Predictive testing for multiple endocrine neoplasia type 2A (MEN 2A) based on the detection of mutations in the *RET* protooncogene

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Background. *The identification of inherited mutations in the RET protooncogene (RET) associated with multiple endocrine neoplasia type 2A (MEN 2A) has enabled the development of a genetic test to identify asymptomatic carriers of disease.*
Methods. *Genomic DNA was extracted from 96 members of an MEN 2A kindred. The polymerase chain reaction was used to amplify the RET exon known to contain the associated mutation. The mutation results in a new restriction endonuclease site and is detected by digestion with the appropriate enzyme. Inheritance of the mutation was verified with a previously developed genetic linkage test.*
Results. *We found that (1) mutations vary among kindreds but are consistently inherited within kindreds, (2) invariable correlation exists between mutation and disease (43 mutations in 43 affected individuals), (3) determination of the genetic status by linkage-based testing was precluded by recombination events and the informativeness of genetic markers, and (4) mutation analysis presymptomatically identified two genetically affected individuals.*
Conclusions. *Direct genetic analysis for mutations in RET circumvents the limitations of linkage-based genetic testing and current biochemical screening assays. This method will be the diagnostic test of choice for the identification of asymptomatic individuals at risk for MEN 2A.*
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MULTIPLE ENDOCRINE NEOPLASIA TYPE 2A (MEN 2A) is characterized by the development of medullary thyroid carcinoma (MTC), pheochromocytomas, and hyperparathyroidism. This syndrome is inherited in a typical Mendelian autosomal dominant fashion and is nearly fully penetrant. Virtually all persons inheriting the MEN 2A mutation have MTC by the third to fourth decade of life.[1] There is, however, variable expressivity in MEN 2A since pheochromocytomas develop in approximately 50% of affected persons and hyperparathyroidism develops in 30% to 40%.[2]

The diagnosis of MEN 2A has depended on biochemical assays based on provocative calcitonin stimulation.[3] In persons at risk for the development of MEN 2A, serum is collected for calcitonin immunoassays before and immediately after the intravenous administration of calcium and pentagastrin. When patients with MEN 2A are screened with provocative testing, almost all affected patients will be diagnosed by 31 years of age.[4] Although the provocative test is highly sensitive for the early detection of MTC,[5, 6] it is bothersome for patients. Repetitive testing is also required, usually on an annual basis. Unaffected children are therefore needlessly subjected to decades of testing to assure their disease-free status. Finally, calcitonin stimulation testing only identifies persons who already have a C-cell disorder (either C-cell hyperplasia or MTC).

Genetic mapping of the MEN2A locus to human chromosome 10 by linkage analysis has recently led to

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the development of presymptomatic genetic testing.[7-10] These linkage-based tests, however, use flanking genetic markers, and their usefulness is therefore limited by potential chromosomal recombination events and the informativeness of the markers. With a suitable family structure, the inheritance of the mutated gene for MEN 2A can be determined with up to 98% accuracy in 94% of patients.[7] Furthermore, to establish cosegregation of the mutant gene and closely linked markers, characterization of large numbers of unaffected and affected members of the given kindred from two or more generations is required. As a result, linkage-based genetic testing can become relatively inefficient, costly, and dependent on the availability and participation of family members.

Recently, mutations in the *RET* protooncogene *(RET)* associated with the inheritance of MEN 2A were identified.[11, 12] *RET* encodes a protein receptor tyrosine kinase with a cysteine-rich extracellular receptor domain, a transmembrane domain, and an intracellular catalytic tyrosine kinase domain.[13, 14] The specific function of the *RET* protooncogene product has yet to be elucidated; however, recent gene target studies with transgenic mice indicate that *RET* may be important in the embryologic development of mammalian genitourinary and enteric nervous systems.[15] The mutations in *RET* associated with MEN 2A result in a nonconservative substitution of cysteine amino acids in a region that encodes the putative extracellular receptor. To date, all identified mutations for MEN 2A have been located in either exons 10 or 11 of *RET* (unpublished data).

By showing the association of these *RET* mutations with inheritance of disease within the MEN 2A families, direct predictive genetic testing for disease is possible. Such direct genetic testing by mutation analysis has previously been reported for diseases such as cystic fibrosis and familial adenomatous polyposis coli.[16, 17] The identification of mutations allows for a more accurate and rapid means for diagnosing disease inheritance. In this study we characterized the *RET* mutation in members of a well-studied MEN 2A kindred and independently assessed the pattern of inheritance of the MEN2A allele by using six flanking genetic markers. We now present a presymptomatic genetic test by direct mutation analysis of *RET* for individuals at risk for developing MEN 2A.

MATERIAL AND METHODS

Kindred collection. Ninety-six individuals from a North American kindred segregating MEN 2A were used in this study. Affected members and individuals at risk for disease were screened for MTC by measurements of serum calcitonin levels after intravenous infusion of calcium and pentagastrin.[3] Also measured were serum calcium concentrations and 24-hour urine excretion rates of catecholamines and metabolites in patients with MTC. Kindred members with an affected parent or sibling, regardless of age, were considered to be at risk for development of MEN 2A.[18]

DNA preparation. DNA was manually prepared from peripheral blood lymphocytes as previously described[19] or by the use of an automated nucleic acid extractor (Applied Biosystems Inc., Foster City, Calif.).

Direct mutation analysis. Oligonucleotide primers to amplify the polymerase chain reaction (PCR) assay referred to as exon 11A of the *RET* protooncogene (previously described as exon 8A) have been reported elsewhere.[11] PCR amplification of 50 ng of genomic DNA was performed in a total reaction volume of 5 μl containing 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 5 mmol/L NH_4Cl, 200 μmol/L each deoxyribonucleoside triphosphate (dNTP), 0.5 units Taq polymerase (AmpliTaq; Perkin Elmer-Cetus Co., Norwalk, Conn.), and 2 μmol/L each of exon 11A primers. The reaction amplification products were denatured at 94° C for 30 seconds, annealed at 68° C for 30 seconds, and extended at 72° C for 1 minute. The reactions were carried out for 30 cycles in a Biometra TRIO-Thermoblock (Emerston Instruments, Richmond Hill, Ontario, Canada).

The exon 11A PCR reactions were incubated with the restriction endonuclease *Cfo* I by use of the buffers and conditions as recommended by the manufacturer (Boehringer Mannheim, Indianapolis, Ind.). Endonuclease digestion products were size separated by electrophoresis through 10% nondenaturing polyacrylamide gels (Mighty Small II; Hoefer Scientific Instruments, San Francisco, Calif.) and stained with ethidium bromide.

Linkage-based genetic analysis. Primers and polymorphisms have been described elsewhere for sTLC-1,[18] D10S141,[20] sTCL-2,[21] and sSSD-2, sSSD-3, and sKMC-2.[22]

One primer for each assay (forward primer) was 5′ end and labeled with [γ-^{32}P] deoxyadenosine triphosphate.[23] PCR amplification of 50 ng of genomic DNA was performed in a total reaction volume of 5 μl. For sTCL-1 and sTCL-2, the reactions were performed in 10 mmol/L Tris-HCl (pH 8.4), 50 mmol/L KCl, 1.2 mmol/L $MgCl_2$, 0.1% gelatin, 2 μmol/L each dNTP, 0.25 μmol/L 5′ end labeled forward primer, 1.8 μmol/L unlabeled forward primer, 2 μmol/L reverse primer, and 0.5 units Taq polymerase. Reactions for sSSD-2 and sSSD-3 were carried out in 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 5 mmol/L NH_4Cl, 200 μmol/L each dNTP, 0.25 μmol/L 5′ end labeled forward primer, 1.5

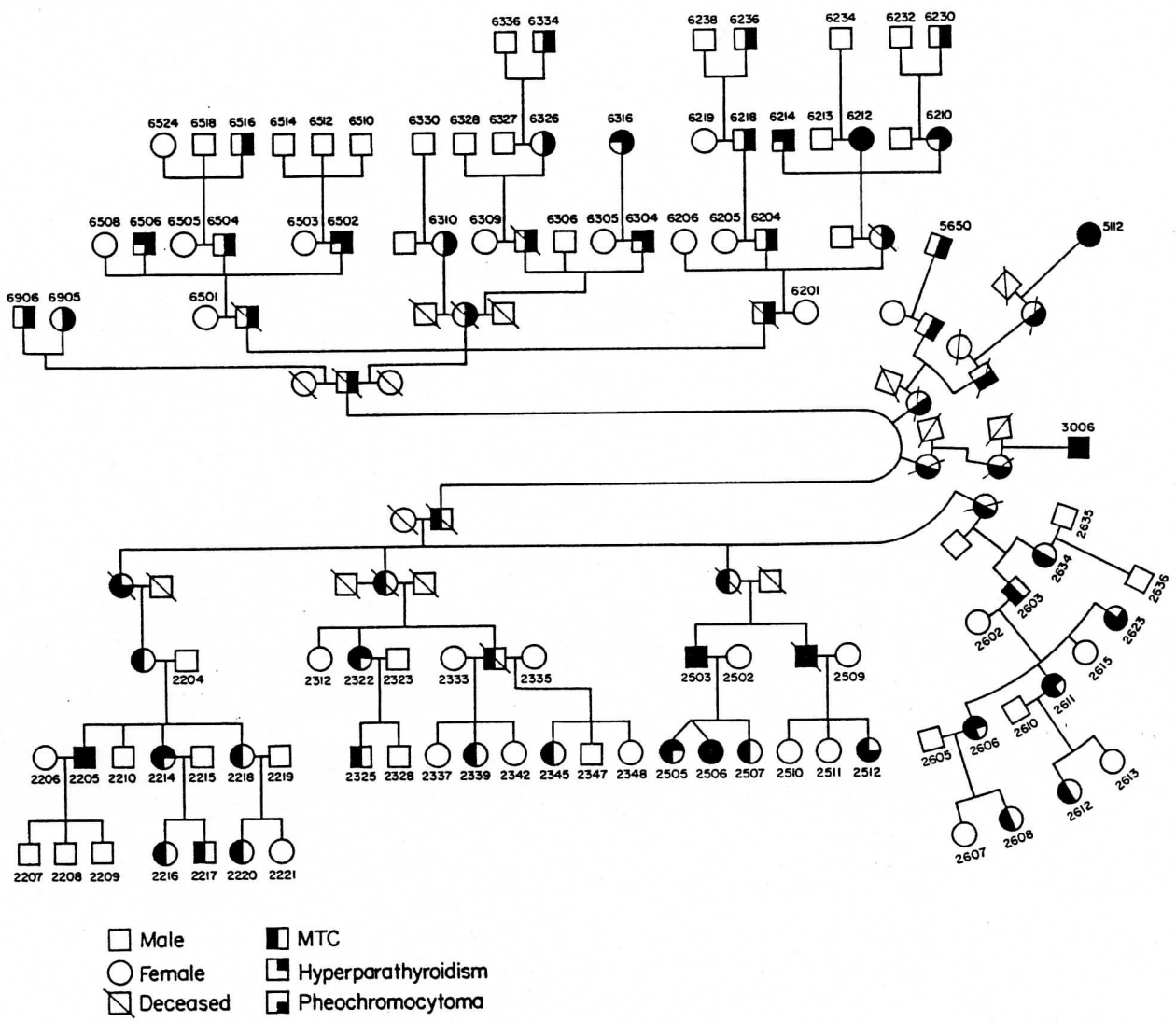

Fig. 1. Partial pedigree for MEN 2A kindred 3 including those family members investigated in this study.

μmol/L unlabeled forward primer, 2 μmol/L reverse primer, and 0.5 units Taq polymerase. For sKMC-2 and D10S141, reactions were performed in 10 mmol/L Tris-HCl (pH 8.4), 50 mmol/L KCl, 1.2 mmol/L $MgCl_2$, 0.1% gelatin, 200 μmol/L each dNTP, 0.25 μmol/L 5′ end labeled forward primer, 1.75 μmol/L unlabeled forward primer, 2 μmol/L reverse primer, and 0.5 units Taq polymerase.

PCR amplifications were carried out in an automated thermocycler (Perkin Elmer-Cetus) with denaturation at 94° C for 1 minute, annealing at 55° C (sKMC-2), 58° C (sTCL-1 and sTCL-2), 63° C (D10S141), or 65° C (sSSD-3 and sSD-2) for 2 minutes, and extension at 72° C for 2 minutes for either 22 cycles (sSSD-3 and sSSD-2), 23 cycles (sTCL-1 and sTCL-2), or 24 cycles (D10S141 and sKMC-2). The reaction products were electrophoresed through 8% polyacrylamide sequencing gels, with [α-^{32}P] deoxyadenosine triphosphate–labeled bacteriophage M13 sequencing ladders as size standards. Gels were vacuum blotted and exposed to Kodak XAR autoradiograph film (Eastman-Kodak, Rochester, N.Y.).

RESULTS

Kindred 3 characterization. The pedigree for the portion of the kindred used in this study is presented in

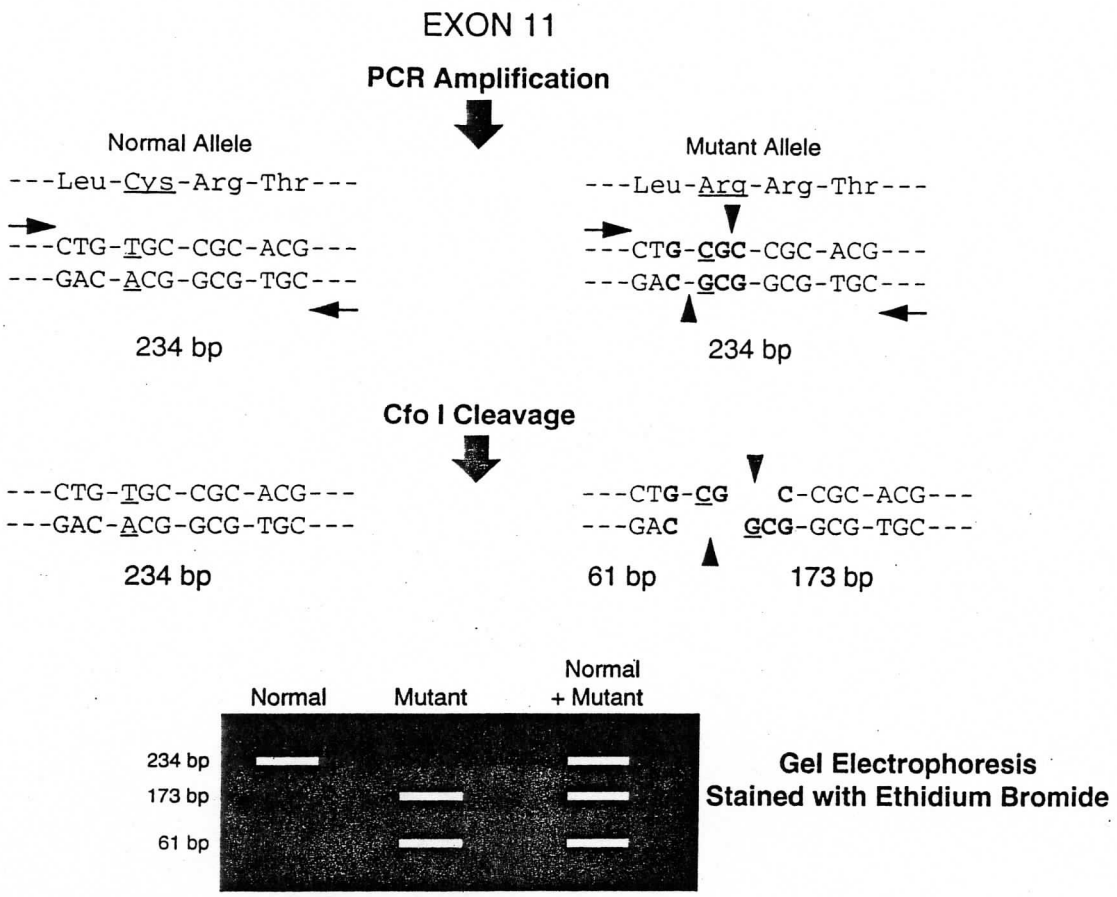

Fig. 2. Direct mutation analysis by PCR amplification and *Cfo* I restriction endonuclease digestion. MEN 2A mutation is indicated by *underlined* sequence and amino acids. *Cfo* I site in DNA sequence of mutant allele is indicated by *bold print*. *Vertical arrowheads* indicate the site for cleavage by *Cfo* I. *Horizontal arrows* indicate 5′ to 3′ direction of DNA. Size separation by gel electrophoresis of normal allele, mutant allele, and both alleles together is drawn.

Fig. 1. A total of 96 individuals from the kindred were included. Of these, 43 persons had MEN 2A documented by either an elevation in provocative serum calcitonin levels or the histopathologic diagnosis of MTC. In some patients the presence of pheochromocytoma, parathyroid hyperplasia, or both was evident. Thirty-one offspring from affected parents were identified as being at risk for development of MEN 2A.

Direct mutation analysis. PCR amplification of exon 11A results in a 234 bp product. The mutation associated with MEN 2A in kindred 3 involves the nonconservative substitution of Cys_{634} to Arg_{634} (TGC –> CGC) within this region of the gene. This nucleotide substitution (T –> C) generates a *Cfo* I restriction endonuclease site (Fig. 2). The normal gene does not contain any *Cfo* I recognition sites in exon 11A. The PCR product resulting from the mutated gene digests with *Cfo* I to produce two DNA fragments of 173 bp and 61 bp. An individual with MEN 2A inherits one chromosome from the affected parent that carries the mutated gene and one chromosome from the unaffected parent that carries the normal gene. Electrophoresis, therefore, separates the *Cfo* I digested DNA from an affected person into three sizes (234 bp, 173 bp, and 61 bp), whereas the unaffected person's DNA remains intact and one product (234 bp) is apparent (Fig. 3).

The mutation in exon 11 of *RET* was found to be present in all 43 clinically affected family members. Furthermore, two of the 31 persons at risk for MEN 2A (3-2208 and 3-2613) were determined to have inherited the allele with the *RET* exon 11 mutation (TGC –> CGC) from the affected parent.

Linkage-based genetic analysis. All members of kindred 3 were analyzed with six genetic markers (simple sequence repeat polymorphism [SSRP]), which are ordered along chromosome 10 as follows: 10p

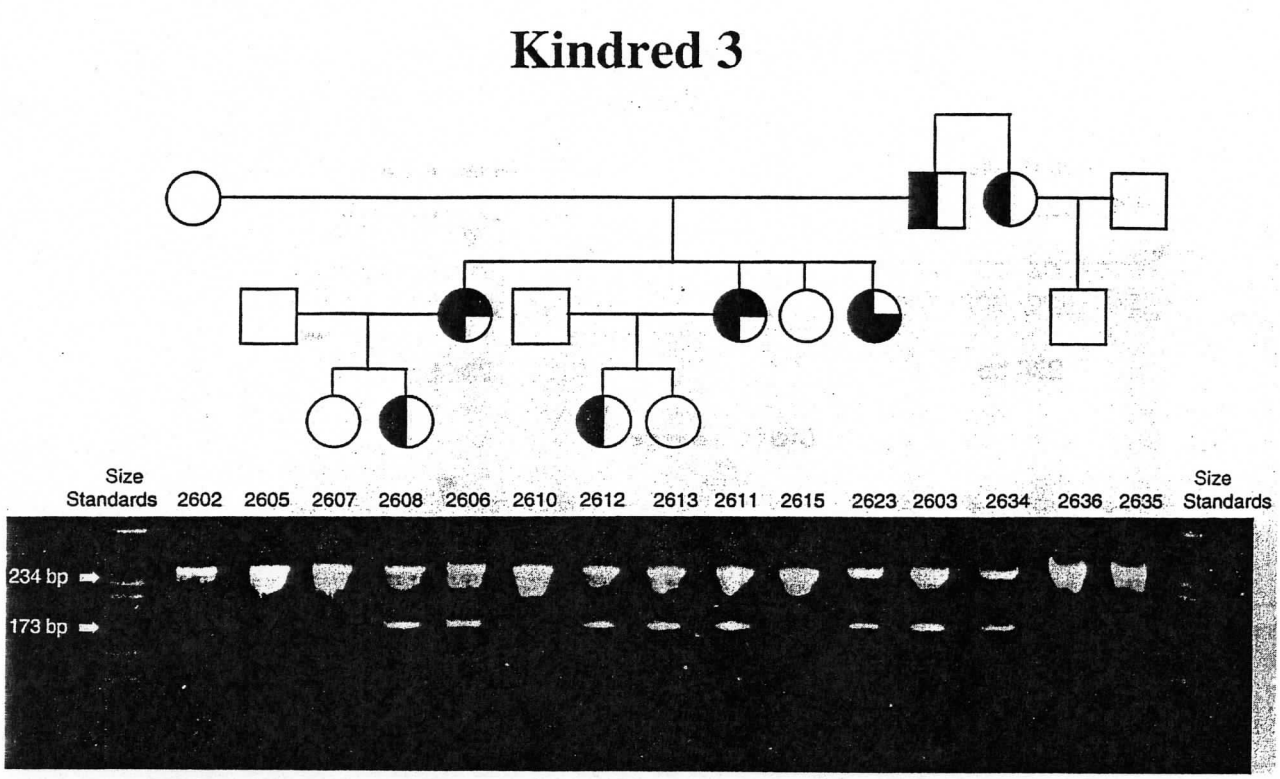

Fig. 3. Direct analysis of *RET* mutation in a portion of kindred 3. The 61 basepair DNA fragment is not shown in this gel.

telomere—TCL-1 (D10S176)—10 centromere—sSSD-3 (D10S1100)-D10S141-MEN2A/*RET*—sTCL-2 *(RET)* —sSSD-2 (D10S1099)—sKMC-2 (D10S1098)—10q telomere (Table I). Three of these markers (sTCL-1, sSSD-3, and D10S141) flank *RET* on the p-telomere side, and three markers (sTCL-2, sSSD-2, and sKMC-2) flank *RET* on the q-telomere side.

All members of the kindred studied were informative for at least one genetic marker. However, 11 of these individuals were only informative for markers on one flanking side of RET (Table II). Of the 43 affected members of the kindred, two were found to be uninformative for markers on the q-telomere side, whereas five were found to be uninformative for the markers on the p-telomere side of *RET*. Twenty of the 22 affected members with offspring at risk for disease were informative for markers on both sides of *RET*.

Haplotypes (the arrangement of the alleles of the genetic markers for each chromosome) for all members of the kindred were constructed based on the transmission of six microsatellite markers from parents to offspring (Fig. 4). The haplotype found to be associated with inheritance of MEN 2A in kindred 3 is sTCL-1, 97 bp; sSSD-3, 195 bp; D10S141, 123 bp; sTCL-2, 165 bp; sSSD-2, 200 bp; and sKMC-2, 141 bp.

By using the haplotype information, prediction of inheritance for the MEN2A locus was made. Forty-two of the 43 affected members of the kindred were found to possess the haplotype associated with MEN 2A. One clinically affected person (3-2608) inherited a recombinant chromosome from the affected parent. Of the 31 members considered to be at risk for disease, 29 inherited the unaffected haplotype from the parent with MEN 2A, whereas two (3-2208 and 3-2613) were presymptomatically determined to have inherited the disease haplotype.

A recombination event involving the region of the chromosome including the MEN2A locus was found in individual 3-2608. The crossover occurs between the markers sTCL-2 and D10S141 and is illustrated in Fig. 4. Because it is not possible to determine whether the recombinant chromosome included the MEN2A allele, one cannot establish whether this individual inherited the mutated *RET* protooncogene from the affected parent. As a result, although this person is clinically affected, the genetic status cannot be ascertained based on genetic linkage studies.

Inheritance of the parental chromosome carrying the mutated MEN2A allele can therefore be made in 84 persons and is limited in 11 persons because of the lack of informative genetic markers on either the p arm or the

Table I. Microsatellite SSRP markers used for linkage-based analysis

Marker sTCL-1 (locus D10S176, HET 0.70)[18] *Allele*		*Marker sSSD-3 (locus D10S1100, HET 0.57)* Allele*		*Marker D10S141 (locus D10S141, HET 0.85)*[20] *Allele*		*Marker sTCL-2 (locus RET, HET 0.71)*[21] *Allele*		*Marker sSSD-2 (locus D10S1099, HET 0.78)* Allele*		*Marker sKMC-2 (locus D10S1098, HET 0.64)* Allele*	
Size (bp)	*Frequency*	*Size (bp)*	*Frequency*	*Size (bp)*	*Frequency*	*Size (bp)*	*Frequency*	*Size (bp)*	*Frequency*	*Size (bp)*	*Frequency*
97	0.536	223	0.13	118	0.28	155	<0.01	194	0.01	131	0.19
99	<0.010	221	0.06	121	0.04	163	0.01	198	0.01	133	0.01
101	0.008	219	0.01	123	0.17	165	0.28	200	0.40	135	0.06
103	0.008	217	0.18	124	0.03	167	0.43	202	0.01	137	0.01
105	0.028	201	0.01	125	0.06	169	0.03	206	0.02	139	0.55
109	0.069	195	0.62	127	0.08	171	0.07	210	0.09	141	0.09
111	<0.010			129	0.12	173	0.19	212	0.02	143	0.09
113	0.024			130	0.03	175	<0.01	214	0.02		
115	0.065			131	0.03	181	<0.01	216	0.01		
117	0.117			133	0.07			218	0.12		
119	0.089			135	0.04			220	0.13		
121	0.012			137	0.01			222	0.01		
123	0.008			139	0.04			224	0.12		
125	0.020							226	0.02		
127	0.004							228	0.01		

HET, Heterozygosity of the marker.
*Carlson et al.[22]

Table II. Numerical identifiers and disease status of individuals from kindred 3 included in this study

Clinically affected					*Offspring (Clinically unaffected, at risk)*				*Spouse (Unaffected, not at risk)*		
2205	2503	2623	6218	6506	2207	2347	6206	6508	2206	2602	6501
2214	2505	2634	6204	6905	2208*	2348	6306		2215	2610	6213
2216	2506	3006	6304	6906	2209	2510	6336		2219	2635	
2217	2507	5112	6316		2210†	2511	6328		2204‡	6219	
2218‡	2512†	5650	6334		2221†	2607	6330		2323	6205	
2220†	2603	6230	6326‡		2312	2613*	6510		2333	6305	
2322‡	2606	6210	6310		2328	2615	6512		2335	6309	
2325	2608§	6212	6502‖		2337	6232	6514		2502	6327	
2339	2611	6214	6516		2342	6234	6518		2509‡	6503	
2345	2612	6236	6504‖		2636	6238	6524		2605	6505	

All individuals were informative for at least one genetic marker on each side of MEN2A locus unless other specified.
*At risk; affected by genetic linkage and mutational analysis.
†Noninformative for markers on p arm side of *RET*; no offspring.
‡Noninformative for markers on p arm side of *RET*; offspring at risk.
§Recombinant; inheritance of MEN2A allele unknown.
‖Noninformative for markers on q arm side of *RET*; offspring at risk.

q arm. Furthermore, inheritance of the MEN2A allele from an affected parent is confounded by a recombination event between sTCL-2 and D10S141 in one individual.

Relationship between direct mutation analysis and linkage-based haplotypes. All 43 clinically affected kindred members were found to have inherited the Cys_{634} to Arg_{634} (TGC –> CGC) mutation in *RET*. Forty-two of these individuals also inherited the haplotype associated with the disease, and one possessed a recombinant haplotype (Fig. 4). In addition, two of the 31 individuals at risk (3-2208 and 3-2613) were also found to have inherited the same mutation in *RET* and the haplotype associated with the disease. The remaining 29 persons at risk did not inherit the haplotype that cosegregates with MEN2A from the affected parent. However, the lack of informative markers on one side of the disease gene limits the determination of the inher-

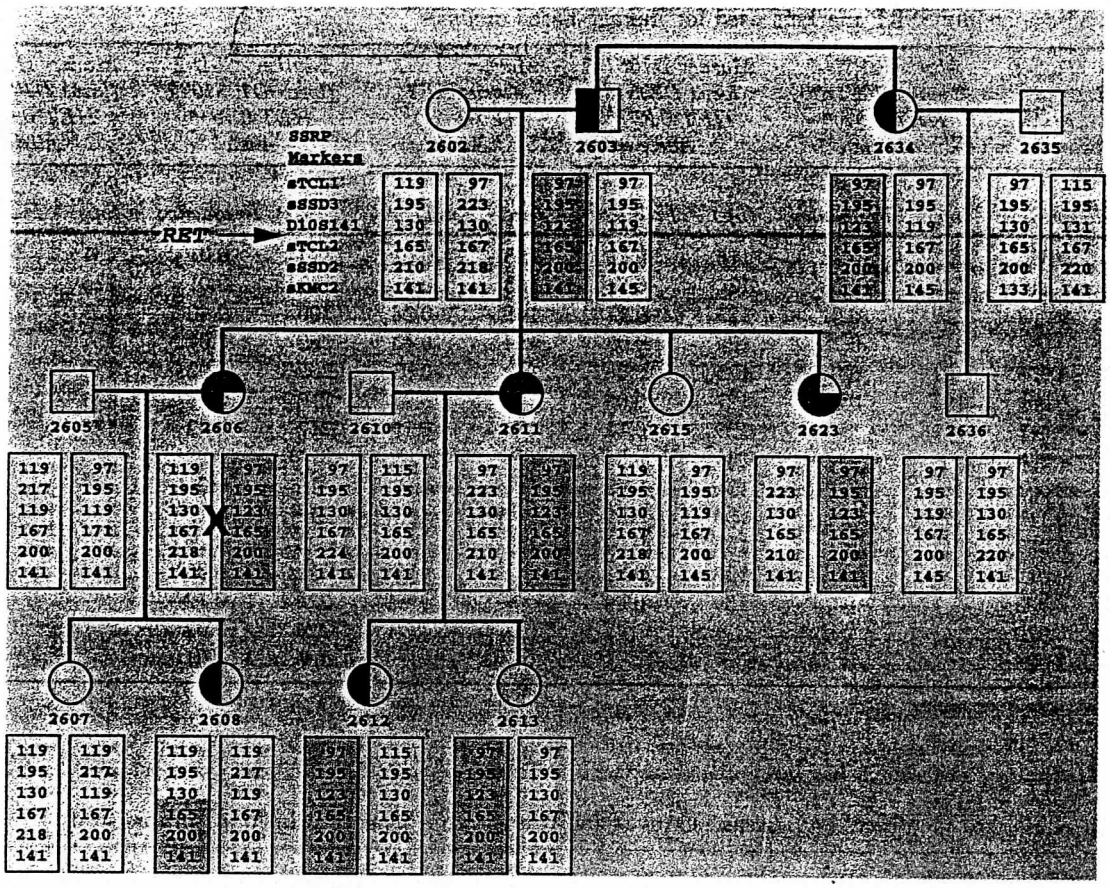

Fig. 4. Haplotype analysis of a portion of kindred 3. *Numbers* in each haplotype indicate size of observed alleles in basepairs. *Darkened box* indicates haplotype associated with MEN 2A. *Half darkened box* indicates recombinant chromosome inherited by individual 2608 from her affected parent (2606). *X* indicates location of crossover in recombination event observed in individual 2608.

itance for disease in the at-risk offspring of 3-2218 (3-2221), 3-2322 (3-2328), 3-6326 (3-6336), 3-6502 (3-6510, 3-6512, 3-6514), and 3-6504 (3-6518, 3-6524) (Fig. 1). Although the inheritance of the MEN2A allele could not be established by these linkage-based studies, direct mutation analysis revealed that these individuals at risk did not inherit the mutation in exon 11 of the *RET* gene.

A recombination event is evident in individual 3-2608, and from the linkage-based genetic study it is not possible to determine whether this person inherited the portion of the chromosome that carries the disease allele. Mutation analysis, however, was able to confirm that this clinically affected person inherited the *RET* mutation from his affected parent.

This mutation is consistently inherited by all genetically positive and clinically affected members within this kindred. Additional *RET* mutations associated with MEN 2A have been described for other kindreds; however, they have not been observed in affected members of this family (data not shown).

DISCUSSION

Early diagnosis of MEN 2A for individuals at risk not only offers the best chance of curative surgery for MTC, it also relieves the anxiety associated with the risk for developing a malignancy. Biochemical screening tests, such as the provocative calcitonin stimulation assay, have been shown to be effective in the early detection of tumors of the thyroid C cells. This method, however, is critically dependent on sufficient C cell mass in the tumors, and the diagnosis may not be established until the fourth decade of life. The introduction of presymptomatic linkage-based genetic testing has made possible the screening of persons at risk for MEN 2A at any age. However, this modality is only possible under certain circumstances. The families studied must be of a size and structure so that cosegregation of the disease gene with documented cases of MEN 2A can be determined. Furthermore, linkage-based genetic testing is dependent on the characteristics of the available closely linked informative genetic markers. Recombination events between the MEN2A locus and the flanking markers may

preclude accurate prediction of inheritance of the disease allele. Finally, genotyping can be technically tedious and requires considerable time to complete. Direct genetic testing for the inherited mutations in *RET* circumvents the limitations associated with linkage-based genetic testing. Mutation analysis can be performed on any individual at risk for disease without the genetic characterization of large numbers of the other members of the kindred. This analysis requires a single assay (such as PCR amplification with restriction endonuclease digestion or direct DNA sequencing) and can be performed rapidly. For example, PCR amplification followed by restriction endonuclease digestion can usually be performed in hours compared with the days or weeks required to determine inheritance by linkage-based genetic analysis. Furthermore, because this genetic test is based on direct mutation analysis, this diagnostic tool is not restricted by recombination events or the need to characterize large numbers of members of the kindred to determine cosegregation.

As is true with linkage-based genetic testing, direct mutation analysis provides several advantages over current methods of diagnosis for disease. First, it only requires a single blood draw for DNA extraction, unlike the provocation calcitonin stimulation test in which annual testing is recommended from 5 years to 45 years of age. Furthermore, the biochemical test is uncomfortable for patients, and young patients tend to become noncompliant after repeated testing. Second, genetic testing allows for the identification of persons who have inherited the mutated gene. Identification of asymptomatic persons at risk for disease can result in more accurate and effective medical, genetic, and operative management of affected and unaffected patients. If the mutations can be identified, the affected patients can be counseled and offered a prophylactic total thyroidectomy. This type of management has been shown to be effective for young patients who were found to be genetically positive by direct genetic testing (unpublished data). Furthermore, individuals who did not inherit the MEN 2A mutation and their descendants can be exempted from further biochemical and genetic testing.

In this study only two of 31 individuals at risk for MEN 2A were found to have inherited a *RET* mutation. This deviates from the expected pattern of inheritance in classical Mendelian genetics. This kindred has been extensively studied and characterized for several decades; therefore most of the genetically affected individuals had been previously identified by biochemical screening efforts and treated. As a result most of the affected kindred members had been removed from the at-risk pool.

Direct genetic testing by mutation analysis is based on two assumptions. The first is that MEN 2A is directly caused by the mutations in *RET*. Our group and others provided evidence that these inherited germline mutations in *RET* are associated with MEN 2A.[11,12] Although these mutations are consistently inherited by all affected members in a given MEN 2A kindred, studies still need to be done to confirm that these mutations directly give rise to MEN 2A or an analogous disease in a biologic model. However, because we have shown that mutation analysis invariably correlates with affected disease status and inheritance of the disease allele containing the MEN2A locus, this represents an effective presymptomatic diagnostic tool. Second, direct genetic testing requires prior knowledge of the specific mutation inherited within each kindred. Once the mutation in *RET* is identified for a given kindred, direct mutation analysis can be performed.

Direct genetic testing by PCR amplification and restriction endonuclease digestion is the preferred method for the detection of mutations because it is straightforward and rapid and requires no radioactivity. Direct sequence analysis of the mutated *RET* exon from individuals at risk should only be done if the mutation does not generate or abolish a restriction endonuclease site because sequencing is more time-consuming and technically difficult. It must be recognized that technology is rapidly changing the way in which mutations can be detected. For example, novel methods for the detection of mutations such as the oligonucleotide ligation assay[24] and dideoxy fingerprinting[25] have been introduced, and it is reasonable to presume that these and similar methods can be extended to include the described mutations in MEN 2A. Regardless of the technique adopted, we advocate the detection of mutations in the *RET* protooncogene as the diagnostic test of choice for the genetic presymptomatic identification of individuals at risk for MEN 2A.

We are grateful to the MEN 2A families and their physicians for the cooperation and support for this study. We thank Mary DeBenedetti and Vicki Amelung for coordinating the screening and collection efforts. Suzanne Cole, Doris Tribune, and Rose Veile assisted in the extraction of the DNA. William G. Dilley provided the data for the calcitonin immunoassays. We thank Michael Skinner, Meghan M. Dierks, and Paul J. Goodfellow for their helpful comments during the preparation of this article. Katrin M. Carlson and Shenshen Dou provided invaluable insight and shared unpublished data.

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DISCUSSION

Dr. Percival Buenaventura (Pittsburgh, Pa.). You mentioned that two exons are possibly affected in the *RET* protooncogene, exons 10 and 11. In the kindred evaluated in this study and in people who have MEN 2A, are both of these exons mutated?

Dr. Chi. Although all affected individuals in this MEN 2A kindred (K3) have a point mutation in exon 11, exon 10 is not mutated. Likewise, in every kindred with an identified mutation in *RET*, only one of these two exons is involved. To date, we and others[11, 12] (unpublished data) have identified 19 mutations in either exons 10 or 11 for the MEN 2A kindreds that have been characterized. These kindreds have a single point mutation that results in the nonconservative substitution of one of five cysteine residues at codons 609, 611, 618, 620, and 634 in the extracellular receptor domain of the gene product. Exon 11 encodes for the entire transmembrane domain and a portion of the extracellular receptor domain. In this exon the involved cysteine residue is located in the extracellular receptor region.

Dr. Buenaventura. All 43 affected patients whom you managed to study had a mutation in exon 11. Can this be interpreted to mean that the members of different kindreds with MEN 2A have the same mutation?

Dr. Chi. One of the observations we made in this study is that all members within a given kindred consistently inherit the same mutation. In the kindred evaluated in this report, the mutation is cysteine at codon 634 to arginine (TGC to CGC). Different point mutations involving the same cysteine residue can result in the substitution to other amino acids. For example, an MEN 2A kindred can have a mutation involving cysteine at codon 618 in exon 10 that is changed to arginine by the mutation of TGC to CGC. On the other hand, another MEN 2A kindred can also have a mutation involving the cysteine residue at the same position (codon 618) in exon 10 that is changed to serine by the mutation of TGC to AGC. In evaluating other kindreds with mutations in exons 10 or 11, the consistent inheritance of mutations within a given kindred is also seen, although not necessarily with the same mutation in this paper. This supports the argument that these mutations in *RET* are associated with MEN 2A.

Dr. Keith D. Lillemoe (Baltimore, MD.). Would you explain how you plan to use this information in the patients you screened to detect the linkage and mutation abnormality?

Dr. Chi. Since verifying the feasibility of this test, we have begun efforts to identify members of the MEN 2A kindreds followed at Washington University who have inherited the