submicroscopic cases, with regard to the motor development and life span, compared to the larger deletions. Obviously, this might be expected for a "contiguous gene syndrome".

Several abnormalities in our three cases were related to the midline. This suggests that gene(s) involved in normal midline development might be located in the deleted region on 1q. The size of the telomeric deletion in two of the cases presented had previously been reported to be between 15.7 and 23.3 cM,[7] which therefore defines the critical region for such a gene(s).

Although several clinical manifestations in the two cases can be observed in other chromosomal disorders, the combination of features seems to be distinctive: severe mental retardation, growth retardation (prenatal onset), severe progressive microcephaly, hypospadias, corpus callosum abnormalities, cardiac anomalies, gastro-oesophageal reflux, and a characteristic facies. Knowledge of the pattern of this "1qter− phenotype" will help clinicians to diagnose this chromosomal abnormality in their patients and to counsel the parents accordingly.

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Pure partial 7p trisomy including the *TWIST*, *HOXA*, and *GLI3* genes

EDITOR—The clinical findings associated with 7p duplication have been well delineated. They comprise large fontanelles and sutures, hypertelorism, large, apparently low set ears, high arched palate, hip joint dislocation or contractures, a high frequency of cardiac septal defect, and mental retardation.[1–5] It usually results from malsegregation of a parental balanced translocation or through abnormal recombination caused by a parental inversion. Some cases, however, result from a partial de novo 7p duplication.[6–15] Because these cases represent pure 7p segmental imbalances, they are of great interest in phenotype-genotype correlation studies.

Here we present a case of pure 7p duplication resulting from an unbalanced inverted insertion of segment 7p13-p21.2 into the short arm of a chromosome 8. A comparative analysis of our case with those published previously suggests that the 7p21.1-p21.2 region might contain a critical region for the 7p duplication syndrome. Moreover, the presence in our patient of some opposite features of Saethre-Chotzen syndrome, which is the result of haploinsufficiency of the *TWIST* gene,[16 17] suggests that these findings may result from a triple dosage of this particular gene.

The patient, a 24 year old man, was referred to us for further investigation because he had dysmorphic features and was mentally retarded. He was the fourth child of healthy, non-consanguineous, Lebanese parents. At birth, the mother was 26 years old and the father 31 years old. The family history was unremarkable. Pregnancy and delivery at term had been uneventful. Birth weight was 3800 g (75th centile) and length 58 cm (97th centile). A right talipes equinovarus was noted at birth. The baby was breast fed and discharged from hospital on the third day of life. A severe delay in developmental milestones was observed as he walked at 5 years of age and said only a few words at 7 years of age. According to the parents, he had a wide open anterior fontanelle that closed only at 4 years of age.

On clinical examination, he was sociable and very affectionate. His height was 170 cm (25th centile), weight 47.5 kg (3rd centile), and head circumference 52.5 cm (60th centile). Physical measurements showed a facial height of 13.5 cm (>97th centile), forehead height 9.5 cm (35th centile), lower facial height 8 cm (>97th centile), arm span 165 cm, total upper limb length 67 cm (35th centile), upper arm length 37 cm (>95th centile), forearm length 27 cm (80th centile), hand length 16.4 cm (3rd centile), and total lower limb length 97 cm (35th centile). The face was long and triangular. There was a long nose with a broad nasal bridge, bushy eyebrows, mild ptosis of the right eyelid, convergent strabismus, and moderate hypertelorism. Ears were low set and protruding, with poorly folded helices. In addition, a deep and short philtrum, a thin upper lip, a small mouth with downturned corners, a high arched and narrow palate, a bifid uvula, and a massive chin were observed. The thorax was narrow with no pectus deformity. A right kyphoscoliosis was present (fig 1). There was a positive thumb sign and mild joint hyperextensibility.

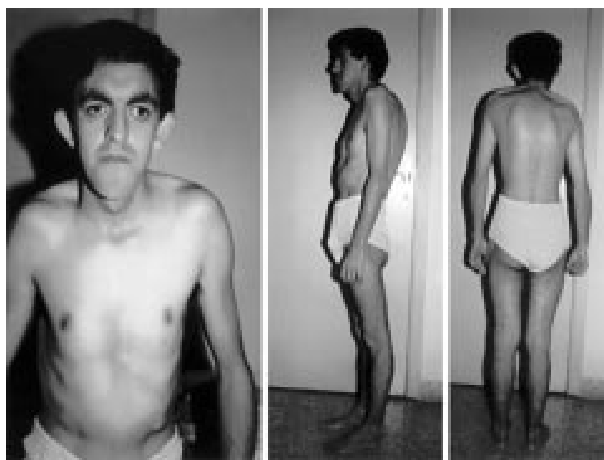

Figure 1 The patient: note long face with hypertelorism and microstomia, large, dysplastic ears, and kyphoscoliosis.

A right single palmar crease was noted. The external genitalia were unremarkable. Heart examination showed a grade 2/6 systolic murmur with maximum intensity in the mitral valve area and a B1 click. Echocardiography showed an ostium secundum atrial septal defect of 27 mm width with probable abnormal pulmonary venous return, a marked dilatation of the right chambers with paradoxical interventricular septum motion, and high pulmonary artery pressure related to pulmonary outflow without any physical obstacle. Full body skeletal radiography was performed and showed a right kyphoscoliosis, thin ribs especially on the right side, and a rectangular form of the vertebrae with broadening of the interpedicular length in L4 and L5. Increased malar angles, long phalanges, and generalised demineralisation were also noted. Magnetic resonance imaging of the brain was unremarkable. Ophthalmological and neurological investigations, abdominal ultrasound, and laboratory tests including liver and thyroid function studies were unremarkable.

High resolution chromosome analysis using RHG, GTG, and replication banding techniques were performed on peripheral blood lymphocyte cultures according to usual procedures. The chromosomes were classified according to the international nomenclature (ISCN, 1995).

Spectral analysis was performed according to the manufacturer's instructions (Applied Spectral Imaging). Briefly, 10 µl of the probe were hybridised to the patient's metaphases. Hybridisation was performed for two days at 37°C. Images were acquired with a SD200 Spectracube (Applied Spectral Imaging) mounted on a Zeiss Axiophot II microscope.

Chromosome 8 painting probe was obtained using *Alu*-PCR from a human-rodent cell line containing chromosome 8 as the sole human material, as previously described.[18]

YAC clone 321d10 and cosmid clones gc550 and gc68 correspond to the *GLI-3* gene locus (7p13).[19] YACs clones 961E5 (7p15) and 933E1 (7p21) encompass the *HOX A* gene complex[20] and the *TWIST* gene locus respectively.[21] The YACs clones 858H6 (D7S2557) and 938A6 (D7S664) (http://carbon.wi.mit.edu:8000/cgi-bin/contig/phys_map), which map to 7p21.2, and 933A5, which maps to the chromosome 8[22] long arm subtelomeric region, were also used. FISH studies were performed as previously described.[23] Comparative genomic hybridisation (CGH) was carried out as previously described.[24] High molecular weight DNA was extracted from the peripheral blood of the patient and a normal male control. One µg of DNA was labelled by nick translation (Vysis, Downers Grove, IL, USA) using FluorX (FluorX Amido 10dCTP) for patient and cyanine 3 (Cy3-AP3-dUTP) (Amersham Life Science, Arlington Heights, IL, USA) for control DNA. For both patient and control, 200 ng of DNA were coprecipitated with 70 µg of unlabelled Cot-1 DNA (Life Technologies, Pasler, Scotland), resuspended in 12 µl of a hybridisation mixture, and hybridised on normal metaphase spreads for two days at 37°C. After post-hybridisation washing, the slides were analysed using a Leica DMRXA epifluorescence microscope. Images were processed and analysed with the Quips CGH Software (Vysis, Downers Grove, IL).

The microsatellite marker D7S2564[25] was studied using the following standard PCR conditions: three PCR reactions were performed in a total volume of 50 µl, containing 80 ng of the father's, mother's and patient's genomic DNA, 50 pmol of each primer, 0.125 mmol/l dNTPs, and 1 unit of *Taq* polymerase. Amplification buffer (1×) contained 10 mmol/l Tris base pH 9, 50 mmol/l KCl, and 1.5 mmol/l $MgCl_2$. Amplifications were carried out for 30 cycles of denaturation (94°C for 40 seconds) and annealing (55°C for 40 seconds). An elongation step (72°C for 40 seconds) ended the process after the final annealing.

Analysis of the patient's chromosomes showed, in all metaphases examined, an abnormal short arm of chromosome 8, with the presence of extra material of unknown origin inserted into band 8p23.1 (fig 2). The chromosomes of the parents were normal.

Molecular cytogenetic analysis was performed to characterise this chromosomal abnormality. FISH using a chromosome 8 painting probe excluded the presence of a chromosome 8 duplication. Spectral karyotyping showed that the extra material originated from chromosome 7 and CGH showed a 7p13-p21 duplication (fig 2A, B). Molecular analysis using microsatellite DNA markers mapping to the inserted chromosome 7p13-p21 region showed that this insertion was of paternal origin (data not shown).

To delineate this chromosomal abnormality further, we performed FISH studies using cosmid and YACs clones encompassing different loci mapping along chromosome 7p. This study showed the presence of an unbalanced inverted insertion of segment 7p13-p21.2 including the *GLI-3*, *HOXA*, and *TWIST* genes into the short arm of the chromosome 8 (fig 2C). In particular, we mapped the *TWIST* gene to the telomeric part of chromosome band 7p21.1. Furthermore, as the critical 7p duplication region has been assigned to 7p21-pter,[26] we decided to map the telomeric breakpoint of our patient's insertion in order to define more precisely the 7p duplication region at the molecular level. For this purpose we performed FISH studies using different chromosome 7p21 YAC clones and showed that the insertion telomeric breakpoint mapped in the 7p21.2 band region between YAC 858 H6 (D7S2557) and YAC 938A6 (D7S664) in a 1 Mb region containing the *MOX/GAX* gene locus (NCBI) (table 1).

Numerous patients with complete or partial 7p duplication have been reported.[26] In infants and children, common findings comprise a large anterior fontanelle, hypertelorism, skull anomalies, large, apparently low set ears, high arched palate, joint dislocation or contractures, a high frequency of cardiac septal defect, and mental retardation. The adult phenotype is less well known. Recognition of the clinical spectrum in patients with smaller duplications has suggested restriction of the critical region to 7p15-pter.[5 27] The most recent review, based on the observation of a patient with an unbalanced translocation resulting in 7p21.2-pter duplication and a characteristic clinical phenotype including a large anterior fontanelle, assigned the critical region of the 7p duplication syndrome to 7p21.2-pter.[26] However, the duplicated chromosome

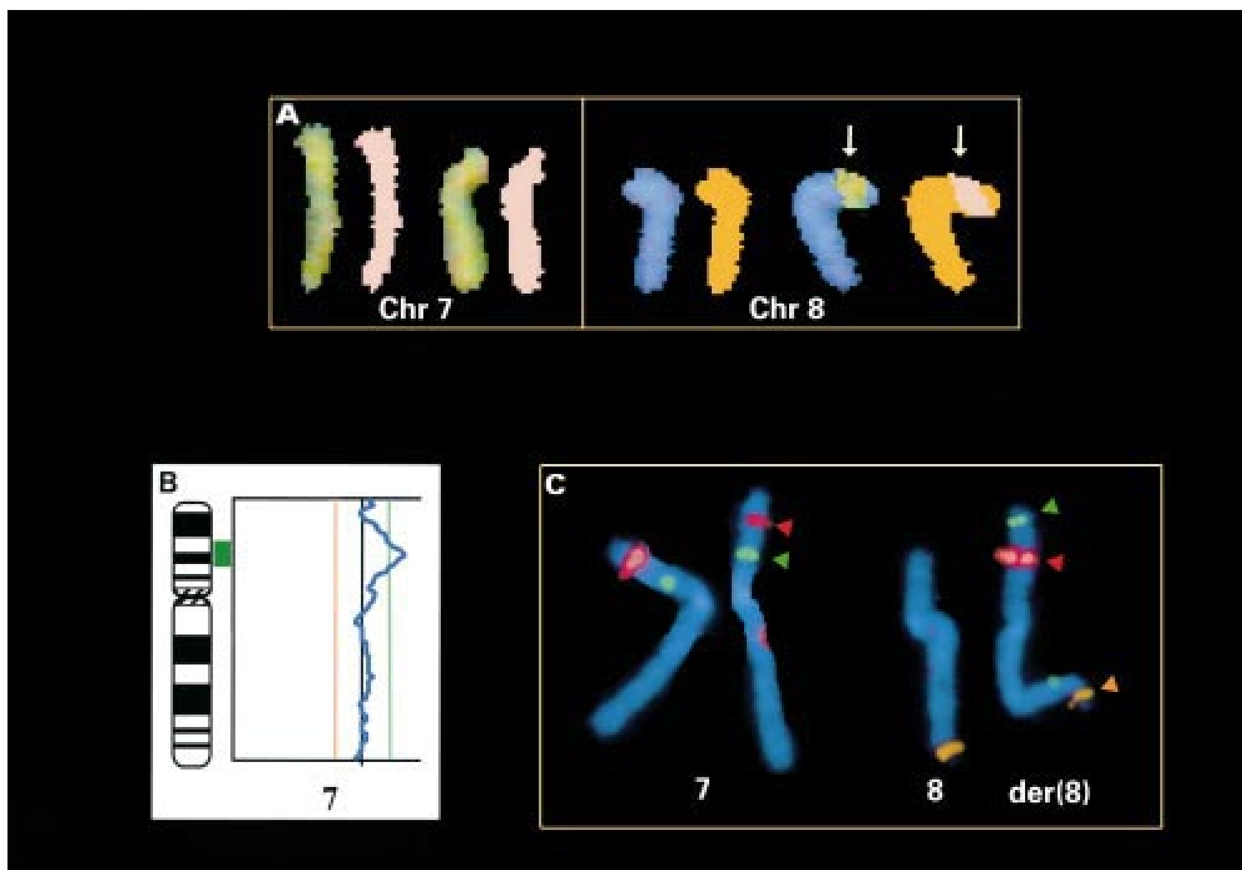

Figure 2 FISH characterisation of the inv ins(8;7). (A) Partial spectral karyotype showing the chromosome 7 insertion into the short arm of chromosome 8. Images were acquired with a SD2000 Spectracube (Applied Spectral Imaging) mounted on a Zeiss Axiophot II microscope. For chromosomes 7 and 8, the two colours correspond to RGB (red, green, and blue) colour and artificial pseudocolour, respectively. The inserted 7p segment is indicated by the arrow. (B) Partial CGH result indicating that the chromosome 7 inserted segment is 7p13-p21. Images were processed and analysed with the Quips CGH Software (Vysis, Downers Grove, IL). (C) FISH with probes corresponding to the GLI-3 (green arrowhead) and the TWIST loci (red arrowhead) as well as the chromosome 8qter region (YAC 933a5, orange arrowhead). The chromosomes were counterstained with DAPI. Note that the order of the GLI-3 and TWIST genes is inverted on the der(8).

segment was not mapped precisely as molecular cytogenetic techniques were not used.

Here we report on a patient with moderate mental retardation and with several clinical features associated with partial 7p duplication, including mild hypertelorism, large, protruding ears, a small mouth with downturned corners, high arched palate, cardiac septal defect, and late closure of a large anterior fontanelle. Detailed molecular cytogenetic analysis showed that the patient carried an unbalanced inverted insertion of the 7p13-p21.2 segment into chromosome 8p23 (fig 3). This observation and previously reported cases suggested that the 7p21.1-p21.2 band region could be critical for the main manifestations of the 7p duplication phenotype.

The 7p21.1-p21.2 band region contains the *TWIST* gene which encodes a transcription factor of the basic helix-loop-helix protein family and plays an important role in mesodermal cell determination. In particular, the *TWIST* gene is involved in membranous ossification occurring during frontal, parietal, and malar bone formation.[28 29] In humans, haploinsufficiency of the *TWIST* gene has been shown to be associated with Saethre-Chotzen syndrome which is characterised by craniosynostosis, a flat face with a thin, long, pointed nose, shallow orbits, plagiocephaly, small, posteriorly rotated ears with long and prominent crus, cleft palate, and often subtle abnormalities of the hands such as mild syndactyly of digits 2 and 3 and bifid terminal phalanges of the hallux, congenital heart defects, and contractures of the elbow and knee.[16 30–32] In addition, mice heterozygous for *TWIST* gene mutations present with craniosynostosis apparently related

Table 1 FISH studies

Clone name	*Origin*	*Localisation*	*Other features*	*Status*
YAC 321d10	CEPH/A Vortkamp	7p13	Gene *GLI3*	T
Cosmid gc550	A Vortkamp	7p13	Gene *GLI3*	T
Cosmid gc68	A Vortkamp	7p13	Gene *GLI3*	T
YAC 961e5	CEPH	7p15	Gene *HOX A*	T
YAC 933e1	CEPH	7p21–22	Gene *TWIST*	T
YAC 858h6	CEPH	7p21	D7S2557	T
YAC 938a6	CEPH	7p21.2	D7S664	N
YAC 933a5	CEPH/T Haaf	8qter	D8S1837	N

The chromosomal map of YACs 961E5, 933E1, 858H6, 938A6, and 933A5 are derived from our own experiments.
T: three signals observed on patient metaphases (two on each chromosome 7 and one on the der(8)).
N: two signals observed on patient metaphases.

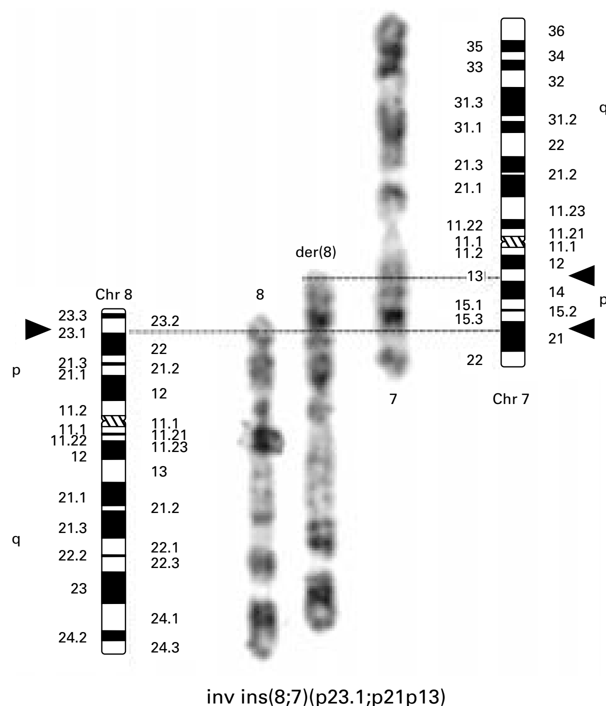

Figure 3 Partial karyotype of the inv ins(8;7) (p23.1;p21.2p13). Chromosome 7 is presented in the inverted position to match the inserted inverted segment into chromosome 8p. Breakpoints are indicated by arrows. Replication R bands were obtained after BrdU incorporation and FPG staining.

to precocious parietal and frontal bone formation as well as abnormal hindlimb development.[29]

Delayed closure of a large anterior fontanelle, a characteristic clinical feature of partial 7p duplication, is the opposite of craniosynostosis, a common clinical finding in the corresponding 7p deletion syndrome,[33] and in the Saethre-Chotzen syndrome.[16] In addition, we mapped the *TWIST* gene precisely in the putative 7p21-1p21.2 duplication syndrome region. Therefore, we would like to suggest that triple dosage of the *TWIST* gene may be responsible for this characteristic clinical feature of the partial 7p duplication syndrome. Indeed, it is not unreasonable to believe that this characteristic may represent a direct reflection of reciprocal gene dosage effects of this particular gene during craniofacial and limb development rather than a mere random event.

Another gene mapping in the putative 7p duplication syndrome region is the *MOX2* gene, which maps in the 7p21.2 band between D75S557 and D7S662 (http://www.ncbi.nlm.nih.gov/Locus.ink/LocRpt.cgi?l=4223) and encodes a homeobox protein implicated in limb muscle and craniofacial development.[34] Interestingly, it has been shown that overexpression of this protein in transgenic mice is associated with decreased cardiomyocyte cell proliferation and abnormal heart morphogenesis.[35] *MOX2* could therefore be a good candidate for heart defects often observed in 7p duplication syndrome. The fact that in our patient the 7p21.2 breakpoint mapped between D75S557 and D7S662 indicates that the *MOX2* gene is likely to be implicated in the duplication.

Finally, in the present observation the duplicated 7p13-p21.1 segment also includes the *GLI3* gene and the homeobox *HOXA* gene complex. Haploinsufficiency of the *GLI3* gene has been associated with Pallister-Hall syndrome,[36] Greig cephalopolysyndactyly syndrome,[37] and postaxial polydactyly type AI,[38] whereas mutations of the *HOXA 13* gene or full deletion of the *HOXA* cluster have been reported in the hand-foot-genital syndrome.[20] No opposite features of the *GLI3* gene or *HOXA* cluster haploinsufficiency were observed in our patient. In particular, the hands, feet, and genitalia are unremarkable. In the present case, the presence of three copies of these genes is not associated with a recognisable impact on the 7p duplication phenotype. It is noteworthy that both of these genes map proximal to the estimated critical segment.

In conclusion, the presence of the *TWIST* gene in triple dosage may be causally related to the presence of a large anterior fontanelle with delayed closure, which is the more characteristic clinical feature of the 7p duplication syndrome. It would be interesting to search for duplication of the *TWIST* gene in patients presenting with a large anterior fontanelle with delayed closure associated or not with mental retardation.

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Suggestive linkage of situs inversus and other left-right axis anomalies to chromosome 6p

EDITOR—Congenital heart disease occurs commonly. One form, heterotaxy, accounts for approximately 3-4% of the total incidence and has a mortality rate approaching 45%.[1] Given that the diagnosis is based on the discordance of the left-right (LR) sidedness between the abdominal viscera and atria,[2] heterotaxy describes a group of malformations arising from the abnormal development of LR asymmetry.[3]

In familial cases one can find subjects with complete, mirror image reversal of normal LR anatomy (situs inversus), and others who manifest the hallmark visceroatrial discordance as well as other laterality malformations (sometimes collectively called situs ambiguus). Moreover other family members with normal LR anatomy (situs solitus) are obligate disease gene carriers by virtue of their pedigree position.

Many genes have been implicated in normal and abnormal LR axis development among non-human vertebrates.[4] Knowledge remains sparse, however, regarding the molecular genetics of human LR malformations. Positional cloning identified a gene, *ZIC3*, on chromosome Xq24-27.1, in which mutations have been found among one sporadic and six familial cases of LR axis malformations.[5] A few mutations have also been found in *LEFTYA* and in the activin receptor type IIB gene (*ACVR2B*), identified on the basis of their homology to the corresponding genes known to cause laterality defects in the mouse.[6 7]

Here we describe a family in which LR malformations segregate across five generations. Although male to male transmission has not occurred, males and females appear to be affected similarly, and linkage analysis has excluded a disease locus on the X chromosome (see below). Both situs inversus and situs ambiguus are found in seven affected subjects and pedigree position implicates four apparently normal subjects as obligate gene carriers. These observations strongly support a model of autosomal dominant inheritance with reduced penetrance. The pedigree comprising 36 subjects is illustrated in fig 1

Seven subjects in five generations manifest laterality defects of multiple organs (fig 1). Of these, four are situs inversus (II.2, III.7, III.10, and IV.6), and three are situs ambiguus (IV.8, V.1, and V.4). There is considerable variability of expression in the situs ambiguus group. IV.8 has mirror image reversal of the heart and of the colon but normal position of the liver, stomach, and spleen, while complex heart malformations were identified in the other two, leading either to prenatal termination (V.1) or surgery (V.4). II.4, III.1, III.6, and IV.4 are obligate disease gene carriers by virtue of their pedigree position but without apparent LR abnormalities. III.9 and V.2 have isolated cardiac defects without any other LR abnormality. The malformation observed in III.9, ventricular inversion in combination with transposition of the great arteries, is usually classified with heterotaxy under the common aetiology of "abnormal looping" defects. Therefore, III.9 was scored as affected in the linkage analysis, while V.2, who showed hypoplastic left heart syndrome (HLHS), which has not been linked embryologically to the cardiac looping defects, was scored as having an unknown disease status. All subjects manifesting laterality defects but III.10, who was unavailable, were included in the linkage analysis and scored as affected. In all subjects, disease status phenotype was assigned before marker genotyping.

Informed consent was obtained from patients participating in this study, which was approved by the Institutional Board at Baylor College of Medicine. Genomic DNA was extracted from whole blood or cell lines (lymphoblast or fibroblast) with the Puregene DNA Isolation Kit (Gentra Systems) according to the manufacturer's protocol. DNA from paraffin embedded tissue was extracted as previously described.[8] Amplifications were performed on HYBAID Omnigene thermocyclers under standard conditions.

The initial screening was performed at the Center for Medical Genetics in Marshfield, WI, using marker screening set 6, consisting of short tandem repeat markers with an