Original Paper

European Journal
of Human Genetics

Eur J Hum Genet 1996;4:267–273

Confinement of PGL, an Imprinted Gene Causing Hereditary Paragangliomas, to a 2-cM Interval on 11q22-q23 and Exclusion of DRD2 and NCAM as Candidate Genes

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Key Words
Chromosome 11q22–q23 · Paraganglioma · Glomus tumour · Haplotype sharing · Linkage

Abstract
Paragangliomas of the head and neck region, also known as glomus tumours, are mostly benign tumours of neuro-ectodermal origin. We mapped the familial form by linkage analysis in 6 families to chromosome region 11q22–q23, between the markers STMY and CD3D which currently span a 16-cM interval. Here, we performed detailed haplotype analysis of this region in a single Dutch multibranch 7-generation family. A region of 2 cM between the markers D11S938/D11S4122 and D11S1885 was shared between all patients of whom disease haplotypes could be reconstructed. In support of this localization, a recombination observed in a small French family with 2 affected nieces places the PGL gene proximal to marker D11S908, genetically coincident with D11S1885.

Introduction

Paragangliomas of the head and neck region, also known as glomus tumours or chemodectomas, are slow-growing, mostly benign tumours of neuro-ectodermal origin. Their incidence has been estimated to be approximately 1:100,000 and they manifest roughly between the age of 18 and 60 [1].

The familial form of the disease displays an autosomal dominant mode of inheritance [1, 2]. However, the tumours only develop in individuals who have inherited the gene paternally, whereas maternal transmission results in non-affected carriers only [2]. This has been interpreted as evidence that the underlying gene defect is subject to 'genomic imprinting' [2], an epigenetic mechanism whereby, in a reversible process, a gamete-specific modification in the parental generation leads to functional differences between maternal and paternal genomes in the offspring [3].

In an earlier study we have mapped the disease gene, termed PGL (OMIM 168000), by linkage analysis in one large Dutch family (FGT01) to chromosome 11q22–q23 [4]. The reported meiotic recombinants in the families positioned PGL between the markers STMY and CD3D,

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1018–4813/96/0045–0267$10.00/0

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http://BioMedNet.com/karger

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Received:
February 29, 1996
Accepted:
May 8, 1996

Fig. 1. Simplified pedigree of family FGT189. Circles are females, squares males. Solid symbols represent patients (according to medical record), open symbols non-affected persons; dashed symbols are persons of whom we were not able to retrieve the medical record, but who should be affected according to the mode of inheritance. Symbols with a dot are obligate (imprinted) carriers. An asterisk indicates the person in the branch (A through K) as far back in genealogy as possible of whom we can deduce the haplotype with certainty. For orientation purposes the year of birth is given below for some individuals. The man in branch G (gray symbol) did not want to cooperate in this study.

separated at a 26-cM genetic distance [4, 5]. More recent estimates fix this distance at 16 cM [6]. Although no statistical evidence was obtained for the involvement of genetic heterogeneity [5], a single family has been shown to link to markers for 11q13, and not to be due to the PGL locus on 11q22–q23 [7, 8]. This suggests that a second locus may exist.

Because paragangliomas are rare, few families become available for recombinant analyses, and the current size of the gene region of 16 cM is too large to seriously attempt the positional cloning of PGL. We present here the detailed haplotype analysis of this region in a single Dutch multibranch 7-generation family. This allowed the definition of a shared haplotype between all affected descendants, narrowing the candidate gene region to an interval of 2 cM between the markers D11S938/D11S4122 and D11S1885.

Materials and Methods

Family Ascertainment

Diagnosis of paraganglioma was based on medical history, physical and otolaryngological examination, and/or determination of free urinary catecholamine excretion [2, 4, 9]. In 94 individuals, magnetic resonance imaging (MRI) of the head and neck region was performed as well [10]. Part of the large family (branches E-H in this study; fig. 1) has been presented earlier as FGT01, in which the initial linkage was obtained [4]. Branches A, C and D were previously presented as family FGT09 [5].

DNA Isolation and PCR Analysis

Blood samples were collected from 190 individuals and genomic DNA was isolated from peripheral blood lymphocytes as described by Miller [11]. PCR conditions to visualize microsatellite polymorphisms were as described [5, 12]. All primer sequences for these markers are retrievable online from GDB (http:/gdbwww.gdb.org/) or Généthon (http:/www.genethon.fr/) databases, and all oligonucleotides were manufactured by Isogen Inc.

Linkage Analysis

Linkage analysis was performed using the LINKAGE program package version 5.1 [13]. Briefly, 8 liability classes were defined to account for age of onset and the absence of penetrance in children of female gene carriers [4, 5]. The population incidence of glomus tumours has been estimated to be 1:100,000, but we believe this is probably an underestimation. In addition, because the disease shows incomplete penetrance, the number of gene carriers is probably higher than the number of patients. Hence we have used a conservative estimate for the disease gene frequency of 0.001 in order not to inflate lod scores. Allele lengths of the markers were determined using an M13 sequence as reference, and were as expected from GDB. Lod scores were computed using allele frequencies that were determined in 41 unrelated Dutch individuals from the same area in the Netherlands from which the 7-generation family originated. Twenty of these were spouses marrying into this family, the others were spouses marrying into 3 families with familial atypical multiple mole melanoma syndrome [14].

For haplotype analysis we used a marker order which was derived from the NIH-CEPH Collaborative Mapping Group [15, 16], complemented with data from the Généthon group [6, 17] and a radiation hybrid map [18]. For all markers, the odds for their mutual order was 1,000:1 unless stated otherwise.

The investigation of the inferred second locus for hereditary paragangliomas on 11q13 was performed with the markers D11S554, D11S905, D11S956, D11S480, PYGM, and FGF3.

Results

In an attempt to further map PGL more accurately, a search for recombinants in the disease-associated haplotype was initiated by typing a total of 19 families with the markers D11S897, NCAM, D11S490 and CD3D, which map to both extremes of the 16-cM candidate region reported previously [4, 5]. Both linkage and haplotype analysis failed to provide evidence for such recombinants, possibly because many of the kindreds analyzed were of relatively small size, and few patients in at least two generations were available for marker typing. By genealogical analysis, we were able to link two of these families into the kindred in which the initial linkage to 11q22–q23 was reported (FGT01 [4]), resulting in a large 7-generation multibranch family derived from a small geographic area in the Netherlands (fig. 1). The comparison of the disease haplotypes of all affected individuals would in principle allow the detection of ancestral recombination events. To this end, a total of 190 individuals, including 25 affected subjects, were genotyped at 21 different polymorphic markers mapping at regular intervals across the entire 16-cM candidate region (fig. 2).

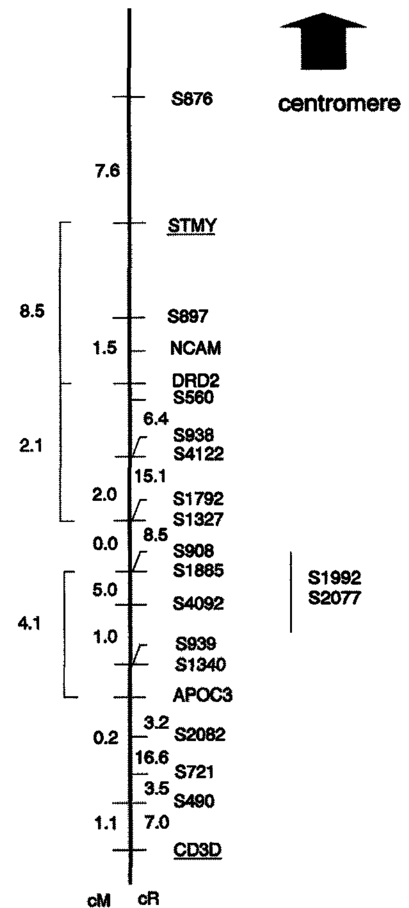

Fig. 2. Map of chromosome 11q22–23 containing the markers used. Odds for order are 1,000:1 for markers with a horizontal line; a vertical line represents markers of which the location is not exactly known. Distances are given in cM (on the left) or in cR (on the right) [6, 15–18]. The markers underlined represent the previous borders of the PGL-containing region [5].

Linkage Analysis

Table 1 shows the 2-point lod score calculations for 12 of the 21 markers used. Seven markers provided significant evidence for linkage, but only D11S908 showed linkage at $\theta = 0.00$ (maximum lod score of 3.99). D11S1327 and D11S1792, which map close to D11S908, showed weak positive scores at $\theta = 0.00$, presumably because their linked alleles are very frequent in the population (64 and 76%, respectively). The frequency of the linked haplotype defined by these three markers was determined to be 18% in our reference population. By recoding the three marker haplotype to a single 'marker', a lod score of 5.77 was obtained at $\theta = 0.00$ (data not shown).

Table 1. Two-point lod scores for family FGT189 (8 liability classes) using population based allele frequencies

Marker[1]	θ						Max lod	θ
	0.000	0.010	0.050	0.100	0.200	0.300		
NCAM	$-\infty$	5.90	6.81	6.66	5.38	3.53	6.83	0.06
DRD2	$-\infty$	0.35	0.96	1.06	0.84	0.47	1.06	0.09
D11S560	$-\infty$	7.93	8.74	8.45	6.90	4.71	8.74	0.05
D11S938	$-\infty$	0.61	1.82	2.03	1.65	0.91	2.03	0.10
D11S4122	$-\infty$	4.29	5.27	5.22	4.19	2.66	5.32	0.06
D11S1792	0.88	0.86	0.79	0.67	0.39	0.14	0.88	0.00
D11S1327	1.42	1.38	1.22	1.03	0.66	0.34	1.42	0.00
D11S908	3.99	3.92	3.60	3.18	2.24	1.28	3.99	0.00
D11S1885	$-\infty$	7.22	7.23	6.61	4.96	3.09	7.36	0.02
D11S2082	$-\infty$	2.86	4.80	5.16	4.51	3.14	5.16	0.10
D11S490	$-\infty$	2.74	2.93	2.61	1.72	0.87	2.96	0.03
CD3D	$-\infty$	2.61	3.58	3.57	2.82	1.74	3.63	0.07

1 Markers are displayed from centromere to 11q telomere.

Table 2. Haplotype analysis in the family FGT189

Marker[1]	Disease haplotype in branch											Freq.[2] %	Alleles[3]
	A	B	C	D	E	F	G	H	I	J	K		
D11S876	6	6	6	6	4	4	4	1	7	7	7	6	11
D11S897	2	2	1	1	1	1	1	1	1	1	1	7	8
NCAM	7	5	3	3	3	3	3	3	3	3	3	7	12
DRD2	2	3	2	2	2	2	2	2	2	2	2	52	5
D11S560	8	4	3	3	3	3	3	3	3	3	3	4	8
D11S938	1	2	3	3	3	3	3	3	3	3	3	58	6
D11S4122	7	7	5	5	5	5	5	5	5	5	5	10	7
D11S1792	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**76**	**4**
D11S1327	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**64**	**4**
D11S908	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**38**	**6**
D11S1885	4	2	2	2	2	2	2	2	2	2	2	11	10
D11S1992	2	3	3	3	3	3	3	3	3	3	3	ND	6
D11S2077	2	2	1	1	1	1	1	1	1	1	1	ND	2
D11S4092	4	4	5	5	3	3	3	3	5	3	3	46	6
D11S939	4	4	4	4	2	2	2	2	2	2	2	45	4
D11S1340	2	2	5	5	2	2	2	2	1	1	1	22	6
APOC3	1	4	5	5	1	1	1	1	9	9	9	2	15
D11S2082	8	4	1	1	10	10	10	10	10	10	10	6	13
D11S721	7	4	4	4	11	11	11	11	11	11	11	ND	ND
D11S490	4	6	6	6	6	6	6	6	6	6	6	32	11
CD3D	2	4	1	1	4	4	4	4	4	4	4	27	6
Patients[4]	1	2	2	1	1	3	4	8	1	2	3		

1 Markers are shown from centromere to 11q-telomere.
2 Freq. = Percentage of the most frequent disease-allele among 82 chromosomes marrying into the family. ND = Not determined.
3 Total number of different alleles within this family.
4 Number of patients in which the haplotype is found.
The marker STMY (previous border) is located between the markers D11S876 and D11S897.

Haplotype Analysis

A total of 29 affected persons were ascertained in the lower 3 generations, and the disease-linked haplotypes were reconstructed with data from 21 markers in 25 of these (table 2). No recombinants were detected in the disease haplotype in those cases where marker data were available for affected or carrier parents as well as their affected child(ren). Therefore, for the sake of comparison, all sibships with an affected case were considered a separate branch of the family, designated A through K (fig. 1), and each could be represented by a single disease-associated haplotype (table 2).

Since all these branches descend from the same ancestral female, all patients are presumed to carry the same gene defect and alleles of markers closely bordering this gene are expected to be identical by descent. This phenomenon was observed with the same 3 markers that showed tight linkage, i.e. D11S1327, D11S1792, and D11S908 (table 2). This suggests that ancestral recombinants have occurred between PGL and D11S938/D11S4122 (these markers are genetically not separated) and between PGL and D11S1885. These markers span a sex-average genetic distance of 2 cM [6]. Notably, the haplotypes C through K appear identical over a much larger region of chromosome 11q, namely between the markers D11S876 and D11S4092, a distance of approximately 10 cM. Haplotypes A and B are most divergent from this, and share only the region D11S938-D11S1885 between them.

While this work was in progress, a small family of French origin (FGT21) was referred to our lab. After haplotyping, the 2 affected nieces (ID number 8 and 10 in fig. 3) appear only to share the region proximal to marker D11S908. This implies a recombination between markers D11S1327 and D11S908 in either one of the parents. Unfortunately, we do not have DNA samples from the grandmother to determine where this recombination occurred. These results suggest that D11S908 can be excluded from the region containing PGL.

Locus Heterogeneity

A second locus for PGL has been mapped to 11q13 in a single family between the markers D11S956 and PYGM, a sex average genetic region of 5 cM [7, 8]. These and several other markers, including D11S480, the only marker showing complete allele-sharing in that family, were investigated here. No common haplotype could be defined among the branches E-H and among the branches A, C and D (data not shown). Also in family FGT21 no shared haplotype could be detected between the 2 affected individuals, of whom marker D11S480 is shown in figure 3.

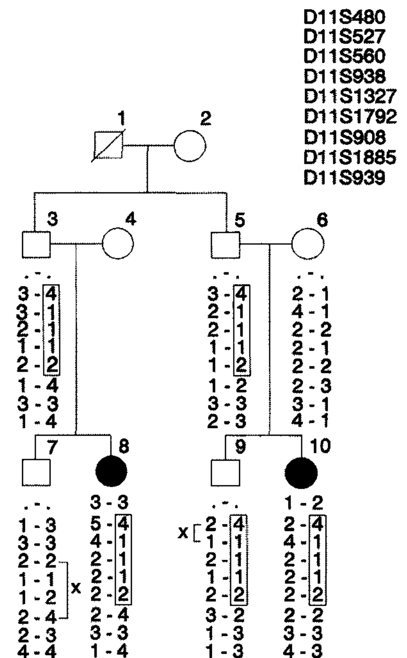

Fig. 3. Pedigree of family FGT21. The symbols used are as in figure 1. Chromosome 11q markers are displayed from centromere to telomere. The shared segment of chromosome 11q23 between the two patients is boxed. Marker D11S4122 is not informative in this family.

Discussion

We have presented evidence that PGL maps to a 2-cM interval on 11q22–q23 by haplotype sharing between affected persons in a large Dutch family and a small French family. Both kindreds are consistent with the sex-dependent modification of gene expression ('imprinting'). Recently, genomic imprinting in families with paragangliomas of the head and neck region was also confirmed independently for 9 US families [19]. Our results represent a significant reduction of the candidate gene region, which stood at 16 cM after recombinant analysis of 6 families [5, 6], and now excludes the dopamine receptor 2 gene (DRD2) and neural cell adhesion molecule gene (NCAM) as candidates for the disease. The PGL region maps between the markers D11S938/D11S4122 and D11S908/D11S1885. The physical localization of these marker pairs in relation to each other is not yet known.

The order of the markers used for the reconstruction of haplotypes was compiled from different types of maps: genetic maps [6, 15–17] and a radiation hybrid map [18], which overlap partially in terms of markers used. One inconsistency of particular concern for this study was the position of D11S1327. RH mapping placed it at the same position as D11S1792 [18], which is supported by characterization of YACs in this region [M. James, unpubl.]. Meiotic recombinant analysis in CEPH families, however, located this marker 1 cM proximal to D11S938 [6], i.e. 3 cM proximal to D11S1792/D11S908. On the basis of the depth of the YAC contig at this position, the RH map, our own cosmid map [unpubl.], and the potential errors in CEPH family analysis, we have here assumed that D11S1327 and D11S1792 are tightly linked.

A potential pitfall in the analysis of the haplotype sharing might be that all family members are from a small isolated community living in a small geographic area in the Netherlands. Thus an identical haplotype might have been brought into the family by an unsuspected consanguineous relationship. Consequently, certain recombination events might have been missed. Indeed, the two large blocks of shared haplotypes interrupted by a discordant block in branches E–K (table 2) might be reflecting such an event. However, since all affected members of the family carry the same mutation by descent, this can only lead to overestimation of the candidate region, but not to a false candidate region. On the other hand, underestimation of the candidate region could occur by mutations in the markers bordering this region. Since frequencies for such events are low (10^{-3}–10^{-4} [20]) and any 2 patients in the large family are at most separated by 14 meiotic events, this seems less likely here. The power of linkage disequilibrium mapping and/or haplotype sharing for gene isolation in founder populations has been demonstrated before [21, 22]. Due to the high frequencies of the linked alleles at the three shared loci, the statistical support for this region is just significant. The isolation of additional markers within the current gene region with a low frequency of the disease-linked allele should increase this significance. The localization reported here is supported independently by our analysis of loss of heterozygosity in several glomus tumours, both sporadic and familial [23]. These tumours all seem to affect the region distal to marker D11S560, which includes the currently defined 2-cM candidate gene region.

The absence of meiotic recombinants in 17 families, although they were of various sizes, is unexpected. At least 50 informative (i.e. of paternal origin) meioses could be scored, and hence about 8 recombination events were expected, especially considering that a significant excess of male over female recombination has been noted in this region [16]. On the other hand, regions as large as 10 cM have been found to be transmitted randomly without recombination through multiple generations in several extended families [14, 24, 25]. Our inability to identify recombinants in the initial screen of 17 families might thus be purely coincidental, particularly as we found evidence for at least a few ancestral recombination events. It is nonetheless tempting to consider that if recombination suppression existed in this region, it may be related to the disease-causing mutation. A link has been proposed between genomic imprinting and sex-specific recombination [26], while a class of mutations has been proposed that might interfere with the process of genomic imprinting [27, 28]. We cannot exclude that the mutation in PGL would render an otherwise non-imprinted gene susceptible for genomic imprinting, concomitantly affecting meiotic recombination.

We are unable to obtain evidence for the involvement of an inferred second locus for PGL at 11q13 [7, 8], not in the two families reported here, nor in other families [5, our unpublished data]. This suggests that, if a second exists, it will play a minor role in inherited paraganglioma, but formal heterogeneity analysis will be required to confirm this.

Although our results represent significant progress towards identifying PGL, a genetic distance of 2 cM would imply a physical size of approximately 2 Mb and therefore a further reduction of this region is still required. A detailed physical map of the region, in conjunction with linkage disequilibrium measurements should enable this.

Acknowledgements

We thank all family members for their cooperation. We thank Dr. M. Schlumberger (Institut Gustave-Roussy, Villejuif, France) for referring family FGT21 and Dr. L. Sandkuijl for his statistical advice. This work was supported by the Dutch Organisation for Scientific Research (NWO 900-715-816) and the Dutch Cancer Society (KWF 93-537).

References

1 Zak FG, Lawson W: The Paragangliomic Chemoreceptor System: Physiology, Pathology and Clinical Medicine. New York, Springer, 1982, p 583.

2 Van der Mey AGL, Maaswinkel-Mooy PD, Cornelisse CJ, Schmidt PH, van de Kamp JJP: Genomic imprinting in hereditary glomus tumors: Evidence for new genetic theory. Lancet 1989;ii:1291–1294.

3 Barlow DP: Imprinting: A gamete's point of view. Trends Genet 1994;10:194–199.

4 Heutink P, van der Mey AGL, Sandkuyl LA, van Gils APG, Breedveld G, Bardoel A, van Vliet M, Cornelisse CJ, Oostra B, Devilee P: A gene subject to genomic imprinting and associated with hereditary paragangliomas maps to chromosome 11q23-qter. Hum Mol Genet 1992;1:7–10.

5 Heutink P, van Schothorst EM, van der Mey AGL, Bardoel AFJ, Breedveld G, Pertijs J, Sandkuijl LA, van Ommen GJB, Cornelisse CJ, Oostra BA, Devilee P: Further localisation of the gene for hereditary paragangliomas (PGL), and evidence for linkage in unrelated families. Eur J Hum Genet 1994;2:148–158.

6 Dib C, Fauré S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Marc S, Hazan J, Seboun E, Lathrop M, Gyapay G, Morissette J, Weissenbach J: A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 1996;380:152–154.

7 Mariman ECM, van Beersum SEC, Cremers CWRJ, van Baars FM, Ropers HH: Analysis of a second family with hereditary non-chromaffin paragangliomas locates the underlying gene at the proximal region of chromosome 11q. Hum Genet 1993;91:357–361.

8 Mariman ECM, van Beersum SEC, Cremers CWRJ, Struycken PM, Ropers HH: Fine mapping of a putatively imprinted gene for familial non-chromaffin paragangliomas to chromosome 11q13.1: Evidence for genetic heterogeneity. Hum Genet 1995;95:56–62.

9 Oosterwijk JC, Jansen JC, van Schothorst EM, Oosterhof AW, Devilee P, Bakker E, Zoeteweij MW, van der Mey AGL: First experiences with genetic counselling based on predictive DNA diagnosis in hereditary glomus tumors (paragangliomas). J Med Genet 1996;33:379–383.

10 Van Gils APG, Falke THM, van Erkel AR, Arndt J-W, Sandler MP, van der Mey AGL, Hoogma RPLM: MR imaging and MIBG scintigraphy of pheochromocytomas and extra-adrenal functioning paragangliomas. Radiographics 1991;11:37–57.

11 Miller SA, Dykes DD, Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1214–1215.

12 Weber JL, May PE: Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 1989;44:388–396.

13 Lathrop GM, Lalouel JM: Easy calculations of lod scores and genetic risks on a small computer. Am J Hum Genet 1994;36:460–465.

14 Gruis NA, Sandkuijl LA, van der Velden PA, Bergman W, Frants RR: CDKN2 explains part of the clinical phenotype in Dutch familial atypical multiple mole-melanoma (FAMMM) syndrome families. Melanoma Res 1995;5: 169–177.

15 NIH-CEPH Collaborative Mapping Group: A comprehensive genetic linkage map of the human genome. Science 1992;258:67–102.

16 Litt M, Kramer P, Kort E, Fain P, Cox S, Root D, White R, Weissenbach J, Donis-Keller H, Gatti R, Weber J, Nakamura Y, Julier C, Hayashi K, Spurr N, Dean M, Mandel J, Kidd K, Kruse T, Retief A, Bale A, Meo T, Vergnaud G, Warren S, Willard HF: The CEPH Consortium linkage map of human chromosome 11. Genomics 1995;27:101–112.

17 Gyapay G, Morisette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S, Bernardi G, Lathrop M, Weissenbach J: The 1993–94 Généthon human genetic linkage map. Nat Genet 1994;7:246–339.

18 James MJ, Ricard CW III, Schott J-J, Yousry C, Clark K, Bell J, Terwilliger JD, Hazan J, Dubay C, Vignal A, Agrapart M, Imai T, Nakamura Y, Polymeropoulos M, Weissenbach J, Cox DR, Lathrop GM: A radiation hybrid map of 506 STS markers spanning human chromosome 11. Nat Genet 1994;8:70–76.

19 McCaffrey TV, Meyer FB, Michels VV, Piepgras DG, Marion MS: Familial paragangliomas of the head and the neck. Arch Otolaryngol Head Neck Surg 1994;120:1211–1216.

20 Weber JL, Wong C: Mutation of human short tandem repeats. Hum Mol Genet 1993;2: 1123–1128.

21 Hästbacka J, de la Chapelle A, Mahtani MM, Clines G, Reeve-Daly MP, Daly M, Hamilton BA, Kusumi K, Trivedi B, Weaver A, Coloma A, Lovett M, Buckler A, Kaitila I, Lander ES: The diastrophic dysplasia gene encodes a novel sulfate transporter: Positional cloning by fine-structure linkage disequilibrium mapping. Cell 1994;78:1073–1087.

22 Ramsay M, Williamson R, Estivill X, Wainwright BJ, Ho M-F, Halford S, Kere J, Savilahti E, de la Chapelle A, Schwartz M, Schwartz M, Super M, Farndon P, Harding C, Meredith L, Al-Jader L, Ferec C, Claustres M, Casals T, Nunes V, Gasparini P, Savoia A, Pignatti PF, Novelli G, Bennarelli M, Dallapiccola B, Kalaydjieva L, Scambler PJ: Haplotype analysis to determine the position of a mutation among closely linked DNA markers. Hum Mol Genet 1993;2:1007–1014.

23 Devilee P, van Schothorst EM, Bardoel AFJ, Bonsing B, Kuipers-Dijkshoorn N, James MJ, Fleuren G, van der Mey AGL, Cornelisse CJ: Allelotype of head and neck paragangliomas: Allelic imbalance is confined to the long arm of chromosome 11, the site of the predisposing locus PGL. Genes Chrom Cancer 1994;11:71–78.

24 Nyström-Lahti M, Sistonen P, Mecklin J-P, Pylkkänen L, Aaltonen LA, Järvinen H, Weissenbach J, de la Chapelle A, Peltomäki P: Close linkage to chromosome 3p and conservation of ancestral founding haplotype in hereditary nonpolyposis colorectal cancer families. Proc Natl Acad Sci USA 1994;91:6054–6058.

25 Houwen RHJ, Baharloo S, Blankenship K, Raeymaekers P, Juyn J, Sandkuijl LA, Freimer NB: Genome screening by searching for shared segments: Mapping a gene for benign recurrent intrahepatic cholestasis. Nat Genet 1994;8: 380–386.

26 Pàldi A, Gyapay G, Jami J: Imprinted chromosomal regions of the human genome display sex-specific meiotic recombination frequencies. Curr Biol 1995;5:1030–1035.

27 Reik W, Brown KW, Schneid H, Le Bouc Y, Bickmore W, Maher ER: Imprinting mutations in the Beckwith-Wiedemann syndrome suggested by an altered imprinting pattern in the IGF2-H19 domain. Hum Mol Genet 1995;4: 2379–2385.

28 Buiting K, Saitoh S, Gross S, Dittrich B, Schwartz S, Nicholls RD, Horsthemke B: Inherited microdeletions in the Angelman and Prader-Willi syndromes define an imprinting centre on human chromosome 15. Nat Genet 1995;9:395–400.