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Genetic Analysis of 24 French Families with Multiple Endocrine Neoplasia Type 2A

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Summary

The gene for multiple endocrine neoplasia type 2A (MEN2A) has been mapped to the pericentromeric region of chromosome 10 by linkage analysis. Thirty-four families with multiple cases of medullary carcinoma of the thyroid (MTC), including 24 families with origins in France, have been typed with nine polymorphic markers spanning the centromere of chromosome 10. No recombination was observed between the MEN2A locus and either of the four loci D10Z1 (lod score 12.79), D10S102 (lod score 6.38), D10S94 (lod score 7.76), and D10S34 (lod score 5.94). There was no evidence for genetic linkage heterogeneity in the panel of 34 families. Haplotypes were constructed for a total of 11 polymorphisms in the MEN2A region, for mutation-bearing chromosomes in 24 French families and for 100 spouse controls. One haplotype was present in four MEN2A families but was not observed in any control ($P < .01$). Two additional families share a core segment of this haplotype near the MEN2A gene. It is likely that these six families have a common affected ancestor. Because the incidence of pheochromocytoma among carriers varies from 0% to 74% within these six families, it is probable that additional factors modify the expression of the MEN2A gene.

Introduction

Multiple endocrine neoplasia type 2A (MEN2A) is a dominantly inherited cancer syndrome consisting of medullary carcinoma of the thyroid (MTC), pheochromocytoma, and, in some families, parathyroid hyperplasia. The lifetime penetrance of medullary thyroid cancer is above 80%, but the precursor lesion, C-cell hyperplasia, is detectable in carriers from childhood on, by endocrine testing (pentagastrin-challenge test). In families where it appears, the penetrance of pheochromocytoma is roughly 50%, but the adrenal tumor is not present in all families with hereditary MTC. The gene for MEN2A has been mapped to the centromeric region of chromosome 10 (Mathew et al. 1987; Simpson et al. 1987), and several polymorphic DNA markers are now available to facilitate early carrier diagnosis (Sobol et al. 1989; Mathew et al. 1991). The gene for MEN2B, an unusually aggressive form of familial MTC and pheochromocytoma, associated with mucosal neuromas of the lips and tongue and with a Marfanoid appearance, is linked to the same region (Norum et al. 1990; Lairmore et al. 1991).

Figure 1 shows a map of the region around the centromere of chromosome 10 and includes the loci studied here. The relative position of FNRB and D10S34 on the short arm is not yet known (Goodfellow et al. 1989; Wu et al. 1989; Mathew et al. 1991). No recombinants have yet been observed between MEN2A and either the alphoid centromeric sequence D10Z1 (Wu et al. 1990*a*) or the two anonymous loci D10S94 and D10S102 (Goodfellow et al. 1990; Mathew et al. 1991). D10S94 has been assigned to proximal 10q11.2 through the analysis of somatic cell hybrids (Goodfellow et al. 1990). D10S102 also maps

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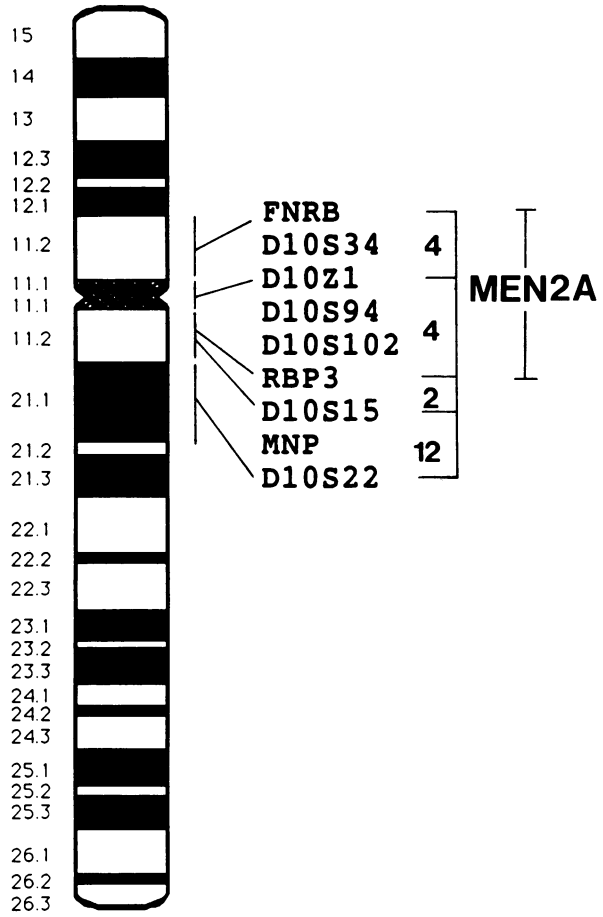

Figure 1 Map of pericentromeric region of chromosome 10. Sex-averaged map distances are taken from Wu et al. (1990*b*).

to the long arm of chromosome 10, telomeric to D10S94, by somatic cell hybridization (P. J. Goodfellow, unpublished data) and by linkage (K. Kidd, personal communication). RBP3 is the closest marker known to flank the MEN2A gene on the side opposite to FNRB and D10S34 (Nakamura et al. 1989; Wu et al. 1990*a*). Other informative loci include D10S15 (Nakamura et al. 1988*a*), mannose-binding protein (MNP) (Schuffenecker et al. 1991), and D10S22 (Bragg et al. 1988). It is not yet known whether MEN2A maps to the long or the short arm of the chromosome 10.

We have typed 34 MEN2A families by using 11 polymorphisms from these nine chromosome 10 loci. DNA haplotypes for the chromosomes carrying MEN2A mutations have been constructed in 24 French families to facilitate the mapping of the cancer-susceptibility gene and to help clarify the genetics of MEN2A in France.

Families, Material, and Methods

Families

Families with a history of MTC are identified in France through a national registry established by the Groupe d'Etude des Tumeurs à Calcitonine (GETC) (Calmettes 1984) and through interested referring physicians. A total of 34 families, from France, Italy, Portugal, and North Africa were studied for linkage. The haplotype analysis was restricted to the 24 families from France because there were adequate numbers of French controls and because putative links could be supported by genealogy. Diagnosis of MEN2A was made when at least two first-degree relatives were documented with MTC. Pheochromocytoma was present in one or more members of 20 families, and in 14 families MTC segregated alone. At-risk individuals were screened with the pentagastrin-challenge test, which identifies about 90% of gene carriers by age 25 years (Easton et al. 1989). An individual with a positive pentagastrin-stimulation test was considered to be affected when MTC or C-cell hyperplasia was confirmed histologically. A small number of individuals with elevated calcitonin levels but for whom pathological confirmation was lacking were identified. For the purposes of linkage analysis these were considered to be of ambiguous disease status. Individuals with MTC were screened for pheochromocytoma by a 24-h urinary vanillylmandelic acid and catecholamine collection.

Genealogic investigations were undertaken according to the methods of the Institut National d'Etudes Démographiques (Chaventré et al. 1991) by using demographic information on dates and places of birth, of marriage, and of death. In 53% of the families it was possible to obtain genealogic information on members of five generations.

Probes

Members of all families were typed for alleles of nine polymorphic probes (table 1) by using techniques and conditions described elsewhere (Narod et al. 1989). For the alpha-satellite repeat D10Z1, two dominant morphs were apparent on digestion with *Pst*I. These were coded as dominant alleles at zero recombination to each other. The probe IRBP.H4, on digestion with *Bgl*II and *Msp*I, identifies two independent polymorphisms at the RBP3 locus. These are

Table 1

Probes Used in Analysis

Probe	Locus	Enzyme	No. Tested	Allele	Allele Frequency		Reference
					Observed	Described	
1. pGEM32	FNRB	*Hin*fI	64	A	.73	.74	Wu et al. 1989
				B	.27	.26	
2. TB14.34	D10S34	*Taq*I	64	A	.43	.40	Nakamura et al. 1988*b*
				B	.57	.60	
3. pα10RP8	D10Z1	*Pst*I	70	A1	.92[a]	.93[a]	Wu and Kidd 1990
				A2	.08[a]	.07[a]	
			60	B1	.57[a]	.55[a]	
				B2	.43[a]	.45[a]	
4. pCl1/A15-6c23	D10S94	*Pvu*II	74	A	.63	.67	Brooks-Wilson et al. 1990
				B	.37	.33	
5. MEN203	D10S102	*Bgl*II	62	A	.68	.63	Mathew et al. 1991
				B	.31	.37	
6. IRBP.H4	RBP3	*Bgl*II	69	A1	.85	.85	Liou et al. 1987
				A2	.15	.15	
		*Msp*I	53	B1	.54	.61	Chin et al. 1988
				B2	.46	.39	
7. pMCK2	D10S15	*Msp*I	64	A	.13		Nakamura et al. 1988*a*
				B	.14		
				C	.11		
				D	.72		
8. p48-11	MNP	*Taq*I	54	A	.24[b]		Schuffenecker et al. 1991
				B	.76[b]		
9. TB10.16	D10S22	*Msp*I	64	A	.27	.26	Bragg et al. 1988
				B	.59	.60	
				C	.14	.14	

Note.—The individuals tested to calculate allele frequencies are unrelated.

[a] Based on the frequency of the two dominant morphs, under the assumption of Hardy-Weinberg equilibrium.

[b] Data available from the present study only.

treated as a single polymorphic locus in these analyses. The allele frequencies entered were those observed in a panel of unrelated individuals taken from our families and are in close agreement with frequencies published elsewhere (table 1).

Linkage Analysis

Hereditary MTC was modeled as an incompletely penetrant autosomal dominant trait. We estimate the frequency of MEN2A mutant alleles to be 1.5×10^{-5}, on the basis of the following criteria: in whites (Connecticut), the cumulative incidence of thyroid carcinoma to age 75 years is 1/350 (Muir et al. 1987); 4.2% of all thyroid carcinoma is MTC (Bergholm et al. 1989); and 25% of MTC is familial (Saad et al. 1984; Bergholm et al. 1989; Raue et al. 1989). The mutation rate was set at zero. The rate of sporadic MTC was set at 1.0×10^{-4} to allow for the possibility of phenocopies. Linkage was performed using the LINKAGE program (Lathrop et al. 1985). Five liability classes were constructed from age-at-onset data (Easton et al. 1989), corresponding to penetrances of 10%, 30%, 50%, 80%, and 95% for age groups 0–9, 10–14, 15–19, 20–29, and 30 years and above, respectively. Confidence limits were estimated using the 1-lod method (Conneally et al. 1985).

The proportion of linked families was estimated from the admixture test by using the HOMOG program (Ott 1985) by entering lod scores generated, for all families, at recombination fractions of 0, .01, and .05. This program tests whether the joint likelihood for all the families combined is significantly greater when the recombination fraction is allowed to vary between two subgroups of families.

Haplotypes were constructed for the 24 French families by assuming both the gene order in table 1 and

the minimum number of recombinants. Haplotypes of 100 control chromosomes were obtained from the spouses of the French families. The proportions of particular haplotypes (vs. all other observed haplotypes) were compared for mutation-bearing and control chromosomes. The significance of observed differences was assessed with the χ^2 test or by the Fisher exact test when appropriate. Significance levels were not adjusted for multiple comparisons.

Results

Pairwise lod scores for the MEN2A locus and each of the nine marker loci studied are presented in table 2. There was no male recombination with any of the eight closest markers. There was no female recombination with any of the four loci D10S34, D10Z1, D10S94, and D10S102. The highest lod score observed for an individual marker was with RBP3, by using the two polymorphisms defined by the IRBP.H4 probe.

To test for possible genetic heterogeneity among the 34 MEN2A families, multipoint linkage was performed by assuming the map order D10S94–D10S102–RBP3, zero recombination between D10S94 and D10S102, and 2 cM (sex averaged) between D10S102 and RBP3. A maximum lod score of 30.58 was obtained at a distance of 0 cM from MEN2A to D10S94–D10S102 (1-lod confidence interval 0.02). In 27 families linkage to this haplotype was informative, with lod scores ranging from 0.30 to 5.29; in five families linkage was uninformative; and in two families lod scores at 0 cM were negative (− 0.36 and − 1.23). The extended haplotype for family 48, for which the lod score was − 1.23, is shown in figure 2. Individual 3 appears to carry the MEN2A mutation but, at age 45 years, is currently healthy, and a recent pentagastrin test was negative. She is likely to be a nonpenetrant carrier. By age 45 years it is expected that 30% of gene carriers will have clinical symptoms and that 90% will have a positive pentagastrin test (Easton et al. 1989). The pentagastrin-challenge test will be performed on the three children of this individual to further investigate this hypothesis. Testing of additional polymorphisms near the D10S102 locus will help rule out the possibility that individual 3 is a double recombinant.

Both types of families, those with and those without pheochromocytoma, were linked. For the 14 families in which pheochromocytoma was not diagnosed, the maximum lod score was 5.79 at a recombination fraction of 0 from D1S94. The linked fraction of families was estimated to be 100%, with a 95% support interval of 82%–100%.

In family 3, individual 5 inherited the MEN2A gene from the mother, individual 3 (fig. 3). There was a crossover, between D10S94 and RBP3, on this chromosome, and the MEN2A gene was inherited with the portion of chromosome 10 carrying the centromere and D10S94 but not the RBP3 locus (the D10S102 locus was not informative). In addition, two siblings

Table 2

Two-Point Lod Scores for Linkage between Multiple Endocrine Neoplasia Type 2A and Chromosome 10 Loci

Locus	Maximum Lod Score	Recombination Fraction[a]	
		Male	Female
1. FNRB	11.83	.00	.04
2. D10S34	5.94	.00	.00
3. D10Z1	12.79	.00	.00
4. D10S94	7.76	.00	.00
5. D10S102	6.38	.00	.00
6. RBP3	19.10	.00	.04
7. D10S15	17.26	.00	.06
8. MNP	7.94	.00	.06
9. D10S22	5.48	.04	.17

[a] The value shown is estimated to be that at which the lod score is highest.

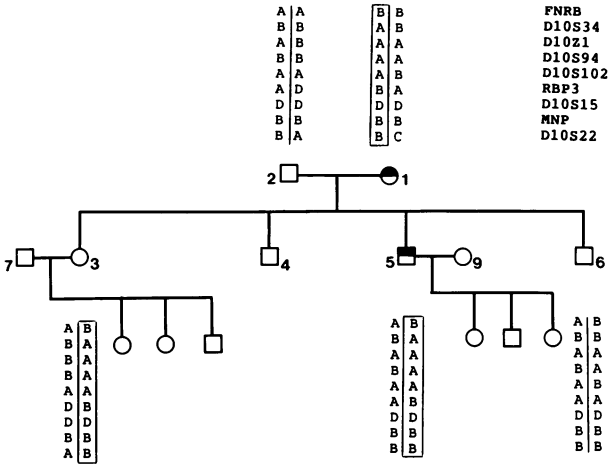

Figure 2 Family 48. Blackened symbols denote individuals with MTC (no individuals in this family had pheochromocytoma). The haplotype of the chromosome 10 homologue carrying the MEN2A mutation is indicated by the vertical boxes.

inherited the same RBP3 allele as did their affected brother, but they were not affected.

To assess whether a particular haplotype was seen in excess among the French families with MEN2A, the observed haplotype frequencies on the mutation-bearing chromosomes were compared with those of controls. Chromosomes were included if the identity of the marker allele at all the relevant loci could be established. The haplotypes described below are constructed based on the order of the polymorphic loci presented in table 1. The majority of haplotypes appeared to be unique (group II), but there were six families with a common core haplotype, for which a common ancestral origin appears likely (group I) (table 3). These shared the D haplotype at the RBP3 locus, which had a frequency of 14.8% in controls. The combination BBA–ADCB– was seen on 4 of 19 MEN2A chromosomes but in none of 56 controls (P = .003). The core haplotype common to six of the group I families, BBA–AD– – –, was present in 1 of 52 controls (P = .001). It is therefore probable that these six families share a common affected ancestor. Chromosomes with mutations in families 43 and 52 also have the infrequent RBP3 allele D. Family 43 has the sequence – – – – –DDBB in common with family 2 and family 193. This was seen in 3 of 72 controls (P = .12). Family 52 carries – – –AADD– –, which was present in 4 of 67 controls (P = .17). The current evidence is insufficient to assign either family 43 or family 52 to group I, but this situation may change when further polymorphisms are tested.

The geographic region of origin of the 24 French

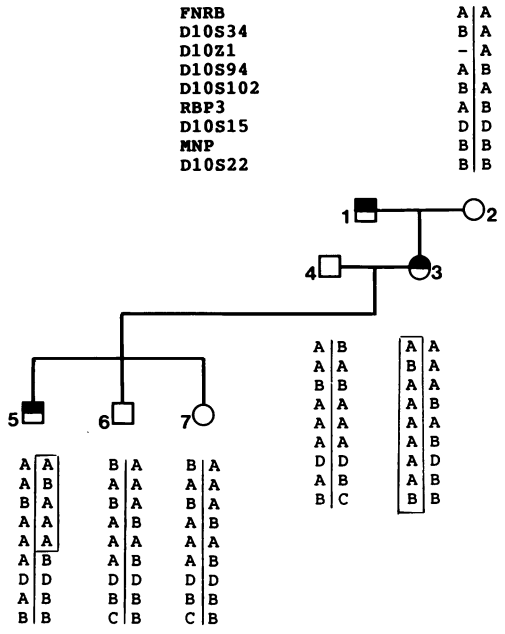

Figure 3 Family 3. Blackened symbols denote individuals with MTC (no individuals in this family had pheochromocytoma). The haplotype of the chromosome 10 homologue which carries the MEN2A mutation is indicated by the vertical box.

Table 3

DNA Haplotypes Characterizing the Mutation-bearing Chromosome in 24 French Families with Hereditary Medullary Thyroid Cancer

Group and Family	Cancer Type[a]	Haplotype of Locus[b]								
		1	2	3	4	5	6	7	8	9
I:										
5	MEN	B	B	A	A	A	D	C	B	B
6	MEN	B	B	A	A	A	D	C	B	C
21	MEN	B	B	A	–	A	D	C	B	–
41	MEN	B	B	A	A	A	D	C	B	B
2	MTC	B	B	A	A	A	D	D	B	B
193 ...	MTC	B	B	A	A	A	D	D	B	B
II:										
43	MEN	A	B	A	A	B	D	D	B	B
52	MTC	A	A	C	A	A	D	D	A	B
3	MTC	A	B	A	A	A	A	A	A	B
322 ...	MTC	A	B	A	A	A	A	A	B	B
31	MEN	A	B	B	A	A	A	D	B	B
184 ...	MTC	A	B	–	A	A	A	D	B	B
214 ...	MEN	B	B	B	A	A	B	D	B	B
8	MEN	B	B	A	A	A	A	D	B	A
42	MTC	A	B	A	A	A	A	D	A	A
426 ...	MTC	A	–	A	A	A	A	D	B	B
48	MTC	B	A	A	A	A	B	D	B	B
92	MEN	A	A	A	A	A	B	D	A	B
44	MEN	A	B	C	B	A	A	A	B	A
24	MTC	B	B	–	B	A	A	D	A	C
32	MEN	B	B	A	B	A	A	D	B	B
13	MEN	A	A	–	A	B	A	D	B	A
22	MTC	A	A	A	A	B	B	C	A	C
10	MTC	B	B	–	A	B	A	D	A	A

[a] MEN = medullary thyroid cancer with pheochromocytoma; and MTC = medullary thyroid cancer without pheochromocytoma.

[b] The haplotypes are those for the chromosome, in each family, that carries the mutation responsible for hereditary MTC. The numbers (1–9) represent the loci in the order listed in tables 1 and 2 (1 = FNRB; 2 = D10S34; 3 = D10Z1; 4 = D10S94; 5 = D10S102; 6 = RBP3; 7 = D10S15; 8 = MNP; and 9 = D10S22). The letters (A–D) refer to alleles in descending order of size, with the exception of D10Z1 and RBP3, which are constructed using two polymorphisms. For these two, A = A1B1; B = A1B2; C = A2B1; and D = A2B2.

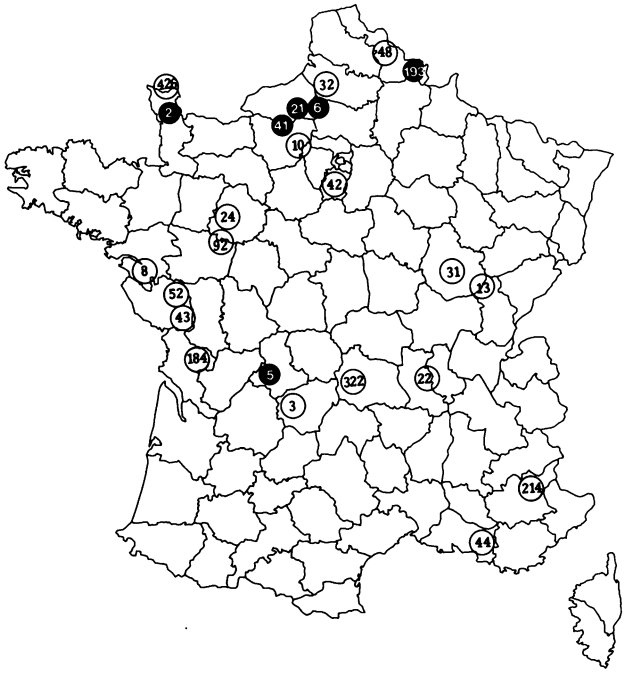

Figure 4 Map of France, indicating region of origin of 25 French MEN2A families. Blackened circles represent group I families.

families is shown in figure 4, with that of the group I families highlighted. Five of the six families in this group live in northern France, in the regions of Normandy and Picardy. Through genealogic records we have been able to identify a common ancestor among the founders of families 21 and 41 (fig. 5), in keeping with their close geographic proximity. In the following discussion these two families will be considered to be a single large family. Family 5 originated in the village of St. Léonard de Noblat in central France. Genealogic reconstructions show that several branches of this family have since migrated to the northern coast (Chaventré et al. 1991). It is possible that families 43 and 52 in our panel also descend from the founder of the others in group I. These two families reside in the western region of Charentes, not far from the origin of family 5 (fig. 4).

Table 4

Clinical Features of Group I Families

Family	No. Affected	No. with Pheochromocytoma	Mean Age at Diagnosis of Pheochromocytoma (years)
5	12	6	32
6	19	14	48
21/41 ...	12	2	54
2	8	0	Not seen
193	7	0	Not seen

The ABAAAAA– – haplotype was seen in families 3 and 322 and in 1 of 51 controls (P = .16). These MTC families are closely situated (fig. 4). In families 31 and 184 the mutation was associated with the AB–AAADBB haplotype, which was seen in 1 of 58 controls (P = .18). No geographic proximity is apparent.

Despite the likelihood of a common mutation in the group I families, the expression of MEN2A differs. Within the families the appearance of pheochromocytoma among those affected with MTC varies from 0% (0 of 8; family 2) to 73.7% (14 of 19; family 6) (table 4). A common proportion for the five families could be rejected (P = .0003). Although the observed prevalences of pheochromocytoma in the different families could be affected by the extent of screening, the great majority of detected pheochromocytomas were symptomatic.

Discussion

We consider hereditary medullary carcinoma with and without pheochromocytoma to be a single genetic

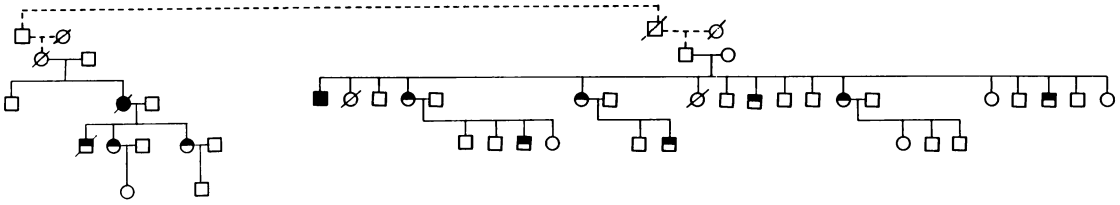

Figure 5 Family 21 (*left branch*) and family 41 (*right branch*), which were ascertained independently. The broken lines indicate links constructed through genealogic records. Symbols whose top halves are blackened denote MTC, and symbols completely blackened denote MTC and pheochromocytoma.

entity (MEN2A). This categorization is supported by our linkage findings and by those of Lairmore et al. (1991), who observed linkage to D10Z1 in two large pedigrees where MTC appeared without pheochromocytoma (lod score 5.88). For the 16 MTC families without pheochromocytoma (the total for their study and our study combined), the total lod score for linkage to D10Z1 is 9.90 at zero recombination. In two additional kindreds with familial MTC without pheochromocytoma a lod score of 3.27 at 15 cM from RBP3 was obtained (Noll et al. 1988).

We estimate the frequency of mutant alleles for hereditary medullary thyroid carcinoma to be 1.5×10^{-5}. This is slightly smaller than the figure of 2.0×10^{-5}, obtained by Carter et al. (1987), which is based on the annual appearance of approximately 30 new cases of MEN2A in England and Wales and on 750,000 annual births. Our estimate incorporates population-based information collected recently from the Swedish National Cancer Registry, where 276 of 6,513 registered thyroid carcinomas were verified as MTC (Bergholm et al. 1989). However, because the MEN2A gene is not completely penetrant (Easton et al. 1989), our estimate may be slightly low.

We did not detect linkage heterogeneity in our panel of 34 families. The 95% lower confidence limit for the proportion of linked families in our panel is 82%, but this figure is conservative if all published reports are taken into account. Among 90 initially reported families with hereditary MTC (Noll et al. 1988; Yamamoto et al. 1989; Norum et al. 1990; Wu et al. 1990*a*; Lairmore et al. 1991; Mathew et al. 1991; present study) consisting of 51 families with MTC and pheochromocytoma, 19 families with MTC alone, and 20 families with MEN2B—there was no report of an unlinked family.

Recently, a family with hereditary MTC without pheochromocytoma was reported to be unlinked to chromosome 10 (Carson et al. 1991). In this family only a single individual had histologically verified MTC; other individuals were considered to be affected on the basis of C-cell hyperplasia alone. This is probably insufficient to make the diagnosis of hereditary MTC, and in our analyses we have chosen not to include families with only a single case of MTC. Stimulated calcitonin screening of relatives of sporadic cases of MTC has been recommended (Ponder et al. 1988). We feel that DNA marker studies should be initiated only when a second case of carcinoma (not C-cell hyperplasia) is discovered. With these criteria, we believe that hereditary MTC can be assumed to be homogeneous with respect to map location, for the purpose of genetic counseling.

On the basis of previous mapping studies, the gene for MEN2A can be placed between FNRB/D10S34 on the short arm and the RBP3 locus at 10q11.2 (Nakamura et al. 1989; Goodfellow et al. 1990). One reported family with MEN2B (Norum et al. 1990) which showed a recombination between D10Z1 and MEN2B in an unaffected 8-year-old male provides suggestive evidence that MEN2B lies on the long arm of chromosome 10 (MEN2B is 99% penetrant in those over the age of 5 years).

The haplotypes constructed for the 24 French families confirm this assignment of MEN2A. The six families in group I can be explained by a single affected founder and subsequent recombination events between RBP3 and D10S15 and between MNP and D10S22 (in family 6). In family 43 a region of the chromosome 10 haplotype, telomeric to D10S102, is shared with families 2 and 193. By testing several additional highly polymorphic markers from this region, it may be possible to confirm that the three families have a common ancestor—in this case the MEN2A gene would map distal to D10S102.

It has been suggested that the three presentations of hereditary MTC represent allelic mutations of the MEN2A gene with variable expression (Lairmore et al. 1991). Our results raise the alternative possibility that the expression of a single MEN2A mutation may vary within an extended family. Similar patterns have been noted for other cancer syndromes, including hereditary retinoblastoma (Matsunaga 1976) and Wilms tumor (Pritchard-Jones and Hastie 1990), where the penetrance of the hereditary tumor varies with the presentation in the carrier parent. An unusual, large Swiss family with hereditary colonic polyposis has also been described (R. J. Scott, personal communication). In one branch of the family classical Gardner syndrome is seen, with a high frequency of desmoid tumors, but in another branch no extracolonic manifestations are detected. This is consistent with recent observations on mutations in the APC (adenomatous polyposis coli) gene (Nishisho et al. 1991). Two individuals with familial adenomatous polyposis had identical germ-line nucleotide substitutions; one presented with a desmoid tumor (Gardner syndrome), and the other had no extracolonic disease.

A possible reason for the variable expression we observe in hereditary MTC is that two closely linked loci are involved, a primary susceptibility locus and a nearby modifier. Different alleles at the modifier locus

may be associated with different penetrances of pheochromocytoma. This hypothesis is consistent with the group I data in table 3. The two families without pheochromocytoma (families 2 and 193) differ in haplotype distal to RBP3 (locus 6). The gene for MEN2A is centromeric to RBP3; it is possible that a different allele of a modifying locus, telomeric to RBP3, has become associated in *cis* in these two MTC families, through an ancestral recombinant. Alternatively, it is possible that families 2 and 193 do not share the same MEN2A mutation with the rest of group I and that allelic variation is the true explanation.

Haplotype analysis is a useful method for mapping genes for diseases with very low rates of new mutation. We have not yet identified a family in France for which MEN2A appears, with a high degree of probability, to be the result of a new mutation. This is in contrast to MEN2B, in which more than 50% of cases appear to be due to new mutations (Norum et al. 1990). Haplotype analysis is best suited to the study of diseases for which it can be assumed that all affected individuals in a population descend from a common founder (e.g., when the disease clusters within a particular geographic region or ethnic group). For diseases such as MEN2A, where incidence has not been shown to vary greatly from country to country, the approach is more limited. Before a minimal mapping interval can be inferred, it must first be established that two families are related, by examining concordance of haplotype over a core region and by applying statistical tests.

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References

Bergholm U, Adami H-O, Bergstrom R, Johansson H, Lundell G, Telenius-Berg M, Akerstrom G, et al (1989) Clinical characteristics in sporadic and familial medullary thyroid carcinoma: a nationwide study of 249 patients in Sweden from 1959 through 1981. Cancer 63:1196–1204

Bragg T, Nakamura Y, Fujimoto E, O'Connell PO, Leppert M, Lathrop GM, Lalouel GM, et al (1988) Isolation and mapping of a polymorphic DNA sequence (pTB10.163) on chromosome 10 (D10S22) Nucleic Acids Res 16:4185

Brooks-Wilson AR, Smailjus D, Myers S, Anderson L, Simpson NE, Goodfellow PJ (1990) Two polymorphisms at the D10S94 locus. Nucleic Acids Res 18:16

Calmettes C (1984) Création d'un groupe d'étude des tumeurs à calcitonine. Bull Cancer 71:266–273

Carson NL, Simpson NE, Myers S, Anderson L, Clarke C, Simpson WJ, Bridge PJ (1991) The mutation in a family with familial medullary thyroid carcinoma is not linked to chromosome 10 pericentromeric markers. Am J Hum Genet 49 [Suppl]: A337

Carter C, Easton DS, Mathew CGP, Welander G, Telenius H, Telenius-Berg M, Ponder BAJ (1987) Exclusion of linkage on chromosome 19 with multiple endocrine neoplasia type 2. Cytogenet Cell Genet 45:33–39

Chaventré A, Calmettes C, Houdent C, Sobol H, Proye C, Bellis G, Allanic H (1991) Role de l'anthropologie génétique (génealogie) dans le diagnostic de la forme familiale du cancer medullaire de la thyroide. In: Calmettes C, Guliana JM (eds) Medullary thyroid carcinoma. Colloque INSERM/John Libbey Eurotext, Paris, pp 163–165

Chin KS, Mathew CGP, Fong SL, Bridges CDB, Ponder BAJ (1988) *Sty*I RFLP recognised by a human IRBP cDNA localised to chromosome 10. Nucleic Acids Res 16:1645

Conneally PM, Edwards JH, Kidd KK, Lalouel JM, Morton NE, Ott J, White R (1985) Report of the Committee on Methods of Linkage and Reporting. Cytogenet Cell Genet 40:356–359

Easton DF, Ponder MA, Cummings T, Gagel RF, Hansen HH, Reichlin S, Tashjian AH, Jr, et al (1989) The clinical and screening age-at-onset distribution for the MEN-2 syndrome. Am J Hum Genet 44:208–215

Goodfellow PJ, Myers S, Anderson LL, Brooks-Wilson AR, Simpson NE (1990) A new DNA marker (D10S94) very tightly linked to the multiple endocrine neoplasia type 2A (MEN2A) locus. Am J Hum Genet 47:952–956

Goodfellow PJ, Nevanlinna HA, Gorman P, Sheer D, Lam G, Goodfellow PN (1989) Assignment of the gene encoding the beta-subunit of the human fibronectin receptor (b-FNRB) to chromosome 10p11.2 Ann Hum Genet 53: 15–22

Lairmore TC, Howe JR, Korte JA, Dilley WG, Aine L, Aine E, Wells SA, et al (1991) Familial medullary thyroid carcinoma and multiple endocrine neoplasia type 2B map to the same region of chromosome 10 as multiple endocrine neoplasia type 2A. Genomics 9:181–192

Lathrop GM, Lalouel JM, Julier C, Ott J (1985) Multilocus linkage analysis in humans: detection of linkage and estimation of recombination. Am J Hum Genet 37:482–498

Liou GI, Li Y, Wang C, Fong SL, Bhattcharaya S, Bridges CDB (1987) *Bgl*II RFLP recognised by human IRBP cDNA localised to chromosome 10 Nucleic Acids Res 15:3196

Mathew CGP, Chin KS, Easton DS, Thorpe K, Carter C, Liou GI, Fong SL, et al (1987) A linked genetic marker for multiple endocrine neoplasia, type 2A on chromosome 10. Nature 328:527–528

Mathew CGP, Easton DF, Nakamura Y, Ponder BAJ (1991) Presymptomatic screening for multiple endocrine neoplasia type 2A with linked DNA markers. Lancet 337:7–11

Matsunaga E (1976) Hereditary retinoblastoma: penetrance, expressivity and age of onset. Hum Genet 33:1–15

Muir C, Waterhouse J, Mack T, Powell J, Whelan S (eds) (1987) Cancer incidence in five continents, vol 5. International Agency for Research on Cancer, Lyon

Nakamura Y, Carlson M, Krapchko K, Gill J, O'Connell PO, Leppert M, Lathrop GM, et al (1988*a*) Isolation and mapping of a polymorphic DNA sequence pMCK2 on chromosome 10 (D10S15). Nucleic Acids Res 16:374

Nakamura Y, Lathrop M, Bragg T, Leppert M, O'Connell P, Jones J, Lalouel J-M, et al (1988*b*) An extended genetic linkage map of markers for human chromosome 10. Genomics 3:389–392

Nakamura Y, Mathew CGP, Sobol H, Easton DF, Telenius H, Bragg T, Chin K, et al (1989) Linked markers flanking the gene for MEN 2A. Genomics 5:199–203

Narod SA, Sobol H, Nakamura Y, Calmettes C, Baulieu J-L, Bigorne J-C, Chabrier G, et al (1989) Linkage of hereditary thyroid carcinoma with and without pheochromocytoma. Hum Genet 83:353–358

Nishisho I, Nakamura Y, Miyoshi Y, Miki Y, Ando H, Horii A, Koyama K, et al (1991) Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. Science 253:665–669

Noll WW, Bowden D, Maurer LH, Muller-Kahle H, Gravius TC, Braeuler C, Chamberlain M, et al (1988) Genetic mapping of familial medullary carcinoma of the thyroid (MTC)/multiple endocrine neoplasia 2A (MEN2A) with polymorphic loci on chromosome 10. Am J Hum Genet 43 [Suppl]: A29

Norum RA, Lafreniere RG, O'Neal LW, Nikolai TF, Delaney JP, Sisson JC, Sobol H, et al (1990) Linkage of the multiple endocrine neoplasia type 2B gene (MEN2B) to chromosome 10 markers linked to MEN2A. Genomics 8: 313–317

Ott J (1985) Analysis of human genetic linkage. Johns Hopkins University Press, Baltimore

Ponder BAJ, Ponder MA, Coffey R, Pembrey ME, Gagel RF, Telenius-Berg M, Semple P, et al (1988) Risk estimation and screening in families of patients with medullary thyroid carcinoma. Lancet 1:397–401

Pritchard-Jones K, Hastie ND (1990) Wilms' tumour as a paradigm for the relationship of cancer to development. Cancer Surv 9:555–577

Raue F, Winter J, Spath-Roger M (1989) Registry of medullary thyroid carcinoma (MTC) in Germany: the German MTC study group. Henry Ford Hosp Med J 37:206

Saad RK, Ordonez NG, Rashid RK, Guido JJ, Stratton Hill C, Hickey RC, Samaan NA (1984) Medullary carcinoma of the thyroid: a study of the clinical features and prognostic factors in 161 patients. Medicine 63:319–342

Schuffenecker I, Narod SA, Ezekowitz RAB, Sobol H, Feunten J, Lenoir GM (1991) The gene for mannose binding protein maps to chromosome 10 and is a marker for multiple endocrine neoplasia type 2A. Cytogenet Cell Genet 56:99–102

Simpson NE, Kidd KK, Goodfellow PJ, McDermid H, Myers S, Kidd KK, Jackson CE, et al (1987) Assignment of multiple endocrine neoplasia type 2A to chromosome 10 by linkage. Nature 328:528–530

Sobol H, Narod SA, Nakamura Y, Boneu A, Calmettes C, Chadenas D, Charpentier G, et al (1989) Screening for multiple endocrine neoplasia type 2A with DNA polymorphism analysis. N Engl J Med 321:996–1001

Wu J, Carson NL, Myers S, Pakstis AJ, Kidd JR, Castiglione CM, Anderson L, et al (1990*a*) The genetic defect in multiple endocrine neoplasia type 2A maps next to the centromere of chromosome 10. Am J Hum Genet 46:624–630

Wu J, Giuffra LA, Goodfellow PJ, Myers S, Carson NL, Anderson L, Hoyle LS, et al (1989) The beta subunit of the human fibronectin receptor: DNA restriction fragment length polymorphism and linkage mapping studies. Hum Genet 83:383–390

Wu J, Kidd KK (1990) Extensive sequence polymorphisms associated with chromosome 10 alpha satellite DNA and its close linkage to markers from the pericentromeric region. Hum Genet 84:279–282

Wu J, Myers S, Carson S, Kidd JR, Anderson L, Castiglione CM, Hoyle LS, et al (1990*b*) A refined linkage map for DNA markers around the pericentromeric region of chromosome 10. Genomics 8:461–468

Yamamoto M, Takai S, Miki T, Motomura K, Okazaki M, Nishisho I, Tateishi H, et al (1989) Close linkage of MEN2A with RBP3 locus in Japanese kindreds. Hum Genet 82:287–288