Neuromas and Prominent Corneal Nerves without MEN 2B

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SUMMARY
Purpose - We studied a family composed of 2 members with the characteristic phenotype of the MEN 2B and without RET proto-oncogene mutations in order to determine whether they had multiple endocrine neoplasia associated with MEN 2B in the 5-year follow-up.

Subjects and methods - The family consisted of a 15 year old female complaining of burning eyes, examined ophthalmologically in 1992 and her mother and sister, who were examined later on in 1992. The proband and the mother were affected with multiple mucosal neuromas and visible corneal nerves. Pentagastrin-stimulated serum calcitonin levels, catecholamines, serum calcium and phosphate levels were measured. Molecular genetic studies were performed on the 2 affected members to look for the specific RET mutation seen in MEN 2B.

Results - Endocrine neoplasia of the syndrome MEN 2B, medullary thyroid carcinoma, pheochromocytoma and hyperparathyroidism, were ruled out in the first examination and after 5-year follow-up. In the 2 cases no mutation at codon 918 for the RET proto-oncogene was found.

Conclusions - We consider that familial multiple mucosal neuromas are a highly distinctive entity of MEN 2B.

INTRODUCTION
When an ophthalmologist finds visible corneal nerves on a clear stroma, or when a clinician detects mucosal neuromas, the diagnosis of MEN 2B should be entertained. Recognition of these ocular or mucosal lesions as components of the MEN 2B is of utmost importance because identification of affected individuals facilitates early treatment of associated endocrine tumors (12). The pathognomonic clinical features of MEN 2B facilitate diagnosis; distinctive features include a marfanoid habitus, mucosal neuromas causing bumpy lips, and ganglioneuromatosis through the gastrointestinal tract.

In 1994, a germ line mutation on codon 918 of exon 16 of the RET proto-oncogene was described in association with MEN 2B. This mutation converts RET into a dominant transforming gene (6) and 95 percent of MEN 2B families from several countries are caused by this mutation (3, 13). Identification of mutation gene carriers by DNA analysis allows early identification for subjects at risk in this familial cancer syndrome and provides the basis for preventive thyroidectomy (1). We reported, some years
ago, a family composed of 3 members with the MEN 2B phenotype and without endocrine tumors (4).

We present 2 members of a new family with the same MEN 2B phenotype, who do not present any endocrine tumors after a 5 year follow-up, and without mutations at codon 918 of the RET gene.

SUBJECTS AND METHODS

- **Case 1.** A 15-year-old woman was first seen in June 1992 by the Department of Ophthalmology complaining of burning and dryness of the eyes. She had prominent lips and nodules on her tongue since childhood (fig. 1). Her past medical history was unremarkable. At examination, the most striking finding was the presence of greatly thickened corneal nerves on slit-lamp biomicroscopy (fig. 2). She had mucosal neuromas in her tongue without marfanoid habitus. The thyroid was normal on palpation and blood pressure was 120/70 mmHg.

- **Case 2.** A 39-year-old woman, mother of case 1, was also studied. She had the characteristic facies with prominent lips, multiple neuroma of the tongue and presence of thickened corneal nerves on slit-lamp biomicroscopy. At examination, the thyroid was normal and blood pressure was 135/80 mm Hg. Further investigation revealed that the other 12-year-old daughter, had no abnormal facies and no thickened corneal nerves. The 2 cases were reexplored in 1994 and 1997 for screening of medullary thyroid carcinoma and pheochromocytoma.

Screening for medullary thyroid carcinoma was performed using serum basal calcitonin (RIA, Diagnostic System Laboratories) and after pentagastrin stimulation (0.5 µg/kg i.v.). When basal or post-stimulation values were higher than 0.5 µg/L, the test was considered abnormal. Screening for pheochromocytoma was performed using 24 h urinary levels of catecholamines (normal values 117-886 nmol/24 h), vanillylmandelic acid (normal values 15-37.8 µmol/24 h) and metanephrines (normal values 0.6-6 µmol/24 h). A high abdominal resolution scanning was performed and was normal. Serum calcium and phosphate levels were normal.

**DNA mutation analysis**

DNA was extracted from peripheral lymphocytes for the patients, according to a previously described method (10). PCR amplification of exons was carried out by extracting peripheral lymphocytes of the 2 cases, according to a previously described method (7). Direct PCR sequence analysis was performed for exons 10, 11 and 16. Amplification of exon 10 was performed in a total reaction volume of 50 µL containing 250 µg of genomic DNA, and was carried out with the oligonucleotide primer Ret17S and Ret10Rb, and Ret5 and 8AR for exon 11, and rRet16 and fRet16 for exon 16. PCR products were purified using the QIAquick PCR purification kit (QUIAGEN). Electrophoresis was performed on 16 % acrylamide gel.

**Restriction enzyme digestion**

Point mutation recognition sites were carried out with restriction enzymes, BsoFI, CfoI and the mutation in codon 918 in exon 16 was studied by digestion with the enzyme Rsal. The fragments were separated by electrophoresis on 12 % acrylamide gel.

In case 1 basal calcitonin levels were 0.02 µg/L and after pentagastrin stimulation 0.05 µg/L in 1992, 0.05 µg/L in 1994 and 0.06 µg/L in 1997. In case 2 calcitonin levels
were 0.04 µg/L and after pentagastrin 0.05 µg/L in 1992, and 0.05 µg/L in 1994 and 0.04 µg/L in 1997. In case 1 urine catecholamine values were 166 nmol/24 h, vanillylmandelic acid 19 µmol/24 h and metanephrines 4 µmol/24 h in 1992, and 144 µmol/24 h, 13 µmol/24 h and 1.8 µmol/24 h respectively, in 1997. In case 2 urine catecholamine values were 176 nmol/24 h, vanillylmandelic acid 27.2 µmol/24-h and metanephrines 2.5 µmol/24 h in 1992 and 112 nmol/24 h, 17.7 µmol/24 h and 2.9 µmol/24 h respectively, in 1997.

Germline DNA extracted from the 2 patients revealed no RET exon, 10, 11 mutation and no mutation in codon 918 in exon 16.

DISCUSSION

Clinical identification of MEN 2B abnormalities, facilitates their early diagnosis (1). DNA analysis has recently been shown to be a sensitive and specific method of identifying individuals carrying the MEN 2B gene in a family. In an individual with some features suggestive of MEN 2B, the absence of the gene mutation would cause the clinician to reconsider the diagnosis. In the rare families with classic clinical manifestations of MEN 2B but no mutation at codon 918, none of the mutations found in MEN 2A or familial medullary thyroid carcinoma was detected either, suggesting that an unidentified mutation in RET proto-oncogene, or another gene, may be involved in causing this disease (13). A large proportion of MEN 2B patients have thickened corneal nerves, 69 percent (14) and 29 percent of patients with MEN 2A (9) ; and this does not occur in any other disease except in familial mucosal neuromas described by some authors previously (2, 4, 11) or in isolated cases of mucosal neuroma syndrome (5). A family recently reported (8) with medullary thyroid carcinoma and prominent corneal nerve thickening was negative for RET exon 10, 11 and 16 mutations, and 4 adults had corneal nerve thickening only. The authors think that there is increasing evidence that the familial mucosal neuroma syndrome may be part of a large family of neural crest disorders.

Corneal nerves may become secondarily thickened following keratoplasties or as a consequence of local corneal inflammation and disease. The thickened corneal nerves had been reported in a case of Refsum syndrome, or in some cases of patients with lamellar ichthyosis, lattice dystrophy and conditions associated with other types of corneal pathology (11).

We reported another family composed of 3 members with MEN 2B characteristic phenotype (4). Initially, we considered that it was an incomplete form of MEN 2B, but this fact was not confirmed in the follow-up of the 2 families. The new family reported is similar and has no endocrine tumors after 5 years of follow-up and without identified genetic basis. These reports based on our experience of 2 families are highly distinctive of the MEN 2B and could be regarded as mucosal disorders not always associated with endocrine tumor development.

REFERENCES

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