Letters to the Editor

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Fine molecular mapping of the 4p16.3 aneuploidy syndromes in four translocation families

EDITOR—Deletions of 4p16.3 have attracted considerable attention, particularly since the introduction of FISH and molecular techniques, and are associated with a variety of clinical pictures. Although all affected subjects are mentally retarded, this can vary from profound to mild and the physical manifestations may be those of the severe, often fatal, Wolf-Hirschhorn syndrome (WHS) or of the relatively milder, usually non-fatal Pitt-Rogers-Danks syndrome (PRDS). Genotype-phenotype correlations are not consistent except for the broad generalisation that the most severe physical abnormalities are more likely to be seen with the largest deletions. There is less information about 4p16.3 duplications. Before FISH, patients with 4p trisomy or duplications were reported to have profound mental retardation with microcephaly, short stature, and other marked physical abnormalities.¹ By contrast, in two translocation families where we have described index cases with PRDS, those sibs with the 4p16.3 duplication had

relatively mild mental retardation and late onset physical overgrowth.² Here we describe the fourth family we have encountered with a translocation in which the index case has PRDS. This boy's father and older brother carried the translocation in a balanced form and his younger brother had an unbalanced karyotype with 4p16.3 duplication.

Patient 1 is the proband, born in 1986, who was diagnosed clinically at the age of 10 years. He was born at 35 weeks' gestation with a birth weight of 1800 g (10th centile). There were early feeding problems and he was in hospital for three months. Pyloric and ureteric stenosis were found and operated on. He was left with only one functioning kidney. In the second year of life, he developed grand mal seizures, up to six per day, but these stopped at the age of 5 years. At 12 years 9 months he was an affable child with some limited conversation. He had developed some expertise in bowling. His height (136 cm) was below the 3rd centile and his head circumference (HC) was 48 cm, some 3.5 SD below the mean. He had abundant curly hair on the head, apparent hypotelorism, slightly prominent eyes with some fullness of the lower lids and the sclera visible below the iris, a pointed nose, some prominence of the glabella, a short philtrum, wide mouth, and small chin (fig 1). He was very slender with little subcutaneous fat.

Figure 1 The PRDS proband (left) and his OGS sib, aged 12 years and 5½ years, respectively.

Figure 2 Family 4. Partial G banded karyotype and FISH using probe D4S96 (Oncor Inc) with marker locus D4S174 of (A) carrier father: 46,XY,ish t(4;6)(p16.3;p25.3)(D4S96-,D4S174+;D4S96+), (B) PRDS proband: 46,XY,ish der(4)t(4;6)(p16.3;p25.3)(D4S96-,D4S174+), and (C) OGS sib: 46,XY,ish der(6)t(4;6)(p16.3;p25.3)(D4S96x3,D4S174x2).

There was fifth finger clinodactyly and accompanying camptodactyly.

The younger brother was born in 1993. His birth weight (3480 g) was on the 50th centile but his length (52 cm) was above average (85th centile) and head circumference (38 cm) at 2 weeks was on the 90th centile. His physical growth was good with heights and weights at the 70th centile. At 5½ years his HC (53.3 cm) was on the 95th centile. By contrast he was slow to pass his developmental milestones. A formal assessment at the age of 4 years put his mental development at 2½ to 3 years but no more than 2 years for his language. He was an energetic boy with a big head, unruly hair, widely spaced eyes (like his father), a short, upturned nose with a deep saddle, a normal mouth with shed central upper deciduous teeth, and plenty of muscle and fat on his sturdy little body (fig 1). The father was born in 1956; his height (182 cm) was on the 75th centile and he had obvious hypertelorism. The mother, born in 1966, had a height of 173 cm (95th centile) and normal facies. The older brother (born in 1984) had a height at 13 years of 171 cm (95th centile); he had obvious hypertelorism and a HC (56 cm) on the 80th centile.

Cytogenetic studies showed that the proband, parents, and sibs had apparently normal GTG banded karyotypes. By FISH, using the probe D4S96 (Oncor Inc), a cryptic translocation was found in the father and the older brother between chromosomes 4p and 6p. The proband had a deletion of 4p16.3 and the younger brother a duplication (fig 2). The translocations found in all four similar families we have studied have involvement of the 4p16.3 regions with different second chromosomes, 6p25.3 (fig 2) in this family, 1q44 (family 1), 8p23.1 (family 2), and 21q22.3 (family 3) as described previously.²

Figure 3 The cosmid contig used in the fine mapping study of family 1; the probes used are in bold. The solid line indicates the region involved in the translocation and localises the proximal breakpoint to be within locus D4S132.

Molecular studies in all four families showed that three different segment sizes were deleted. The smallest translocated region was found in family 1 and this was used in the present study for finer mapping of the breakpoint.

Overlapping cosmids spanning 390 kb, between loci FGFR3 and D4S43 (fig 3), were applied on an obligate carrier of family 1. Hybridisation signals for the probes 184d6, 19h1, 27h9, 58b6, 141a8, and 108f12 were seen on the translocated segment. The probe 10d12 was shown to hybridise to the terminal ends of both chromosomes 4 indicating that the breakpoint was at the ends of 108f12 and 10d12, within locus D4S132.

The interpretation of these findings is not straightforward. Differences between WHS and PRDS have been discussed and debated²⁻⁴ and the validity of the overgrowth syndrome we found in families 1 and 2² questioned.⁵ Whether the younger brother with the 4p16.3 duplication in this present family will show overgrowth features as an adolescent or adult remains to be seen but his current shape and size suggest that he may.

Phenotypic variation resulting from imprinting or partial trisomy of the other chromosome involved in the

Figure 4 Physical map of 4p16.3. The loci used are indicated in bold. Solid patterns indicate the regions translocated and the broken lines show undefined areas. The size of the region involved in each translocation is shown for our families 1, 2, 3, and 4. All deletions overlap with the newly refined WHSCR.⁷

translocation was not evident in our families where the probands had PRDS. At the molecular level both WHS and PRDS seem to have very similar deletions as the proximal breakpoint we have found is the same as that recently defined in WHS patients, that is, within D4S132.⁷

Two recent publications^{6,7} describe molecular studies of one WHS and six PRDS patients with a deletion of 4p16.3 in all cases. Only one of these resulted from an obvious translocation; no details of molecular family studies were given in the other cases. The breakpoints found were proximal to locus D4S180, that is, similar to the translocation breakpoints in our families 2 and 3 (fig 4). One cell line of a PRDS patient (MA117)⁷ showed a

breakpoint between loci D4S166 and D4S43, similar to our family 1.

The overlap in some of the clinical features of WHS and PRDS is probably the result of the overlap in the two critical regions (fig 3). The PRDS critical region may be entirely within the WHS critical region (WHSCR) but smaller, involving fewer gene(s); this could account for the relatively milder phenotype of PRDS compared to WHS. Another explanation might be that the critical regions of WHS and PRDS overlap in the middle, leaving out the distal end of WHS and the proximal end of the PRDS critical regions; the PRDS critical region could then include *FGFR3* which may be relevant for overgrowth. It could be that point mutations in different genes or in a different region of the same gene within the overlapped area of the PRDS and WHS critical regions might account for the difference in severity of the two syndromes. So far, WHS families have not been described with 4p translocations that have sibs with the overgrowth resulting from a duplication of 4p16.3.

There are reports of patients with possible WHS/PRDS who were not deleted for locus D4S96.^{4,8,9} As the critical region of WHS has been recently reduced to 260 kb by WHS patient data,⁷ this now excludes the locus D4S96 and provides one explanation for these patients. However, as the distal breakpoint for PRDS has not been determined, it may be that D4S96 is not included in the critical region either. Just as the WHSCR has been reduced in size to 260 kb, the same could be done with PRDS patients when such are found with interstitial deletions.

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Investigation of meiotic rearrangements in DGS/VCFS patients with a microdeletion 22q11.2

EDITOR—Microdeletions in 22q11.2 are associated in 80-90% of cases with DiGeorge syndrome (DGS, MIM 188400) or velocardiofacial syndrome (VCFS, MIM 192430) and occur with an estimated frequency of 1/4000 live births.¹ Most deletions are the result of a *de novo* event, although probably 6-28% of them are familial.² The phenotype of the patients is mainly characterised by conotruncal heart defect, cleft palate, immune deficiency, neonatal hypocalcaemia, and facial dysmorphism. The number of clinical symptoms varies substantially and their reduced expression can lead to a mild phenotype.³ There does not seem to be a correlation between the presence or the size of a microdeletion and the clinical manifestation of the syndrome. Molecular analyses have shown that most patients have a deletion of about 1.5 or 3 Mb.⁴⁻⁵ The length of the delineated minimal critical region for a DGS/VCFS phenotype, however, is only 480 kb.⁶ Reports of patients with a DGS/VCFS-like phenotype having a deletion in 10p⁷ led to the definition of a second critical region, DGSII. However, the incidence of 10p deletions is low in comparison to the rate of microdeletions in 22q.⁸⁻⁹ The high rate of sporadic microdeletions in 22q11.2 provides evidence for frequent meiotic rearrangements as a molecular basis for the development of this structural aberration.

In order to ascertain such rearrangements in patients with a 22q11.2 deletion, we performed haplotype analyses on five patients and their unaffected relatives using 11 polymorphic STRP markers from the DGS/VCFS critical region in 22q11.2 (fig 1). Furthermore, the haplotype analyses enabled us to determine the extent of the deletions in deletion carriers and the parental origin of the abnormal chromosome.

Microdeletion analysis was performed by fluorescence *in situ* hybridisation (FISH) on metaphase chromosomes prepared from fresh peripheral blood samples using DNA probes D22S75 (Oncor, Illkirch) or TUPLE1 (Vysis, Downers Grove, IL) from the DGS/VCFS critical region on 22q11.2.

In order to haplotype patients and their family members the parents and, if available, the grandparents of origin were analysed with 11 STRP markers using standard methods. Primer information was obtained from the Genome Data Base (GDB). If the grandparents of origin were not available, haplotyping was performed with the results obtained from healthy sibs of the patient and the parent of origin. A total of 30 family members were included in the study. The family pedigrees are shown in fig 1.

The STRP analyses allowed us to determine the deletion sizes in 22q11.2 of the investigated probands (fig 1). Patients F1-7, F2-8, F4-3, F4-8, and F5-3 had deletions of the 3 Mb type and patient F3-4 has a 1.5 Mb deletion. The parental origin of the aberrant chromosome was determined to be four times paternal and once maternal.

Haplotype analyses were performed to ascertain the developmental mechanism of the microdeletion. In four families (F1, F2, F3, F5), a parental unequal crossover was proven by the exchange of parental marker alleles flanking the deleted region (fig 1 and 2). In family F4, the underlying mechanism could be either an unequal crossover or an intrachromosomal rearrangement, as the microsatellite

markers proximal to the monosomic area did not allow us to distinguish between these mechanisms.

Additional crossover events are present in family F5 where proband F5-4 shows a rearrangement between D22S264 and D22S311 (fig 1) and in family F2 in probands F2-6 and F2-7 between D22S303 and D22S257 (fig 1).

In this study we were able to analyse in detail the 22q11.2 deletions present in five patients and one father from five unrelated families. Although there are differences in deletion size, it was not possible to delineate any significant correlation with the phenotypic manifestations. This is especially conspicuous in family F4 in which the father and son share an identical deletion. In this case the father displays only slight dysmorphic facial features and a cleft palate, while his son is more severely affected with ventricular septal defect (VSD), cerebral malformations, T cell defect, and marked developmental delay. This variation may be the result of the different maternal haplotype in each of them (fig 1, patients F4-3 and F4-6).

All proximal deletion breakpoints are flanked by the STRP marker D22S427. The distal breakpoint, however, is variable, being flanked in four cases by D22S306 and in one case each by D22S311 or D22S308. These findings are in agreement with previously defined deletion breakpoints where most patients showed a deleted interval of approximately 3 Mb flanked by D22S247 and D22S306.⁶ The genetic distance over this region is approximately 6 cM according to the Génethon and GDB linkage maps.

In our study the deletions were of maternal (n=1) as well as of paternal (n=4) origin which does not confirm the bias towards maternally derived deletions found in other studies.¹⁰ The haplotype analyses of the investigated families show that four of five deletions are the result of an unequal meiotic crossover event. In these cases the markers flanking the deletion breakpoints are derived from different parental chromosomes (fig 1 and 2). In family F4, it is not clear from the present data if the underlying event involved homologous pairing of chromosomes or exchanges between sister chromatids (fig 1). In our sample of five families, no statistical significance can be calculated, but the data confirm the findings from a previous investigation that the DiGeorge critical region (DGCR), though located near the centromere of chromosome 22, is subject to numerous meiotic recombinations, many of which lead to the formation of a microdeletion.¹¹ Patient F5-4 displays a crossover near D22S264 and D22S311 (fig 1). This is an interesting finding because these markers are located at common distal deletion breakpoints⁶ and underlines the presence of crossover mediating elements at the breakpoints of 22q11.2 deletions.^{5 12-14}

The mechanisms of microdeletion formation have been investigated in other syndromes as well. The critical region for Prader-Willi/Angelman syndrome (PWS/AS) (15q11-q13) is subject to above average rates of recombination and sex specific hotspots have been described.¹⁵ The deletions were caused by both intra- and interchromosomal recombination in the PWS/AS families investigated.^{16 17} The results obtained for deletions in 7q11.23 associated with Williams-Beuren syndrome (WBS) suggest that the majority of microdeletions in this region are caused by unequal crossover events.^{11 18 19} In comparison, in most informative DGS/VCFS families, the microdeletion 22q11.2 was associated with a crossover but an intrachromosomal rearrangement cannot be excluded in the remaining cases.^{5 11}

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Figure 1 Pedigrees of the investigated families. The index patients are indicated by arrows. Bars and numbers symbolise the individual chromosomes and the haplotypes of the tested STRP markers. Marker names are given on the left. In the case of a meiotic crossover, the abnormal chromosome 22 shows STRP markers from both chromosomes of the parent of origin. Unclear recombination breakpoints are indicated by white bars. Black symbol = microdeletion 22q11.2, black spot indicates parent of origin.

Figure 2 STRP analyses of markers from 22q11.2 in family F2 show that the deletion in patient F2-8 (black symbol) has developed from an unequal crossover event. The grandparental marker alleles (a grandpaternal, b grandmaternal) are given below. D22S427 defines the proximal breakpoint, D22S308 the distal breakpoint of the microdeletion, and D22S941 is located within the deleted area. In patient F2-8 the flanking alleles are derived grandmaternally proximal (b) and grandpaternally distal (a) to the deletion which confirms a meiotic crossover in the parent of origin (*F2-3).

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Pure trisomy 20p resulting from isochromosome formation and whole arm translocation

EDITOR—Approximately 33 cases of trisomy 20p have been reported.¹⁻¹⁰ Most cases are the product of reciprocal translocations with a few cases arising from inversions. A trisomy 20p syndrome has been difficult to delineate as many cases involve only partial trisomy, often in the presence of partial monosomy of the partner chromosome. We describe a case of pure trisomy 20p arising from de novo isochromosome formation associated with non-reciprocal translocation. This type of chromosome rearrangement is very rare and to date has been described only for isochromosome formation of chromosomes 4p, 5p, 7p, 9p, 10p, and 12p.¹¹⁻¹³ The rarity of these cases is the result of selection bias as only those partial trisomies compatible with life will be ascertained.

Our case, involving duplication 20p with no other chromosomal imbalance, is important to help delineate this syndrome, which is not yet clearly defined. The boy, now aged 19, had dysmorphic features, mild to moderate learning difficulties, osteopenia, and renal abnormalities. He was the second of two children of unrelated, normal Indian parents aged 35 years (mother) and 37 years (father).

His facial features included epicanthic folds and anteverted, flared nostrils as a baby. As a child and adult he had a low anterior hairline, coarse hair, and laterally arched eyebrows. He had a very prominent, large nose, noticeable as a young child, with a convex nasal bridge and anteverted nostrils, present to a much lesser degree in his father. He had short, upward slanting palpebral fissures, a featureless philtrum, thin vermilion border of the upper lip, and a prominent lower lip. His ears were large and low set with a bilaterally prominent antihelix. He had a high arched palate, moderate micrognathia, and dolichocephalic skull (figs 1 and 2).

He grew normally along the 10th centile, had a pubertal growth spurt at 12 years, and achieved a final height of 170 cm (25th centile), as expected from parental heights, and head circumference of 55 cm (normal).

Developmental delay was apparent with mild hypotonia as an infant, a delay in motor activity with walking achieved at 3 years, and poor coordination. Speech was particularly delayed and there were difficulties with articulation; his first words appearing at 2½ years with slow progression. He received both speech therapy and physiotherapy as an infant and attended a special school for moderate learning difficulties where his achievements were average. He was unable to take any formal examinations, though continued his education after completing his schooling.

He had a congenitally small right kidney, with reflux to the level of the renal pelvis. He had two urinary infections and required prophylactic antibiotics for two years. Serial

Figure 1 (A, B) Front and lateral view of the proband aged 13 months.

radioisotope DMSA (2,3-dimercaptosuccinic acid) renal scans showed that the right kidney contributed 8% of the total renal function. He was normotensive and had normal renal function tests. He also had a glandular hypospadias, slight chordee, and an undescended right testis (orchidopexy at 7 years). He also had an umbilical hernia as an infant, glue ears treated with grommets, and astigmatism of both eyes. He had no congenital heart defect.

At 13 months of age, radiographs showed marked generalised osteopenia with collapse of several vertebrae,

particularly the 7th and 9th thoracic, platyspondyly, biconcave vertebral bodies, and coarsening of the trabecular pattern (fig 3). He had bilateral coxa valga with subluxation of both hips on x ray as an infant. He sustained a Colles (distal radial) fracture when 9 years old. Bone densitometry scans confirmed the osteopenia and showed improvement (though not achieving normal levels) during puberty. Serial measurements of calcium, phosphate, and alkaline phosphatase, parathyroid hormone, and 25-hydroxy-cholecalciferol were unremarkable. Cortisol, testosterone,

Figure 2 (A, B) Front and lateral view of the proband aged 13 years.

Figure 3 Lateral x ray of the spine at 13 months of age.

Figure 4 *Der(4)t(4;20)(pter;q11.1),i(20)(q11.1)* shown by (A) G banding. (B) Whole chromosome paints for chromosomes 4 (Cy3) and 20 (FITC). (C) Subtelomeric probes for 20p (FITC) and 20q (Cy3) showing 20q signal on *der(4)t(4;20)*. (D) Subtelomeric probes for 4p (FITC) and 4q (Cy3), showing interstitial 4p signal near the breakpoint junction on *der(4)t(4;20)*. (E) All human telomeres probe (FITC) showing interstitial signal on *der(4)t(4;20)*. (F) Probe for D20Z1 (rhodamine) showing similar signals on the normal and isochromosome 20, but lack of signal at the breakpoint junction on *der(4)t(4;20)*. Chromosome 4 was identified by the centromeric signal of D4Z1 (rhodamine). (G) All human centromeres probe (FITC) showing signal at the breakpoint junction of *der(4)t(4;20)*. Chromosome 4 was identified by previous hybridisation with D4Z1 (rhodamine), resulting in a yellow signal.

24 hour urinary calcium, and urinary amino acid measurements were all normal.

G banded metaphase chromosomes were karyotyped after routine PHA stimulated peripheral blood culture. Chromosome analysis showed a male karyotype with an isochromosome for the short arm of chromosome 20 and translocation of the chromosome 20 long arm to the short arm of one chromosome 4 (fig 4A).

Examination of 100 cells showed no evidence of telocentric 20p, 20q, or other mosaicism. Both parents of the patient had a normal (46,XX and 46,XY) karyotype.

Further characterisation of the chromosome rearrangement was obtained from fluorescence in situ hybridisation (FISH) studies, in all cases performed following the manufacturer's instructions. Application of whole chromosome paints (WCP) (Cambio) for chromosomes 4 and 20 showed hybridisation of WCP 20 to the isochromosome and to the distal p arm of the *der(4)t(4;20)* confirming that the translocated material was derived from chromosome 20 (fig 4B).

FISH with probes (Cytocell) mapping to the subtelomere regions of 20p and 20q (fig 4C) showed 20q subtelomeric sequences on the *der(4)t(4;20)*, confirming that the translocated material was derived from the chromosome 20 long arm, and 20p signals were seen at both ends of the isochromosome, confirming it to be an isochromosome for the 20 short arm.

FISH with probes (Cytocell) mapping to the subtelomere regions of 4p and 4q (fig 4D) showed the signal near the breakpoint junction suggesting the possibility of a telomeric breakpoint in 4p. FISH with a probe specific to the Wolf-Hirschhorn region, mapping to D4S96 (Oncor), showed signal on both the normal and abnormal copies of

chromosome 4, indicating that there was no deletion of the Wolf-Hirschhorn critical region.

The all human telomeres probe (Oncor) showed an interstitial signal in addition to the expected terminal signals on the *der(4)t(4;20)*, confirming the presence of interstitial telomeric TTAGGG repeat sequence at the *t(4;20)* breakpoint junction (fig 4E). The presence of interstitial telomeric sequence confirmed that the rearrangement involved either breakage of the 4p telomere or fusion between the 4p telomere and sequence from chromosome 20.

C banding suggested a monocentric isochromosome although C band positive material was present in both arms of the isochromosome and was therefore larger than in the normal 20. No C band positive material was seen on the *der(4)* at the *t(4;20)* breakpoint junction.

Application of a chromosome 20 alpha satellite probe (D20Z1, Oncor), showed similar signals on the normal chromosome 20 and the isochromosome 20p, again suggesting a monocentric isochromosome. The *der(4)t(4;20)*, identified by hybridisation with a chromosome 4 alpha satellite probe (D4Z1 Oncor), showed no signal with D20Z1 at the breakpoint junction (fig 4F).

The all human centromeres probe labelled with FITC (Oncor) gave a very small but unambiguous signal at the *der(4)t(4;20)* breakpoint junction in a proportion of cells (fig 4G). Signal was seen on both chromatids in nine of the 27 cells, on one chromatid in seven cells, and six cells were negative. This variation in signal is almost certainly because of its small size and is not considered to be suggestive of mosaicism. *Der(4)t(4;20)* was identified by sequential hybridisation with the rhodamine labelled chromosome 4 alpha satellite probe resulting in a yellow signal.

Table 1 Clinical features of 32 cases of trisomy 20p*

	Fraction	Percentage	Our case
Sex	16F/16M		M
Gestation	21/25 (21=term)		Term
Birth weight (g)	1800–4300		2500
Growth normal	15/21	71	+
Mental retardation	29/31 (moderate/severe)	94	+ (moderate)
Poor coordination	18/19	95	+
Language delay	21/22	95	+
Occipital flattening	11/22	50	–
Low posterior hairline	3/4	75	–
Coarse hair	14/18	78	+
Thick, arched eyebrows	2/2	100	+
Epicanthus	8/26	31	+
Strabismus	9/23	39	–
Increased inner canthal distance	11/21	52	–
Upward slanting palpebral fissures	19/29	66	+
Round face, prominent cheeks	24/29	83	–
Short, upturned nose	17/30	57	–
Large flared nostrils	16/30	53	+
Large/abnormal ears	11/11	100	+
Moderate microgathia	16/22	73	+
High arched/cleft palate	8/11	73	+
Short neck	5/5	100	–
Cardiac anomalies	10/28	36	–
Renal anomalies	6/8	75	+
Vertebral anomalies	17/21	81	+
Osteopenia	2/2	100	+
Hip/pelvis anomalies	6/6	100	+
Digital anomalies	12/13	92	–
Feet anomalies	14/15	93	–
Dental anomalies	13/19	68	–
Umbilical/inguinal hernia	6/7	86	+

+ : present, – : absent.

*Taken from 32 cases described.^{1–6 8–10}

The karyotype was interpreted as 46,XY,der(4)t(4;20)(pter;q11.1),i(20)(q11.1). The translocation is non-reciprocal and the patient therefore appears to be trisomic for 20p without any concomitant loss of material from 4p.

We have described a patient with pure trisomy 20p as a result of a rare type of de novo non-reciprocal chromosome rearrangement involving formation of an isochromosome by a whole p arm and translocation of the residual q arm to another chromosome. Rearrangements of this type are thought to arise from centromere fission, or pericentric breakage followed by isochromosome formation by the p arm and translocation (or fusion) of the acentric or telocentric q arm to the telomere of another chromosome.¹⁴ They are thought to be mediated by the presence of intrachromosomal telomere-like repeats which have been detected at many sites including the centromeres of chromosomes 1–6, 8–11, 16, 17, and 20.¹⁵ The presence of both alphoid sequences and interstitial telomere sequence has been shown in three cases of telomere-centromere fusion. In two cases the translocation involved an acrocentric chromosome and loss of the acrocentric short arm.¹⁶ The third case involved translocation of 12q to 8p with i(12p) formation.¹³

We were able to show both centromeric repeat sequence and interstitial telomeric sequence at the t(4;20) breakpoint junction. Our results support the interpretation of a break in the pericentric long arm of chromosome 20 close to the end of the alphoid sequences, leading to formation of an isochromosome with fused centromeres and therefore a monocentric appearance. The absence of mosaicism involving normal cells or telocentric 20p or 20q supports a single step aetiology.

As our patient is an example of pure trisomy 20p, the features are of particular importance in helping to delineate the syndrome. Although there are many similarities with previously described patients with trisomy 20p, the most striking differences in our patient are the very prominent nose and the osteopenia (table 1). Van Langen *et al.*¹⁷ produced a table of the clinical picture seen in trisomy 20p; however, 15 cases were included twice in their table

(Centerwall and Francke,¹ 13 cases; Delicado *et al.*,¹⁸ one case; Schinzel,¹⁹ one case), thus producing inaccurate figures.

Moderate mental retardation and poor coordination are fairly consistent findings, as is a marked speech delay (with mutism reported in two cases by Taylor *et al.*²⁰), all of which were present in our case. The dysmorphic features of our case have similarities with some previously reported cases. The distinctive nose is an unusual feature, as many other cases had a short, upturned nose (10 reported in younger children and seven in older children or adults). However, the nose in our patient has a similar appearance to the cases described by Grammatico *et al.*⁴ (case 1) and Balesstrazzi *et al.*²¹

The thick, high arched eyebrows seen in our case were also noted by Grammatico *et al.*⁴ Thick eyebrows were noted by Rudd *et al.*²² and thin, high arched eyebrows by Funderburk *et al.*²³ Epicanthic folds and upward slanting palpebral fissures are frequently seen. Short palpebral fissures have been occasionally described.^{9 19 24}

Renal anomalies have been seen before, but a congenitally hypoplastic kidney, as in our case, has not been seen. Previously reported renal anomalies include duplicated urinary tract and hydronephrosis,²⁰ bilateral polycystic kidneys,^{8 25} ectopic kidney,²⁴ and duplication of the left renal collecting system.² Hypospadias, as seen here, has been reported in two previous cases.^{20 26} Cryptorchidism was previously reported by Schinzel *et al.*¹⁹ A case involving macro-orchidism has been described,²¹ and one involving a ventrally positioned clitoris and anus.²²

Our patient had striking osteopenia first noted at a very young age (13 months). Two previous reports exist of a generalised osteoporosis.^{2 19} Coxa valga deformity of the hips as seen in our case has been previously reported.^{19 20 25} Skeletal anomalies in trisomy 20p appear to be a variable phenomenon, with vertebral abnormalities the most commonly reported, including fusion of vertebrae, reduction of intervertebral spaces, spina bifida, scoliosis, and kyphosis.

Our case is unusual in being the first reported case of a centromere-telomere fusion resulting in trisomy 20p.

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A *SALL1* mutation causes a branchio-oto-renal syndrome-like phenotype

EDITOR—The Townes-Brocks syndrome (TBS, MIM 107480) is an autosomal dominantly inherited association of imperforate anus, supernumerary/triphalangeal thumbs, and dysplastic ears. In addition to this, sensorineural or conductive hearing loss, renal malformations, cardiac defects, and mental retardation maybe present in affected subjects. TBS is caused by mutations of the putative zinc finger transcription factor gene *SALL1*.² *SALL1* has four double zinc finger domains which are evenly distributed over the protein.³ The majority of *SALL1* mutations identified to date in TBS patients are located 5' to the first double zinc finger encoding region.⁴⁻⁵ Most mutations (nonsense mutations, small insertions/deletions, and one larger deletion) have been predicted to result in prematurely truncated proteins lacking all double zinc finger domains presumed to be essential for *SALL1* gene function or to result in unstable transcripts, thus causing TBS via haploinsufficiency.⁴⁻⁵

TBS is known to overlap phenotypically with other conditions like Goldenhar syndrome, VACTERL association, or oculo-auriculo-vertebral spectrum.¹ However, a *SALL1* mutation has so far only been reported in one patient with a clinical picture attributable both to Goldenhar syndrome and TBS.⁶ This patient, as well as the TBS patients in whom mutations were detected, showed at least two out of three major criteria for TBS, that is, malformations of the thumbs, ears, or anus.⁴⁻⁵ Therefore, the phenotypic spectrum associated with *SALL1* mutations seemed to be quite characteristic.

Here we report the first family in which a *SALL1* mutation is associated with a phenotype which is different from TBS. The two affected daughters, their affected father, and the unaffected mother (fig 1A) were examined for *SALL1* mutations after giving informed consent to the analysis. In all affected subjects, chromosome analysis before DNA studies had shown a normal karyotype. The older girl, now aged 19, was admitted to hospital as a baby because of failure to thrive which turned out to be because of renal failure. On examination, both kidneys were found to be hypoplastic. After treatment, renal function recovered but remained impaired. Besides the renal problems, mildly dysplastic ears with slight overfolding of the superior helices were seen (fig 1D). She also showed pes planus and an unusual bony fusion in one foot. Further findings include mild developmental delay with an IQ of 71 at the age of 18 (verbal 73, performance 74, assessed by WISCII), mild sensorineural hearing loss, mild hypermetropia, gastro-oesophageal reflux resulting from a small hiatus hernia, and chronic abdominal pain. She has no anal or thumb malformation (fig 1E). Her sister, now aged 13, was born with bilateral dysplastic ears (fig 1F) and preauricular tags on one side. As a baby, she had bilateral grade 3 vesicoureteric reflux and bilateral hypoplastic kidneys. Her kidney function is mildly impaired. Like her sister, she has hypermetropia and gastro-oesophageal reflux. She also has dental crowding, mild developmental delay (IQ 71), and mild bilateral high frequency hearing loss, but no anal or thumb malformation (fig 1G). The mother has no health problems, whereas her husband shows impaired renal function, based on thin membrane disease with focal glomerulosclerosis, and dysplastic ears. He also has a Barrett ulcer resulting from chronic gastro-oesophageal reflux.

Genomic DNA was prepared from peripheral lymphocytes by routine procedures. *SALL1* mutation analysis

Figure 1 (A) Pedigree of the family reported here. Both daughters (II.1 and II.2) and their affected father (I.1) show the *SALL1* mutation (B, upper part), whereas the mother (I.2) shows the wild type sequence (B, lower part). The position of the mutation with respect to the *SALL1* protein is shown in (C) possibly resulting in a truncated protein lacking double zinc finger domains 2-4 (zinc fingers are indicated by oval symbols). (D-G) Ears and hands of the affected girls: (D, E) II.1 aged 19, (F, G) II.2 aged 13. Note typical overfolded superior helices (D, F), but absence of thumb malformations (E, G) in both patients, which was also confirmed by x ray (not shown).

was performed by PCR amplification and direct sequencing of PCR products as described previously.⁷ Both affected children as well as the affected father had a heterozygous 1819delG *SALL1* mutation, which is located in exon 2 between the coding regions for the first and the second double zinc finger unit (fig 1C).

Most *SALL1* mutations previously reported in TBS reside in exon 2 5' of the coding region for the first double zinc finger domain and are predicted to result in *SALL1* haploinsufficiency.^{4,5} 1819delG is yet another short deletion but is located 3' of the region where most previously known mutations cluster. While this mutation could result in a prematurely terminated *SALL1* protein lacking double zinc finger domains 2-4, it remains unclear if the mutated transcript and the corresponding protein are indeed expressed. Mutations at similar positions have been reported by Marlin *et al.*⁴ Here, the patients with the mutations 1565delC or 1966del10 showed at least two out of three major criteria for TBS. Both mutations should have a similar effect (translation stop at nucleotides 1624-1626 and 2074-2076) as compared to the mutation reported here (translation stop at nucleotides 1924-1926). Therefore, if proteins were translated from these mutated alleles, all would be missing the double zinc finger domains 2-4. The most likely effect, however, is that such mutations would lead to an unstable transcript and thereby to *SALL1* haploinsufficiency.

The clinical presentation in the family reported here is quite unusual, since all affected family members do not have thumb or anal malformations. Therefore, TBS could not be diagnosed. While the features in the patients

carrying the 1565delC or 1966del10 mutation⁴ can be explained by haploinsufficiency for *SALL1*, the clinical picture in the family shown here could suggest that the 1819delG mutation has a different effect. As seen with mutations in *GLI3*, truncating mutations at similar positions may lead to either Pallister-Hall syndrome (PHS), different polydactylies, or Greig cephalopolysyndactyly syndrome (GCPS), suggesting that some might result in *GLI3* haploinsufficiency while others lead to truncated proteins with aberrant functions.^{7,8}

Based on these observations one could speculate that, unlike 1565delC or 1966del10, the 1819delG mutation does not result in an unstable mRNA but in a truncated *SALL1* protein. On the other hand, TBS is known to vary even between families with the same mutation, thus pointing to a strong influence of modifying genes or environmental factors. Yet, while families with *SALL1* mutations may differ from each other with respect to the occurrence of renal or cardiac malformations, no family has been reported to date in which all affected members have a *SALL1* mutation but not TBS.

Patients like those reported here may instead carry the diagnosis of branchio-oto-renal (BOR) syndrome (MIM 113650), as was initially suspected in our patients based on the combination of dysplastic ears, hearing loss, and hypoplastic kidneys. However, BOR syndrome patients commonly present with cup shaped ears, preauricular pits, and branchial fistulae,⁹ none of which was seen in our patients. In addition, gastro-oesophageal reflux and borderline mental retardation are not typical of BOR syndrome.⁹

Therefore, our report shows that a phenotypic overlap not only exists between TBS and VACTERL association, oculo-auriculo-vertebral spectrum, or Goldenhar syndrome, but also between TBS and BOR syndrome. *SALL1* mutation analysis should therefore be considered for patients who present with dysplastic ears, hearing loss, and renal malformations but do not have a causative mutation in the *EYA1* gene.¹⁰

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New MR/MCA syndrome with distinct facial appearance and general habitus, broad and webbed neck, hypoplastic inverted nipples, epilepsy, and pachygyria of the frontal lobes

EDITOR—We present the clinical histories and physical findings in two unrelated, severely mentally retarded males, now 14 and 11 years old.

Patient 1, a male, was born as the second and youngest child of healthy, unrelated Flemish parents with normal family histories. Pregnancy and delivery at 38 weeks' gestation were normal. Birth weight was 3200 g, length 47 cm, and head circumference 34 cm. Immediately after birth a number of dysmorphic signs were noted by the paediatrician, including facial oedema with ptosis of both eyelids, temporal flattening, hypertelorism, webbed neck, broad thorax with widely spaced, small, inverted nipples, shallow scrotum, and testes in the inguinal canal. The hands were broad and short with permanent oedema on the dorsum and the skin was loose and hyperextensible, especially on the arms. The diagnosis of Noonan syndrome was considered. Cardiac and renal echography was normal. Prometaphase chromosome studies on a peripheral blood lymphocyte culture showed a 46,XY normal male karyotype after G and R banding. Except for excessive weight loss, down to 2600 g, no major problems were noted in the neonatal period. In the first two years of life mild psychomotor retardation was noted with discrete hypertonia of the lower limbs. He started to walk without support on tiptoes at the age of 19 months. At the age of 2 years mental age was 15 months on the Bayley Developmental Scale.

At the age of 3 years, the first episodes of epileptic attacks were noted with variable clinical presentation of the grand

mal, petit mal, and myoclonic types. Seizures were resistant to anti-epileptic therapy and, from that age onwards, severe behavioural problems were noted with chaotic and destructive tantrums. EEG was diffusely disturbed with generalisation from a right frontotemporal focus. Brain MRI showed diffuse pachygyria, most evident in the frontal lobes. Ophthalmological examinations and x ray skeletal survey were normal. At that age, weight was 14.5 kg (25th centile), length 91 cm (25th centile), and head circumference 48 cm (3rd centile). At the age of 6 years mental retardation was severe (mental age of 2 years, SON-R)F. Neurological examination showed fine motor coordination problems and mild signs of spastic paraparesis. He walked without support with 20-30° extension deficit of both knees and 20° extension deficit of both elbows. Now, at the age of 11 years, he is severely mentally retarded with no progress in psychomotor development and has persistent epileptic fits.

Figs 1A and 2A and C illustrate the craniofacial dysmorphism, the general habitus, and the chaotic behaviour. Craniofacial appearance is distinct with oedema, narrowing of the frontal part of the skull, arched eyebrows, trigonocephaly, bilateral ptosis, hypertelorism, a large mouth with a fine upper lip and everted lower lip, prominent upper central incisors, posteriorly rotated ears with underdeveloped antihelix, and a high arched palate. The neck is broad, short, and webbed with a low posterior hairline. The upper part of the thorax is narrow, and the nipples are widely spaced, hypoplastic, and inverted. The hands are broad with tapering fingers.

Extensive metabolic screening has been performed over the years with normal results. Chromosome studies on a fibroblast culture after skin biopsy of the left upper arm were normal. Genital development is prepubertal with small testes (5 ml) in the inguinal canal. Hormone studies (LH, FSH, and plasma testosterone) showed normal prepubertal results. Height, weight, and head circumference are on the 3rd centile for age.

Figure 1 Similar craniofacial dysmorphism in both males, (A) patient 1 and (B) patient 2. Note the facial oedema.

Patient 2, a male, is the third child of a non-consanguineous European couple. He has two normal brothers, 16 and 9 years old respectively, and a 7 year old normal sister. Pregnancy was unremarkable. Birth weight was 3200 g. He had facial oedema and it was noted that he had significant redundant skin over the nape of the neck. Furthermore, he had significant weight loss in the neonatal period, losing more than 500 g in weight. He did not have any feeding difficulties. He crawled at 9 months and walked on tiptoes by 18 months. He had mild speech delay and his development was reasonable until the age of 5 years when he began to have persistent seizures which so far are not totally controlled with anti-epileptic medication. A brain CT scan showed pachygyria of the frontal lobes. His mental development deteriorated from the onset of the seizures and currently his mental function is at the 5 year level. In the past he has had surgery for squint, as well as for ptosis. Bilateral vesicoureteral reflux with hydronephrosis was also diagnosed in infancy and required bilateral ureteral reimplantation.

At present (figs 1B and 2B, D), height is 136 cm (3rd centile is 143 cm) and head circumference 53.5 cm (25th centile). Craniofacial appearance is distinct with thick hair, low frontal hairline, significant narrowing of the frontal part of the skull, facial oedema, bushy, arched eyebrows, a broad root and bridge of the nose, and persistent bilateral ptosis. He has a long, flat philtrum, a thin upper lip, micrognathia, and everted lower lip. The palate is high arched and the upper central incisors are prominent. The ears are protuberant, posteriorly rotated, and with underdeveloped antihelices. The neck is broad, short, and webbed with a low posterior hairline. The upper part of the thorax is relatively small with broadly spaced, hypoplastic, and inverted nipples. The hands are broad with proximally placed thumbs. He is able to walk without support, with 20–30° extension deficit of the knees and some difficulties in fully extending the elbows.

Chromosome studies on a peripheral blood lymphocyte culture showed a 46,XY normal male karyotype on G banding.

The two unrelated males present, as described above, a remarkably similar MCA/MR syndrome and their clinical

history is also identical. Patient 1 has been followed in Leuven since the neonatal period and patient 2 has been followed in Auckland. The striking resemblance between the two patients was recognised almost by coincidence on the occasion of the exchange of data on patients with distinct but hitherto unidentified MCA/MR syndromes. As described, their craniofacial appearance is particularly similar. Both males presented at birth with facial oedema, and it is still evident at their present respective ages of 14 and 11 years. The significant weight loss in both patients in the neonatal period indicates that they had more generalised fetal oedema and, especially in patient 1, in the postnatal period the skin was loose and hyperextensible, most evident on the arms. Based on the combination of facial oedema, ptosis of the eyelids, webbed neck with low posterior hairline, and broad thorax with widely spaced nipples, the diagnosis of Noonan syndrome was considered in patient 1.¹ However, the clinical follow up and evolution with age were not compatible with this diagnosis. Another remarkable finding in both males was their lack of psychomotor evolution with age. At the respective ages of 3 and 5 years, epileptic attacks began, which so far cannot be controlled despite a great variety of anti-epileptic medication. In both patients brain CT and MRI scan showed pachygyria, most pronounced in the frontal lobes. Up to the start of the complex epileptic fits, psychomotor development was only mildly to moderately retarded, but since the onset of seizures mental development has deteriorated. At the present time, both males are severely to profoundly mentally retarded and, especially in patient 1, major behavioural problems are now present. Also the general habitus of both males is identical. Whereas no specific neurological abnormalities are present, except walking on tiptoes with mild signs of spastic diplegia at a young age, both males walk independently but with 20–30° extension deficit of both knees, and both have difficulties in fully extending their elbows.

The MCA/MR syndrome present in these two males thus combines the following major symptoms: (1) distinct facies with oedema and notable postnatal weight loss;

Figure 2 The general habitus with similar dysmorphic signs and behaviour, (A, C) patient 1 and (B, D) patient 2.

(2) broad and webbed neck; (3) hypoplastic, inverted nipples; (4) limited extension of elbows and knees resulting in a characteristic general habitus; and (5) complex epilepsy in early childhood with deterioration of mental development and pachygyria on brain imaging.

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The mitochondrial genome in Wolfram syndrome

EDITOR—Wolfram syndrome is the association of juvenile onset diabetes mellitus and optic atrophy,¹ also known as DIDMOAD (diabetes insipidus, diabetes mellitus, optic atrophy, and deafness). This is a progressive, neurodegenerative disorder, with diabetes mellitus and optic atrophy presenting in the first decade,² cranial diabetes insipidus, and sensorineural deafness in the second, and neuropathic bladder in the third, followed by neurological complications (cerebellar ataxia, myoclonus) and psychiatric illness in the fourth decade. The clinical phenotype is consistent with an ATP supply defect, suggesting a mitochondrial mediated disease.³ Mitochondrial genome deletions and pathogenic point mutations^{4,5} have been described in Wolfram patients. A nuclear gene *WFS1*, wolframin,^{6,7} was recently identified, encoding a polypeptide of 890 amino acids. Wolfram syndrome thus appears to be genetically heterogeneous. Recently, a distinct “mitochondrial haplotype” was described.⁸ Because recombination does not appear to be characteristic of mtDNA, the accumulation of polymorphisms can be used as a “genetic clock” to estimate diversity within and between populations.⁹ A cluster of nucleotide exchanges at nucleotide positions 4216 and 11 251 roughly distinguished a series of 6/8 Wolfram patients from controls and patients with Leber hereditary optic neuropathy (LHON). The authors suggested that these mtDNA variants may predispose to Wolfram syndrome.⁸ We investigated our cohort of 50 Wolfram syndrome patients¹⁰ for evidence of a distinct mitochondrial haplotype and mitochondrial DNA rearrangements.

Patients for this study were included from a cohort recruited nationally in the UK.¹⁰ Minimal ascertainment criteria were juvenile onset (less than 30 years of age) diabetes mellitus and optic atrophy. These were chosen as the only features consistently present and earliest to develop in 166/168 case reports.¹¹ Diabetes mellitus was defined as a fasting plasma glucose of more than 6.0 mmol/l (>3 SD above the mean of the normal population). All affected patients had been examined, with pupils dilated, by an experienced ophthalmologist. All patients were visited at home; blood samples were obtained from all available family members after informed consent was given. DNA was extracted from whole blood using Puregene DNA extraction kits (Gentra Systems), according to the manufacturers' instructions, and diluted to stock solutions of 500 ng/μl.

The mitochondrial haplotype of the Wolfram patients was investigated using PCR and direct sequencing of the region between 16 050 and 16 400 in the first hypervariable region of the large non-coding region as previously described.¹² Mitochondrial haplotype analysis was carried out on 32 Wolfram families of European origin. The presence of mtDNA variants at bp 4216, 11 251, and 15 257 were assessed using PCR and restriction digestion.^{9,13} Mean pairwise differences¹⁴ were estimated and compared with a control data set comprising 100¹⁵ and 60 UK white subjects.¹⁶ An unrooted tree was drawn based on previous studies of Europeans. A PCR *ApaI* restriction site assay was used to screen for the mitochondrial tRNA Leu (UUR) A to G (3243) mutation¹⁷; this has been associated with maternally transmitted diabetes and deafness.¹⁸ The final cycle of PCR was labelled with ³³P-dCTP permitting

detection of the mutation at levels of heteroplasmy below 1%. DNA from whole blood was also screened for the 11778A:T and 3460A:T mutations associated with Leber's hereditary optic neuropathy (LHON) using allele specific PCR.¹⁹ These mutations were chosen as they account for 75% of all cases of LHON. The 14484 mutation was not investigated as it is phenotypically milder and has a better outcome.²⁰ Mitochondrial DNA was also analysed for the presence of major rearrangements by long range PCR²¹; PCRs were performed in a final volume of 50 μl with 0.25 μl each of primers L1 (nt2695–2720) and H3 (nt16459–16436), 2.5 mmol/l MgCl₂, 0.25 mmol/l of each dNTP, 1 × Bio-Optiform 111 buffer (Bioline), and 1.5 U Bio-X-ACT *Taq* polymerase (Bioline). PCRs were hot started at 80°C by the addition of 20–50 ng DNA and denatured at 95°C for 10 seconds and 68°C for 10 minutes plus 30 seconds for each subsequent cycle (25 cycles). PCR products were run on 0.7% Seakem agarose gels. The resolution for long range PCR is about 1 kb (sometimes 0.5 kb depending on the gel).

Needle muscle biopsies under local anaesthetic were undertaken on nine adult Wolfram patients. Informed, written consent was obtained, after a written and verbal explanation. Respiratory chain activity was analysed in fresh muscle biopsy samples from six patients by two separate methodologies. Flux experiments measuring rotenone sensitive cytochrome c reductase with glutamate, 2-oxoglutarate, and pyruvate + malate as substrates to assay complexes I and III, and antimycin A sensitive succinate cytochrome reductase for complexes II and III, were carried out.^{22,23} Flux experiments on cytochrome oxidase for complex IV were also performed. Specific assays using n-decylubiquinone for complex I and the reduced form for complex III were also used.²⁴ The reference values were calculated from a control population of 100, which included normal muscle obtained from orthopaedic procedures, and muscle biopsy tissue from patients with neuromuscular disease including non-mitochondrial metabolic abnormalities, such as McArdle disease. Histochemistry results were available for three additional patients for cytochrome oxidase, succinate dehydrogenase, and NADH reductase. The study was approved by the ethics committee of South Birmingham district health authority.

The clinical features of our cohort of 45 patients have been described.¹⁰ There were 29 index patients (14 male, 15 female) and 16 secondary patients (all sibs, seven male and nine female). Twenty seven of the 29 families were white UK patients. In addition, we included three affected sibs from Ireland and two affected sibs from New Zealand. The mitochondrial haplotypes of 32 white UK Wolfram patients are shown in the “unrooted tree” (fig 1). There was no evidence of clustering of the Wolfram haplotypes by eye or by calculating the mean number of differences between subjects in the Wolfram and control populations.¹⁴ Only 4/32 (12%) fell into haplogroups 2A and 2B (equivalent to J and T of Torroni *et al*²⁵ and collectively to 4216+11251 of Hofmann *et al*⁶), which was less than the expected 6 based on prevalence of these lineages in our control population. This proportion was significantly different from the 7/8 (88% p=0.0001, Fisher's exact test) Wolfram patients found in this haplogroup by Hofmann *et al*.⁸ Hence there was no evidence of a founder mitochondrial haplotype in our patients. The patients were then divided into those from families showing genetic linkage to the Wolfram locus on chromosome 4p and sporadic cases. Neither group showed evidence of haplotype clustering either on the unrooted tree or by comparison of the mean

Table 1 Mitochondrial respiratory chain activities

	Patients						
Assays	4	14	30	32	23	34	Reference value
Flux experiments							
Complexes I and III							
Pyruvate + malate cytochrome oxidase	0.245	0.327	0.426	0.164	0.418	0.139	0.1–1.0
Glutamate cytochrome reductase	0.009	0.019	0.098	0.147	0.262	0.025	0.1–1.0
Oxoglutarate cytochrome reductase	0.296	0.164	0.426	0.115	0.303	0.115	0.1–1.0
Complexes II and III							
Succinate cytochrome reductase	0.475	0.139	0.672	0.409	0.409	0.254	0.1–6.0
Complex IV							
Cytochrome oxidase	0.395	0.299	0.901	0.655	0.984	0.59	0.1–5.0
Specific assays							
Complex I			0.836	0.409	0.861	0.091	0.1–1.0
Complex II			4.918	9.835	10.359	1.967	3.0–15.0

Respiratory chain activities expressed as $\mu\text{mol/g/min}$ (rate constant k/g/min for COX). Complexes I and III are specific assays. Patients 4, 14, 30, and 32 have been previously reported.²⁶

were reported at nucleotide positions 4216 and 11 251 (designated haplogroup B by Hofmann *et al.*,⁸ haplogroup 2B by Richards *et al.*,²⁸ and haplogroup T by Torroni *et al.*²⁹), which was found in 6/8 (75%) of Wolfram patients but in only 13/67 (19%) controls. Only 2/28 (7%) of our patients fell within this haplogroup and there was no evidence of polymorphism clustering in the remainder of our cohort of 40 patients. We conclude that because of the small size of the previous study, the results most probably occurred by chance.⁸ On the other hand, it is clear that in LHON the 11 778 A:T mutation is associated with sequence changes at base pairs 4216 and 13 708 (which define haplogroup A of Hofmann *et al.*,⁸ 2A of Richards *et al.*,²⁸ and J of Torroni *et al.*²⁹). This suggests that mtDNA sequence changes found in this haplogroup (not necessarily the 4216 and 13 708 variants) may play an aetiological role in LHON. Similarly, recent studies suggest that a common variant in the non-coding region of mtDNA at bp 16189 is associated with insulin resistance in adult life.²⁹ It remains possible that an unrecognised mitochondrial DNA variant may predispose to Wolfram syndrome.

Secondly, we found no abnormal mitochondrial function or mtDNA mutations in our series. Our search for mtDNA rearrangements only excluded them from one tissue (lymphocytes); one might expect patients to harbour mutations in other non-dividing tissues such as brain or pancreas. Wolfram syndrome is a neurodegenerative disease; it is known that mtDNA mutations normally accumulate with age in postmitotic tissues, and this may be increased in neurodegenerative disease.³⁰ We believe that the phenotypes of patients in whom mtDNA mutations have been reported are not typical of the majority of our cases and will not prove to have mutations in *WFS1*. The recent identification of a Wolfram syndrome gene (*WFS1*, wolframin), allowed us to screen our patients for mutations.²⁷ We found loss of function mutations in *WFS1* in 16 of the 17 families investigated. At present, the intracellular location of the *WFS1* protein is not known. It is expressed in most organs, but exact localisation awaits the development of specific antibodies. The amino acid sequence does not have significant homology with other proteins in the databases and there is no evidence that the *WFS1* protein has a mitochondrial targeting sequence.

Our study has implications for clinical practice; definition of inherited diabetes syndromes at a molecular level will help us to distinguish overlapping clinical phenotypes such as Wolfram syndrome and mitochondrial diabetes and deafness. In addition, exclusion of a role for mitochondrial DNA should simplify genetic counselling for families with Wolfram syndrome.

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Two further cases of Sener syndrome: frontonasal dysplasia and dilated Virchow-Robin spaces

EDITOR—Case 1, a male, was born at 38 weeks' gestation by normal delivery to unrelated parents. Mild bilateral renal pelvis dilatation was noted on prenatal scans as was a slight increase in liquor volume. An amniocentesis, which was performed for maternal age, showed a 46,XY karyotype. His birth weight was 2593 g (9th centile), length 47.5 cm (50th centile), and OFC 32.7 cm (50th centile). Features noted at birth included neck oedema, a large anterior fontanelle (6 cm × 7 cm), a short penis with a large scrotum, and an anteriorly placed anus. Echocardiography showed a patent ductus arteriosus with mild septal and right ventricular wall hypertrophy. His cranial ultrasound was reported to show partial agenesis of the corpus callosum. Postnatal renal ultrasound showed mild dilatation of the left pelvicalyceal system. At 7 months his growth parameters were weight 6.37 kg (0.4th centile), length 64.5 cm (2nd centile), and OFC 44 cm (25th centile).

Bilateral inguinal herniae were present from 3 months and repaired at 4 months of age. Ophthalmological examination showed a hypoplastic left disc and a small coloboma of the right disc. He also has hypermetropic astigmatism and bilateral entropion which required surgery. During his first year he developed eczema and persistent diarrhoea. He sat at 7 months, crawled at 1 year, and walked independently at 2 years. His linear growth progressed close to the 3rd centile and his OFC followed the 90th centile. The facial features of note are his wide mouth, long, smooth philtrum, and small posteriorly angulated ears (fig 1). His hair grows well but is brittle and coarse. He has one extra tooth in the mandible and all his teeth are irregular and pointed. He was due to attend mainstream school. He had required speech therapy for delayed language. Urinary mucopolysaccharides, oligosaccharides, and white cell enzymes were normal. The triglycerides were at the upper range of normal at 1.4 mmol/l (range 0.5-1.8 mmol/l) and the cholesterol was at the lower range of normal (2.8 mmol/l, normal range approximately 1.7-5.2 mmol). A 7-dehydrocholesterol result was not available as the child left the country before this test became available. In addition, there were no records of the parental cholesterol levels. Thyroid function at 10 months

showed T4 107 µmol/l and TSH 5.7 IU/l. Blood chromosomal analysis showed a normal 46,XY karyotype. Skeletal survey was normal.

Case 2, a male, was born at 32 weeks' gestation, weighing 2000 g, to unrelated parents, following spontaneous onset of labour and a normal delivery. There was a history of polyhydramnios during the pregnancy. He has one normal female sib. He was admitted to the special care baby unit for five weeks because of his prematurity. Dysmorphic features noted at birth included hypertelorism, a persistent large posterior fontanelle, a large anterior fontanelle, a narrow, high arched palate with midline cleft of the upper alveolar margin, two neonatal teeth, and a right inguinal hernia (fig 2).

Motor development was noted to be delayed; he sat at 10 months and walked at 18 months. He has had both inguinal and umbilical hernia repairs. He has required speech therapy and requires special help at school. At the age of 8 years, he is hyperactive and is said to have an

Figure 1 (A, B) Front and side view of case 1 showing hypertelorism, a wide mouth, and posteriorly rotated ears.

Figure 2 (A, B, C) Front and side view of case 2 aged 7 months and 5 years showing hypertelorism, a wide mouth, and posteriorly rotated ears.

attention deficit disorder. Ophthalmological assessment showed hypermetropic astigmatism. He was also noted to have tortuosity of the fundal vessels. On examination aged 8 years, his weight (24.3 kg) and head circumference (51 cm) were on the 25th centile and his height was <25th centile (118.5 cm). He had coarse hair with two crowns. In addition, he was noted to have abnormally shaped, slender teeth. An orthopantomogram showed abnormalities of several of the teeth in the position of the incisors and canines. Many were slender and canine-like. In addition, some deciduous teeth were absent. At the age of 8, none of his adult teeth have erupted. The possibility of an ectodermal dysplasia was raised, although following consultation with a dermatologist this was excluded.

Urinary amino and organic acids (including glycosaminoglycans), performed on two separate occasions, excluded a mucopolysaccharide disorder. Cholesterol and triglyceride levels, measured on two occasions, were normal. A 7-dehydrocholesterol level was also normal. A skeletal survey was normal. Chromosomal analysis showed a normal 46,XY karyotype.

In case 1, a CT scan was reported to show diffuse low density areas in the cerebral white matter bilaterally. The corpus callosum was hypoplastic. MRI scan aged 18 months showed multiple cystic areas within the white matter radiating out at right angles from the ventricles into all lobes, but especially the parietal, occipital, and temporal lobes (fig 3) Their signal intensity paralleled that of CSF.

Figure 3 Case 1, aged 4 years. MRI of the head/sagittal T₁W + coronal/axial T₁W, showing multiple, well defined areas of CSF signal intensity throughout both cerebral hemispheres, especially in the periventricular white matter. They are of varying sizes, being seen as linear streaks in the cortex but oval or rounded areas in the white matter. All tend to be orientated at right angles to the lateral ventricles.

The basal ganglia and brain stem were spared with very little involvement of the corpus callosum. A repeat MRI scan aged 4 years showed an increase in the number and size of the spaces suggesting a progressive process. The ventricles were mildly dilated suggesting some atrophic change.

In case 2, CT brain scan showed multiple cystic spaces (fig 4). MRI scan at the age of 6 years confirmed the presence of these cystic spaces. These were noted to be more confluent in the occipital region (fig 5). The ventricles were of normal size. The findings were similar to case 1.

Figure 4 (A, B) Case 2 aged 6 years 9 months. CT of the head showing multiple areas of low density within the cerebral white matter bilaterally, most prominent posteriorly.

Figure 5 (A, B) MRI of the head (sagittal/coronal T₁W and axial T₁W) showing a similar appearance to that shown in fig 3. The CSF density areas in the cerebral white matter are most prominent in the parieto-occipital regions where there is associated localised ventricular dilatation. Some involvement of the corpus callosum is also noted. The brain is otherwise normal.

Frontonasal dysplasia is a developmental field defect of midfacial development. Clinical features include a broad nose, hypertelorism, low anterior hairline, and sometimes bony defects of the forehead. It is usually a sporadic malformation and is known to occur with a high frequency in twins. There are reports of this malformation occurring with CNS anomalies, in particular frontal encephaloceles and agenesis of the corpus callosum. Dobyns *et al*¹ have reported the association of frontonasal dysplasia with bilateral periventricular nodular heterotopia. Additional features including mental retardation and epilepsy were present in their cases. However, the CNS findings in our cases are distinct with no evidence of nodular heterotopia.

Virchow-Robin spaces are invaginations of the sub-arachnoid space containing cerebrospinal fluid that accompanies small arteries and arterioles as they perforate the surface of the brain. The distribution of the abnormalities seen on the MRI scans of these two cases mirrored the perivascular distribution of the Virchow-Robin spaces. Dilatation of Virchow-Robin spaces have been described in a number of conditions including old age dementia, HIV encephalopathy, and multiple sclerosis (MS).² In children, similar MRI findings are seen with mucopolysaccharidosis.³ Neither of our cases had any other clinical features of a mucopolysaccharide disorder and screening of urinary amino acids was negative. In addition, the basal ganglia and corpus callosum are frequently involved in the mucopolysaccharide disorders. In our cases, only case 1 had some involvement of the corpus callosum and the basal ganglia were spared in both cases. The pathogenesis of large Virchow-Robin spaces remains unknown. Increased CSF pulsations, vascular ectasia, or an abnormality of arterial wall permeability have all been postulated as possible mechanisms.² These spaces may also have an important immunological role in reactions to foreign antigens that gain access to them.² It is felt that this immunological role may have implications for the understanding of the pathogenesis of a number of

neuropathological conditions including encephalitis, MS, and HIV encephalopathy. Both HIV encephalopathy and MS are progressive in nature. The fact that case 1 had features suggesting a progressive process may indicate that the abnormality seen in our cases has a metabolic or autoimmune basis.

Rollins *et al*⁴ described the prevalence of dilated Virchow-Robin spaces in 1250 children having consecutive MRI scans. Thirty seven children (3%) were shown to have such abnormalities on MRI scanning. Five of these children were said to have coarse or dysmorphic features and the remainder were referred with headaches, seizures, or developmental delay. All of the children with dysmorphic features had a negative urinary amino and organic acid screen. Four of the 37 were said to be hyperactive, although none of these had dysmorphic features. In their study, the size and number of the dilated Virchow-Robin spaces appeared to be static in contrast to case 1. Unfortunately, the authors did not describe the dysmorphic features of the five cases in detail, nor were there any clinical photographs so it is not possible to determine whether their cases had similar clinical features to our own. A number of other case reports have described similar MRI findings but without a dysmorphic phenotype.⁵⁻⁸

It is difficult to establish the significance of the low normal cholesterol level in association with high normal triglyceride level in case 1. Cholesterol has a role in myelination of the brain in early fetal life. The child has left the country and is therefore no longer available for further testing. The fact that case 2 had consistently normal levels suggests that the association may be coincidental.

Blepharocheilodontic syndrome shares some of the ectodermal characteristics in common with our cases, but the degree of hypertelorism is milder and cleft lip/palate is a common finding in BCD.⁹ In contrast, Sener¹⁰ described a female child with similar dysmorphic features and identical MRI findings to our own cases. This child was said to have normal development up to the age of 5 years. Subsequently she developed mild developmental delay and had to leave normal school aged 13 years. She was noted to have dental anomalies with hypodontia, dental occlusions, and several buccal frenulae. She was also noted to have thin hair and nail abnormalities. The author suggested that her features were consistent with ectodermal dysplasia and that her condition was slowly progressive in nature. No karyotype was reported for this patient. Slaney *et al*¹¹ reported a boy with dysmorphic features similar to the case reported by Sener.¹⁰ However, this child, a boy, had different abnormalities on MRI scan with evidence of periventricular grey matter heterotopia. All three cases (that of Sener¹⁰ and our two) have occurred sporadically with no parental consanguinity. Both males and females have been affected. The genetic basis remains unknown although the similarities on scan to the mucopolysaccharide storage disorders and the suggestion of a progressive nature raises the possibility of autosomal recessive inheritance.

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The psychological impact of a cancer family history questionnaire completed in general practice

EDITOR—On the basis of family history, it is possible to identify subjects at significantly increased genetic risk of breast or colorectal cancer.^{1,2} Evaluation of the benefits of screening these patients to facilitate early diagnosis and treatment forms the subject of continuing studies. For colorectal cancer, the benefits of colonoscopic surveillance have been reported,³ but for breast cancer more data are needed to confirm the value of mammographic screening.⁴ At present, patients with a significant family history who seek advice from their general practitioner are likely to be referred to a cancer genetics clinic and offered screening. If further research confirms the benefits of screening for patients at increased genetic risk, effective strategies for their ascertainment in primary care will be needed. One possible method is a postal family history questionnaire sent to the patient by their general practitioner. We report elsewhere on the effectiveness of this approach.⁵ An important issue is whether this method of ascertainment raises anxieties, particularly among the majority of patients who do not have a significant family history. The collection of cancer family history information constitutes a form of screening. There is a large body of evidence that health related screening can have unintended adverse effects, the most studied of which is raised anxiety, particularly among those found to be at an increased risk.⁶ As knowledge of the genetic component of common diseases increases,⁷ more patients may be asked to provide information about their family history. It is therefore timely to consider whether such a task may inadvertently raise general levels of anxiety or worries about the disease in question. To our knowledge, there have been no previous studies of the psychological consequences of screening using a postal questionnaire to obtain information about relatives affected by cancer. The purpose of the present study was to determine the psychological impact of completing a cancer family history questionnaire and receiving an assessment of personal genetic risk of breast or colorectal cancer.

General anxiety was assessed using the six item, short form of the state scale of the Spielberger State-Trait

Anxiety Inventory (STAI).⁸ This yields a single score ranging from 20 to 80. The mean for the adult population is 36. Worry about cancer was measured using the shortened version of the Cancer Worries Scale,⁹ which assesses (1) people's perceptions of their own chances of developing cancer, (2) their frequency of cancer related thoughts, (3) the frequency with which they perceive their mood to be affected by such thoughts, and (4) the frequency with which such thoughts affect the performance of their daily tasks.

The participants in this study were patients completing a cancer family history questionnaire as part of a separate study to evaluate its use in general practice.⁵ For that study, patients aged between 35 and 65 years registered with a single general practice in Cambridge were invited to participate. They were sent an information sheet explaining that the purpose of the study was to identify the small minority of patients whose family history would put them at sufficiently increased risk of breast or colorectal cancer to warrant the offer of screening to facilitate early diagnosis and treatment. A consent form and short questionnaire to measure baseline levels of general anxiety and worry about cancer were also enclosed. Those wishing to participate were asked to complete and return the consent form and the baseline measure. They were then sent the family history questionnaire (for details see <http://www.jmedgenet.com>). On the basis of their responses, the majority of patients were judged not to be at significantly more than the population risk of breast or colorectal cancer (lower risk group). These patients were sent a letter telling them that, on the basis of their stated family history, their personal risk of developing breast or colorectal cancer was below the level at which extra screening tests would be recommended. A small number of patients were assessed to be at potentially increased risk where one of the following applied: (1) their family history as reported met local screening criteria for breast or colorectal cancer (table 1) or (2) their family history approached screening criteria so closely that it was considered advisable to check crucial details such as age at onset in relatives, or (3) the information provided on the questionnaire was ambiguous or incomplete and there remained a possibility that the screening criteria might be met, or (4) their family history did not meet screening criteria but suggested an increased risk to the GP assessor. Almost all of these patients were

Table 1 Criteria used to define increased genetic risk sufficient to warrant referral and the offer of screening

For breast cancer, females with one of the following:

- (1) Three first or second degree relatives with breast or ovarian cancer
- (2) Two first or second degree relatives with breast cancer diagnosed under 60 years of age or ovarian cancer at any age
- (3) One first degree relative with (i) breast cancer diagnosed under 40 years of age, or (ii) bilateral breast cancer, or (iii) male breast cancer

For colorectal cancer, one of the following:

- (1) A first degree relative plus two other relatives with colorectal cancer and (i) one case diagnosed under 50 years of age, and (ii) one case a first degree relative of the other two, and (iii) at least two generations affected
- (2) A first degree relative with colorectal cancer diagnosed under 45 years of age

Table 2 General anxiety and cancer worries at baseline and follow up for paired responses in (1) patients assessed not to be at significantly increased risk (lower risk group), (2) patients with potentially significant family histories subsequently shown not to be at significantly increased risk (false positive group), (3) patients confirmed to be at significantly increased risk (higher risk group)

	Mean scores (95% CI)					
	Lower risk group		False positive group		Higher risk group	
	Baseline	Follow up	Baseline	Follow up	Baseline	Follow up
<i>Spielberger State-Trait Anxiety Inventory</i>						
General anxiety	35.8 (34.6–36.9) n=427	35.1 (33.9–36.2)	34.8 (26.6–42.9) n=7	34.3 (24.1–44.5)	36.3 (31.7–40.9) n=18	38.9 (32.8–45.0)
<i>Cancer Worries Scale</i>						
Perception of own chances of developing cancer	2.95 (2.88–3.01) n=534	2.83 (2.76–2.91)	3.55 (3.19–3.90) n=11	3.27 (2.53–4.01)	3.56 (3.18–3.94) n=25	3.40 (2.99–3.81)
Frequency of cancer related thoughts	1.63 (1.57–1.69) n=564	1.61 (1.55–1.67)	1.73 (1.29–2.16) n=11	1.64 (1.09–2.18)	1.96 (1.59–2.33) n=25	1.76 (1.36–2.16)
Effect on mood	1.21 (1.17–1.26) n=560	1.22 (1.17–1.26)	1.45 (1.10–1.81) n=11	1.27 (0.84–1.71)	1.29 (1.10–1.49) n=24	1.25 (1.03–1.47)
Effect on performance of daily tasks	1.12 (1.08–1.15) n=562	1.11 (1.08–1.15)	1.00 * n=11	1.09 (0.89–1.29)	1.17 (1.01–1.33) n=24	1.08 (0.96–1.20)

CI = confidence interval.

*All respondents scored 1 for this item.

interviewed but in a few cases minor uncertainties were resolved over the telephone. Most were confirmed to be at significantly increased genetic risk (higher risk group) but a minority were deemed not to be at increased risk (false positive group). An explanation of their final risk assessment was given to all these patients, usually at personal interview (all patients assigned to higher risk as a result of the study were informed of this at interview), but in a few cases by telephone or letter. Patients in the higher risk group who had not previously received genetic advice were offered referral to the cancer genetics clinic. All participants were asked to complete a follow up anxiety and cancer worries questionnaire four to six weeks after feedback of their personal risk. In the group of patients referred for genetic advice, this measure was completed after the consultation at which referral was offered but before the appointment at the cancer genetics clinic. Statistical comparisons were made using the Wilcoxon signed rank test for paired data and the Mann-Whitney U test for independent samples. The Cambridge Local Research Ethics Committee gave ethical approval for the study.

The effective practice population for the study of the cancer family history questionnaire was 2265 patients. A total of 666 patients (29%) completed that questionnaire and are the participants in the present study. They differed from the practice population in terms of both gender and age.⁵ A total of 62.2% were women, compared to 50.2% of the practice population ($\chi^2=37.8$, $p<0.001$) and a lower proportion were aged 35–44 (30.2% compared with 40.7%, $\chi^2=30.3$, $p<0.001$).

A total of 604 patients (91%) returned baseline and follow up measures of anxiety and cancer worry. The gender and age distribution of respondents did not differ significantly from the study population. Paired responses were obtained from 568 patients assessed not to be at significantly increased risk of breast or colorectal cancer (lower risk group) and 36 patients judged to be at potentially increased genetic risk on the basis of their family history questionnaires. The latter group comprised 25 patients who were subsequently confirmed to be at significantly increased risk (higher risk group) and 11 deemed not to be at significantly increased risk after further investigation of their family history (false positive group). Some patients failed to answer all six items of the Spielberger State-Trait Anxiety Inventory or all four items of the Can-

cer Worries Scale. Table 2 gives the numbers of valid paired responses in each patient group.

The scores for general anxiety and cancer worries at baseline (before completion of the family history questionnaire) and follow up (four to six weeks after receipt of their risk assessment) for all three groups are shown in table 2. In the lower risk group, the only difference in paired responses between the two time points assessed was in patients' perceptions of their personal risk of developing cancer, which showed a small reduction ($p<0.001$). For the other two groups there were no differences in paired responses for general anxiety or cancer worries. For both the higher risk group and the false positive group, baseline responses showed that their pre-existing perception of their risk of developing cancer was higher than that of the lower risk group ($p<0.001$ and $p=0.003$, respectively). For the false positive group, the frequency with which cancer related thoughts affected their mood was also higher ($p=0.02$).

The results of this study suggest that completion of a cancer family history questionnaire and receipt of an assessment of personal genetic risk for breast and colorectal cancer does not make patients more anxious or worried about cancer. This conclusion is based on a substantial number of subjects, but should be tempered by the fact that only a minority of practice patients returned the family history questionnaire and constituted a self-selected group.

Responses to the Cancer Worries Scale showed that most patients rarely worried about their risks of developing cancer either before or after the study. Indeed, receipt of information that their personal risk was below the level at which extra screening tests would be offered was associated with a small but significant reduction in perceived risks of developing cancer. This raises the question of whether this knowledge could influence health related behaviour. For example, it might reduce the incentive to participate in health related activities, such as attendance for routine mammographic screening or eating a fibre rich diet. This effect has been reported for other forms of screening^{10 11} and merits further investigation.

For patients assessed to be at potentially increased risk on the basis of their family history questionnaire, baseline responses showed that their pre-existing perceptions of their own risk of developing cancer were significantly higher than those of other patients. This suggests that many

of these patients already understood the implications of their family history. For patients confirmed to be at significantly increased risk and advised accordingly, their follow up responses showed that they continued to perceive themselves at increased risk with no significant change from baseline. There was no indication that completion of the cancer family history questionnaire and subsequent discussion of their cancer risk exacerbated existing concerns. For the small group of patients assessed not to be at significantly increased risk after further evaluation of their family history, their baseline responses showed that before the study they too perceived themselves to be at increased risk on account of their family history. After being advised that their personal risk of developing cancer was below the level at which extra screening tests would be recommended, their responses at follow up show a mixed reaction. Four patients regarded themselves at lower risk than before, three saw themselves at increased risk, and four were unchanged. The numbers are too small to draw firm conclusions, but suggest that at least some of these patients still regarded their family history as putting them at somewhat increased risk.

Much recent work on the psychological impact of genetic screening has focused on the impact of DNA testing¹² where a positive result usually implies a much greater than population risk. There is evidence that, in women undergoing predictive DNA testing for breast cancer, a positive result has little impact on general levels of anxiety or depression.^{13 14} One possible explanation for this is that these women already perceived themselves to be at high risk and were understandably anxious before testing. The result did not, therefore, alter their psychological status appreciably. The present study assessed anxiety in patients participating in a questionnaire survey designed to identify subjects at moderately increased risk with a view to offering extra mammographic or colonoscopic screening rather than DNA testing. Again, no significant change in psychological well being was detected and there was evidence that those at increased risk already perceived themselves at risk, although they may not have sought advice, while those who perceived themselves to be at lower risk had their view endorsed by the process.

If improving knowledge about familial cancer risk is to benefit all patients and not just the better informed, it will be necessary to develop effective ascertainment strategies in primary care. The results of this study suggest that it

should be possible to do this without increasing anxiety either in those at increased genetic risk or in those at no more than the population risk.

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Attitudes to genetic testing for breast cancer susceptibility in women at increased risk of developing hereditary breast cancer

EDITOR—The localisation of the two breast cancer susceptibility genes *BRCA1* and *BRCA2* made possible the use of mutation detection as a susceptibility test for people who wish to learn whether they carry a risk conferring mutation.¹⁻⁴ Several studies have assessed attitudes to genetic testing for breast cancer susceptibility,⁵⁻¹¹ most of which involved either community samples or women with just one first degree relative with breast cancer. The objective of our study was to assess attitudes to genetic testing for breast cancer susceptibility in a large sample of

women at high risk of developing hereditary breast cancer on the basis of family history. The majority of women included in our sample (80%) had a family history consistent with a dominantly inherited predisposition to breast cancer (lifetime risk of 1 in 4 to 1 in 2),¹² and the remainder (20%) was at moderately increased risk of developing breast cancer (lifetime risk of 1 in 8 to 1 in 4).¹²

The findings reported here are based on a sample of 461 unaffected women with a family history of breast cancer. Women who approached one of 14 familial cancer clinics and six associated outreach clinics in five Australian states between November 1996 and January 1999 were eligible for participation. Women were considered ineligible for study participation if they had a previous diagnosis of ovarian or breast cancer, were unable to give informed consent, or had limited literacy in English, since data were collected using self-report questionnaires. The study was approved by 16 institutional ethics committees.

A very important factor in deciding about whether I would have a gene test or not would be...

Figure 1 Percentages of women endorsing perceived benefits of genetic testing.

A very important factor in deciding about whether I would have a gene test or not would be that...

Figure 2 Percentages of women endorsing perceived limitations of genetic testing.

Familial cancer clinic staff invited women to participate in the study during the preclinic telephone call, where possible. Questionnaires, consent forms, and reply paid envelopes were then mailed out by the coordinating research centre. Women were subsequently telephoned by the central research staff and given further information about the study and issues of informed consent. Women were asked to return the completed questionnaire and consent form before attending the familial cancer clinic, if possible. Reminder calls were made as required.

Sex, age, educational level, marital status, number and sex of biological children, and referral source were assessed. To provide an estimate of objective risk, clinic staff were asked to make a judgment on whether a participant's family history was either consistent or not consistent with a dominantly inherited predisposition to breast cancer, and participants were thus classified as being at "high risk" or "moderately increased risk", respectively. Following the risk assessment interview at the familial cancer clinic and once pedigree information^{13 14} and relatives' diagnoses confirmed by medical records were available, clinic staff categorised participants into objective risk groups. For women from high risk families, clinic staff made a judgment on whether the participant was at either 50% or 25% mutation carrier risk. Risk of being a mutation carrier, rather than the estimated lifetime risk of developing breast cancer, was used as a measure of objective risk. The expert opinion of clinical geneticists was used as a gold standard, since there are currently no universally accepted standards to estimate breast cancer risk in high risk women. The number of first and second degree relatives who developed breast or ovarian cancer were collected from study participants.

One item asked participants to select their approximate perceived lifetime breast cancer risk from the following response options: 1%, 4%, 8%, 12%, 16%, 25%, 33%, 50%, 85%, and 100%. Risk was expressed both as a percentage and as odds (for example, 1 in 8).

One item measured perceived likelihood of being a mutation carrier, with five response options ranging from "Certain that I will not have gene" to "Certain I will have gene". The phrase "have the gene" was used, because of the common misconceptions regarding heterozygosity among the lay population and to facilitate ease of understanding.

The Impact of Event Scale is a 15 item, validated scale that measures anxiety responses about a specific stressful event.^{15 16} Subjects at increased risk of developing hereditary breast cancer may construe their being at risk as a continuous, rather than specific, trauma. Although the scale has not previously been specifically validated among subjects at high risk of developing breast cancer, it has been used in several studies as a measure of breast cancer anxiety^{7 17} and has previously been shown to be predictive of interest in genetic testing.¹⁸ In the current study the particular stressor was concern about being at risk of developing breast cancer. Participants were asked to rate symptoms of anxiety (for example, "I had strong waves of feelings about being at risk of breast cancer") on a scale ranging from "Not at all" to "Often".

The Monitoring-Blunting Style Scale is an eight item, validated scale measuring individual differences in coping styles in threatening situations.¹⁹ The scale measures a person's tendency either actively to seek out threatening information ("monitoring") or to ignore it and distract oneself ("blunting"). The Monitoring-Blunting Style scale was

Table 1 Sociodemographic and family history variables of study sample (n=461)

Variable	Level	No	%
Sociodemographics			
Age	<30	97	21
	30–39	157	34
	40–49	124	27
	50+	81	18
Marital status	Married	295	64
	Not married	164	36
Biological children	Yes	329	72
	No	130	28
Educational level	Post-school qualifications	301	68
	No post-school qualifications	143	32
Family history variables			
Objective risk	Moderate risk	85	20
	High risk - 25% MCR*	48	10
	High risk - 50% MCR*	303	70
No of FDR & SDR with BrCa or OvCa†	1–2	135	34
	3–4	176	44
	5–18	87	22

*MCR = mutation carrier risk.

†Number of first and second degree relatives with breast or ovarian cancer.

included to assess individual differences in responding to genetic risk information.

Six items each assessed perceived benefits and limitations of genetic testing for breast cancer susceptibility. Figs 1 and 2 show items relating to benefits and limitations respectively. These items have been used in related studies.^{20, 21} Women endorsed perceived importance of each benefit or limitation on three response options ranging from “Not at all important” to “Very important”.

Interest in genetic testing was the outcome variable and asked women whether they would be interested in a genetic test. Four response options ranged from “Yes, definitely” to “No, definitely not”.

The outcome variable “interest in genetic testing” was recoded as a binary variable. Only eight participants (2%) reported being “probably not” interested in genetic testing and none reported being “definitely not” interested. It is generally recommended to collapse categories which contain less than 5% of cases to achieve a more balanced distribution.²² Therefore, rather than collapsing the two “yes” and two “no” categories, the new variable was defined as “yes, definitely” versus “yes, probably”, “probably not” and “definitely not”.

To assess associations of predictor variables with the outcome variables, contingency table analysis with χ^2 tests were performed for categorical predictor variables, Mann-Whitney U, and Kruskal-Wallis tests for ordinal and non-normal interval variables and two sample *t* tests for normally distributed interval data. For the outcome variable “interest in genetic testing” hierarchical logistic regression was used to identify variables associated independently with interest in genetic testing. All independent variables with a bivariate association of $p < 0.1$ with

Table 2 Hierarchical regressions on attitude to genetic testing for breast cancer predisposition (n=384)

Model variable	Reference group	-2 log likelihood (p value)	OR*	95% CI OR	p
Final model		428.31 (p<0.040)			
Objective risk					0.57
	25% MCR†		0.71	0.30, 1.72	0.45
	50% MCR†		0.73	0.40, 1.32	0.30
Perceived carrier risk	Moderate risk				0.017
	Not have gene		0.38	0.11, 1.29	0.12
	Unsure		0.37	0.18, 0.73	0.0044
	Have gene				

*OR = odds ratio, refers to comparisons with the category listed last.

†MCR = Mutation carrier risk.

the outcome variable were entered as predictors and age and objective risk as covariates.

Of the 520 women who met the eligibility criteria, 59 women declined participation or never returned the questionnaire (response rate of 89%). Table 1 summarises sociodemographic and family history variables of the study sample.

The mean age of breast cancer onset in the youngest person in the family was 41 years (SD=9.6). The number of self-reported first and second degree relatives with a diagnosis of breast or ovarian cancer ranged from 1 to 18, with a median of three. Ninety five women (21%) had a family history which included ovarian cancer in addition to breast cancer. All women were assessed before receiving a genetic testing result. Seventy percent of women reported being “definitely”, 22% “probably”, and 2% “probably not” interested in genetic testing, with the remainder (6%) being unsure.

Bivariate analyses between predictor variables and interest in genetic testing showed that subjective carrier risk was significantly associated with interest: 86% of women who reported being “quite certain” or “certain” that they were carriers were “definitely interested” in testing, compared to 71% of those who were uncertain ($\chi^2=8.20$, $p=0.017$). Women who were “definitely interested” in genetic testing had significantly higher breast cancer risk perceptions ($Z=-2.08$, $p=0.038$). Neither educational level ($\chi^2=0.82$, $p=0.37$), referral status ($\chi^2=0.32$, $p=0.57$), having daughters or not ($\chi^2=0.22$, $p=0.63$), breast cancer anxiety ($Z=-1.48$, $p=0.14$), nor monitoring score ($Z=-0.062$, $p=0.95$) were significantly associated with interest in genetic testing. Table 2 shows the final regression model. Only perceived mutation carrier risk was significantly associated with interest in genetic testing ($p=0.0017$), in that women who were uncertain if they were mutation carriers were less likely to be definitely interested in genetic testing (OR 0.37, 95% CI 0.18–0.73, $p=0.0044$), compared to women who were quite certain or certain that they were carriers.

Fig 1 shows the percentages of participants who endorsed each perceived benefit as a “very important” factor in deciding about whether or not to have a genetic test. The item endorsed by the highest percentage of women as very important (87%) related to perceiving genetic testing as helpful in understanding what steps to take to reduce one’s cancer risk. Learning about one’s children’s risk was the second most frequently endorsed item, with 77% of women who had children reporting it to be a very important factor.

Fig 2 shows the percentages of participants who endorsed each perceived limitation as a “very important” factor in deciding about whether to have a genetic test. The item endorsed by the highest percentage of women as very important (16%) related to concerns about the effect of genetic testing on the family. Interestingly, only 4% and 11% of women reported that worry about losing insurance was a very important or somewhat important factor, respectively.

Table 3 provides an overview of the results of bivariate analyses to identify associations between demographic and psychological variables and individual perceived benefits and shortcomings of testing. It shows that women who had daughters, compared to women who had sons only, were significantly more likely to report that learning about one’s children’s risk was an important factor (84% versus 61%, $\chi^2=29.31$, $p<0.0001$). Women who reported that not being able to handle the knowledge emotionally was a very or somewhat important factor had significantly higher breast cancer anxiety than women who reported that this was not at all important ($Z=-6.91$, $p<0.0001$). None of the other

Table 3 Factors associated with particular perceived benefits and limitations of genetic testing for breast cancer predisposition

Variable	No*	Level (independent variable)	Percentage very important or mean (SD)	χ^2	p
To learn about my children's risk†	242	Daughters	84.3	29.31	<0.0001
	81	Boys only	60.5		
To make child bearing decisions‡	200	≥ 1 in 3 (high)	16.5	3.99	0.14
	112	<1 in 3 (low)	9.8		
To help me understand what steps to take to reduce my risk of developing cancer	291	Married	87.6	0.61	0.74
	164	Not married	86.0		
To plan for the future	295	Post-school	59.3	0.58	0.79
	145	No post-school	55.9		
I am worried about losing insurance	273	Post-school	3.4	0.03	0.99
	137	No post-school	3.6		
Mean (SD) (dependent variable)			Z score		
I couldn't handle the knowledge emotionally	15	Very important	26.8 (22.9)	-6.91	<0.0001
	131	Quite important	22.9 (16.3)		
	285	Not at all important	11.5 (12.1)		

*Numbers vary owing to missing data.

†Only includes women with children.

‡Excludes women who endorsed "not applicable".

variables was significantly associated with interest in genetic testing.

This study found that interest in genetic testing for breast cancer susceptibility is very high in a familial cancer clinic population, with 92% of women indicating that they would definitely or probably be interested. This percentage is consistent with previous findings.^{7-9 23 24} Owing to the clinic based recruitment, the sample may not be representative of high risk women as a whole and is likely to be self-selected for interest in genetic testing. Thus, the high percentage of women reporting interest in genetic testing is perhaps not surprising. Equally, since interest in genetic testing was self-reported, it may not necessarily translate into actual use.

In contrast to findings from other studies,^{6 8} we found that breast cancer anxiety was not associated with interest in genetic testing. Our results suggest that interest in genetic testing in women with a strong family history of breast cancer is unlikely to be motivated by psychological distress as the primary factor, and contrast with those of related studies.^{9 18} We found that interest in genetic testing was associated with perceived likelihood of being a mutation carrier, but not objective risk, confirming earlier results from the two studies that assessed attitudes to genetic testing of people with a strong family history of breast cancer.^{23 24}

Perceiving genetic testing as helpful in understanding what steps to take to reduce one's cancer risk and learning about one's children's risk were the most commonly reported reasons for considering testing. These findings are consistent with several studies which assessed reasons for undergoing genetic testing for breast cancer susceptibility,^{7 9 21 23 25-27} and underline the importance women attribute to both reasons.

Understanding what steps to take to reduce one's cancer risk was endorsed by 87% of women as a very important factor. In contrast, several surveys of attitudes to genetic testing for Huntington's disease identified wanting "to be certain" as the most commonly reported reason.²⁸⁻³¹ In the breast cancer scenario, the salience attributed to understanding how to reduce one's breast cancer risk reflects the potential of genetic testing to provide women with a more informed basis for decision making about prophylactic strategies. Perhaps not surprisingly, women with daughters, compared to those with sons only, attributed greater importance to learning about one's children's risk. The greater risk to daughters, compared to sons, seems to be a strong motivator to undergo genetic testing.

Only 15% of participants endorsed worries about insurance as a somewhat or very important shortcoming of genetic testing, compared to 34% of subjects undergoing

BRCA1 testing in the United States.²¹ Contrasting findings are likely to reflect differences in medicolegal factors and government policies for health and life insurance. Health insurance premiums in the United States are risk based, while there is universal government health coverage and even risk based private health insurance in Australia. These differences highlight the ethical issues faced by countries such as the United States, where insurance issues may be among the most important factors influencing a woman's decision concerning genetic testing.

On the whole, the degree of importance attributed to perceived shortcomings was much lower than that attributed to benefits, as has also been observed in related studies in the United States.^{7 25} This finding suggests that women believe that the benefits of genetic testing outweigh its risks. It also indicates that women may benefit if counsellors provide comprehensive information on the limitations of genetic testing to ensure that decisions about genetic testing are informed decisions. Full deliberation of positive and negative consequences of alternate choices is considered one of the most important features of informed decision making.^{32 33} A randomised trial showed that a genetic counselling approach, but not a purely educational approach, achieved significant increases in perceived limitations and decreases in perceived benefits of *BRCA1* testing.³² Thus, an approach to genetic services provision that relies exclusively on conveying factual information may be less likely to succeed in achieving comprehensive consideration of both benefits and shortcomings of testing.

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Attitudes of von Hippel-Lindau disease patients towards presymptomatic genetic diagnosis in children and prenatal diagnosis

EDITOR—Von Hippel-Lindau (VHL) disease is an autosomal dominant disorder characterised by a predisposition to develop a wide variety of benign tumours and malignant neoplasms, most frequently haemangioblastomas of the cerebellum and spinal cord, retinal haemangioblastomas, pheochromocytomas, renal cysts, and clear cell carcinomas, pancreatic cysts and tumours, epididymal cystadenomas, and endolymphatic sac tumours.¹⁻³ The combination of affected organs and the sequence of organ involvement vary considerably among families and even among individual patients in a family. Two types of families may, however, be distinguished according to the presence or absence of pheochromocytoma.⁴ Central nervous system haemangioblastoma remains the main cause of death although renal cell carcinoma could represent a major problem in the future.⁵ Recent progress in research methods will render possible the identification of VHL gene mutations in virtually all families.⁶

Between 1995 and 1997, we have evaluated the views of 24 women aged 20 to 41 and 17 men aged 20 to 50 with VHL about presymptomatic genetic diagnosis in their children as well as prenatal diagnosis and termination of pregnancy. All were informed of the hereditary nature of their disease. Patients, having previously agreed over the telephone, were interviewed in the French VHL Care Centre (Necker Hospital in Paris and Kremlin-Bicêtre Hospital) where they were currently being followed. It was decided to have a full discussion with each subject after completion of the questionnaire, but not to interrupt its completion or correct erroneous answers immediately. The oral discussion allowed us not to ask a specific question, or even to interrupt the interview, if the patient showed a strong emotional reaction. All were included in the national French VHL register which includes 650 patients to date. Family pedigrees and medical records were reviewed.

The participants belonged to 34 families. In 28 families, one subject was interviewed. In the remaining six families, two or three sibs (two brothers in two families, a brother and sister in three families, and two sisters and a brother in one family) were interviewed. At the time of interview, 31 patients (76%) had developed central nervous system haemangioblastoma, 25 (61%) pancreatic cysts, 19 (46%) retinal haemangioblastoma (one woman was blind, another was amblyopic, and three had unilateral blindness), 14 (34%) renal cell carcinoma (one brother-sister pair

Table 1 Knowledge about transmission and risk for children to be affected at the time of interview

	Men (n=17)	Women (n=24)
Current knowledge about transmission		
That both genders can be affected	15	21
That both parents can transmit the disease	15	19
Current knowledge about the risk in children		
That risk for the first child is 50%	12	15
That risk for a second child is identical	10	13
Overall adequate knowledge* of transmission and risk	9(53%)	11(46%)

*Four correct answers.

being treated by dialysis because of bilateral nephrectomy and another man showing chronic renal failure), and seven (17%) phaeochromocytoma (one man and four women requiring substitutive treatment because of bilateral adrenalectomy). Differences between men and women were not significant. Percentages of retinal haemangioblastoma were, however, higher in women. According to a personal medical score (evaluating the severity of the disease considering the harm possibly resulting from symptoms, periodic examination, treatment, and residual disabilities), VHL disease was considered mild in five patients, moderate in 15, intense in 17, and severe in four. During the interview, 75% of women and 47% of men declared that their lives were modified by manifestations directly linked to the illness and treatments and/or to psychological distress, that is, anxiety or depression, sometimes prominent and requiring specific treatment. When comparing the patient's personal perception of burden with personal medical score, it appears that life modifications were acknowledged by many, but not all patients who had severe or intense disease and also by patients (especially women) who had moderate or mild disease and mainly suffered from psychiatric symptoms.

In studying family structures, it appears that VHL disease had an impact on the patients' reproductive intentions. Only three men and 11 women (34%) have had children. There were more childless men than women (82% *v* 54%). Most patients had only one child, but one woman had two children, two others have had three children, and a fourth woman with two children was pregnant at the time of interview. Two thirds of the pregnancies had occurred either when the parent was not ill or when he (or she) did not know that the disease was hereditary and that children would be at risk. Furthermore, four patients (three women and one man) who already have children and six childless patients (three women and three men) have decided not to have children in the future because of the potential risk for them. Although all patients claimed that they had heard of inheritance of the disease, half of them did not correctly remember the genetic risks (table 1). This misunderstanding was not influenced by educational background. One part of the discussion we had with each subject after com-

pletion of the questionnaire focused on helping them to understand the present state of medical and genetic knowledge so as to be able to make better informed decisions in the future.

Before the recent progress in DNA based testing, the diagnosis of VHL led to recommending screening of at risk members throughout their lifetime to determine whether they had manifestations of the disease. This recommendation imposed a considerable burden on asymptomatic family members.¹ On the one hand, it is not known at what age screening can be discontinued for at risk members, assuming that they do not have the disease. On the other hand, it is not known at what age screening should begin. It may be guided by the possible age at onset of symptoms since each lesion has its own onset age.^{1,2,7} In this series, disease onset occurred between 10 and 15 years in six patients. Arterial hypertension related to a phaeochromocytoma was discovered in a 10 year old boy and neurological symptoms related to a cerebellar haemangioblastoma occurred in a 9 year old boy. A 12 year old girl and two 13 and 15 year old boys presented with ocular symptoms. In addition, visual field loss was discovered at school in a 12 year old girl. Similarly, there are several other published reports of childhood onset. For example, retinal haemangioblastoma, phaeochromocytoma, and cerebellar haemangioblastoma have been detected in children as young as 4,⁸ 5,⁹ and 10 years,¹⁰ respectively. Furthermore, renal cell carcinoma was discovered in a 12 year old child included in the French VHL register (Richard, personal communication). Consequently it could be recommended to start regular screening of asymptomatic patients for retinal lesions and phaeochromocytoma at the age of 5, for renal cell carcinoma at the age of 10, and for central nervous system haemangioblastomas at the age of 15.

The rapid development in genetic technology is changing the practice of medicine. Once the VHL gene mutation has been identified for a given family, at risk members can be tested. If no mutation is identified, it is not necessary to subject a person to periodic medical testing. If a mutation is identified, that person should be periodically examined for manifestations of the disease, taking the specific mutation into account, as well as the age specific incidence of VHL tumours. As indicated by the American Society for Clinical Oncology, genetic testing constitutes a help for families presenting with syndromes predisposing to cancer (for example, familial adenomatous polyposis, multiple endocrine neoplasia type 2, retinoblastoma, and VHL disease) for which either a positive or negative result will change medical care, and for which it may be considered part of the standard management of affected families.¹¹ Various situations have to be distinguished, however. Whereas presymptomatic surgical treatment is proposed for patients with familial adenomatous polyposis and multiple endocrine neoplasia type 2, preventive treatment cannot be offered for VHL. Carriers with a mutant gene causing VHL syndrome are treated only after expression of the disease. However, identification of the mutation may have implications for guiding specific screening for phaeochromocytoma and renal cell carcinoma.¹²⁻¹⁴

In our group of 14 VHL patients with children, most, but not all, were willing to have their children tested as soon as possible or had already asked for testing (table 2). In six of these families, nine children aged between 3 and 18 years had already been clinically tested (no abnormality had been detected). However, there was a discrepancy between attitudes regarding themselves and their children since three of the 10 patients with onset after 20 years declared that they would not have appreciated knowing earlier that they were affected and three others did not know whether they would have appreciated it. Asked if they would tell the

Table 2 Attitudes of the 14 patients with children towards genetic testing for their children

	Men	Women
Willing to have their children studied	n=3	n=11
Yes	2	9
Undecided	1	2
No	0	0
Would tell the truth to their children if the test is positive	n=2*	n=9*
Yes	2	8
No	0	1
Would have liked to have known earlier through genetic testing whether they were affected	n=2†	n=8†
Yes	0	4
Did not know	2	1
No	0	3

*Patients willing to have their children studied.

†Patients in whom initial symptoms had occurred after 20 years of age.

truth to their children in the case of a positive test, all answered yes, except one woman with three children (aged 15, 13, and 10 years), who had already refused presymptomatic clinical testing. Finally, asked when they would tell the truth, they could not specify the age. Although all authors agree on the need for testing at risk children for VHL, the age at which they should be tested is still under discussion. As shown by the reports of an international consensus meeting, answers vary. Fifty six percent of the participants from different disciplines favoured DNA analysis in VHL before the age of 5 and 5% prenatally, 15% between the ages of 5 and 10, and 18% at an age when children can make their own choice.¹⁵

With the use of genetic testing, the child and his family will be informed on the carrier state probably years before the initial clinical manifestations. Many authors recommend careful weighing of potential benefits and psychological harm to the child or adolescent and his family before making a decision about genetic testing.¹⁶⁻¹⁹ They mentioned the impact of testing on the child's own development as well as the emotional reactions of both the affected and the unaffected parent. Moreover, in large families, the reactions of the non-carrier sib(s) should also be considered. As mentioned by Fanos,¹⁸ psychosocial support should be provided to at risk families before making a decision. This view is strengthened by our observation of a high frequency of psychological distress in our patients (47% of the men and 67% of the women).

The use of DNA studies introduces new options for couples who may have to consider whether the abortion of an affected fetus is morally acceptable given the partially treatable nature of VHL disease. Most reports on patients' attitudes towards prenatal diagnosis have focused on autosomal recessive diseases where unaffected parents are grieving the loss of an affected child. Few have dealt with dominant diseases such as VHL disease where one parent is affected. In our series, 22 of the 31 VHL patients who wished to have children intended to use prenatal diagnosis in the case of a pregnancy (table 3). However, half of this group either disapproved of the idea of termination of an affected pregnancy or were undecided. The other 11 patients considered abortion of a fetus with the VHL disease acceptable. When studying Alport syndrome, an X linked dominant condition, we have already pointed out the discordant attitudes existing between the choice of prenatal testing and termination of pregnancy.²⁰ Of the 21 VHL patients in favour of termination in the case of severe malformations (independent of VHL), 10 considered abortion of a VHL fetus as acceptable. The eleventh patient, a woman, who would choose abortion in the case of a VHL fetus, was reluctant to terminate a pregnancy for severe malformations. This patient presented with the highest score of severity, but denied any modifications in life style. Neither the VHL patient's own perception of the illness (that is, the associated physical, psychological, social, and financial problems) nor the patient's own attitude towards termination of pregnancy appeared to influence the decision making process. As indicated by the discordant sibs' answers, attitudes appear to depend upon personal motivation, independent of a common cultural and religious environment. Decisions of VHL patients appear to be quite individual and difficult to predict. Most patients who were against termination of pregnancy should prenatal diagnosis show an affected fetus based their feeling on the hope that progress in treatment would prevent symptoms as severe as their own in their children.

Table 3 Attitudes of the 31 patients wishing to have children towards prenatal diagnosis and termination of pregnancy

	Men	Women
Prenatal diagnosis for VHL	n=13	n=18
For	11	11
Undecided	0	2
Against	2	5
Termination of pregnancy of an affected VHL fetus	n=11*	n=11*
For	4	7
Undecided	3	2
Against	4	2
Termination of pregnancy if serious malformations	n=13	n=18
For	9†	12‡
Undecided	4	5
Against	0	1‡

*Patients in favour of prenatal diagnosis for VHL.

†Four of the nine men and six of the 12 women were in favour of termination of an VHL affected fetus.

‡Woman in favour of termination of a VHL fetus.

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CORRECTION

The following is a correction to the paper by Loukola *et al* (*J Med Genet* 1999;36:819-22). An algorithm was introduced by Wijnen *et al* to predict the probability of finding a disease causing *MLH1* or *MSH2* mutation in patients with HNPCC or possible HNPCC. The variables of this formula are the mean age of colorectal cancer (CRC) diagnosis in the family, fulfilment of the so called Amsterdam criteria, and the presence of endometrial cancer in the family. In addition to this basic formula, an alternative formula to be used in small families was introduced. The variables of this alternative formula are the mean age of CRC diagnosis in the family, the number of patients with CRC in the family, and the number of patients with endometrial cancer in the family. Applying these algorithms, a probability (p_1 for the basic formula and p_2 for the alternative formula) of 20% or higher for a germline mutation was proposed to justify mutation analyses. We tested these formulae in a series of 509 consecutive colorectal adeno-

carcinoma samples.² MSI analysis and genomic sequencing of *MLH1* and *MSH2* for the MSI positive samples had been previously performed.³

Unfortunately, we used an inaccurate factor in the alternative formula. We used $L=1.4 + (-0.09)V_1 + 0.27 V_2 + 0.75 V_3$, when the first factor should have been 1.8. Thus, the results obtained using the alternative formula were not accurate. We proposed that the alternative formula was able to identify only three out of 10 mutation positive patients when first degree pedigrees (on average eight family members) were used and six out of 10 when extensive pedigrees (on average 39 family members) were used. The correct results are: the alternative formula was able to detect six out of 10 (with first degree pedigrees) and eight out of 10 (with extensive pedigrees) mutation carriers.

To test the algorithm of Wijnen *et al* further, we have now analysed 535 additional consecutive colorectal adenocarcinoma samples. The subjects ranged in age from 29 to 91 years with a mean age of 67 years. MSI analysis and genomic sequencing of *MLH1* and *MSH2* for MSI positive samples were performed previously (Salovaara *et al*, submitted); 66 out of 535 (12%) samples were MSI positive and 18 of these patients had an *MLH1* or *MSH2* germline mutation. We then

Table 1 Calculations using the algorithms of Wijnen *et al* to determine the probabilities of finding a germline *MLH1* or *MSH2* mutation in 28 mutation carrier probands

Patient	Mean age of diagnosis of CRC in the family	Fulfilment of Amsterdam criteria	Endometrial cancer in the family	No of patients with CRC	No of patients with endometrial cancer	No of family members with verified cancer status	p_1	p_2
(A) Limited pedigrees (proband and first degree relatives)								
c35	49	No	No	2	0	6	3	11
c54	36	No	No	1	0	4	10	24
c64	53	No	No	2	0	5	2	8
c125	52	No	No	2	0	9	2	9
c145	42	Yes	Yes	3	1	12	78	40
c219	50	No	Yes	1	1	7	13	16
c340	61	No	Yes	3	2	7	5	20
c430	38	No	No	2	0	4	8	25
c558	57	No	Yes	4	2	8	7	32
c564	45	No	Yes	2	1	7	19	28
c138	39	No	No	1	0	5	8	19
c275	55	No	No	2	0	9	2	7
c587	36	No	Yes	2	1	4	37	46
c614	50	No	Yes	3	2	6	13	40
c615	38	No	No	2	0	5	8	25
c661	54	No	Yes	1	1	6	9	12
c676	46	No	Yes	2	1	7	18	26
c698	43	No	Yes	1	1	6	22	26
c700	61	No	No	1	0	5	1	3
c811	54	No	No	2	0	14	2	7
c870	56	No	No	2	0	5	1	6
c899	50	No	No	2	0	6	3	10
c952	52	No	Yes	2	1	10	11	17
c953	45	No	Yes	6	3	9	19	83
c955	53	No	No	2	0	7	2	8
c1027	53	No	No	1	0	8	2	6
c1077	45	Yes	No	3	0	8	34	19
c1095	53	Yes	Yes	3	1	8	55	20
(B) Extended pedigrees								
c35	47	Yes	No	4	0	24	30	21
c54	35	No	No	1	0	13	11	25
c64	53	Yes	No	4	0	24	10	16
c125	48	Yes	No	4	0	14	28	19
c145	56	Yes	Yes	13	3	85	48	93
c219	52	No	Yes	7	2	49	11	62
c340	56	Yes	Yes	5	2	33	48	40
c430	44	Yes	Yes	9	1	33	75	73
c558	57	Yes	Yes	5	3	39	45	57
c564	45	Yes	Yes	12	4	77	73	98
c138	38	No	Yes	1	1	8	32	35
c275	46	Yes	Yes	10	4	87	71	97
c587	42	No	Yes	3	1	14	24	40
c614	49	No	Yes	3	2	42	14	42
c615	40	Yes	No	3	0	16	46	27
c661	54	No	Yes	1	1	24	9	12
c676	44	Yes	Yes	12	4	74	75	98
c698	46	Yes	Yes	10	4	87	71	97
c700	49	No	Yes	13	5	97	14	99
c811	48	Yes	No	5	0	29	28	24
c870	56	No	No	2	0	20	1	6
c899	50	No	No	2	0	8	3	10
c952	64	No	Yes	3	1	11	3	8
c953	44	Yes	Yes	12	4	74	75	98
c955	47	No	No	3	0	25	4	17
c1027	43	No	Yes	2	1	20	22	31
c1077	44	Yes	No	7	0	46	37	43
c1095	53	Yes	Yes	3	1	12	55	20

p_1 = probability of finding a germline mutation in *MLH1* or *MSH2*.

p_2 = probability of finding a germline mutation in *MLH1* or *MSH2*; the alternative formula.

combined the results with the previous set of samples and describe here the results from 1044 consecutive colorectal cancer patients. The results of all 28 mutation positive samples are presented in table 1. The basic formula identified five out of 28 (18%) mutation carriers when first degree pedigrees (on average eight family members) were used, and 18 out of 28 (64%) when extensive pedigrees (on average 39 family members) were used. The corresponding results for the alternative formula are 13 out of 28 (46%) and 21 out of 28 (75%). In addition, these formulae identified 11 patients in whom no *MLH1* or *MSH2* mutations could be found. They have all been tested and found negative for the two most common Finnish founder mutations. One of them was found to be a FAP patient and another one had juvenile polyposis. Three were sequenced for *MLH1* and *MSH2* mutations with negative results. The remaining six patients were all MSI negative, diagnosed before the age of 40, and had no family history of cancer.

Extensive pedigree data are difficult to obtain in clinical practice. When relying on first degree pedigrees, which are generally easily obtained during patient interview, the mathematical algorithms proposed by Wijnen *et al*¹ were able to detect 18% (p₁) and 46% (p₂) of mutation carriers. Even with extensive pedigree information, the formulae were able to detect only 61% (p₁) and 75% (p₂) of carriers, meaning that every third or every fourth were missed. Based on the analysis of 1044 colorectal cancer probands, we conclude that these mathematical formulae alone are of limited value and use of additional tools such as MSI screening is warranted.

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- 2 Loukola A, de la Chapelle A, Aaltonen L. Strategies to screen for hereditary nonpolyposis colorectal cancer. *J Med Genet* 1999;**36**:819-22.
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NOTICE

The Tenth International Clinical Genetics Seminar

"Genetics in Primary Care" is the main theme of the Tenth International Clinical Genetics Seminar to be held in Amman, Jordan on 20-24 October 2000. Further information may be obtained from Professor Mohammed El-Khateeb, Department of Pathology and Microbiology, University of Jordan/NCDEG, PO Box 13002, Amman 11943, Jordan. Fax: 00 962 6 535 5655. E-mail: Mkhateeb@ju.edu.jo