A Novel Missense Mutation in *CYLD* in a Family with Brooke–Spiegler Syndrome

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Brooke–Spiegler syndrome (BSS, familial cylindromatosis or turban tumor syndrome) is an inherited disease characterized by neoplasms of the skin appendages such as cylindroma, trichoepithelioma, and spiradenoma. The disease has been mapped to 16q12-13, and mutations in the *CYLD* gene have been identified in families with this disorder. Of interest, multiple familial trichoepithelioma (MFT) has been described as a distinct disorder characterized by the familial occurrence of trichoepitheliomas. MFT has been mapped to 9p21; however, to date a candidate gene has not been identified. In this report, we describe a four-generation family with BSS presenting predominantly with trichoepitheliomas (resembling MFT phenotype). We identified a novel missense mutation in the *CYLD* gene, designated E474G, in the affected individuals of this family. Our findings exemplify clinical heterogeneity within BSS and extend the body of evidence that mutations in *CYLD* are implicated in this disease. Although not conclusive, these findings suggest that BSS and MFT may represent a single entity. ***Key words: familial cylindromatosis/turban tumor syndrome/cylindroma/trichoepithelioma/genodermatosis. J Invest Dermatol 121:732–734, 2003***

Brooke–Spiegler syndrome (BSS), also known as familial cylindromatosis or turban tumor syndrome (OMIM 132700, 605041) (Brooke, 1892; Spiegler, 1899), is an autosomal dominantly inherited disease characterized by predisposition to neoplasms of the skin appendages. The most commonly observed tumors are cylindromas, trichoepitheliomas, and spiradenomas. Typically, these tumors are located in the head and neck region, appear in early adulthood, and gradually increase in size and number throughout life. Cylindromas located on the scalp may eventually cover the entire scalp, resulting in so called "turban tumors." In addition to tumors of the skin appendages, patients with BSS are also at risk for developing tumors of the salivary glands. Basal cell monomorphic adenoma and adenocarcinoma of the parotid glands have been described as a rare association in this disease (Antonescu and Terzakis, 1997; Jungehulsing *et al*, 1999). Although cylindromas are usually benign tumors, malignant transformation to cylindrocarcinomas is rare, but well documented (Gerretsen *et al*, 1993). Cylindrocarcinoma or malignant cylindroma is a locally aggressive tumor that often metastasizes, requiring careful monitoring of these patients (Gerretsen *et al*, 1995; Pizinger and Michal 2000; Durani *et al*, 2001).

The gene for familial cylindromas (thus for BSS) was mapped to chromosome 16q12-13 by linkage analysis (Biggs *et al*, 1995). Subsequently, the *CYLD* gene was discovered by positional cloning, and mutations in *CYLD* were identified in families with this disease (Bignell *et al*, 2000). In this report, we describe a large family with BSS in which a novel missense mutation in *CYLD* has been identified. Of interest, the affected individuals of the family described here exhibit a phenotype that resembles multiple familial trichoepithelioma (MFT).

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Abbreviations: BSS, Brooke–Spiegler syndrome; MFT, multiple familial trichoepithelioma.

MATERIALS AND METHODS

Genomic DNA was extracted from whole blood using the PureGene DNA isolation kit (Gentra Systems, Minneapolis, MN). The 17 coding exons of the *CYLD* gene were amplified by PCR using specific primers (the sequences of the primers are available upon request from the authors). For mutation detection, the PCR products were sequenced using an automated sequencing system (310, Applied Biosystems, Foster City, CA). The PCR products were digested with *Mnl*I at 37°C for 12 h and analyzed on 1.5% agarose/TBE minigels. The study has been approved by the institutional IRB and patient blood samples were collected after signing the consent forms.

RESULTS

Clinical data The family is of Turkish descent. The pedigree is consistent with an autosomal dominant mode of inheritance of the disease in this family (**Fig 1**). The affected individuals began developing skin tumors in the late teenage years. On examination, all affected persons had numerous papules predominantly on the nasolabial folds, nose, and upper lip (**Fig 2*a***). These papules were flesh colored, measuring 0.2 to 0.4 cm, and the histologic examination showed findings of trichoepithelioma. Whereas most of the affected persons were noted to have skin lesions on the nasolabial folds, one family member (II-3) had numerous papules on his entire back, of which the histology was trichoepithelioma as well. None of the affected persons showed classic turban tumors and lacked

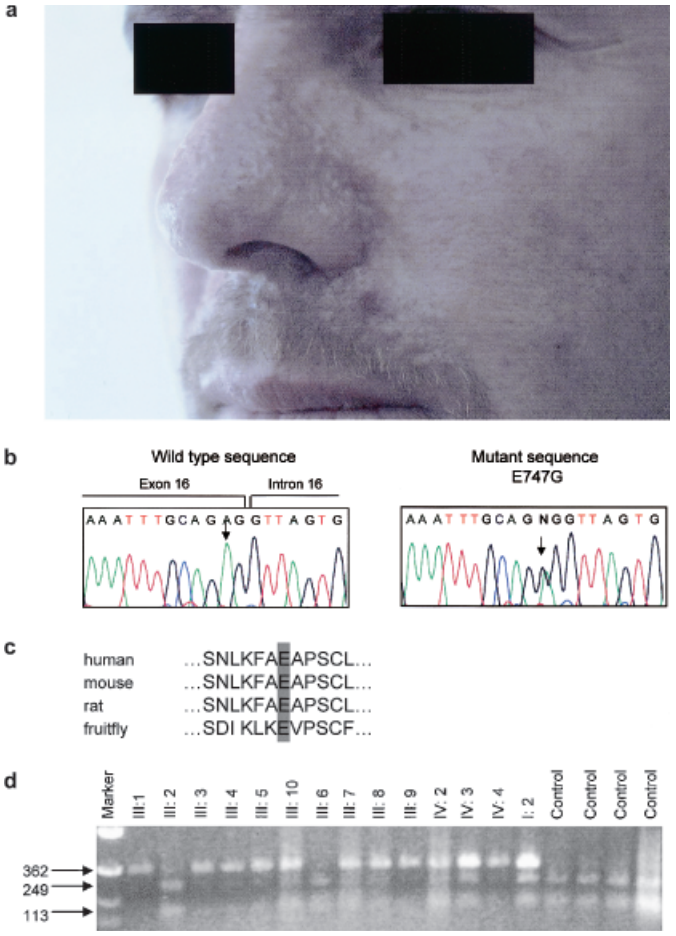

Figure 1. Clinical data and genetic analysis of the family with BSS. (*a*) Biopsy-proven multiple trichoepitheliomas on the nose, nasolabial folds, and the upper lip. (*b*) DNA sequence of exon 16 of *CYLD* from control and affected individuals. (*c*) The alignment of human *CYLD* protein (amino acids 741–752) and its orthologs. The conservation of glutamic acid (E) at codon 747 is indicated. (*d*) The figure shows *Mnl*I digestion of the PCR samples, which cleaves the wild-type allele in 249- and 113-bp fragments. The mutation E747G abolishes the *Mnl*I site, and therefore the mutant allele does not cleave and shows the intact 362-bp PCR product.

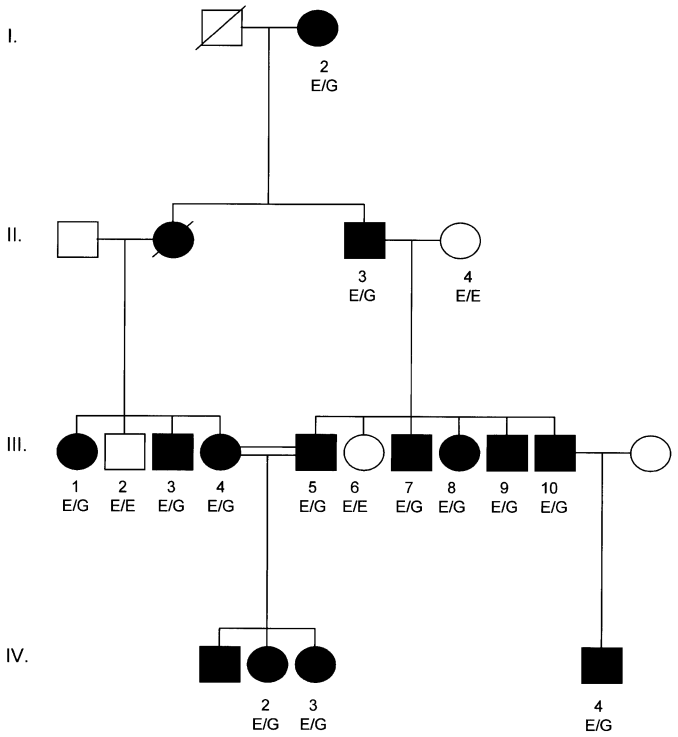

Figure 2. Pedigree of the family. For individuals whose DNA samples have been analyzed, the allele sequences at codon 747 have been indicated as E (glutamic acid) or G (glycine). The unaffected persons encode for an E on both alleles (E/E), whereas the affected persons are heterozygous for the mutation and encode a G on the mutant allele (E/G).

skin lesions on the scalp. Nevertheless, upon careful skin examination, one individual was noted to have isolated papules on the scalp. The biopsies obtained from the scalp lesions showed histologic findings of cylindroma.

Mutation detection DNA from 13 affected and 3 unaffected family members were tested for mutations in the *CYLD* gene. A transition of an adenine to a guanine at nucleotide 2240 in exon 16 was identified (**Fig 2*b***). The mutation results in replacement of glutamic acid (GAG) by glycine (GGG) at amino acid 747. The mutation, designated as E747G, cosegregates with the disease in the family (**Fig 1**). The mutation E747G abolishes a restriction endonuclease site for the enzyme *Mnl*I, which was used to confirm the mutation (**Fig 2*d***). The sequence alteration found in this family was not observed in 200 unrelated, unaffected controls.

DISCUSSION

The *CYLD* gene consists of 20 exons, of which the first three are untranslated. When one copy of the gene is inactivated in the germline, affected individuals are predisposed to developing neoplasms of the skin appendages. Moreover, loss of heterozygosity at the *CYLD* locus has been found in these tumors, suggesting that *CYLD* functions as a tumor suppressor (Bignell *et al*, 2000).

To date, there have been two reports describing mutations in families with BSS. Bignell *et al* (2000) reported mutations in *CYLD* in 21 of 25 families with BSS. Recently, Gutierrez *et al* (2002) described a family with a mutation in *CYLD*. All mutations were located in the 3′ two-thirds of the *CYLD*-coding sequence (exons 9–20), a region that is well conserved among its orthologs. The mutation E747G described here is also within this region. E747G missense mutation is predicted to be pathogenic in this family owing to perfect cosegregation of the mutation within the family and its absence in the control group, as well as conservation of this region of the protein among its orthologs (**Fig 2*c***). It is possible, but unlikely, that E747G represents a rare polymorphism within *CYLD*. Nevertheless, we have not encountered this sequence variation in 200 healthy control patients. Of interest, we have not noted any polymorphisms within exon 16 of *CYLD*.

A major and unique feature of BSS is the presence of heterogeneity of tumors in the affected families. Whereas some families present with cylindromas and trichoepitheliomas (Burrows *et al*, 1992), other families presenting with spiradenomas and trichoepitheliomas (Weyers *et al*, 1993) have been described. Of interest, MFT (OMIM 601606) described as a distinct syndrome is inherited in an autosomal dominant pattern and is characterized by multiple trichoepitheliomas. MFT has been mapped to 9p21 (Harada *et al*, 1996); however, to date a candidate gene has not been identified in this region. Moreover, loss of heterozygosity in sporadic trichoepitheliomas was demonstrated at 9q22.3 (48%), but not at 9p21 (Matt *et al*, 2000). In a different study, loss of heterozygosity at 16q around the *CYLD* locus was shown in one of two sporadic trichoepitheliomas studied (Leonard *et al*, 2001). Inter- and intrafamilial phenotypic variability has been well documented in BSS. Gerretsen *et al* (1995) suggested that both BSS and MFT may be caused by the same genetic defect,

because both cylindroma and trichoepithelioma can occur in the same patient or in different patients within a single family. Recently, a four-generation family exhibiting phenotypic variability with a single germline mutation in *CYLD* was described (Gutierrez *et al*, 2002). In this family, some affected members had cylindromas, whereas others had trichoepitheliomas as the predominating tumor type. Of interest, the affected individuals of the family described here exhibit a phenotype that resembles MFT. All of the affected individuals of this family presented with multiple trichoeptiheliomas, with the exception of only one individual who had cylindromas on the scalp. While not conclusive, our findings and currently known data suggest that BSS and MFT may represent phenotypic variability of a single entity. Evaluation of families with MFT phenotype for mutations in *CYLD* will help to clarify this issue.

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