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Germline mutation analysis in the *CYLD* gene in Chinese patients with multiple trichoepitheliomas

Z.L. Li[1,2], H.H. Guan[3], X.M. Xiao[1,2], Y. Hui[3], W.X. Jia[1,2], R.X. Yu[1,2], H. Chen[1,2] and C.R. Li[1,2]

[1]Institute of Dermatology,
Chinese Academy of Medical Sciences and Peking Union Medical College, Nanjing, China
[2]Jiangsu Key Laboratory of Molecular Biology for Skin Diseases and STIs, Chinese Academy of Medical Sciences, Nanjing, Jiangsu, China
[3]Department of Dermatology, Jinling Hospital,
Nanjing University School of Medicine, Nanjing, Jiangsu Province, China

Corresponding author: C.R. Li
E-mail: nylcr72@163.com

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ABSTRACT. Trichoepithelioma is a benign neoplasm that primarily shows follicular germinative differentiation. Classic trichoepithelioma typically presents as a skin-colored papule or nodule on the face or upper trunk; lesions have a predilection for the nose. Trichoepithelioma can be sporadic or familial and solitary or multiple. Most previously reported multiple trichoepithelioma cases are familial, and germline *CYLD* mutations could be detected in some patients. We performed mutational analysis of the germline *CYLD* gene in 8 Chinese multiple trichoepitheliomas patients, 6 of which were sporadic cases. A heterozygous missense mutation (c.1112C>A) in the 9th exon of the *CYLD* gene was detected in some mother-daughter patients. However, the germline *CYLD* mutation could not be detected in the 6 non-

familial cases. The results suggest that the pathogenesis of sporadic multiple trichoepitheliomas may differ from that of familial cases. Our findings also further confirmed the genetic heterogeneity of multiple trichoepitheliomas.

Key words: *CYLD*; Mutation; Trichoepithelioma

INTRODUCTION

Trichoepithelioma is a benign tumor originating from hair follicles. The multiple lesions are characterized by skin-colored papules and nodules located around the nose, nasolabial folds, upper lip, forehead, and occasionally on the scalp, neck, and upper trunk. Although malignant changes are rare in these lesions, cosmetic issues can affect a patient's mental health. Histopathogically, the tumor consists of nodules of basaloid cells in a fibrous stroma with papillae, small horny cysts, and conspicuous follicular germs. Trichoepithelioma occurs as either solitary or multiple and non-familial or familial cases. In general, multiple lesions are associated with genetic predisposition, whereas solitary lesions involve sporadic cases. In 2004, Zhang et al. (2004) identified the pathogenic gene responsible for multiple familial trichoepitheliomas (MFT) on chromosome 16q12-13 and identified *CYLD* as the target gene. The relationship between MFT and the *CYLD* gene was further confirmed in many other pedigrees (Zuo et al., 2007; Chen et al., 2011; Ying et al., 2012). We recruited 8 multiple trichoepitheliomas patients for the present study, including 6 sporadic cases and 2 mother-daughter relationships. We then conducted genetic analysis of the *CYLD* gene in the 8 patients to determine whether these cases were related to abnormalities in the *CYLD* gene.

MATERIAL AND METHODS

Subjects

The study included 8 Chinese patients with multiple trichoepitheliomas, 6 females aged 8-40 years, and 2 males with ages of 9 and 46 years, respectively. All 8 patients showed numerous skin-colored, dome-shaped, firm nodules and papules on the face and nose, with a size range of 2-8 mm (Figure 1 and Table 1). All patients were examined by experienced dermatologists and were diagnosed with trichoepitheliomas based on clinical features; 3 patients (patients 1, 6, and 7) were also confirmed by pathological examination.

Mutational analysis

After obtaining written informed consent from patients, blood was collected from the patients. Genomic DNA was extracted using Wizard® Genomic DNA Purification Kit (Promega; Madison, WI, USA) according to the manufacturer protocol. In addition, genomic DNA from 100 normal healthy Chinese individuals was used as control. All exons of the *CYLD* gene with intronic flanking sequences were amplified by polymerase chain reaction (PCR). Primer sequences for the 9th exon were designed as follows: forward, 5'-TCCTGTTTCCCTCCTATCT TGACT-3' and reverse, 5'-CAGCAGTACGGTTCAAACTGTGTA-3'. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen; Hilden, Germany) and sequenced

using an ABI Prism 3730 automated sequencer (Applied Biosystems; Foster City, CA, USA). Sequence comparisons and analyses were performed using Phred-Phrap-Consed version 12.0 (http://www.phrap.org).

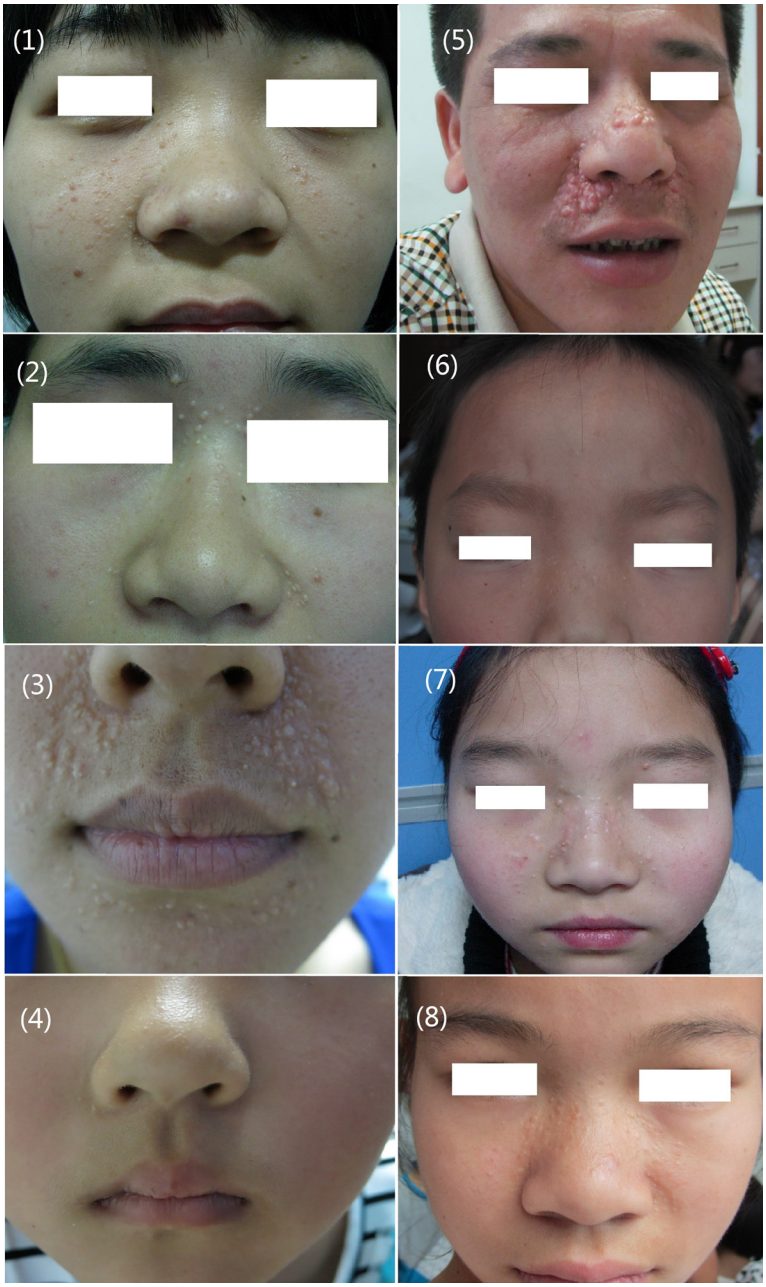

Figure 1. Multiple papules or nodules on the face and nose of the 8 patients.

Table 1. Salient clinical features and molecular biology findings in the 8 multiple trichoepithelioma patients.

Pat.	Clinical features	Gender/age	Family history	Germline *CYLD* mutations
1	Multiple papules on the face	F/30	Negative	Wt
2	Multiple papules in nasolabial fold and glabellum	F/35	Negative	Wt
3	Multiple papules on the nose	F/40	Yes	1112C>A
4	Multiple papules on the nose	F/12	Yes	1112C>A
5	Multiple papules and nodules on the nose and in nasolabial fold	M/46	Negative	Wt
6	Multiple papules on the nose	M/9	Negative	Wt
7	Multiple papules on the nose	F/13	Negative	Wt
8	Multiple papules and nodules on the nose and in nasolabial fold	F/8	Negative	Wt

Pat = patient; F = female; M = male; Wt = wild-type.

RESULTS

Sequencing analysis of the coding regions flanking the exons of the *CYLD* gene in the 6 sporadic multiple trichoepitheliomas cases and 100 controls showed no germline mutations, whereas in the mother-daughter patients, we detected a heterozygous missense mutation (c.1112C>A) in the 9th exon of the *CYLD* gene (Figure 2).

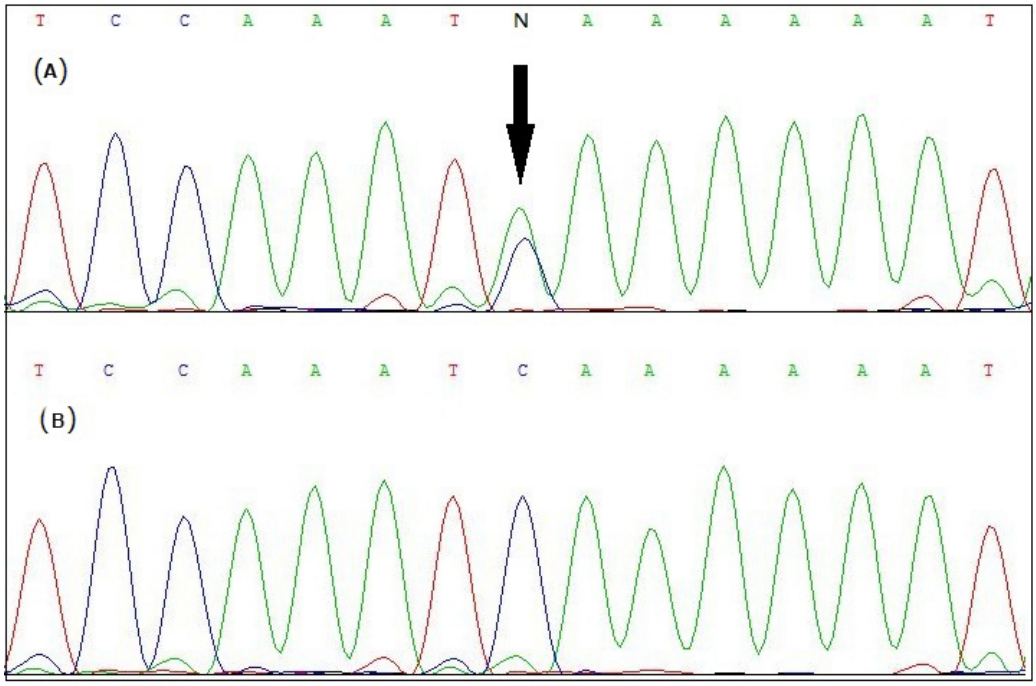

Figure 2. A. Sequencing results of exon 9 of *CYLD* from the mother-daughter patients revealed a heterozygous missense mutation (c.1112C>A). **B.** Equivalent *CYLD* genomic sequence in a normal individual.

DISCUSSION

In 1996, Harada et al. (1996) located a pathogenic gene of MFT on 9p21, but its target gene has not been identified, and there are many tumor suppressor genes in this location. In 2004, Zhang et al. (2004) found that the *CYLD* gene located on chromosome 16q12-13 was the pathogenic gene for MFT in a Chinese pedigree. The expression product of *CYLD* belongs to the deubiquitinating enzyme family and plays a pivotal role in the negative regulation of nuclear factor-κB and c-Jun N-terminal kinase signal. *CYLD* was initially identified as the target gene of Brooke-Spiegler syndrome (BSS) and cylindroma. The adnexal tumors involved in BSS include trichoepitheliomas. This suggests that uncomplicated trichoepitheliomas without other adnexal tumors represent a pole within the spectrum of BSS.

Previously reported trichoepitheliomas cases with germline mutations in *CYLD* were familial; however, the germline mutation could not be detected in all familial cases. Ponti et al. (2012) reported an MFT patient without abnormalities in *CYLD*. Saggar et al. (2008), Kazakov et al. (2011), and Grossmann et al. (2013) reported 9, 13, and 18 MFT patients, respectively, in which a germline mutation in *CYLD* was found in only 4 (44.4%), 6 (46%), and 8 (44.4%) cases, respectively. There have been few studies examining multiple trichoepitheliomas without a family history. Six of the 8 patients who participated in our study had multiple lesions, whereas germline mutations were not detected. We speculated that the onset of these patients may be related to the following factors. Some mutations may lie in non-coding regions, as was observed in a case of BSS reported by Kazakov et al. (2010) in which a deep intronic mutation was identified. Gross rearrangements and chromosomal deletions are also potential factors. van den Ouweland et al. (2011) identified a gross chromosomal rearrangement spanning approximately 5 kb involving the *CYLD* gene in a patient with familial cylindromatosis. Somatic mutation may also be important. Vorechovský et al. (1997) reported 2 cases with frameshift mutations and in-frame somatic deletions in the *CYLD* gene. Somatic mutations were detected in 2 and 3 of the 7 and 10 cases lacking germline *CYLD* mutations, respectively, as reported by Kazakov et al. (2011) and Grossmann (2013). However, equally important is that trichoepitheliomas displays genetic heterogeneity and may thus contain gene abnormalities other than those in *CYLD*, such as some *CYLD*-regulating genes that may map to the undetermined 9q21 or 9p22 regions. Other results indicate that sporadic trichoepitheliomas are not related to BSS and that the pathogenesis must be further examined (Kacerovska et al., 2013).

The c.1112C>A mutation identified in the mother-daughter patients has been reported in many other studies, indicating that this mutation is a potential hotspot. However, this mutation has not been reported in Chinese patients. Further studies are required to determine whether the c.1112C>A mutation is also a hotspot in Chinese multiple familial trichoepitheliomas patients. The c.1112C>A mutation may be related to both cylindromatosis and trichoepitheliomas, and the mechanism remains unclear regarding how the same mutation can cause different phenotypes in BSS.

In conclusion, we reported 8 cases of trichoepitheliomas, 6 of which lack a family history. Because germline *CYLD* mutations were not detected in the 6 sporadic cases, further studies must be conducted to confirm the genetic heterogeneity of the disease. The somatic mutation should be detected in tumor tissues and other possible pathogenic genes should be further explored.

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