Phenotype Diversity in Familial Cylindromatosis: A Frameshift Mutation in the Tumor Suppressor Gene CYLD Underlies Different Tumors of Skin Appendages

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Familial cylindromatosis (turban tumor syndrome; Brooke–Spiegler syndrome) (OMIM numbers 123850, 132700, 313100, and 605041) is a rare autosomal dominantly inherited tumor syndrome. The disorder can present with cutaneous adnexal tumors such as cylindromas, trichoepitheliomas, and spiradenomas, and tumors preferably develop in hairy areas of the body such as head and neck. In affected families, mutations have been demonstrated in the CYLD gene located on chromosome 16q12–13 and reveal the characteristic attributes of a tumor suppressor. Here, we studied familial cylindromatosis in a multigeneration family of German origin. Clinically, some individuals only revealed discrete small skin-colored tumors localized in the nasolabial region whereas one family member showed expansion of multiple big tumors on the trunk and in a turban-like fashion on the scalp. Histologically, cylindromas as well as epithelioma adenoides cysticum were found. We detected a frameshift mutation in the CYLD gene, designated 2253delG, underlying the disorder and were able to show that a single mutation can result in distinct clinical and histologic expression in familial cylindromatosis. The reasons for different expression patterns of the same genetic defect in this disease remain elusive, however.

Identification of mutations in the CYLD gene enables us to rapidly confirm putative diagnoses on the genetic level and to provide affected families with genetic counseling. Key words: adnexal and skin appendage neoplasms/apocrine glands/DNA mutational analysis/ eccrine glands. J Invest Dermatol 119:527–531, 2002

In 1892 and 1899, respectively, Brooke and Spiegler for the first time described the familial occurrence of multiple tumors of skin appendages, a disease nowadays referred to as familial cylindromatosis, turban tumor syndrome, or Brooke–Spiegler syndrome (OMIM numbers 123850, 132700, 313100, and 605041) (Brooke, 1892; Spiegler, 1899).

This uncommon tumor syndrome is observed twice as often in females as in male individuals (Knoth, 1978). Usually in the first or second decade of life, dermal papules and nodules arise that are mainly localized in hairy areas of the body with approximately 90% on the head and neck, rarely on the face or trunk. The tumors are pink to red in color and their consistency ranges from smooth to firm, thus sometimes mimicking neurofibromas or hypertrophic scars. They can increase in number as well as size and result in marked disfiguration. Malignant transformation, primarily to basal cell epitheliomas, has been described but seems to be rare (Gerretsen et al., 1993, 1995; Sybert, 1997).

Cylindromas show a typical histopathology. In the dermis, mosaic-like masses of epithelial cells are surrounded and separated from each other by thin layers of periodic acid–Schiff (PAS) positive stroma. Two cell types can be distinguished by electron microscopy: on the one hand large cells with a moderate amount of cytoplasm and a vesicular nucleus, and on the other hand small cells with little cytoplasm and a compact nucleus (Reynes et al., 1976; Weber et al., 1984).

To date, the cell-type-specific origin of familial cylindromas is a topic of ongoing debate. Whereas some investigators believe in an eccrine origin, others were able to show apocrine derivatives or argue for pilar precursors (Reynes et al., 1976; Michal et al., 1999).

Familial cylindromatosis is inherited in an autosomal dominant fashion with apparently complete penetrance but variable expression. Recently, linkage of familial cylindromatosis with a locus on chromosome 16q12–13 was demonstrated (Biggs et al., 1995; Takahashi et al., 2000) and several groups were able to show loss of heterozygosity at 16q in sporadic cylindromas as well as familial cylindromatosis (Biggs et al., 1995; Thomson et al., 1999; Leonard et al., 2001). In 2000, Bignell and colleagues reported the first mutations in the CYLD gene causing familial cylindromatosis (Bignell et al., 2000). The gene consists of 20 exons and obviously functions as a tumor suppressor (Bignell et al., 2000).

There is increasing evidence that familial cylindromatosis is a clinically, genetically, and histologically heterogeneous disorder as the simultaneous occurrence of cylindromas and other tumors of skin appendages such as trichoepitheliomas and spiradenomas within affected individuals and families is well documented (Reynes et al., 1976; Schirren et al., 1995; Puig et al., 1998; Michal et al., 1999). To date, however, it has not been demonstrated on the molecular level that these different tumors can indeed arise from the same mutation in one single gene.
Here, we studied a four-generation family of German origin by polymerase chain reaction (PCR), reverse transcriptase (R.T)-PCR, heteroduplex analysis, automated sequencing, and haplotype analysis. Histologic examination of various tumors from affected family members revealed cylindromas as well as multiple trichoepitheliomas, so-called epithelioma adenoides cysticum. Interestingly, however, mutation analysis performed on leukocyte DNA as well as tumor DNA and RNA revealed only one molecular defect in the CYLD gene, designated 2253delG. These molecular genetic studies for the first time confirm the previous clinical notion that one single mutation in a recently identified tumor suppressor gene underlies phenotypically and histologically distinct tumors of skin appendages.

MATERIALS AND METHODS

Family and control individuals We studied seven individuals from a four-generation family of German origin (Fig 1) and 100 unrelated, unaffected control individuals. In four family members, the diagnosis of familial cylindromatosis was made by typical clinical and histopathologic findings.

Clinical material and DNA/RNA extraction Ethylenediamine tetraacetic acid (EDTA) anticoagulated blood samples were drawn from family members and controls, after informed consent for inclusion in the study, in accordance with guidelines set forth by the local institutional review board. Blood samples could not be obtained from individuals I-1 and II-1 (Fig 1) because they were already deceased. From individual I-1 (Fig 2), however, paraffin-embedded tumor tissue was available. Thus, we isolated DNA from skin specimens originally obtained for histologic examination after excision biopsy.

Further, DNA and RNA was isolated from tumor material of individuals III-2 and IV-1 (Figs 3, 4) directly after excision. In total, we isolated and sequenced DNA samples obtained from two paraffin-embedded skin specimens of individual I-1, one located on the nasolabial region and the other on the scalp. Both skin specimens revealed the histologic characteristics of cylindromas. From individual III-2 we obtained three skin samples, all of them taken from the
nasolabial region and demonstrating cylindromas upon histologic examination. From one of these specimens we isolated DNA and RNA, which was subjected to automated sequencing analysis. A total of three skin biopsies were taken from individual IV-1, one from the nasolabial region, one from the back of the nose, and one from the scalp. The specimen obtained from the nasolabial region showed the histologic attributes of cylindroma; the two other samples revealed epithelioma adenoides cysticum. DNA and RNA was isolated from all three skin samples and subjected to sequencing analysis.

Isolation of genomic DNA from blood specimens was performed according to standard techniques (Sambrook et al., 1989). For extraction of DNA and RNA from freshly obtained skin biopsies (individuals III-2 and IV-1; Fig 1) we used PUREGENE DNA purification kits and PURESCRIPT total RNA purification kits from Gentra (Gentra Systems, Minneapolis, MN) following the manufacturer’s instructions. For DNA extraction from paraffin-embedded skin specimens these samples were first de-paraffinized. Subsequently, DNA and RNA were isolated as aforementioned.

PCR, RT-PCR, and mutation detection. PCR was performed on the basis of DNA extracted from EDTA blood where available (individuals III-1, III-2, III-3, IV-1, and IV-2) or from tumor specimens (individual I-1). Amplification of all coding exons of the CYLD gene and the adjacent splice sites was carried out using PCR primers that were either reported previously (Bignell et al., 2000) or specifically designed by us for this study because the originally published primer pairs did not yield satisfactory amplification results in our hands. In detail, we designed new primer pairs for exons 4 and 14 of the CYLD gene. Primer sequences were CYLD 4bF 5¢-GTCACTTCAACCTACTGG-3¢, CYLD 4bR 5¢-AGCAATTCAACTCCAAAAG-3¢, CYLD 14F 5¢-TCCAGCCTGAGTGATAGAGT-3¢, and CYLD 14R 5¢-ACA-AAAAGAGGAGCTAACCA-3¢. PCR was carried out according to the following program: initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 45 s, annealing at primer-specific temperatures for 1 min (Bignell et al., 2000), and extension at 72°C for 1 min and 15 s; followed by a final extension at 72°C for 10 min, in a Biometra TGradient thermal cycler (Whatman Biometra, Göttingen, Germany). Each amplification reaction contained 100 ng of genomic DNA, 50 ng per μl of each forward and reverse primer, and 45 μl of Platinum Taq PCR Super Mix (Invitrogen Life Technologies, Karlsruhe, Germany), in a total volume of 50 μl.

For mutation detection, PCR products were subjected to conformation sensitive gel electrophoresis (CSGE) analysis as described previously (Ganguly et al., 1993). PCR products displaying a heteroduplex on CSGE analysis were purified in a first step, using the High Pure PCR product purification kit (Roche, Basel, Switzerland). In a second step, PCR fragments were purified on Edge Centriflex columns (Edge BioSystems, Gaithersburg, MD) and sequenced directly with POP-6 polymer using an ABI Prism 310 Genetic Analyzer from Applied Biosystems (Foster City, CA).

To verify the frameshift mutation in exon 17 identified in this study, a combination of heteroduplex analysis and automated sequencing was performed. We studied 200 chromosomes for the presence of this mutation (data not shown) and thereby excluded it as a common polymorphism.

To further study tumor tissue on the RNA level, total RNA extracted from skin specimens was reverse transcribed into cDNA using ThermoScript RNase H-Reverse Transcriptase (Invitrogen Life Technologies, Karlsruhe, Germany) according to the manufacturer’s instructions. Amplification of a cDNA fragment containing exons 16±18 of the CYLD gene was performed following the aforementioned PCR protocol using 10 μl of cDNA, and primers RT-CYLD 16F 5¢-GT-TTCATCATATTTTTAAAGGG-3¢ and RT-CYLD 18R 5¢-TATTTTAT-GATTCAGGCTCTTT-3¢. RT-PCR products were subcloned using the TOPO TA cloning kit (Invitrogen Life Technologies, Karlsruhe, Germany) according to the manufacturer’s instructions. Direct sequencing of the cloned fragments into the forward direction was performed using the M13 forward primer provided with the kit.

Haplotype analysis. To identify a common haplotype segregating with the disease, thereby possibly ruling out unaffected family members, microsatellite typing was performed. The microsatellite markers used were D16S517, D16S411, and D16S416, flanking the CYLD gene on...
obtained from blood samples and from all tumor specimens and was not detectable on the RNA level by cDNA sequencing.

RESULTS

Histology Skin specimens obtained from the nasolabial region and scalp of individual I-1 (Fig 2) revealed the typical histologic features of cylindromas with numerous isolated islands of epithelial cells that differed in form and size and were surrounded and separated from each other by hyaline material that showed a PAS positive staining (Fig 5).

The same histopathologic results were found upon microscopic examination of tumor material from her granddaughter (Fig 3). In contrast, however, skin biopsies taken from the nasolabial region, the dorsum of the nose, and the scalp of her great-grandson (Fig 4) not only showed cylindromas but also revealed the typical histologic characteristics of epithelioma adenoides cysticum.

Here, well-demarcated nodules could be seen consisting of small basoloid cells that were arranged in a palisade-like manner at the margins (Fig 6).

Haplotype analysis The pedigree of the nuclear family and the haplotypes obtained with the aforementioned microsatellite markers are depicted in Fig 1. The results indicate that these markers were not informative and sufficient to point out a common haplotype distinguishing unaffected and affected individuals in this family, and in the case of individual III-3 they were even misleading. Individual IV-2 could already be excluded as a mutation carrier, however.

Mutation analysis Heteroduplex analysis of the PCR fragment containing exon 17 of the CYLD gene revealed a complex heteroduplex formation in individuals I-1, III-2, and IV-1 (Fig 7, top panel). This heteroduplex formation was found in DNA obtained from blood samples and from all tumor specimens, and was also used to prove the absence of this mutation in 100 unrelated control individuals (data not shown). Interestingly, direct sequencing of subcloned RT-PCR products encompassing exons 16–18 of the CYLD gene did not reveal any sequence deviation in affected individuals. Thus, the mutation was not detectable on the RNA level by cDNA sequencing.

DISCUSSION

In this study, we identified a frameshift mutation in the CYLD gene underlying multiple tumors of skin appendages in a German four-generation family. For the first time we were able to demonstrate on the molecular level that a hitherto not described mutation in CYLD can result in phenotypically and histologically distinct tumors, thereby confirming earlier clinical and histologic observations.

Cylindromas have been reported to occur either in a familiar or in a sporadic fashion. In a recent report, mutations in CYLD, a gene with the characteristic features of a tumor suppressor, were shown to cause familial cylindromatosis. In all studied families, disease penetrance was apparently complete. It was further demonstrated that CYLD mutations could be found even in sporadic cylindromas (Bignell et al, 2000). Thus, these sporadic forms most probably arise due to de novo mutations, either in the germ line or somatically.

In previous clinical reports, the simultaneous occurrence of cylindromas and other tumors of skin appendages such as trichoepitheliomas and spiradenomas has been described in familial cylindromatosis (Weyers et al, 1993; Schirren et al, 1995; Puig et al, 1998). To date, the nonsyndromic Mendelian transmission of isolated spiradenomas has not been reported to our knowledge. There are several reports, however, that indicate that a familial type of trichoepithelioma (OMIM 601606) exists in which trichoepitheliomas occur exclusively without other accompanying adnexal skin tumors. This disease was previously linked to chromosome 9p21. It is not allelic to familial cylindromatosis and therefore has to be distinguished as a unique entity (Harada et al, 1996). Interestingly, a variable clinical and histologic expression was also observed in our study with different members of the same family presenting with cylindromas either exclusively or in combination with epithelioma adenoides cysticum. Subsequently, we were able to demonstrate on the molecular level by mutation analysis performed on leukocyte DNA as well as tumor DNA and RNA that the identical mutation in one single gene can give rise to the
development of clinically and histologically distinct tumors in a family suffering from familial cylindromatosis.

The frameshift mutation identified in this study consists of a one basepair deletion at position 2253 of the CYLD cDNA, designated 2253delG (Fig 5, bottom lane), that has not been described previously. It leads to a premature termination codon 28 nucleotides downstream of the deletion site. As we were not able to detect the mutated allele upon RT-PCR studies performed on RNA extracted from cylindromas as well as trichoepitheliomas of our patients, this premature termination codon will most probably perturb CYLD protein function through nonsense mediated messenger RNA decay and might act by haplo-insufficiency (Hentze and Kulozik, 1999). It is also, however, possible that the mutation might exert a dominant negative effect as CYLD is believed to be a cytoskeletal protein.

Mutational changes in the CYLD gene could affect the normal regulation of apocrine or eccrine stem cell populations, which then give rise to the growth of distinct tumor types because the physiologic function of the encoded protein is disabled. Thus, CYLD may play a crucial role in governing cell fate decisions. This hypothesis by itself, however, cannot explain the variable clinical expression observed here in three affected family members carrying the mutation 2253delG. It is therefore possible that either modifying genes or other hitherto unknown environmental factors might have an important influence on the distinct differentiation patterns of tumor cells encountered in familial cylindromatosis. This notion is also supported by the previous report of Bignell and colleagues (Bignell et al, 2000).

In affected families, the identification of mutations in the CYLD gene has greatly enhanced our understanding of familial cylindromatosis. Once a mutation has been identified in a patient suffering from this disorder, the detection of questionable mutation carriers with a less severe clinical phenotype is easily accomplished using molecular biologic techniques. Polymorphic microsatellite markers flanking the CYLD locus on chromosome 16q12-13 and the locus for familial trichoepitheliomas on chromosome 9p21 will facilitate clear assignment in those patients with difficult clinical differential diagnosis and presenting a histologic diagnosis of trichoepithelioma and/or epithelioma adenoide cysticum. The earlier a diagnosis on the basis of genetic analyses is made, the higher the chance of providing affected individuals with regular examinations and small surgical interventions before the full picture of devastating turban-tumor-like growth can develop, which not only disables those patients physically but can also lead to psychologic stress. In our study, genetic analysis allowed exclusion of the tumor predisposition in a 6-y-old boy (individual IV-2 in Fig 1) and in another family member (individual III-3 in Fig 1) who, being afraid of suffering from the disease, had already undergone surgical excision of some skin lesions on her head and neck in the past.

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