

## Loss of heterozygosity at cylindromatosis gene locus, CYLD, in sporadic skin adnexal tumours

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### Abstract

**Aim**—The gene for familial cylindromatosis (CYLD) has been localised to chromosome 16q, and has recently been cloned. Loss of heterozygosity (LOH) at 16q has also been demonstrated in sporadic cylindromas. The aim of this study was to investigate whether CYLD plays a role in the development of other skin appendage tumours.

**Methods**—A total of 55 cases of skin adnexal tumours, comprising 12 different types, and a control group of 14 squamous cell carcinomas (SCCs) and basal cell carcinomas (BCCs) were studied. Three microsatellites (D16S407 (16p), D16S304 (16q), and D16S308 (16q)) were analysed for LOH after microdissection from paraffin wax embedded sections using laser capture microdissection.

**Results**—In keeping with previous data, a proportion of cylindromas exhibited LOH at markers on 16q, but not at 16p. The skin adnexal tumours showing a similar pattern included apocrine hydrocystomas, eccrine spiradenomas, and sebaceous adenoma. One case of syringoma showed LOH at 16q, and a further case at 16p, but not 16q. One case of eccrine hydrocystoma showed loss at 16p, but not 16q. The remaining tumours were either negative or non-informative. All tumours in the control group were either negative or non-informative, except for a single case of BCC showing LOH at 16q.

**Conclusion**—CYLD may be involved in the development of skin adnexal tumours other than cylindromas.

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Keywords: cylindromatosis locus CYLD; loss of heterozygosity; skin adnexal tumours

The two most common skin tumours are squamous cell carcinomas (SCCs) and basal cell carcinomas (BCCs), which together account for 95% of non-melanoma skin cancers.<sup>1</sup> Although both are keratinocyte derived tumours, they have important biological and clinical differences. BCCs are typically slow growing, locally aggressive tumours that very rarely metastasise. In contrast, SCCs generally grow faster, are also locally invasive, but have metastatic potential. Genetic alterations on chromosomes 3, 9, 13, and 17 have been described in SCCs, whereas BCCs show alterations predominantly on chromosome 9.<sup>2</sup>

Adnexal skin tumours are an uncommon but related group of benign neoplasms, which differentiate towards epidermal appendages

rather than surface epidermis. They are a perplexing and difficult group of tumours, comprising different morphological types with confusing nomenclature and overlapping histological appearances. The overlap in histological features has led some authors to postulate that adnexal skin tumours represent aberrant differentiation from pluripotential basaloid cells.<sup>3</sup> Features of these neoplasms include a tendency to develop multiple tumours, especially within a familial setting, low incidence of malignant transformation, and a good prognosis with little morbidity.

The gene for familial cylindromatosis (CYLD) has been localised to chromosome 16q using linkage analysis,<sup>4</sup> and loss of the wild-type allele in tumours is consistent with the gene acting as a tumour suppressor. CYLD has recently been cloned<sup>5</sup> using standard fine mapping and a positional cloning technique. Germline mutations have been detected in families affected by cylindromatosis, and somatic mutations have been seen in both sporadic and familial cylindromas. Loss of heterozygosity (LOH) at this site has also been demonstrated in a high proportion of sporadic cylindromas and it seems to be the only important site of genetic alteration.<sup>6</sup> Studies on skin adnexal tumours other than cylindromas are sparse, and few genetic alterations have been demonstrated in these lesions.<sup>2</sup> We hypothesised that the gene for cylindromatosis might play a role in the development of all skin appendage tumours, and have therefore investigated LOH on chromosome 16 in a range of such tumours.

### Materials and methods

A total of 55 cases of skin adnexal tumours were obtained from the histopathology archives of the Royal Victoria Infirmary, Newcastle Upon Tyne Hospitals NHS Trust. All cases were identified and reviewed by an experienced histopathologist (NL) with an interest in dermatopathology. Formalin fixed, paraffin wax embedded tissues comprising cylindroma (four), trichoepithelioma (two), apocrine hydrocystoma (four), syringoma (seven), eccrine spiradenoma (seven), sebaceous adenoma (four), eccrine hydrocystoma (five), trichofolliculoma (eight), eccrine poroma (eight), trichilemmoma (four), eccrine carcinoma (one), and desmoplastic trichoepithelioma (one) were obtained. Five SCCs and nine BCCs were also studied because 16q LOH is not a feature of these tumours.

Tumour cells and normal tissue (overlying normal epidermis, lymphoid aggregates, and

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inflammatory infiltrates) were obtained using laser capture microdissection<sup>7</sup> (PixCell II; Arcuturus, Mountain View, California, USA) from 5 µm sections stained with haematoxylin and eosin. Tissue was pooled from up to five serial sections depending on the size of the lesion. DNA was extracted by standard protocols in a total volume of 15–20 µl using proteinase K, as described previously.<sup>8</sup>

Three microsatellite markers—D16S407 (16p) (forward 5'-CTCGCGCTGGGTACAG TTAT-3', reverse 5'-AGATCAGAGGAGTG GGTTC-3'), D16S304 (16q) (forward 5'-GTCAGTGCAATGGAGGTAAGAAAAA G-3', reverse 5'-GATCAGATGAGATAGGG CAT ATTCATGG-3'), and D16S308 (16q) (forward 5'-CAGCCAGGGTAGTAAGGCT AGACCT-3', reverse 5'-TGGGTGGCAGAG TGAGACCCTGTCT-3')—were used in our study. The 16q markers were chosen because these lie close to the region of the recently cloned CYLD gene.<sup>5</sup> The 16p marker was used as a negative control. The forward strand of each primer pair was fluorescently labelled using either the FAM or TET dye (Oswel, Southampton, UK and Genosys, Cambridge, UK, respectively). The polymerase chain reaction (PCR) was performed in a total reaction volume of 12.5 µl. The PCR mixture contained 1× PCR buffer (Gibco BRL Paisley, UK); 200µM each dNTP; 1.25 pmol (D16S407), 0.75 pmol (D16S304), or 0.63 pmol (D16S308) of each primer; 1.5mM MgCl<sub>2</sub> (D16S407 and D16S304) or 1.0mM MgCl<sub>2</sub> (D16S308); 0.25 U of platinum Taq DNA polymerase (Gibco BRL); and 2 µl (from total extraction volume of 15–20 µl) of DNA template. Amplification was carried out for 40 cycles at 94°C for one minute, 55°C for one minute, and 72°C for one minute, with a final extension step at 72°C for 10 minutes.

The products were analysed for LOH on an ABI 373A automated fluorescent DNA sequencer (Applied Biosystems Warrington, UK) using Genescan analysis software (version 2.1) (Applied Biosystems) and imported into Genotyper (version 1.1) (Applied Biosystems). The data were assessed by comparing tumour to normal allele intensity ratios, and a value of ≤ 0.5 was assigned as indicative of LOH.<sup>8</sup>

Table 1 Loss of heterozygosity (LOH) in skin adnexal tumours

Type (no.)	16p (D16s407)	16q (D16s308)	16q (D16s304)	Overall LOH at 16q
Cylindroma (4)	0/4 (0%)	2/4 (50%)*	2/3 (67%)*	75%
Trichoepithelioma (2)	0/1 (0%)	NI	1/2 (50%)	50%
Apocrine hydrocystoma (4)	0/3 (0%)	0/3 (0%)	1/3 (33%)	33%
Eccrine spiradenoma (7)	0/5 (0%)	0/5 (0%)	1/5 (20%)	20%
Sebaceous adenoma (4)	0/2 (0%)	1/3 (33%)	1/3 (33%)	66%
Syringoma (7)	1/5 (20%)	0/3 (0%)	1/5 (20%)	20%
Eccrine hydrocystoma (5)	1/4 (25%)	0/5 (0%)	0/4 (0%)	0%
Trichofolliculoma (8)	0/6 (0%)	0/8 (0%)	0/5 (0%)	0%
Eccrine poroma (8)	0/6 (0%)	0/7 (0%)	0/7 (0%)	0%
Trichilemmoma (4)	0/3 (0%)	0/3 (0%)	0/1 (0%)	0%
Eccrine carcinoma (1)	NI	0/1 (0%)	0/1 (0%)	0%
Desmoplastic trichoepithelioma (1)	0/1 (0%)	NI	0/1 (0%)	0%
Basal cell carcinoma (9)	0/5 (0%)	1/8 (13%)	0/5 (0%)	13%
Squamous cell carcinoma (5)	0/3 (0%)	0/4 (0%)	0/3 (0%)	0%

The data are presented as number of cases exhibiting LOH/number informative for that marker (%).

\*One case showed LOH at both 16q markers.

NI, not informative; no, number of cases studied.

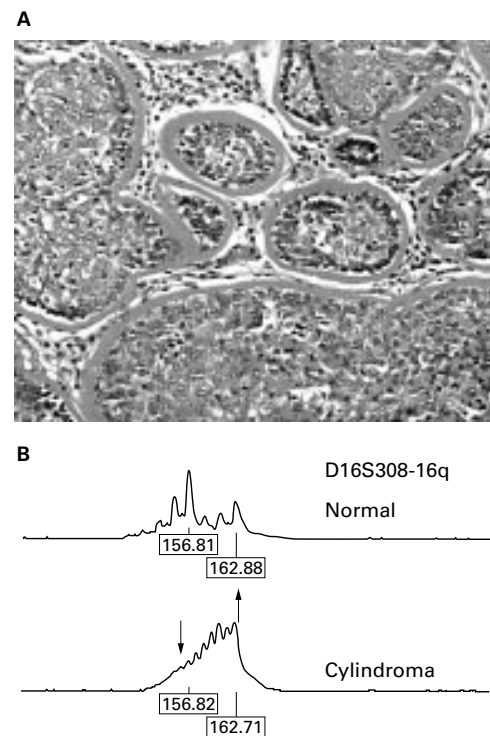


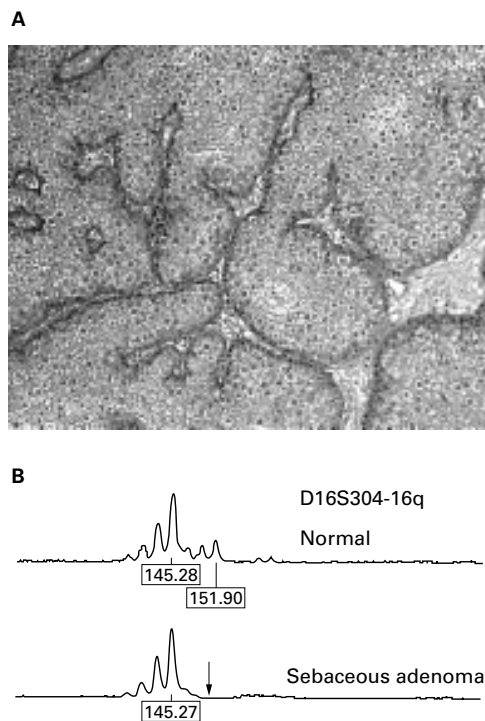
Figure 1 (A) Characteristic morphology of cylindroma, showing lobules of basophilic epithelial cells, surrounded by thick hyaline membranes (haematoxylin and eosin stain; original magnification, ×200). (B) Loss of heterozygosity at 16q (D16S308). There is an imbalance at the 156 bp allele (allele ratio of 0.2) compared with the normal DNA.

## Results

Fifty five skin adnexal and 14 keratinocyte derived tumours (BCCs, SCCs) were analysed for LOH on chromosome 16 using three microsatellite markers. Table 1 summarises the data. In keeping with previous data, three of the four cylindromas exhibited LOH at one or other of the 16q markers (D16S308 or D16S304), but not at 16p (D16s407) (fig 1). The skin adnexal tumours showing a similar pattern (LOH at 16q but not at 16p) included one of two trichoepitheliomas, one of three apocrine hydrocystomas, one of five eccrine spiradenomas, and two of three sebaceous adenomas (fig 2). One of five syringomas exhibited LOH at 16q, and one further case showed LOH at 16p, but not 16q. One case of eccrine hydrocystoma also showed loss at 16p, but not 16q. All cases of trichofolliculoma, eccrine poroma, trichilemmoma, eccrine carcinoma, and desmoplastic trichoepithelioma were either non-informative or negative at the markers studied. One of eight informative cases of BCC showed LOH at 16q (D16S308), but not at the second 16q marker or at 16p. All five SCCs were either negative or non-informative for all markers.

## Discussion

Adnexal skin tumours are an uncommon but related group of neoplasms, which differentiate towards epidermal appendages rather than surface epidermis. They can differentiate towards several different cell lineages, namely hair follicles, sebaceous glands, apocrine glands, and eccrine glands. Individual tumours



**Figure 2** (A) Histology of sebaceous adenoma (haematoxylin and eosin stain; original magnification,  $\times 200$ ). (B) Loss of heterozygosity at 16q (D16S304). There is a loss of the 151 bp allele (allele ratio of 0).

can also exhibit more than one type of differentiation, further complicating the nomenclature. Other features in common include a tendency for the development of multiple tumours and the occurrence of familial syndromes.<sup>4-9</sup>

Hereditary cylindromatosis is an autosomal dominant disease characterised by the development of multiple skin cylindromas, a type of adnexal tumour. Occasionally, other skin adnexal tumours, such as trichoepithelioma and spiradenoma have been reported.<sup>10-11</sup> The gene responsible was localised in 1995 to chromosome 16q12–q13.<sup>4</sup> Consistent loss of the wild-type allele was observed, indicating that the gene is probably a tumour suppressor. Unlike familial cylindromatosis, sporadic cylindromas occur later in life and are less likely to be multiple. LOH at 16q has also been demonstrated in sporadic cylindromas,<sup>6</sup> and it appears that it might be the only tumour suppressor gene involved in the syndrome. Recently, the familial cylindromatosis gene, *CYLD*, has been identified and cloned by detecting 21 different germline mutations in families affected by cylindromatosis, and six somatic mutations from both familial and sporadic cylindromas.<sup>5</sup> The gene for multiple familial trichoepithelioma has been mapped to chromosome 9p21.<sup>12</sup> Clearly, it would be interesting to investigate whether trichoepitheliomas arising within the setting of familial cylindromatosis show alterations at this locus.

In our study, we have shown that LOH at 16q is present in several morphologically distinct skin adnexal tumours, including cylindromas, sebaceous adenomas, trichoepitheliomas, apocrine hydrocystomas, syringomas, and

eccrine spiradenomas. The finding of LOH in these lesions showing diverse adnexal differentiation provides tentative evidence for their origin from a common pluripotential cell. Furthermore, the data provide support for the hypothesis that *CYLD* plays a role in the development of at least a proportion of skin adnexal tumours. Further support for this hypothesis comes from a report of 16q22 loss in an eccrine spiradenoma,<sup>13</sup> although this was an unusual case with lymph node metastases (but not distant metastases).

Takata and co-workers<sup>2</sup> have examined a range of adnexal tumours for LOH at multiple loci, but did not investigate chromosome 16q. The overall frequency of LOH in their series was 1.6% (four of 247 informative loci). Examples include LOH at 17q in sebaceous epithelioma and eccrine porocarcinoma, and LOH at 9q in trichoepithelioma. These alterations in skin adnexal tumours were different to those identified in BCCs and SCCs.

The only other important data in adnexal tumours has been in p53 expression. p53 accumulation was found in 10 of 14 sweat gland carcinomas and three of 60 sweat gland adenomas.<sup>14</sup> p53 expression has been documented in spiradenomas, but this is only in the malignant portions of malignant spiradenomas.<sup>15</sup> The benign components of the lesion remain negative. Malignant sweat gland tumours have a poor prognosis and the finding of abnormal p53 protein expression might reflect increasing genetic instability with accumulation of chromosome damage.

In Muir Torre and Cowden's syndromes, multiple skin adnexal tumours are associated with underlying malignancy—colonic cancer in Muir Torre syndrome, breast and thyroid carcinomas in Cowden's syndrome.<sup>16-17</sup> Cylindromatosis does not have such systemic manifestations, and defects in mismatch repair enzymes (Muir Torre syndrome) or mutations in PTEN (Cowden's syndrome) are not likely to play an important role in this disease.

Pilomatricomas are skin tumours of unknown origin. A recent paper<sup>18</sup> suggests that they arise from hair matrix cells and that many have mutations in the  $\beta$ -catenin gene. The role of  $\beta$ -catenin in skin adnexal tumours is unknown.

SCCs are easy to distinguish from adnexal tumours at a morphological and molecular level. Compared with BCCs and skin adnexal tumours, they exhibit LOH at multiple sites including 3p, 9p, 9q, 13, and 17p.<sup>19</sup> A relation between the accumulation of genetic abnormalities and the clinical behaviour of a neoplasm has been suggested.<sup>20</sup> This might account for the relatively more aggressive behaviour and metastatic potential of SCCs.

In our series, a single case of BCC showed LOH at one marker on 16q. This case has been re-reviewed in light of these data. It is still felt to be a BCC, although there are some unusual features in the form of organoid growth pattern and focal adnexal differentiation. There is no evidence in the literature to suggest that 16q alterations play a role in the development of

BCCs. Molecular genetic analysis has demonstrated mutation at 9q22–31 in 70% of sporadic BCCs.<sup>21 22</sup> This mutation is not found in adnexal tumours.<sup>2</sup> Whether the 16q LOH is a pathogenetic alteration or a non-specific random deletion is unclear in this case. Most of the data in the literature suggest that the evolution of BCCs and skin adnexal tumours occurs via different genetic pathways.

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