A novel germline mutation in the *CYLD* gene in a Slovak patient with Brooke-Spiegler syndrome

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SUMMARY
The authors report a 64-year-old female with Brooke-Spiegler syndrome who presented with multiple cutaneous nodules and tumors mostly involving the scalp. Histopathological examination of one of the lesions located in a periauricular area revealed a typical cylindroma. In some neoplastic nodules ductal differentiation and occasional bilayered glands composed of the dark abluminal basal/myoepithelial cells and luminal mucinous cells might be recognized. Apocrine secretion was focally noted. Molecular biologic study of the *CYLD* gene performed from the peripheral blood identified a novel splice site c.2041+1 G>T mutation. This new germline mutation in the *CYLD* gene of a Slovak patient with Brooke-Spiegler syndrome extends the catalogue of known *CYLD* germline mutations in this condition.

Keywords: adnexal neoplasms – Brooke-Spiegler syndrome – *CYLD* – cylindroma – apocrine secretion – basal cell carcinoma

Nová zárodečná mutace v *CYLD* genu u slovenského pacienta s Brookeovým-Spieglerovým syndromem

SOUHRN
Autoři prezentují případ 64 leté ženy s Brookeovým-Spieglerovým syndromem s mnohočetnými kožními noduly a tumory lokalizovanými ve kštici. Histopatologické vyšetření projevu z periaurikulární krajiny odhalilo typický obraz cylindromu. V některých nádorových uzlech mohla být navíc detekována duktální diferenciace a příležitostně i přítomnost dvouřadých žlázek složených z tmavých bazálních/myoepitelálních buněk uložených periferně a luminálních mucinózních buněk. Místy byla detekována apokrinní sekrece. Molekulárně-biologická studie genu *CYLD* provedená z periferní krve prokázala mutaci v sestřihovém místě c.2041+1 G>T. Jedná se o zcela novou zárodečnou mutaci genu *CYLD* poprvé popsanou u slovenského pacienta s Brookeovým-Spieglerovým syndromem, která tak rozšiřuje spektrum dosud známých zárodečných mutací u tohoto onemocnění.

Klíčová slova: adnexální tumory – Brookeův-Spieglerův syndrom – *CYLD* – cylindrom – apokrinní sekrece – bazocelulární karcinom

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Brooke-Spiegler syndrome (BSS) is an inherited autosomal dominant disease characterized by the occurrence of multiple adnexal cutaneous neoplasms, including spiradenoma, cylindroma, spiradenocylindroma and trichoepithelioma (cribriform trichoblastoma) (1–7). In its phenotypic variant, multiple familial trichoepitheliomas (MFT), only trichoepitheliomas without accompanying cylindromas, spiradenomas and spiradenocylindromas are seen (8,9). Rarely, malignant tumors develop from preexisting benign cutaneous neoplasms (10–15). In addition to cutaneous lesions, the affected patients present on rare occasions with salivary gland neoplasms that are histopathologically similar to their cutaneous counterparts (10,16–21). Exceptionally rare is the occurrence of cylindroma in the breast (20,22).

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BSS/MFT is characterized by a common genetic alteration, namely mutations in the *CYLD* gene, a tumor suppressor gene located on chromosome 16q12-q13 (23–29). The *CYLD* gene contains 20 exons (the smallest being 9 bp), of which the first 3 are untranslated, and extends over approximately 56 kb of genomic DNA. Exon 3 (in the 5` untranslated region) and the 9-bp exon 7 (which is coding) show alternative splicing. *CYLD* encodes a deubiquitinating enzyme that negatively regulates the nuclear factor-kappaB and c-Jun N-terminal kinase pathways by removing lysine 63-linked polyubiquitin chains from several specific substrates. The CYLD protein contains 2 essential domains: 3 cytoskeletal-associated protein-glycine-conserved (CAP-Gly) repeats, which are found in proteins that coordinate the attachment of organelles to microtubules and one zinc-finger-like B-box motif within the ubiquitin carboxy-terminal hydrolases (UCH or USP; Ub-specific proteases) domain. In addition, CYLD contains 2 conserved proline-rich segments that can potentially mediate interactions with Src homology 3 (SH3) domains found in other proteins. It has been suggested that the CYLD protein may play a role in immunity, lipid metabolism, spermatogenesis, osteoclastogenesis, antimicrobial defense, and inflammation (25).

To date, a total of 85 distinct germline *CYLD* mutations have been reported in over 100 BSS families originating from the USA, UK, Russia, Belorussia, Ukraine, Czech Republic, France, China, Ire-

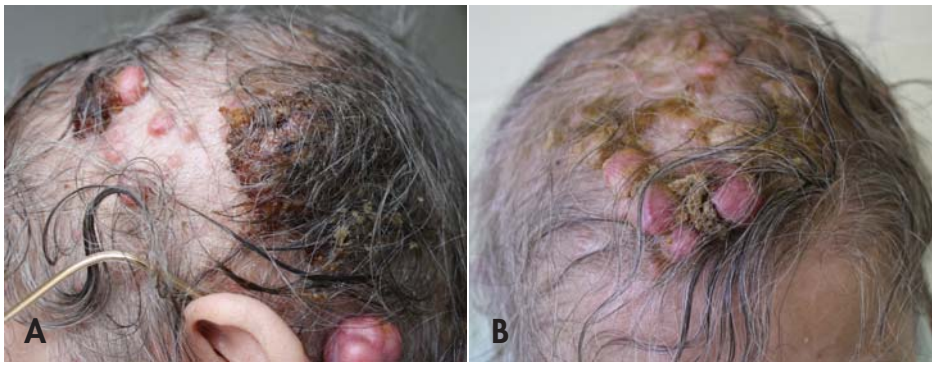

Fig. 1. Clinical findings: multiple cutaneous nodules and tumors involving the scalp **(A)**. Most neoplasms have a smooth surface but some are impetiginized and covered with yellow crusts **(B).**

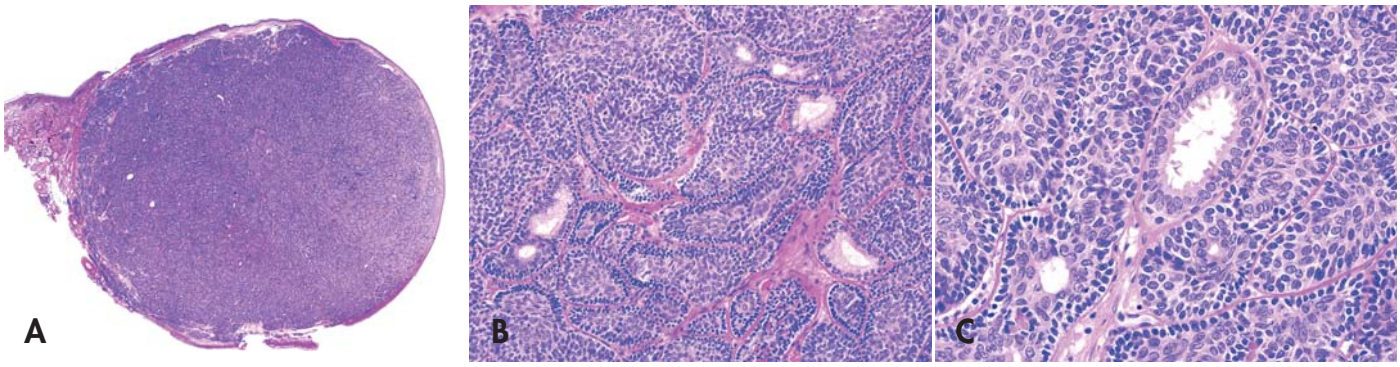

Fig. 2. Histopathologic findings: Whole–mount of the lesion revealing a typical cylindroma composed of nodules arranging in a jigsaw puzzle pattern and surrounded by variably thick eosinophilic hyalinized basement membrane material **(A)**. In some nodules ductal differentiation and occasional bilayered glands composed of the dark abluminal basal/myoepithelial cells and luminal mucinous cells could be identified **(B)**. Focally apocrine secretion was seen **(C)**. HE, 12,5x (A), 100x (B), 200x (C)

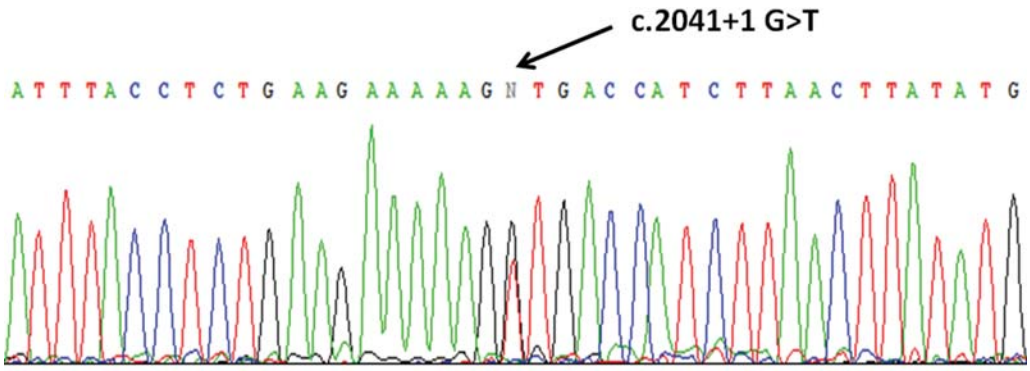

Fig. 3. Molecular biologic finding: A novel germline mutation c.2041+1 G>T in 5` canonical splice-site of intron 14 of the *CYLD* gene.

land, Spain, Germany, Austria, Hungary, Australia, Switzerland, Algeria, Turkey, Italy, and Japan (30,31). Here, we report a Slovak patient affected with BSS with a novel germline *CYLD* mutation.

CASE REPORT

The patient was a 64-year-old female who presented with multiple cutaneous nodules and tumors mostly involving the scalp (Fig. 1). The lesions ranged in size from 1 to 4 cm, were elastic on palpation, and some were situated on a short stalk. Rare lesions were seen in a periauricular area. Most neoplasms had a smooth surface but some were impetiginized and covered with yellow crusts or showing pustules atop of them. Focally, the skin between the lesions showed changes consistant with impetigo and was malodorous. Few 0.5 cm papules were detected on the trunk and extremities.

According to the patient, the lesions had been present for more than 25 years and had been prominently increasing in size and number during the last 5 years. The patient had no children and, reportedly, there was no history of similar cutaneous lesions in her family. Specifically, the patient has a sister, and both her parents died at the age of 82 years and, allegedly, none had any skin changes. The patient medical charts indicated two previous skin biopsies performed in an outside hospital 27 years earlier with the diagnoses of cylindroma and basal cell carcinoma (slides were not available for review).

MATERIAL AND METHODS

One tumor from the periauricular area was excised and submitted for pathological examination. The material was fixed in for-

malin and embedded in paraffin, and histopathological slides were prepared and stained with hematoxylin and eosin and PAS using standard protocols.

After obtaining the patient's informed consent, analysis of *CYLD* mutations was performed as described previously (32–34). Briefly, DNA was extracted from the peripheral blood using the Nucleo-Spin Tissue Kit (Macherey Nagel, Duren, Germany). Coding sequences and exon-intron junctions (exons 4 – 20) were amplified using HotStar Taq DNA polymerase (QIAgen, Hilden, Germany), PCR products were purified and then sequenced bidirectionally using the Big Dye Terminator Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA).

RESULTS

Histopathological findings

Histopathological examination revealed a typical cylindroma composed of nodules of small uniform basaloid cells located at the periphery and paler cells in the center. The nodules were arranged in a jigsaw puzzle pattern and were surrounded by variably thick eosinophilic hyalinized PAS-positive basement membrane material. Ductal differentiation was recognized in some nodules, and occasional bilayered glands composed of the dark abluminal basal/myoepithelial cells and luminal mucinous cells were seen. Apocrine secretion was focally noted (Fig. 2).

Molecular biologic findings

The sequencing of coding exons and exon-intron junctions detected a novel germline mutation c.2041+1 G>T in 5` canonical splice-site of intron 14 of the *CYLD* gene (Fig. 3).

DISCUSSION

The identified germline *CYLD* mutation c.2041+1 G>T is a novel splice-site mutation. Of the known to date germline *CYLD* mutations in patients with BSS, about 50 % are frameshift, ~25 % are nonsense, ~15 % are missense, and only ~10 % (putative) splice-site. The vast majority (over 85 %) of the mutations are predicted to result in truncated proteins. There is no hot spot but the most common sites for mutations are exon 17 (~20 %) followed by exons 10 and 16 (~10 % each) and the mutations have been almost exclusively identified in the C-terminal two-thirds of the gene (exons 9 – 20) despite the fact that exons 4 – 8 are translated. Avoidance of a dominant-negative effect that may occur with more N-terminal truncation has been suggested as a possible explanation for the lack of reported mutations in exons 4 – 8 (5) but a mutation in exon 5 has recently been described (35). Using a PCR based approach with analysis of exonic sequences and exon–intron junctions of the *CYLD* gene, germline mutations are detected in about 80 – 85 % of patients with the classical BSS phenotype and in about 40 – 50 % of the individuals with the MFT phenotype (14,34). Large deletions in *CYLD*, mutations in the intronic or within the promoter region of the *CYLD* gene are suggested mechanisms explaining an absence of a demonstrable *CYLD* sequence mutation (33,36).

An interesting pathological feature in our case is the presence of well-developed glands with apocrine secretion. These areas were less prominent compared to the previously reported cases of cylindroma and related neoplasms such as spiradenocylindroma and spiradenoma, in which glandular areas were conspicuous and formed an adenomatous or adenomyoepitheliomatous component (37–39). Noteworthy also is the history of basal cell carcinoma, which apparently has developed among the scalp lesions. Occurrence of malignant neoplasm de novo or malignant transformation of preexisting benign neoplasms is rare in patients with BSS/MFT. Basal cell carcinoma in the setting of BSS/MFT is usually seen in patients with the MFT phenotype and only rarely does it occur in the individuals with the classic BSS phenotype (2,33,40–46).

In conclusion, we have reported a new germline mutation in the *CYLD* gene of a Slovak patient with BSS which extends the catalogue of known *CYLD* germline mutations in this condition.

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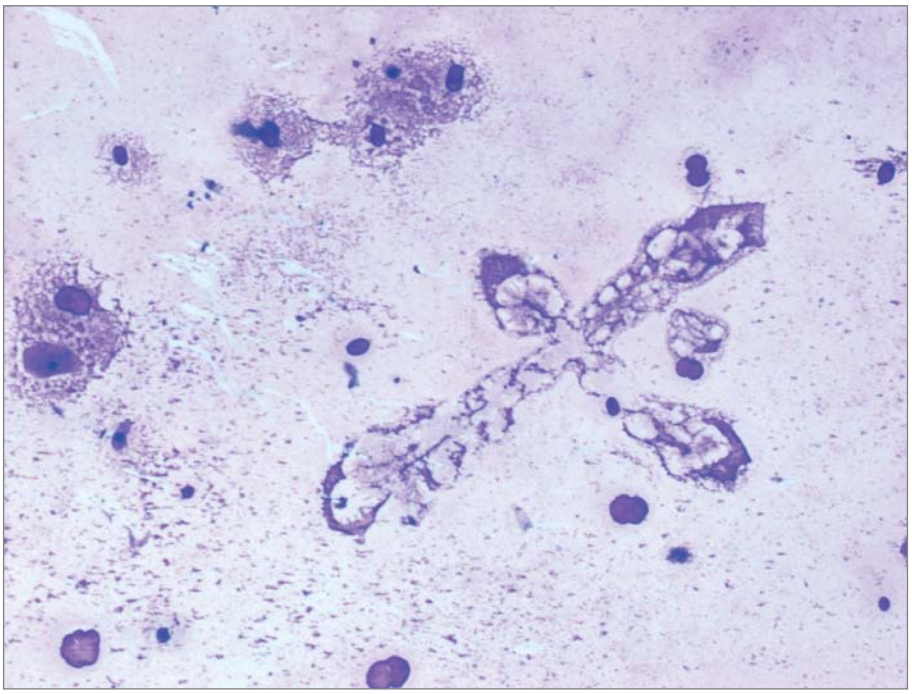

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