

A Mutational Hotspot in *CYLD* Causing Cylindromas: A Comparison of Phenotypes Arising in Different Genetic Backgrounds

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Brooke-Spiegler syndrome (BSS; OMIM 605041) has been described as an autosomal dominant disease characterized by the development of a wide variety and number of skin appendage tumours not commonly found in the general population, such as cylindromas, trichoepitheliomas and spiradenomas (1, 2). The tumours grow slowly in size and number throughout life and may give rise to a large confluent mass on the head, historically referred to as turban tumours (1, 2).

The gene responsible for BSS, the *cylindromatosis gene* (*CYLD*), is localized on 16q12-q13 (2). So far more than 79 mutations have been identified, with clustering at the 3' end of the *CYLD* gene, which encodes for the catalytic (exons 8–20) (3, 4). These mutations have been identified in patients with phenotypic features of either BSS, familial cylindromatosis (FC; OMIM 132700) or multiple familial trichoepithelioma type 1 (MFT1; OMIM 601606), suggesting that these 3 syndromes are phenotypic variations of the same genetic disease (5, 6).

A Hungarian pedigree from Bukovina (Romania) affected by BSS and an English pedigree from northern England were included in this study.

MATERIALS AND METHODS

After ethical approval from Hungarian ethics committee (ETT TUKEB and REC REF: 06/059), blood samples were taken from the affected and unaffected family members, as well as from unrelated controls for genetic analysis. Genomic DNA has been isolated by a BioRobot EZ1 DSP Workstation (Qiagen; Hilden, Germany). After the amplification of the coding regions of the *CYLD* gene and the flanking introns (primers were used as displayed on the UCSC Genome Browser www.genome.ucsc.edu), DNA sequencing was performed.

For the haplotype analysis, common polymorphisms (rs117347778, rs118122197, rs28705891, rs28654666, rs112993837, rs60077744, rs2160683, rs115042932, rs117998712, rs116979331, rs74822565, rs117713908, rs3743781, rs117537927, rs116971974, rs114552144) located in the 3' and 5' prime region of the identified mutation were genotyped using direct sequencing of the flanking coding and non-coding regions of the *CYLD* gene. Assay conditions and flanking sequences for the polymorphisms are available on request.

RESULTS

We identified 21 affected family members in the 7-generation Bukovinian family. The affected individuals had extensive skin appendage tumours, with some

demonstrating “turban tumours” from numerous cylindromas as well as trichoepitheliomas on the face (Fig. 1). The tumours appeared in early life as small nodules and progressively enlarged, and also developed on the back and on the extremities of the patients.

The Anglo-Saxon BSS pedigree from the north of England contained 8 affected family members spanning 5 generations. The affected individuals had a comparatively milder phenotype, with cylindromas and spiradenomas on the scalp and trichoepitheliomas on the face (Fig. 2).

Direct sequencing of the coding regions and the flanking introns of the *CYLD* gene revealed a heterozygous nonsense mutation (c.2806C>T, p.Arg936X) in exon 20 in affected family members of the Hungarian and Anglo-Saxon pedigrees (Fig. S1a; available from: <http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1590>). Clinically unaffected family members carried the wild-type sequence (Fig. S1b). Delineation of the surrounding common polymorphisms indicated different haplotypes in the investigated BSS pedigrees (Fig. S1c).

DISCUSSION

The identified mutation (c.2806C>T, p.Arg936X) was first reported by Bignell et al. (2) and later by Bowen et al. (7) in a European and a Canadian BSS pedigree. Haplotype analysis of the Hungarian and the Anglo-Saxon BSS pedigrees demonstrated that the same mutation carried by the 2 geographically distant pedigrees was the result of 2 independent mutational events. Previous haplotype analyses have demonstrated examples when identical *CYLD* mutations in independent BSS families were associated with the same haplotypes (c.2469+1G>A) and also when identical *CYLD* mutations in independent BSS families were associated with different haplotypes and different founder events (c.2272C>T p.Arg758X) (2). It is notable that the previously reported mutation (c.2272C>T p.Arg758X) in independent BSS pedigrees with different haplotypes is also a nonsense mutation affecting an arginine (2), as is the case for the mutation reported in the investigated Hungarian and Anglo-Saxon BSS pedigrees (c.2806C>T, p.Arg936X). We hypothesize that these positions may be mutational hotspots on the *CYLD*

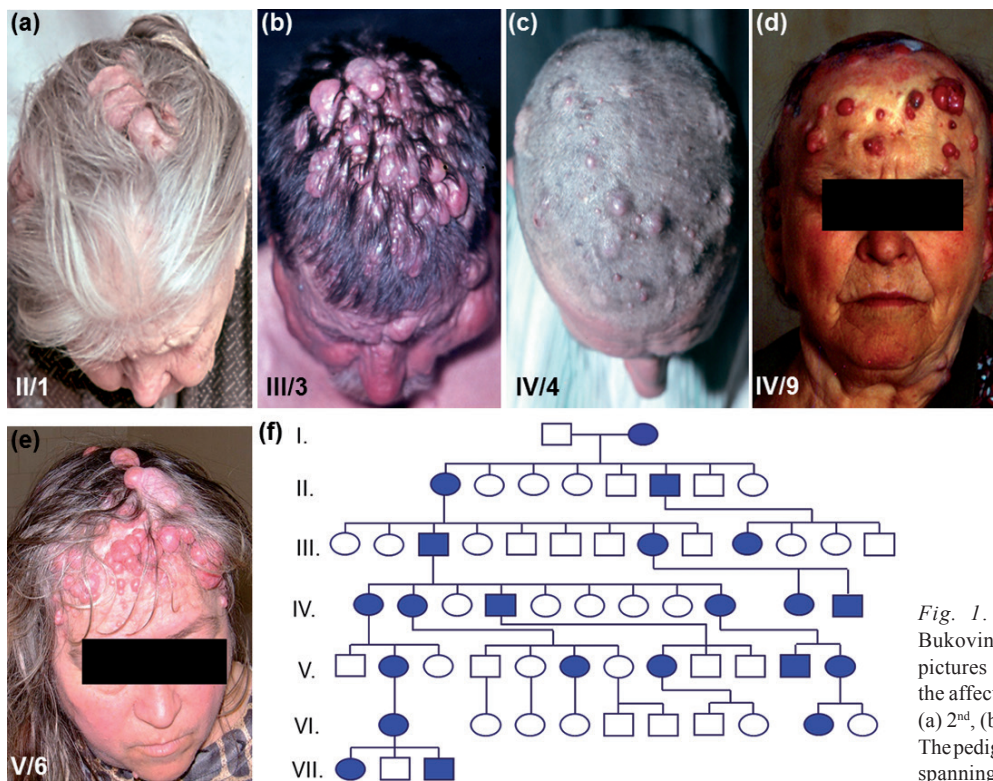


Fig. 1. The Hungarian BSS pedigree of Bukovinian (Romanian) origin. The clinical pictures of the severe hairy scalp symptoms of the affected individuals are represented from the (a) 2nd, (b) 3rd (c, d) 4th and 5th (e) generations. (f) The pedigree contains 21 affected family members spanning 7 generations.

gene. Notably, these hotspots occur in sequence of the gene that encodes the catalytic residues of *CYLD*, suggesting that a dominant negative effect may be important in manifesting a phenotype (8, 9).

There are previous studies in the literature that have also reported huge phenotypic heterogeneity even within the same BSS pedigrees (10–12). This raises the

putative role of either environmental factors or modifying genes, which influence the clinical phenotype of the BSS patients (2, 11). A recent report by Rajan et al. (12) provided the first clinical evidence that hormonally sensitive hair follicles may be predisposed to tumour formation, potentially implicating the role of hormonal factors in tumour induction in BSS patients. It is anticipated that further genetic research may highlight genetic or epigenetic variation that could predict the development of a severe phenotype.

The identification of such changes are important in the prognostic counselling of *CYLD* mutation carriers and could influence the early treatment of these patients with approaches such as kinase inhibition with TRK inhibitors to prevent the development of the disfiguring “turban tumour” phenotype (6).

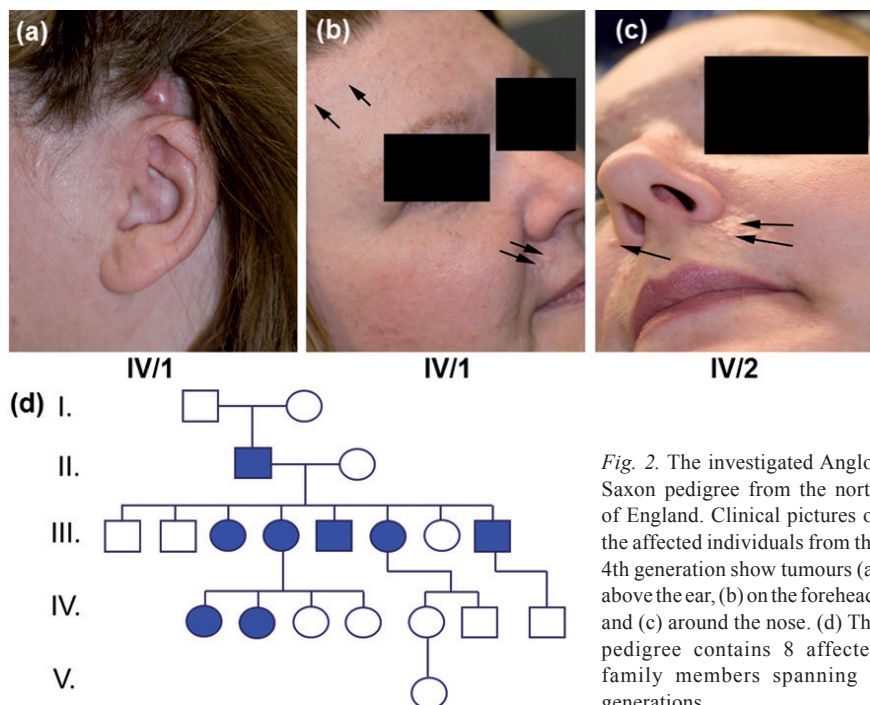


Fig. 2. The investigated Anglo-Saxon pedigree from the north of England. Clinical pictures of the affected individuals from the 4th generation show tumours (a) above the ear, (b) on the forehead, and (c) around the nose. (d) The pedigree contains 8 affected family members spanning 5 generations.

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