Linkage of the Multiple Endocrine Neoplasia Type 2B Gene (MEN2B) to Chromosome 10 Markers Linked to MEN2A


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The syndrome of multiple endocrine neoplasia type 2B (MEN 2B) resembles that of MEN 2A in that both include medullary carcinoma of the thyroid gland, pheochromocytoma, and autosomal dominant inheritance, but is distinct in that MEN 2B patients have neureomas of the mucous membranes. MEN2A has been linked to RBP3, D10S5, FNRRB, D10S15, and D10Z1 near the centromere of chromosome 10. We examined linkage between MEN2B and RFLPs on chromosome 10 in all available members in two or three generations of 14 kindreds. The centromere marker D10Z1 was linked to MEN2B with a peak lod score of 5.42 at θ = 0.02. One possible recombinant was observed between D10Z1 and MEN2B. Multipoint analysis of RFLPs at FNRRB, D10Z1, RBP3, and D10S15 gave a peak lod score of 7.12 at the midpoint between D10Z1 and RBP3 on the long arm (band q11). The most likely gene order FNRRB–D10Z1–MEN2B was 27 times more likely than MEN2B–FNRRB–D10Z1 and 32½ times more likely than FNRRB–MEN2B–D10Z1. Additional data will be required to establish the order of these loci with confidence.

INTRODUCTION

Multiple endocrine neoplasia type 2B (MEN 2B) is a syndrome of medullary carcinoma of the thyroid gland (MTC), tumors of the medulla of the adrenal gland, and neuromas of the oral mucosa and is inherited as an autosomal dominant trait. The gene for a similar syndrome of multiple endocrine neoplasia, type 2A (MEN 2A), has been located in the pericentromeric area of chromosome 10 by linkage to several restriction fragment length polymorphisms (RFLPs) (Mathew et al., 1987; Simpson et al., 1987; Sobol et al., 1988, 1989a; Nakamura et al., 1989; Yamamoto et al., 1989; Wu et al., 1990). MEN 2B is distinguished from MEN 2A by the presence in MEN 2B of the mucosal neuromas, earlier onset and faster growth of the thyroid cancers, and often skeletal abnormalities including the Marfanoid habitus with arachnodactyly (Williams and Pollack, 1966; Gorlin and Mirkin, 1972; Khairi et al., 1975; Carney et al., 1976; Jones and Sisson, 1983; Jackson et al., 1984).

We have reported initial linkage studies with MEN2B (Jackson et al., 1988) that were suggestive of linkage to the centromere marker D10Z1 (lod score of 2.68 at θ = 0), although only limited data on linkage to the same markers to which MEN2A had been linked were available then. No other reports of linkage analysis of MEN2B have appeared. To test whether genes causing MEN 2A and MEN 2B might be in the same area of the genome, we have examined linkage of MEN2B to RFLPs that have been linked to MEN2A.

MATERIALS AND METHODS

Families

Fourteen kindreds with MEN 2B were examined (Fig. 1). Each of the 44 affected individuals in these kindreds had the bumpy lips and/or tongue typical of the oral mucosal neuromas seen in MEN 2B (Williams and Pollack, 1966; Gorlin and Mirkin, 1972; Khairi et al., 1975; Carney et al., 1976). Each had had proven medullary thyroid cancer except for two obviously affected individuals: a newborn girl in pedigree 4 with bumpy lips and feeding difficulties, and a man

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21 22 22 34 22
19 12 11

FIG. 1. Abbreviated pedigrees of the 14 MEN 2B families studied. Solid circles and squares indicate affected individuals. DNA was not available from deceased individuals in families 1, 5, 7, and 12 indicated by the slashed lines through the symbol, from the youngest unaffected child in family 13, or from an unaffected spouse in families 8, 9, 11, 13, and 14. All others were typed for some or all of the polymorphisms.

in pedigree 10 who had refused surgery for his thyroid enlargement although pheochromocytomas had been removed. Nineteen of the 44 had pheochromocytomas and/or adrenal medullary hyperplasia. Pedigree 1 (O'Neal, 1983), pedigree 5 (Khairi et al., 1975), pedigree 8 (Eyer et al., 1988), pedigree 11 (Sobol et al., 1989b), and pedigree 13 (Kullberg and Nieuwenhuiizen Kruseman, 1987) have been reported. In pedigree 5, the younger affected male was examined at age 6 and reported (Khairi et al., 1975) to have no mucosal neuromas and normal catecholamines in plasma and urine. Although his mother had noticed in infancy the characteristic lip changes that she had noted in his older sister and brother, his thyroid was not removed until age 9. On examination at age 20 he also had definite mucosal neuromas of the tongue.

DNA Markers and Genotyping

DNA was extracted from peripheral blood (Gustafson et al., 1987) and digested with restriction enzymes as recommended by the suppliers. Agarose electrophoresis was performed as described (Devilee et al., 1988), except that Tris–borate buffer replaced Tris–acetate buffer. Fragments were blotted on Nytran filters and hybridized with a probe for D10Z1 as described (Devilee et al., 1988) or with probes for the other RFLPs as described (Meinkoth and Wahl, 1984). The following DNA markers were used: p9-12A/IdHII2.5/TaqI (McDermid et al., 1987); pH4/MspI or BglII (RBP3) (Liou et al., 1987); p(alpha)10RP8/MspI (D10Z1) (Devilee et al., 1988; Wu and Kidd, 1990); p7A9/MspI or TaqI/D10S24 (Wu et al., 1988); pMCK2/RsaI or MspI (D10S15) (Lathrop et al., 1988); gEM1-P32/KpnI,BanII,HinfI(FNRB) (Wu et al., 1989), and pB/R2/MspI (FNRB) (Goodfellow et al., 1989).

Linkage Analysis

Linkage analysis was performed with the LINKAGE program package version 5.0 (Lathrop et al., 1984) run on an IBM PS/2 Model 50Z computer with DOS version 3.30. The RFLP contribution of missing individuals relevant to analysis of linkage to MEN2B could not be inferred from the genotypes of the offspring or parents without reference to population allele frequencies in four instances (deceased spouse in pedigree 5, at D10S5; the mother in pedigree 12 at D10S15; the father in pedigree 13 at D10S15; the mother in pedigree 14 at D10S15). For analysis of these pedigrees the population frequency of the TaqI RFLP at D10S5 was from Simpson et al. (1987), and the frequencies of three alleles at the D10S15 RFLP with either RsaI or MspI were 0.79, 0.12, and 0.09. The frequency used for the MEN2B gene was 0.0001, and the mutation rate assumed at this locus was 0.00001. The fraction of MEN2B carriers manifesting any aspect of the syndrome was assumed to be 80% between birth and age 5 and 99% over age 5. The rarity of MEN2B has prevented observations that would provide estimates of age-of-onset of the accuracy available in MEN2A provided by Gagel et al. (1982). For multipoint analysis the distances between loci used were from Wu et al. (1990). Because the multipoint analysis of all the pedigrees exceeded the capacity of our computer, pedigrees 1–10 (Fig. 1) were analyzed as one group, and pedigrees 11–14 were analyzed as a second group. The lod scores from the two groups were added at each recombination fraction.

RESULTS

Table 1 shows the results of analysis of linkage between MEN2B and DNA markers. These pairwise results indicate that MEN2B is linked to D10Z1 (with a lod score of 5.42 at $\theta = 0.02$ and a 1-lod confidence interval of 0–0.13) but is not closely linked to D10S24 on the short arm. The results are only slightly positive for linkage to RBP3 but strongly positive for linkage to D10S15 (which is tightly linked to RBP3) because RBP3 was informative for a probable recombinant with MEN2B but not informative in several other off-
TABLE 1
Pairwise Linkage of MEN2B to Chromosome 10 Markers

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>0.001</th>
<th>0.05</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>δ</th>
<th>Lod at δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>D10S24</td>
<td>-7.57</td>
<td>-5.16</td>
<td>-1.06</td>
<td>-0.39</td>
<td>0.06</td>
<td>0.11</td>
<td>0.04</td>
<td>0.28</td>
<td>0.12</td>
</tr>
<tr>
<td>FNRB</td>
<td>1.63</td>
<td>1.67</td>
<td>2.06</td>
<td>1.96</td>
<td>1.51</td>
<td>0.93</td>
<td>0.37</td>
<td>0.05</td>
<td>2.06</td>
</tr>
<tr>
<td>D10Z1</td>
<td>5.22</td>
<td>5.25</td>
<td>5.29</td>
<td>4.83</td>
<td>3.60</td>
<td>2.23</td>
<td>0.93</td>
<td>0.02</td>
<td>5.42</td>
</tr>
<tr>
<td>RBP3</td>
<td>-1.51</td>
<td>0.17</td>
<td>1.50</td>
<td>1.44</td>
<td>0.99</td>
<td>0.47</td>
<td>0.09</td>
<td>0.06</td>
<td>1.51</td>
</tr>
<tr>
<td>D10S15(MCK2)</td>
<td>0.63</td>
<td>2.58</td>
<td>3.74</td>
<td>3.48</td>
<td>2.63</td>
<td>1.67</td>
<td>0.74</td>
<td>0.04</td>
<td>3.75</td>
</tr>
<tr>
<td>D10S0</td>
<td>-1.95</td>
<td>-0.02</td>
<td>1.13</td>
<td>1.13</td>
<td>0.83</td>
<td>0.44</td>
<td>0.44</td>
<td>0.07</td>
<td>1.16</td>
</tr>
</tbody>
</table>

spring in whom D10S15 was informative. Because no DNA marker was informative in every kindred, we used multipoint linkage analysis to combine results on one map (Fig. 2) which shows the peak lod score of 7.12 at the midpoint between D10Z1 and RBP3. Our results with MEN2B compared with a multipoint map of MEN2A (Wu et al., 1990) show overlapping confidence limits. The 1-lod confidence interval for MEN2B extends from D10Z1 9 cM on the q arm to 6 cM on the p arm in the female map. The most likely gene order FNRB–D10Z1–MEN2B was 27 times more likely than MEN2B–FNRB–D10Z1 and 3 \times more likely than FNRB–MEN2B–D10Z1. Additional data will be required to establish the order of these loci with confidence.

A possible crossover in pedigree 2 between MEN2B and both D10Z1 and FNRB provides evidence that MEN2B lies on the long arm flanked by D10Z1 and RBP3/MCK2. This involved an unaffected 8-year-old male who shows none of the phenotypic features of MEN2B which his mother and two brothers manifested at a much earlier age. Additionally, strong evidence for his unaffected status is provided by a recent unequivocally negative pentagastrin-stimulated calcitonin test for MTC. This result effectively excludes MTC, which is present and often metastatic in MEN2B patients by age 4 (Jones and Sisson, 1983). A single crossover between RBP3, D10S15, and D10S5 on one side and MEN2B, D10Z1, FNRB, and D10S24 on the other side probably occurred in pedigree 1 (Fig. 1). This resulted in different DNA marker types in two people who have MEN2B and thus is unlikely to be an artifact of misclassification of MEN2B genotype. In both of these possible crossovers the phase in the affected parent is unknown, so the lod scores for linkage at the marker are not strongly negative. The locations of the probes used are shown on the physical map of chromosome 10 (Fig. 3) modified from Smith and Simpson (1989).

DISCUSSION

Although our initial linkage studies with MEN2B (Jackson et al., 1988) were only suggestive of linkage to the centromere marker D10Z1 (lod score of 2.68 at $\theta = 0$), limited data on linkage to the same markers to which MEN2A had been linked were available then. No other reports of linkage analysis of MEN2B have appeared. Wu et al. (1990) have recently reported evidence for linkage between MEN2A and D10Z1 (lod score of 12.0 at $\theta = 0$). The data reported here confirm that MEN2B, like hereditary medullary thyroid cancer without pheochromocytoma (Sobol et al., 1989b), is closely linked to the same DNA markers near the centromere of chromosome 10 to which MEN2A has been linked and suggest a close relationship of the similar syndromes but do not establish allelism of the genes.

This study illustrates well the dependence of gene localization on the accurate identification of affected individuals on the basis of clinical data. If the crossover male in pedigree 2 (Fig. 1) is correctly identified as not having the disease gene, MEN2B is likely local-
ized to the proximal long arm of chromosome 10 by studies of this one family. Although our clinical experience has shown that the diagnosis of MEN2B can be made in early childhood by observation of the mucosal neuromas by experienced parents and/or physicians and by conversion of negative to positive pentagastrin-stimulated calcitonin testing for the thyroid cancer by age 5, insufficient data on this rare disease are available at present to exclude all doubt.

Although these findings are compatible with the contiguous gene theory for tumors of the syndromes (Jackson et al., 1989; Jackson and Norum 1989), further evidence of the nature of the actual genes involved is needed. The evidence for the genes for MEN 2A and MEN 2B being located close to each other would suggest that MEN 2B is probably a better term for the mucosal neuroma phenotype than the MEN 3 syndrome. These findings suggest that studies of the gene for either MEN 2 syndrome may contribute knowledge of the gene for the other syndrome.

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