1038G, S1040N, K1183R, S1436S, and S1613G.

codon 1436; RNA from the consensus (TCT) allele was lost. The question remained whether the lost allele came from the same chromosome that also contained IVS20ins12. A single nucleotide polymorphism haplotype analysis of patient samples addressed this question.

Our anonymised BRCA1 sequence variation database contained 19 observations of IVS20ins12 and they all shared SNP haplotype 2 (fig 2). There were six homozygous haplotype 2 carriers. The rest contained a haplotype 2 chromosome along with a chromosome with one of the five previously described common haplotypes. Therefore, IVS20ins12 is found solely on a haplotype 2 background. This finding agrees with the previous observation that the vast majority of infrequent sequence variations within the BRCA1 gene are only found on single SNP haplotype backgrounds.13-15 Indeed, from that population includes 3550 subjects who do not have a single copy of haplotype 2, and none of these carries an insertion in IVS20, there is 90% confidence that the frequency of IVS20ins12 on non-haplotype 2 backgrounds does not exceed 0.033%. In contrast, 0.77% of 2452 instances of haplotype 2 carry IVS20ins12. Since IVS20ins12 is present on the background of haplotype 2, for which codon 1436 is encoded by TCC, the RNA detected by Robledo et al14 was transcribed from the chromosome carrying the insertion. It appears that the loss of transcript resulted from a hitherto unidentified BRCA1 mutation on the other chromosome.

Germline mutations in the β-catenin gene are not associated with the FAP phenotype without an APC mutation

EDITOR—Familial adenomatous polyposis (FAP) is generally considered a typical monogenic disease caused by germline mutations within the adenomatous polyposis coli (APC) gene. Despite applying several screening techniques, however, mutational studies worldwide have failed to identify a germline mutation within the APC gene in up to 50% of all FAP cases (called APC negative below).1,2 Intrinsic alterations, mutations within the regulatory regions causing altered APC gene expression, or large scale rearrangements of the APC gene could account for the failure to identify an APC mutation in some FAP families. Assuming no hot spots, however, such mutations are not likely to cause a high percentage of APC negative cases.

Fifty families which were referred to our department with the clinical diagnosis of FAP were tested for APC germline mutations. In 14 of them (28%), no APC mutation could be identified after screening the entire coding region of the gene (APC negative group) using the protein truncation test (PTT, the whole coding region), single strand conformation polymorphism analysis (SSCP, the affected PTT segment), and direct DNA sequencing (the affected SSCP exon).3 As these families were either too small or only a limited number of family members were available, linkage analysis with respect to the APC locus could not be performed. They all fulfil the major criteria for the clinical diagnosis of FAP (table 1). We have shown previously that these families differ phenotypically from those with an APC mutation, suggesting that they might represent a distinct genetic entity.4 In summary, the APC negative group tended to have less severe disease characteristics with significantly increased age at diagnosis, fewer colonic polyps, and fewer extracolonic manifestations, so they are similar to those with attenuated polyposis coli (AAPC). However, all three regions of the APC gene which have been reported to correlate with AAPC were analysed without detecting DNA abnormalities.

A candidate which might be involved in the locus heterogeneity in FAP is the β-catenin gene, a member of the same cellular pathway as APC. Free β-catenin is targeted for degradation by the glycogen-synthase kinase (GSK) β and the APC protein. An increase in the stability of free β-catenin is triggered by the Wnt signal as well as by the absence of APC protein or the presence of mutated APC protein. Finally, if APC is intact, mutations within the β-catenin gene itself could also result in an increased level of free β-catenin. In recent studies, somatic mutations in the β-catenin gene were found in colorectal and other types of tumours.5,6 However, none of the studies observed a germline mutation within the β-catenin gene in tumour matched constitutional DNA.

Based on the observation that somatic mutations in the β-catenin gene can mimic mutations in the APC gene in colonic tumours, the possibility arises that, similarly, germline β-catenin mutations could mimic germline APC mutations giving rise to an FAP-like phenotype. In the present study this hypothesis was tested.

In 14 APC negative FAP families, the entire coding region (16 exons) of the β-catenin gene (CTNNB1) was screened in DNA isolated from peripheral blood. SSCP analysis with PCR primers allowing investigation of intron/exon bounda-

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Simpson-Golabi-Behmel syndrome and attention deficit hyperactivity disorder in two brothers

EDITOR—Simpson-Golabi-Behmel syndrome (SGBS, MIM 312870) is an X linked condition characterised by pre- and postnatal overgrowth, coarse facial appearance, large mouth, predisposition to embryonic neoplasia, 3 and a variety of vis- ceral and skeletal abnormalities. Psychomotor development in the syndrome is extremely variable, ranging from normal intelligence, 4 to moderate impairment, to severe impairment evident from birth. 5 We report the cases of two male sibs with normal psychomotor development, diagnosis at 6 and 7 years of age with SGBS, who manifest significant behavioural disturbances consistent with a diagnosis of attention deficit hyperactivity disorder (ADHD). This is the first report of an association between SGBS and a specific behavioural phenotype (ADHD).

Case 1, the older of the two boys, was the first born to non-consanguineous, healthy, white parents. The pregnancy was complicated at 36 weeks by polyhydramnios and pregnancy induced hypertension. An ultrasound performed at this time discovered a left sided diaphragmatic hernia. No other fetal abnormalities were reported. Labour was induced at 39 weeks and the child was delivered by forceps assisted vaginal delivery. Birth weight was 4400 g (well above the 97th centile) and immediate transfer for stabilisation and surgical repair of the diaphragmatic hernia was undertaken. Other birth indices were not recorded. The primary surgical repair was successful but was complicated by a left pneumothorax on day 4 of life. This complication was successfully managed and the subsequent postsurgical course was uneventful. The child was discharged from hospital aged 14 days.

At 5 months bilateral inguinal hernias were noted and direct DNA sequencing using the same primers as for SSCP, the Thermosequenase labelled primer cycle sequencing kit (Amersham), and an automated DNA sequencer (LI-COR) without identifying any changes in the nucleotide sequence. As there were no differences detected in the coding region, β-catenin gene expression in patients' lymphocytes was examined using reverse transcription PCR. PCR amplification of cDNA was performed in two separate PCR reactions using PCR primer pairs 3.1/6.2 and 6.1/13.2, as listed in table 2. This showed that in all persons tested the expected β-catenin transcript was present and of expected size, suggesting that differential splicing did not occur. However, as our experiments regarding β-catenin expression were performed on lymphocytes, differential expression in colonic or other tissues affected in FAP, which were not available for our study, cannot be excluded.

Taken together, using a combination of several techniques for the mutational analysis, in our group of 14 FAP APC negative families, no hereditary alterations were identified in the β-catenin coding sequence or gene expression, suggesting that β-catenin germline mutations do not account for APC negative FAP cases. Even though these results need confirmation in a larger sample of FAP negative families, they indicate that β-catenin might play a different role in the pathogenesis of hereditary colon carcinoma compared to sporadic colorectal cancer.

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1 Nagase H, Nakamura Y. Mutations of the APC (adenomatous polyposis coli) gene. Hum Mutat 1993;2:425-34.

References
The dose of methylphenidate was increased to 10 mg morning and midday shortly after this consultation with marked improvement in overall behaviour and concentration over the next month.

Case 2, the second child of these parents, was born by emergency caesarean section at 39 weeks because of a prolapsed cord after an uneventful pregnancy. Birth weight was 5100 g (well above the 97th centile). Birth length and head circumference were not documented. The baby was noted to have macroglossia on neonatal examination. Karyotype was performed and was normal male. There were no neonatal complications. He had recurrent chest and ear, nose, and throat infections in the first 11 months of life. At 16 months he was referred for genetic opinion and the diagnosis of Beckwith-Wiedemann syndrome (BWS) was proposed and 3 monthly abdominal ultrasound surveillance instituted. At 2 years a tongue reduction was performed. Owing to ongoing recurrent ear infections and night time snoring he was reviewed by an ear, nose, and throat specialist. A submucous cleft palate was discovered and he underwent adenotonsillectomy and insertion of tympanostomy tubes. The submucous cleft was managed conservatively as his speech development was normal. At 5 years, a single renal cyst was found on surveillance ultrasound. His behaviour and sleep patterns (from infancy) were strikingly similar to those described in his brother and a similar developmental assessment documented age appropriate functioning in all areas apart from attention, sequencing tasks, and behaviour. He also fulfilled the criteria for diagnosis of ADHD. He was started on the same medications (and dosage) as his sibling with a similarly poor response. He was referred (with his brother) for review aged 6 years. Growth parameters were height 128 cm (>97th centile), head circumference 55 cm (>98th centile), and weight 30 kg (>97th centile). He shared similar dysmorphic features with his brother comprising coarse facial features, hypertelorism, broad nasal bridge, short nose, macrostomia, prominent jaw (fig 1), two right sided supernumerary nipples, a pectus excavatum chest deformity, and short fingers with broad, short thumbs (fig 2). Urine mucopolysaccharide screen was normal. His behaviour and attention span also markedly improved in response to an increased dosage of methylphenidate (10 mg, twice daily).

The boys had a sister, aged 4 years, who had no medical or behavioural problems nor any abnormalities on examination. The boys’ mother was also normal on physical examination and the family history was unremarkable.

Simpson-Golabi-Behmel syndrome is an X linked overgrowth syndrome first reported by Simpson et al in two male cousins from an Ashkenazi Jewish kindred. Both of these males had normal developmental milestones and normal intelligence. Nine years later, Golabi and Rosen reported four males with a “new X-linked mental retardation-overgrowth syndrome”. The proband in this kindred had “moderate” mental retardation at 8 years and another male was developmentally delayed at 4 months of age. The other two males died in the newborn period and...
no comment was made on their development. Opitz coined the term “Golabi-Rosen” syndrome and added the reports of a further three males with similar features, but no prominent overgrowth. Shortly after this, Behmel et al. reported a five generation family with 13 affected males with features of X linked overgrowth and pointed out the similarities between these cases and the patients described initially by Simpson et al. Neri et al. emphasized the similarities between the above authors’ reports and proposed the designation “Simpson-Golabi-Behmel” syndrome to encompass this clinical entity. To date, at least 40 patients have been reported with the syndrome, and mutations in GPC3, a glypican gene, have recently been found to cause the condition. GPC3 is an extracellular proteoglycan and is inferred to play a major role in growth control of mesodermal tissues, possibly by modulating the actions of insulin-like growth factor 2.

The two patients reported here both displayed marked neonatal macrosomia and the typical facial features of SGBS. The additional clinical findings of short, broad hands, pectus excavatum chest deformities, and supernumerary nipples, present in both boys, add further credibility to the diagnosis. The congenital diaphragmatic hernia, reported in case 1, has been previously described in association with SGBS and the inguinal hernias (case 1) are a well established feature of the condition. Similarly, the macroglossia, submucous cleft palate, and single renal cyst, reported in case 2, are all features consistent with the diagnosis of SGBS. The main differential diagnostic consideration in the same cases was Beckwith-Wiedemann syndrome, given the prenatal macrosomia and macroglossia seen in both boys and macroglossia, seen in case 2. This boy was initially considered to have BWS and the clinical overlap between SGBS and BWS is highlighted by similar examples of diagnostic confusion in published reports. The particular constellation of clinical features in these two male sibs in addition to the presence of supernumerary nipples (a feature not reported in patients with BWS) clearly differentiates them as having SGBS. Another diagnostic possibility, given the coarse facies and behavioural problems present in these cases, was a mucopolysaccharide storage disorder. This diagnosis was excluded in both boys by appropriate urine testing.

Our two cases are distinct from all previously reported cases with SGBS because of their striking behavioural disturbances, not present in their unaffected 4 year old sister. There is relatively little mention of the behavioural phenotype in the published cases of SGBS. In the initial report by Simpson et al., one of their cases (case 1, DG) was evaluated psychologically at 30 months of age and reported to have an “attention span of short duration”. In the report of Behmel et al., passing comment is made of the “severe emotional and behavioural troubles during adolescence” in one of their patients (II.3 in family 2) and “behavioural difficulties during school attendance” (that necessitated psychological treatment) in his nephew. These comments are not further elaborated and comprise the few references to behavioural phenotype in SGBS.

This is the first report of a specific behavioural pattern (ADHD) in patients with SGBS. It raises the question of whether other patients with SGBS are at risk of developing this (or other) neurobehavioural problems. The well recognised neurobehavioural patterns seen in patients with Prader-Willi and velocardiofacial syndromes serve as examples of other multisystemic disorders with clinically significant associated behavioural profiles. If SGBS is associated with a predisposition to specific behavioural disturbances, as indicated by the two cases reported here, it is important that the parents and clinicians who care for these children are aware of this. This knowledge will then allow the opportunity for anticipatory guidance to be provided for these families and for intervention and treatment strategies to be put in place.

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Presence of a deletion in the 5\' upstream region of the GALT gene in Duarte (D2) alleles

Editor—Galactosaemia is an autosomal recessively inherited metabolic disorder caused by a defect in the galactose-1-phosphate uridyltransferase (GALT) enzyme. Absence or severe reduction of GALT activity results in classical galactosaemia (G/G) while an approximately half reduction of enzyme activity leads to the Duarte variant of galactosaemia (D/D). Mutation Q188R was found to be the most common molecular defect among classical galactosaemia patients, whereas N314D was predominantly detected in Duarte galactosaemia patients. In recent studies, the Duarte (D2) allele with 50% of normal GALT activity and the Los Angeles (D1) allele with 110-130% of normal GALT activity were characterised as having
nucleotide alterations in addition to N314D. A study of the GALT gene from 31 unrelated galactosaemia families and from 504 control subjects is reported.

Thirty three patients and their relatives from the Czech and Slovak Republics were investigated (100% of all known galactosaemia patients). All families were white. There is no galactosaemia newborn screening in our country. Clinical onset of classical galactosaemia began in all patients in the neonatal period. The patients were identified with typical symptoms of classical galactosaemia (vomiting, failure to thrive, icterus, sepsis, hepatosplenomegaly, cataracts) and diagnosed by erythrocyte GALT assay (residual GALT activity less than 3% of the control value). A total of 504 non-galactosaemic subjects from the Czech Republic, including healthy donors and parents of α-1-antitrypsin deficiency patients, were used as a control group for population screening of the Duarte (D2) and Los Angeles (D1) alleles.

Mutation analysis was performed for the 5′ upstream region and the whole coding region with flanking intronic sequences of the GALT gene using PCR/digestion, denaturing gradient gel electrophoresis (DGGE), heteroduplex analysis (HA), and sequencing methods. A total of 11 sequence variations in six mutated alleles was found. The two most common molecular defects were the mutations Q188R (45.2%) and K285N (27.5%). Two novel mutations in the coding region of the GALT gene, Y209S (3.2%) and 2142delGCC (1.6%), were detected; both were associated with severe reduction of enzyme activity. The previously described mutation L195P was found in exon 7 on three mutant alleles (4.8%). An unusual molecular genotype was observed in three classical galactosaemia alleles (4.8%), with six variations from the normal nucleotide sequence presented in cis (V151A, N314D, −119del4, 1105G→C, 1323G→A, and 1391G→A). A novel deletion of four GTCA nucleotides in the 5′ promoter region, in a position 119 nucleotides upstream from the initiation codon (−119del4), was found in Duarte (D2) alleles (fig 1, above), in addition to N314D, 1105G→C, 1323G→A, and 1391G→A. The deletion abolished a DdeI restriction site in the amplification created restriction site (ACRS) detection system (fig 1, below). In addition, during analysis of the 5′ promoter region, a discrepancy was found between published and detected sequences (fig 2).

Using PCR/restriction digestion assay, we screened a sample of 1008 control alleles, obtained from 504 subjects, for N314D plus accompanying intron and exon variations. The control subjects had no clinical symptoms of galactosaemia (biochemically and electrophoretically untested). N314D was found on 82 of 1008 (8.2%) different control alleles examined. From these, 54 (5.4%) were Duarte (D2) alleles contained in cis N314D plus −119del4, 1105G→C, 1323G→A, and 1391G→A. Twenty eight (2.8%) were Los Angeles (D1) alleles carrying in cis mutation N314D plus the silent mutation L218L. From previously obtained results, as well as the study presented here, it is evident that the Duarte (D2) and Los Angeles (D1) alleles are widespread among various populations. In both Czech galactosaemics V151A + Duarte (D2) and control Duarte (D2) alleles, the promoter deletion −119del4 and the intronic variations 1105G→C, 1323G→A, and 1391G→A were in linkage disequilibrium with N314D. Based on these results, we assume that the Duarte (D2) allele contains the promoter deletion −119del4 and the three intron substitutions, 1105G→C, 1323G→A, and 1391G→A, together with N314D. N314D appears to be an ancient genetic variant of the GALT gene, a background on which several other sequence variations were created.

The mechanism of partial GALT activity impairment in the Duarte alleles still remains to be fully understood. Two possible explanations have been described. Lai et al. showed that the N314D mutation reduces the biological stability of the GALT dimeric protein in human lymphoblastoid cell lines; they stated, however, that no nucleotide changes other than N314D had been found in the GALT genes they studied. This finding is, though, in conflict with results obtained from the yeast expression system, in which the N314D subunit dimerises well both with wild type GALT and with itself. The reduced stability was not seen when N314D containing GALT protein was overexpressed via exogenous promoters in yeast. On the other hand, Podskarbi et al. reported that, besides N314D, Duarte
alleles (D2) carry two “intrinsic mutations”, 1105G→C and 1391G→A. They suggested that these intron alterations might be “regulatory mutations” involved in regulation of GALT gene expression.

In the present work, we have described a new DNA alteration on Duarte alleles, the deletion of four nucleotides (GTCA) in the 5′ promoter region of the GALT gene (−119del4). There is a high probability that this deletion is located in the transcription factor binding region.

For this reason a computer search for potential regulatory DNA elements in the area of the deletion was performed.10 Two Homo sapiens binding factors (activator proteins AP1 Q2 and AP1 Q4), which lose their binding motifs in Duarte (D2) alleles, were found. We conclude that the −119del4 promoter mutation is perhaps the main factor in Duarte allele enzyme activity reduction caused by a decrease in the synthesis of mRNA. This hypothesis will be tested further; however, Shin et al11 reported that in competitive RT-PCR, the RNA level from homozygous Duarte (D2) cultured human lymphocytes was lower than that obtained from control cultured human lymphocytes.

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Familial congenital diaphragmatic hernia: is an imprinting mechanism involved?

EDITOR—Isolated congenital diaphragmatic hernia (ICDH) may be sporadic or familial. The mode of inheritance in familial cases (IFCDH) is a matter for debate and different patterns have been proposed. For example, multifactorial inheritance was suggested by Wolff5 and by Norio et al.3 However, autosomal recessive inheritance has been suggested in clinical studies4,5 and also in animal studies.6,7 Also, there have been various families reported to date in which other patterns of inheritance are possible (table 1).

If all the published pedigrees with familial CDH are analysed, autosomal dominant, autosomal recessive, and X linked inheritance patterns can be seen. We propose a hypothesis which unifies these various mechanisms, which is to consider imprinting as involved in the inheritance pattern of this condition.

Table 1 Published isolated familial congenital diaphragmatic hernia (IFCDH) cases

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In 1994, two non-consanguineous girls with isolated CDH and balanced translocations involving 8q22.3 were reported by Temple et al.21 More recently, Tokuhara et al.22 have cloned the gene HFZ6 and localised it to 8q22.3–q23.1. HFZ6 corresponds to the human frizzled-6 gene and is a member of the family of frizzled genes that encode receptors for Wnt proteins, which are secreted proteins involved in cell-cell interaction during embryonic development and tumorigenesis.23 Moreover, these frizzled genes are homologous to the Drosophila frizzled gene family, a group of homeobox genes determining morphogenesis and planar polarity phenotypes both in Drosophila and vertebrates.14–19 Frizzled gene deletions have already been associated with other genetic disorders, such as Williams syndrome,20 in which they could have a role in early brain developmental anomalies.
As can be seen in the cases reported by Temple et al., one girl inherited the cytogentic defect from her mother, the translocation in the other girl was de novo, and uniparental disomy was excluded in both. This suggests the presence of a major gene involved in normal diaphragmatic development at this locus. Major genes are involved when the development of complex structures is thought to be controlled by various genes, with one of them having a more important role in phenotype determination. In this case, if such a gene (for example, Hfz6) is involved, as suggested by the patients with balanced translocations, genomic imprinting could be involved in its regulation, with the maternal gene being normally expressed and the paternal gene being normally silenced, producing the disease phenotype when an abnormal gene is inherited from the mother. This could have happened in families 1, 2, 3, and 5 in table 1, as well as in the numerous cases reported by other authors and ourselves with two or more sibs affected. Although there are not many examples of these genes, the theoretical presence of a major gene regulated by imprinting is possible. Various genes, now clearly recognised as being regulated by the imprinting mechanism, initially had a poorly understood pattern of inheritance because of the apparently contradictory data. It is also known that many imprinted genes are involved in development, such as the one we have mentioned here. There is evidence of imprinting of at least one of the frizzled gene family members, Xfz3, which is maternally expressed in Xenopus. In humans, there is no clear evidence of imprinting on chromosome 8, but some authors have found different parental origin of a deletion in chromosomal region 8q24 in Langer-Giedion syndrome, which is located contiguously to the chromosome band containing HZF6.

The case of Frey et al. (father to daughter transmission, family 4, table 1) could be explained if paternal uniparental disomy had occurred. This was not ruled out, unlike in the study of Temple et al., where uniparental disomy of chromosome 8 was studied and excluded.

In spite of this evidence, we could not exclude the possibility that different patterns of inheritance could be occurring in different familial cases, as happens in other diseases. Consanguineous kindreds, such as those reported by Farag et al. and Mitchell et al. (table 1, fig 1E), could support our hypothesis but are more likely to represent other mechanisms of inheritance.

However, molecular and segregational analyses of more families are needed to verify this hypothesis.

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Genetic counselling: do we recognise and meet the consultands’ agenda?

Editor—Agreeing the issues that are to be addressed is central to the process of genetic counselling. We undertook a study to document the consultand’s agenda at the genetic clinic and whether the geneticist recognises that agenda. We also addressed whether failure to recognise the agenda is the prime factor when consultands think that issues important to them have been dealt with badly.

Ethical approval was given by the Newcastle and North Tyneside Health Authority Joint Ethics Committee to invite new consultands over 18 years of age, attending a general genetic clinic to take part in this study. Consultands attending follow up appointments or those who had had a home visit before the hospital appointment were not included. Consultands were given an information sheet with details of the study and a verbal explanation on arrival at the clinic. Those wishing to take part were given a questionnaire to complete in the waiting room before seeing the geneticist. The first question was whether they had asked for the appointment or it had been suggested to them, and the second question asked if they knew the name of the medical condition they had come about. These two questions were followed by a list of issues (table 1) that they might want to address in the consultation. The consultands were asked to mark boxes indicating whether a topic was important, not important, or not applicable to them. There was a space at the end of the questionnaire to add further issues. The study design was based on self completion questionnaires because there are no spare rooms available at the clinics and therefore no privacy for interviews. The benefits of the method are that it is simple and does not intrude unduly on the consultation or the consultand’s time. The major disadvantage is that the list one gives may influence the consultand’s initial agenda.

The questionnaire related to a single counselling situation and so could be completed by a single person or several people. For example, a couple attending the clinic with an affected child were considered as one consultation and asked to complete the questionnaire together. However, when a number of family members at risk of developing the same condition were seen together each was given a questionnaire. When a couple was seen because one of them was at risk of developing a disorder they were asked to complete the questionnaire together.

After the consultation the same 12 statements were given to the geneticist (nurse or doctor) who was asked to mark whether they had felt an issue was important, not important, or not applicable in that consultation. If the geneticist marked a statement important they were asked to mark a box to indicate how well they felt they had addressed the issue, the options being: very well, well, neither badly nor well, badly, or very badly. The geneticist was also given space at the end of the questionnaire to add other information.

Four to five weeks after the clinic appointment the consultands were visited at home by AS. They were asked to complete a third questionnaire which had the same list but the statements that the consultands had thought important before their clinic appointment were highlighted. For these highlighted topics the consultands were asked to mark how well they felt the issue had been addressed using the same box system as the geneticist, so that direct comparisons could be made. There was also a recorded interview at this stage. The time delay of four to five weeks was chosen to ensure that they would have received the letter summarising the consultation. Comparison of responses between groups was analysed using the chi-squared test.

One hundred consultands were enrolled after approaching 110 consultands. The questionnaire was completed by 60 male/female couples and 40 individuals, 28 women and 12 men. A corresponding questionnaire was completed by the geneticist for each of the consultand questionnaires. Eighty four of the consultations were with doctors and 16 with nurses. The consultations were undertaken by seven doctors and three nurses.

Sixty three of the consultations were about childhood illness and 34 about adult onset disease. Three consultations did not fit into this simple classification; two were for infertility and one for genetic risk to a consanguineous couple. Thirty one of the respondents said that they had asked to be referred to the genetic clinic. Sixty four said it had been suggested to them and five did not complete the question.

The number of consultands who thought an issue important is shown in fig 1. The difference between consultand and geneticist thinking an issue important was significant (p<0.001) for recurrence risk, current treatments, future developments, prognosis, medical/genetic testing, prenatal diagnosis, cause, how the condition is inherited, and written information. Fig 2 shows the discrepancies between consultand and geneticist about whether an issue was important or not. The largest discrepancy (73%) between consultands and geneticists was that consultands rated treatment important more frequently than geneticists. While treatment and development of new treatment were clearly important to the consultands, they might not have expected them to be addressed at the genetic clinic if it had not been itemised in the questionnaire. It is debatable whether discussion of treatment should be part of the geneticists’ remit. However, the geneticist should recognise the importance of this information in the consultand’s decision making process, if only to clarify whether they will be addressing it or not, and if not perhaps making suggestions about how the consultand can obtain information.

Comparison of the responses of those who had requested the appointment with those to whom it had been suggested identified significant differences in response to two of the statements. Those who had requested the appointment were more interested in personal risk (16/31 v 19/64, p<0.05) and in support groups (16/31 v 6/64, p<0.001). When comparing consultands relating to adult onset disorders with childhood illness, personal risk (28/34 v 6/63, p<0.001), prognosis (32/34 v 44/63, p<0.01), and

<table>
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<tr>
<th>Table 1 List of issues on each of the questionnaires</th>
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<tr>
<td>(1) I would like to know if I am going to get this condition.</td>
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<td>(2) I would like to know how likely my child(ren) are to get this condition.</td>
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<td>(3) I would like to know about treatments for this condition available in the moment.</td>
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<td>(4) I would like to know what is happening in the developments of new treatments for this condition.</td>
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<td>(5) I would like to know what will happen to someone with this condition as time goes by.</td>
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<tr>
<td>(6) I would like to know if there is a medical or genetic test to see if I or others will get this condition.</td>
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<tr>
<td>(7) I would like to know if there is a test for this condition in pregnancy.</td>
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<tr>
<td>(8) I would like to know what causes this condition.</td>
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<td>(9) I would like to know a medical name for this condition.</td>
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<td>(10) I would like to be able to contact other families affected with this condition for support and advice.</td>
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<tr>
<td>(11) I would like an explanation of the way this condition is passed on from one generation to the next.</td>
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<tr>
<td>(12) I would like the information discussed today written down so that I can look at it again when I want to.</td>
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current treatments (30/34 v 45/63, p<0.05) were all marked important more often for the adult onset conditions. No issues were marked more important for the childhood onset conditions.

Follow up questionnaires and home interviews were completed for 69 of the 100 consultations. Forty nine of the first questionnaires had been completed by couples and 20 by individuals. Of the 49 initially completed by couples, both partners were present for 35 of the follow up questionnaires. There were no significant differences in responses to the first questionnaire between those who were followed up and those who either declined or could not be contacted. Five consultands (7%) marked that the geneticist had given information about one or more issues very badly and were dissatisfied; these consultations are outlined below.

A couple with a 2 year old child who has developmental delay and dysmorphic features had asked to be referred. No diagnosis was reached. The geneticist (doctor 4) had recognised the important issues but had marked that the information had been given neither badly nor well for both. The couple were disappointed that no diagnosis was forthcoming and, while they understood the geneticist might not have been able to give a diagnosis, they felt they were “fobbed off”. A follow up appointment had been arranged.

Another couple had been referred to the clinic by their GP who thought the child had dysmorphic features. No diagnosis was made when they attended the consultation. The geneticist (doctor 4) recognised the important issues but was unaware of the couple’s perception that they had been dealt with badly, marking all of these topics as having been dealt with well. At interview the couple said they did not think there was anything wrong with the child and were upset that it had been suggested. No follow up appointment was made.

A 44 year old lorry driver was referred by his GP. He thought he had been referred because of acne when in fact the diagnosis was hereditary haemorrhagic telangiectasia. His late mother had bled massively from a pulmonary arteriovenous malformation. The geneticist (doctor 7) had recognised the important issues and that their explanation had been poor. This consultand failed to attend a follow up appointment.

A 28 year old woman was referred because of a family history of colon cancer. The geneticist (nurse 2) recognised the important issues and was aware that she had dealt badly with three of them but marked that she had dealt with the fourth well. The consultand said she “was not told anything for sure....they couldn’t be sure.... I just felt that if I hadn’t gone, nothing would have changed and I

![Figure 1](http://jmg.bmj.com/first-published-as-10.1136/jmg.36.7.571-on-1-July-1998.Downloaded-from-http://jmg.bmj.com-on-November-27,2023-by-guest.Protected-by-copyright.)

**Figure 1** The number of times an issue was marked important by consultands and by geneticists. The number of questionnaires completed by each group was 100.

![Figure 2](http://jmg.bmj.com/first-published-as-10.1136/jmg.36.7.571-on-1-July-1998.Downloaded-from-http://jmg.bmj.com-on-November-27,2023-by-guest.Protected-by-copyright.)

**Figure 2** The discrepancies between consultand and geneticist for each issue on the questionnaire.
wouldn’t have had all this worry”. Screening was recommended and follow up arranged.

Another woman referred because of a family history of breast cancer attended with her husband. The geneticist (doctor 6) had not recognised the issues important to the couple. The consultands felt that too much time was spent talking about tests and genetics and not enough devoted to practical measures such as treatment and how the disease would affect someone. She said “(the geneticist) was going on about genes and DNA and what it would mean if I got a positive test, but I just wanted to know if I was going to get cancer and what I could do about it”. Screening was recommended and no follow up appointment was made. It was only in this fifth consultation that failure to recognise part of the agenda was the crux of the problem.

In two of the consultations, those relating to the dysmorphic children, the geneticist had been unaware of the consultands’ dissatisfaction. Thus one’s subjective opinion of how well a consultation has gone cannot be relied upon. In none of these consultations where the consultands were dissatisfied was the geneticist giving bad news when one might have expected a “shoot the messenger” response. In four of the five consultations the consultands were left with an element of uncertainty. Failure to arrive at a diagnosis in the two children with delay meant that clear answers could not be given. Both of the women with a family history of cancer really wanted to know if they would get it or not rather than have a risk estimate, and although correct information was given and screening arranged both felt dissatisfied. In summary, failure to recognise the agenda was not a major contributor to patient dissatisfaction.

The most important finding of this study was that consultands want to know about available and developing treatments. This puts an onus on the geneticist to stay up to date with treatment modalities for the condition under discussion in any consultation and will require close links with colleagues in other specialities. Where the number of consultands with specific disorders is large enough, the most practical way of addressing treatment issues would be to hold joint clinics with the relevant specialist.

Costello syndrome and rhabdomyosarcoma

EDITOR—Kerr et al reported two children with Costello syndrome who also had embryonal rhabdomyosarcomas. I report a 14 year boy with Costello syndrome and an alveolar rhabdomyosarcoma.

The baby was born after 35 weeks gestation but weighed 3544 g. Polyhydramnios was present. At birth the infant appeared to be somewhat dysmorphic, was oedematous, and had low set ears. He required a respirator for five days. His oral intake was poor and at the age of 2 weeks he was admitted to hospital because of failure to thrive. At the age of 6 months, a diagnosis of alveolar rhabdomyosarcoma of the right foot was made. Treatment consisted of below the knee amputation and chemotherapy (doxorubicin, actinomycin D, vincristine, and cyclophosphamide). Continual follow up by oncologists has not indicated any recurrence of the rhabdomyosarcoma.

Subsequently, he was seen by various geneticists because of the following findings: nystagmus, a low set ear, midface hypoplasia, slightly coarse facial features, bitemporal narrowing, anteverted nares, broad nasal bridge, prominent pouting lower lip, marked joint laxity and hyperexten-
sibility, and developmental delay. At that time the diagnosis of cutis laxa was made. Collagen studies were normal.

As he grew older, his face appeared coarser (fig 1). He remained below the 3rd centile for height but the 10th centile for weight. Other findings consisted of posteriorly positioned ears, large ear lobes, prominent lips, increased chest diameter, deep plantar palmar creases, loose skin on his hands and feet, hyperkeratotic lesions on his palms and soles, and papillomas around his nose. He has remained developmentally delayed and at present requires special classes.

The findings present in this patient are typical of Costello syndrome. In contrast to the two patients reported by Kerr et al who had an embryonal subtype of rhabdomyosarcoma, our patient had an alveolar subtype. Rhabdomyosarcomas and Costello syndrome are rare disorders and to find three patients with such a combination gives credence to the possibility that an association exists between the two entities. Recently, Suri and Garrett reported a patient with Costello syndrome who had an acoustic neuroma.

Of interest, when I informed the mother of my patient who lived in Maine, and now lives in Oregon, about the two patients from England who also had Costello syndrome and rhabdomyosarcomas, she told me she had been aware of this information for more than a year as she communicated with these families through the Internet.

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