Tissue and tumor mosaicism of the myotonin protein kinase gene trinucleotide repeat in a patient with multiple basal cell carcinomas associated with myotonic dystrophy

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We describe the third case (to our knowledge) of multiple basal cell carcinoma associated with myotonic dystrophy and carry out a genetic study of the tumor comparing it with healthy skin. We consider that our results show that this association might be not a purely random phenomenon and that the particular genetic characteristics of this disorder might have a role in the pathogenesis of the tumor. (J Am Acad Dermatol 2004;50:S1-3.)

Myotonic dystrophy (DM) is an autosomal dominant disorder of variable penetrance, characterized by myotonia, muscular dystrophy, cataracts, hypogonadism, frontal balding, and electrocardiographic alterations.[1] Atrophy of masseter muscles, sternocleidomastoid muscles, and the temporal muscles produces a characteristic haggard appearance. Myotonia, delayed muscle relaxation after contraction, is most frequent in the tongue, forearm, and hand.

This genetic defect is found in the 3′ untranslated region of the myotonin protein kinase gene located on chromosome 19q 13.3. The genetic defect consists of expansion on the trinucleotide CTG more than 50 repeats (normal range, 5-37 repeats). The number of repeats is strongly correlated with the severity of the disorder. Thus, mildly affected persons have from 50 to 100 repeats, and more severely affected persons might have from 100 to several thousand repeats. This fact is the molecular basis of the phenomenon of anticipation, which consists of worse and earlier presentation of the clinical condition in successive generations.[1]

Several tumors have been associated with DM.[2-14] The best known is probably pilomatricoma,[2-5] but it has also been associated with thymomas,[6] insulinomas,[7] cancer of the testicle,[8] parotid tumor,[9] cancer of the larynx,[10] and tumor of the ovary.[11] Only two cases of multiple basal cell carcinomas have been reported in association with this condition.[12,13]

The aim of this study is to describe a new case of multiple basal cell carcinomas associated with DM and to carry out a genetic study of the tumor, comparing it with healthy skin, and finally to consider the role played by mutation in the occurrence of basaliomas in DM. Recently, a genetic study of similar characteristics has been carried out in other tumors associated with DM.[9-11,14]

CASE REPORT

A 41-year-old man consulted the Department of Dermatology for multiple cutaneous tumoral lesions that he had had for several years. The patient was moderately mentally retarded, complained of weakness of unknown cause for many years, diabetes mellitus treated with oral antidiabetic drugs, and had had cataract operations 2 years previously. Several members of his family had myotonic dystrophy.

On clinical examination there were many pearly tumoral lesions on his left upper eyelid (Fig 1, *A*), thorax (Fig 1, *B*), and back, all compatible with the clinical diagnosis of basal cell carcinoma. The patient had white skin with no apparent actinic damage and no history of long-term exposure to the sun. His face was expressionless, with marked weakness of the facial muscles, including ptosis of the eyelids, which gave him an aged, tired appearance, and marked frontal balding. He had no hypertelorism, pain in the jaw, toothache, or palmar pitting.

The tumoral lesions were all excised. Pathologic studies confirmed the clinical diagnosis of multiple basal cell carcinomas.

Because the patient had phenotype characteristics of Steinert's myotonic dystrophy, he was referred to the Neurology Department. Neurologic examination and electromyography with a recording typical of the disease confirmed the diagnosis.

Blood tests showed raised triglycerides and gamma-glutamyl transpeptidase. On the echocardiogram, there was concentric hypertrophy of the left ventricle but no other significant alterations. Orthopantomography ruled out the presence of odontogenic cysts. Bone radiographic studies showed no significant change.

Subsequently, DNA studies were done of a sample of leukocytes to obtain genetic confirmation of the disorder. Molecular studies were done according to Southern blotting technique. The patient's DNA was digested using the *Sac*I restriction enzyme, subsequently hybridized with the p5B1.4 probe, and an expan-

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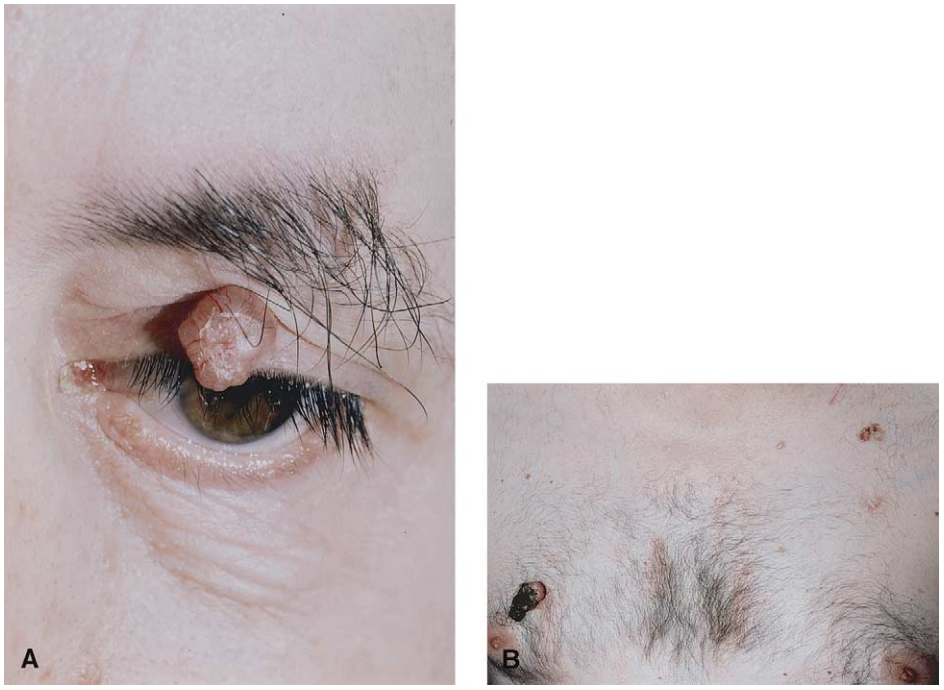

Fig 1. A, Basal cell carcinoma on the left upper eyelid and another smaller one in the left cheek. **B**, Multiple basal cell carcinomas on the thorax of the patient.

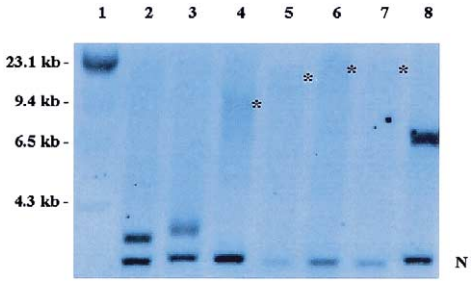

Fig 2. Southern blot analysis showing myotonic dystrophy–associated expanded SacI restriction fragments. Normal allele size (N) has approximately 3.6 kb. Lanes 2, 3, and 8 are mutant expanded controls with myotonic dystrophy, with 0.2 kb, 0.5 kb, and 4.0 kb, respectively; lanes through 4 to 7 represent the patient's tissues; all of them show mosaicism. Lane 4 is leukocytes (~7 kb CTG expansion), lane 5 is normal skin (~20 kb expansion), and lanes 6 and 7 are two different basal cell carcinomas (>20 kb expansion). The patient's expanded bands are indicated by an asterisk (*). Weak signals on lanes 6 and 7 are due to the wide tissue mosaicism. Molecular size patterns are showed on lane 1 and indicated on the side. Genomic DNA (10 μg) from each sample was digested with SacI run in 0.8% agarose gel and blotted onto nylon membrane (Nytran). The blot was hybridized with digoxigenin-deoxyuridine triphosphate–labeled p5B1.4 probe. After a higher stringency wash with 0.5× standard saline citrate and 0.1% sodium dodecylsulfate for 15 min at 65°C, the blot was detected by chemoluminescence.

sion of the CTG triplet of 7 kb (approximately 2300 repeats) was obtained. Tissue mosaicism was observed (Fig 2).

Once the diagnosis of DM had been confirmed clinically, electromyographically, and finally genetically, we considered studying CTG triplet expansion in healthy skin and in the skin with basal cell carcinoma to compare them. We therefore excised a piece of healthy skin from internal aspect of the left arm and two new basal cell carcinomas from the nose and right nasogenial sulcus. DNA analysis of the healthy skin showed expansion of the CTG triplet of approximately 20 kb (Fig 2). Analysis of DNA from two basal cell carcinomas showed very large CTG expansions in the upper limit of the Southern blot resolution. In both tumors, expansion was more than 20 kb. Again, somatic mosaicism was detected (Fig 2).

DISCUSSION

It is well known that the genetic defect of DM consists of more expansion of the CTG trinucleotide repeats than usual variable polymorphism (5-37 repeats).[1] The association of this disease with a long list of tumors has until recently only been studied from the descriptive point of view.[2-8,12,13] Over the past 3 years, several Japanese investigators have studied this mutation in tumors of patients with DM and have observed that the CTG repeat expansion is more marked in them than in nontumoral tissue.[9-11,14]

A large series of mutations have been considered to be involved in the genesis of many cancers. For instance, it has been shown that in hereditary nonpolypoid colorectal cancer, the repeat sequences of DNA or "short tandem repeat sequences" make the DNA unstable, and this instability might become a factor for progression of the cancer.[15,16]

The genetic findings in this patient are similar to those seen in other tumors of patients with DM. Thus, the triplet expansion was far greater in the basal cell carcinomas than in healthy skin. Even expansion size difference between normal skin and blood was evident. The increased CTG repeats might be interpreted as the high degree of cell division seen in tumor tissue. It could be argued that these repeat sequences might lead to instability of the DNA and cause increased predisposition to cancer in these patients.

The association of multiple basal cell carcinomas and myotonic dystrophy has only been reported twice before,[12,13] but no

genetic studies have yet been done. We, therefore, consider that the results of this study show that this association might be not a purely random phenomenon and that the particular genetic characteristics of this disorder might have a role in the pathogenesis of the tumor.

REFERENCES

1. McKusick VA, Antonarakis SE, Francomano CA, Hurko O, Scott AF, Smith M, et al. Mendelian inheritance in man: a catalog of human genes and genetic disorders. Baltimore and London: The John Hopkins University Press; 1998.
2. Harper PS. Calcifying epithelioma of Malherbe and myotonic dystrophy in sisters. Birth Defects Orig Artic Ser 1971;7:343-5.
3. Chiaramonti A, Gilgor RS. Pilomatricomas associated with myotonic dystrophy. Arch Dermatol 1978;114:1363-5.
4. Berberian BJ, Colonna TM, Battaglia M, Sulica VI. Multiple pilomatricomas in association with myotonic dystrophy and a family history of melanoma. J Am Acad Dermatol 1997;37:268-9.
5. Betlloch I, Morell A, Silvestre JF, Sevila A, Bañuls J, Alfonso R. Pilomatricomas y distrofia miotónica. Rev Neurol 1997;25:306-7.
6. Murakami S, Sakakibara K, Yamada A, Kuriowa Y, Kitamura S, Sakurai M, et al. Dystrophia myotonica associated with thymoma: 2 autopsy cases. Nippon Rinsho 1978;36:3037-8.
7. Sugio T, Jinnai K, Ohara T, Nishida Y, Satake S, Yamamamoto H, et al. Myotonic dystrophy associated with insulinoma. Intern Med 1999;38:504-6.
8. Kerbrat P, Toussaint C, Ben Hassel M, Guerin D. Association of Steinert's disease and testicular cancer. Press Med 1987;16:1434.
9. Ogata K, Takahashi A, Oguchi N, Ishitoya J, Fuse S, Shimpo T. Somatic mosaicism of p(CTG)n expansion in a case of myotonic dystrophy with parotid tumor. Rinsho Shinkeigaku 1998;38:736-8.
10. Osanai R, Kinoshita M, Hirose K, Homma T, Kawabata I. CTG triplet repeat expansion in a laryngeal carcinoma from a patient with myotonic dystrophy. Muscle Nerve 2000;23:804-6.
11. Kinoshita M, Igarashi A, Komori T, Tamura H, Hayashy M, Kinoshita K, et al. Differences in CTG triplet repeat expansions in a ovarian cancer and cyst from a patient with myotonic dystrophy. Muscle Nerve 1997;20: 622-5.
12. Stieler W, Plewig G. Multiple Basaliome bei Myotonia atrophica Curschmann-Steinert. Hautarzt 1986;37:226-9.
13. Azurdia RM, Verbov JL. Myotonic dystrophy and basal cell carcinoma-a true association? Br J Dermatol 1999;141:941-2.
14. Jinnai K, Sugio T, Mitani M, Hashimoto K, Takahashi K. Elongation of (CTG)n repeats in tumors associated with myotonic dystrophy patients. Muscle Nerve 1999;22:1271-4.
15. Panzer S, Kuhl DP, Caskey CT. Unstable triplet repeat sequences: a source of cancer mutations? Stem cells 1995;13:146-57.
16. Peltomaki PT. Genetic basis of hereditary nonpolyposis colorectal carcinoma. Ann Med 1994;26:215-9.