Application of MEN 203 As a Polymorphic DNA Probe in Screening Multiple Endocrine Neoplasia 2a

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Abstract. Multiple endocrine neoplasia 2a (MEN 2a) is known to be genetically linked to a locus on chromosome 10. The application of polymorphic DNA probes for the region has made it possible to identify carriers of the disease susceptible gene. We performed DNA analysis for a newly found non-Caucasian MEN 2a family using MEN 203 as a probe. Data from DNA analysis of the family members were concordant with the results of conventional endocrinological tests. Furthermore, DNA analysis discriminated four individuals out of fifteen as non-carriers of the gene with a high degree of certainty. The results relieved these people from taking screening tests for years. DNA analysis employing suitable markers such as MEN 203 appears to be useful for a screening program of MEN 2a in Japanese as well as Caucasians.

Key words: MEN 2a, DNA analysis, RFLP.

MULTIPLE ENDOCRINE neoplasia 2a (MEN 2a) is a genetic disorder characterized by an association of medullary thyroid carcinoma and pheochromocytoma [1]. It is possible for affected individuals to have a widespread metastatic cancer in their lifetime. It is inherited in an autosomal dominant manner, yet the penetrance of the disease is incomplete [2, 3, 4], making prediction of the carrier state difficult especially in the young. Fortunately, epidemiological data in the literature show that thyroid C cell hyperplasia or a carcinoma precedes, hence, serum calcitonin is a sensitive marker for the morbidity of the disease [5, 6]. Periodical measurement of serum calcitonin has been recommended to detect this cancer syndrome early [7]. Assays employing monoclonal antibodies [8] in combination with a calcium and pentagastrin provocative test [9–11] seem to have increased the sensitivity. However, the definite criteria as to when to cease the check up have not yet been established. This is important from a practical point of view since epidemiological data indicate that the probability of the carrier state never decreases to less than 60% even at the age of seventy [2]. Considering all the efforts to achieve biochemical tests in all the family members on a regular basis, a single and sensitive screening method has been sought and has now become available.

Recent progress in molecular biology has made it possible to detect disease responsive genes on a specific chromosome by applying polymorphic DNA probes and analyzing restriction-fragment-length-polymorphism (RFLP). In 1987, using IRBP H.4 and D10 S5 as probes, linkage between the MEN 2a gene and interstitial retinol binding protein 3 (IRBP3) on chromosome 10 was first demonstrated [12, 13]. Since then, various probes which seem to be tightly linked with the MEN 2a gene have been explored and applied to MEN 2a families registered mainly in European countries [14–18]. In their studies done on a large scale, the potential usefulness of RFLP analysis in screening...
MEN 2a was demonstrated.

We have introduced RFLP analysis to a newly found Japanese MEN 2a family using MEN 203 as a new probe. Screening tests by conventional biochemical methods were performed concomitantly and the results from hormonal and genetic studies were obtained. The usefulness of genetic screening by DNA analysis in Japan is discussed.

Subjects and Methods

MEN 2a family

A 75-year-old male was referred to our hospital because of a neck tumor. He was diagnosed as having hypertension in his fifties. At the age of 72, a right adrenal mass was found incidentally, and removed surgically. Histological examination revealed a pheochromocytoma. He has been normotensive since the operation. Physical examination suggested a thyroid tumor with swelling of cervical lymph nodes. Biochemical tests revealed increased serum calcitonin and carcinoembryonic antigen (CEA). A diagnosis of MEN 2a was made. Family members were called up and registered, except for those who refused the tests.

Biochemical screening

In addition to routine biochemical examination, serum calcitonin, CEA, and intact PTH were measured by RIA. Urine catecholamines and their metabolites (metanephrine and normetanephrine) were measured by fluorometric assay employing HPLC. In some of the family members, calcium (2 mg/kg/min) and pentagastrin (0.5 µg/kg/5s) administration [11] as a provocative test was performed. An increase in basal serum calcitonin or a more than three fold increase in serum calcitonin after the administration of calcium and pentagastrin was defined as an indication of medullary thyroid carcinoma [8, 11]. For those who were proved to have medullary thyroid carcinoma, measurements were done in duplicate and imaging studies by X-ray CT or ultrasonography were done. The proband had a left adrenal tumor suggesting recurrent pheochromocytoma on the opposite

DNA analysis

DNA was extracted from peripheral blood lymphocytes. DNA electrophoresis and hybridization methods were as described previously [19]. In brief, extracted DNA was digested with the restriction endonuclease Taq I (Molecular Biology Resources, Milwaukee) and separated by electrophoresis. The DNA was hydrolized and then transferred to nylon membrane filters. After the transfer, the DNA was hybridized with radiolabelled probe MEN 203 and the filters were exposed for autoradiography.

The probe MEN 203 was isolated from a chromosome 10 cosmid library and identifies two polymorphic alleles (9.0 and 6.3 kb) after digestion with Taq I. An analysis of this marker done previously suggests a close linkage with the MEN 2a gene [16].

Results

Clinical and biochemical characterization

Two individuals of the II generation in the panel (Fig. 1) had died of a stroke in their forties. Another had died of a sudden unknown cause at the age of seventeen. Fifteen individuals including the proband were examined. Among them five were shown to have increased calcitonin concentrations ranging from 132 to 228100 pg/ml, thus a diagnosis of medullary thyroid carcinoma or C cell hyperplasia was made. Serum CEA was increased in these five cases. Thyroid tumors were palpable in three of the five.

Plasma and urine catecholamines and their metabolites were within the normal range in all individuals including the proband. Among those with medullary thyroid carcinoma, measurements were done in duplicate and imaging studies by X-ray CT or ultrasonography were done. The proband had a left adrenal tumor suggesting recurrent pheochromocytoma on the opposite

Fig. 1. DNA typing of MEN 2a family. A, B=alleles distinguished by probe MEN 203. Circles denote female family members and squares males; when solid, they represent those affected. Slashes denote deceased persons.
side, although he was asymptomatic. Thus, except for the proband, none of the members were proved to have pheochromocytoma.

DNA analysis

To perform linkage analysis by RFLP, DNA samples from at least two generations must be obtained and the parents must be heterozygous for the marker, i.e. MEN 203. In this regard, the present family was informative. First, the proband family was examined. The proband (II ②) allele was typed as AB, whereas that of his offspring, III ⑤, who was proved to be affected in the clinical study, was typed as AA. From these data, III ⑤ must have inherited the high risk allele, i.e. A from the parent II ②. In this regard, both III ④ and III ⑥ are supposed to be unaffected, since their allele A must have been inherited from their mother who is apparently unaffected. Next, eleven DNA samples from the family panel were tested. Seven individuals were typed as AB and the rest as BB.

Comparison of the biochemical and genetic screening tests

Results from biochemical and genetic tests are summarized in Table 1. From the results done on the proband family, it was assumed that the disease gene might be inherited in close linkage with the A allele distinguished by the probe MEN 203. All the affected individuals had allele A in their RFLP pattern. Based on this assumption and the characterization of the marker MEN 203 done previously, the kindred typed as BB (III ②③⑤⑧⑩) should have reduced risk and the probability of being a gene carrier seems to be less than 1% [15]. These four individuals were found to be normal in the conventional screening tests. For those typed as AB (III ①⑦⑨), the prediction of the carrier state by linkage analysis with MEN 203 was impossible because of incomplete DNA sampling. In routine examinations none of these three was proved to be affected, but serial screening efforts must be continued.

Discussion

An approach to DNA analysis using RFLP was introduced to the screening of a Japanese MEN 2a family. Specifically, we employed MEN 203 as a new marker and for the first time examined a non-Caucasian MEN 2a family with this new probe. MEN 203 is a suitable marker for predictive screening, since the close location of MEN 203 to MEN 2a gene has been established by studies of numbers of Caucasian MEN 2a families and the lod score is increased to 13.7 provided that the recombination fraction (θ) is 0 [16]. In a strict sense, however, it is possible that the gene responsible for MEN 2a is different from race to race and that some of the MEN 2a are inherited in a different manner. At present, there is no conclusive evidence that supports these possibilities. Moreover, our results indicate that MEN 203 can be used as a useful marker in non-Caucasian
subjects as well as in Caucasian subjects.

The present results confirmed the advantages of DNA analysis over the conventional screening tests mentioned in previous studies [14–18]. First, it can be done by a single blood sampling and is independent of the age of the subject and the results are quite reproducible. Second, and more importantly, some of the family members were proved to have greatly reduced probability of gene carriers of the disease.

In the present family, all the affected individuals had A allele, which MEN 203 distinguishes as a high risk allele. On the other hand, four individuals (III 2, 3, 8, 10) were typed as BB and were determined to be at low risk. We therefore assessed that these four members are non-carriers. This is important from clinical aspects. For example, case III 1 is clinically unaffected at the age of 30. The risk estimated at birth is 0.5. According to the data accumulated by CRC Medullary Thyroid Group register [2], the probability that a gene carrier will still appear unaffected at age 30 is 0.9. Conversely, the probability that III 1 is not a carrier is 0.5. Thus, standard risk estimation indicates that the probability of case III 1 being a gene carrier is 47.4% (0.5 × 0.9/(0.5 × 0.9) + 0.5). When negative results are obtained in repetitive stimulation tests, the probability of the disease decreases substantially, but it never decreases to less than 5% [2]. Another aspect of the disease is that only two-thirds of all MEN 2a gene carriers will be symptomatic by the age of 70 [2–5]. For such complicated situations, it is difficult to make a correct clinical decision to exclude the subject from screening tests. Practically, the recommendation for screening young offspring of a MEN 2a family is to take an annual calcitonin measurement after the age of five [20]. The four individuals in the present family, who are in their twenties or thirties, have become free of the need for such frequent screening tests. Thus, the marker was useful in discriminating non-carriers of the disease.

The third advantage of genetic diagnosis is that DNA analysis is capable of making a presymptomatic diagnosis. Unfortunately, the prediction of asymptomatic carriers was not possible in the present study. One of the reasons is that the number of family members employed in the present study was too small. For the IV generation in the panel, who are too young to take an unpleasant provocative test, using RFLP analysis as a single screening test is promising.

In MEN 2a, the incidence is low but data in the literature show shorter life expectancy of MEN 2a patients [20]. The need for central registration must be emphasized in our country and a supervision program including genetic screening must be constructed [21].

In summary, DNA analysis with MEN 203 was performed in a newly found MEN 2a family and the usefulness of the method was proposed. Since the onset and severity of the disease and association of pheochromocytoma are variable, a second mechanism in addition to the single gene defect must be present [22]. The characterization of the predisposing gene(s) awaits further studies and is expected to bring out a more accurate diagnostic method.

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References


