A Novel Splicing Mutation of the CYLD Gene in a Taiwanese Family with Multiple Familial Trichoepithelioma

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Multiple familial trichoepithelioma (MFT) is an autosomal dominant skin disorder that is characterized by childhood onset of numerous skin-colored papules and nodules on the central face that originate from hair follicles. Mutations in CYLD, the disease gene of familial cylindromatosis, have recently been detected in several families presented with MFT phenotype. CYLD has been shown to be a tumor suppressor gene. CYLD functions as a negative regulator of the transcription factor NF-kB, which protects against apoptosis, and inactivation or mutation of the CYLD gene contributes to oncogenesis. Here, we report a novel splicing mutation (IVS16+1G>T) in the CYLD gene in a Taiwanese family with MFT. (Dermatol Sinica 25: 128-131, 2007)

Key words: Multiple familial trichoepithelioma, Familial cylindromatosis, CYLD gene, Splicing mutation

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INTRODUCTION

Multiple familial trichoepithelioma (MFT; MIM 601606) is an autosomal dominant disease characterized by childhood onset of numerous rounded, skin-colored, firm papules and nodules located on the central face, mainly around the nasolabial folds. Histopathologically, the tumor consists of nodules of basaloid cells in a cribiform or lace-like reticular pattern in a fibrous stroma with conspicuous follicular germs and papillae, and small horn cysts. Based on the histologic findings, this disorder has been thought to represent a benign hamartoma of the pilosebaceous apparatus.

Brooke-Spiegler syndrome (BSS, MIM 605041), also known as familial cylindromatosis (FC) or turban tumor syndrome, is also an autosomal dominant disease characterized by multiple neoplasms of the skin appendages such as cylindroma, trichoepithelioma, and spiradenoma. The gene for FC has been mapped to chromosome 16q12-13 by linkage analysis.1 The CYLD gene was later identified as the disease gene (MIM 605018) for FC.2

More recently, CYLD has been identified as the susceptibility gene in several families with only MFT phenotype,3–6 suggesting that mutations of CYLD gene may cause the classical phenotype of MFT without overlapping with cylindromas. The coexistence of both cylindroma and trichoepithelioma occurring in the same patient or in different members within a single family had been reported. This finding suggested that the two types of disorders may be caused by dysfunction of the same gene.7 In this report, we described a novel splicing mutation of the CYLD gene in a Taiwanese family with MFT.

CASE REPORT

The proband was a 21-year-old girl who developed numerous asymptomatic, 2-4 mm flesh-colored, dome-shaped papules with smooth shiny surface over her central face since childhood. (Fig. 1) Her mother and a younger brother also had similar but milder skin lesions. Her father and elder sister had no such tumors. The pedigree was consistent with an autosomal dominant mode of inheritance. (Fig. 2) None of the affected family members had skin tumors on the scalp.

Biopsy of a facial papule from the proband showed features of trichoepithelioma characterized by aggregates of basaloid cells within the dermis surrounded by a concentric fibrotic and slightly sclerotic stroma. In some foci, there are hints of hair bulb formation or tumor nodules with keratinous microcysts.

![Fig. 1](image1.png)

Fig. 1
The proband presents with numerous small flesh-colored dome-shaped papules on the central face.

![Fig. 2](image2.png)

Fig. 2
The pedigree of the family indicates autosomal dominant mode of transmission.
GENE ANALYSIS AND RESULT

Genomic DNA was extracted from peripheral blood of the patient and other family members with informed consent. DNA samples were then subjected to mutation screening by amplification of segments of CYLD gene using primers synthesized on the basis of intronic sequences. The PCR products were purified using a QIAquick PCR Purification Kit (Qiagen, Chatsworth, USA), followed by direct DNA sequencing with an ABI 377 automatic sequencer (Advanced Biotechnologies, Columbia, MD, U.S.A.).

A novel splicing mutation (IVS16+1G>T) in the CYLD gene was detected in the proband and her mother and younger brother. (Fig. 3A) No such mutation was found in her father and elder sister who had normal-appearing skin on the face. (Fig. 3B)

DISCUSSION

In search of MFT gene, Harada et al. (1996) initially mapped the locus for MFT to chromosome 9p21 with linkage analysis in three American MFT families. Nevertheless, the finding could not be replicated by other studies. In 2003, Hu et al reported a novel missense mutation in the CYLD gene in a family with BSS presenting predominantly with trichoepitheliomas resembling MFT phenotype. Subsequently, mutations in the CYLD gene are detected in families with MFT. To date, eight different CYLD gene mutations in MFT have been reported. (Table 1) The splicing mutation (IVS16+1G>T) found in the present study is novel. Most pathogenic inactivating mutations of CYLD gene result in truncations or frameshift alterations of the C-terminal region of the molecule. In the present study, the mutation (IVS16+1G>T) is predicted to alter the canonical splice acceptor sequence and thereby prevents proper splicing of the transcript.

The CYLD gene encodes a protein with three cytoskeletal-associated protein-glycine-conserved (CAP-GLY) domains, and two ubiquitin carboxy-terminal hydrolases (UCH) catalytic domains. UCH catalyses the hydrolysis of ubiquitin, resulting in de-ubiquitination and reduces degradation of target proteins by the proteosome. Therefore, inactivation of the CYLD gene may contribute to oncogenesis by enhancing the degradation of proteins that suppress cell proliferation or promote apoptosis.

For cellular homeostasis, proteins are inactivated by the attachment of ubiquitin moieties (the process is called ubiquitination). The ubiquitin moieties facilitate rapid cellular degradation of proteins that are important for cell-cycle progressions, including transcription factor NF-kB. Different heterodimers of NF-kB bind to specific promoters to initiate transcription of a wide range of genes that influence the inflammatory response, tissue repair, and cell death and survival. Tumor necrosis factor -a (TNF-α) pathway is implicated in inflammation and oncogenesis. Binding of TNF-α to its receptor initiates a series of events, such as the ubiquitination of TNF-receptor-associated factor 2 (TRAF-2), which in turn leads to activation of IκB kinase (IKK) complex, followed by activation of NF-κB. CYLD has been identified as a tumor suppressor gene. CYLD functions as a negative regulator of NF-κB activation in the TNF-α signalling pathway via de-ubiquitinating...
of TRAF-2, thus prevents the downstream activation of NF-κB. Loss of the de-ubiquitinating activity of CYLD correlates with tumorigenesis.

Although BSS, FC and MFT are originally described as distinct entities, they share overlapping clinical features. The results of mutation analysis further support that these disorders not only share a common genetic basis but also may represent phenotypic variation of a single entity. However, it has also been pointed out that a clear correlation between genotype and phenotype is lacking. The 3 affected family members in the present study also showed variable clinical severity despite having the same mutation.

REFERENCES

Table 1. CYLD Mutations in Patients with Multiple Familial Trichoepithelioma

<table>
<thead>
<tr>
<th>Case</th>
<th>Mutation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Amino acid change&lt;sup&gt;b&lt;/sup&gt;</th>
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<td>9</td>
<td>IVS16+1G&gt;T</td>
<td>Splicing mutation</td>
<td>Present report</td>
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<sup>a</sup>nucleotide numbers refer to CYLD cDNA sequence; <sup>b</sup>amino acid numbers refer to deduced peptide sequence.