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 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).

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 MnlI 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 2a). 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...
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 2b). 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 MnlI, which was used to confirm the mutation (Fig 2d). 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 germ line, 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 within the family and its absence in the control group, as well as conservation of this region of the protein among its orthologs (Fig 2c). 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 \textit{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 trichoepitheliomas, 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 \textit{CYLD} will help to clarify this issue.

\textbf{REFERENCES}


Spiegler E: Ueber endotheliome der haut. \textit{Arch Derm Syph} 50:163–176, 1899


We appreciate the participation of the patients in the study. This work was supported in part by the Dermatology Foundation (J.T.C.), the Waterbor Burn and Cancer Foundation (J.T.C.), and the Irving Center for Clinical Research at Columbia University (J.T.C.).