Mosaicism of activating FGFR3 mutations in human skin causes epidermal nevi

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Epidermal nevi are common congenital skin lesions with an incidence of 1 in 1,000 people; however, their genetic basis remains elusive. Germline mutations of the FGF receptor 3 (FGFR3) cause autosomal dominant skeletal disorders such as achondroplasia and thanatophoric dysplasia, which can be associated with acanthosis nigricans of the skin. Acanthosis nigricans and common epidermal nevi of the nonorganoid, nonepidermolytic type share some clinical and histological features. We used a SNAPSHOT multiplex assay to screen 39 epidermal nevi of this type of 33 patients for 11 activating FGFR3 point mutations. In addition, exon 19 of FGFR3 was directly sequenced. We identified activating FGFR3 mutations, almost exclusively at codon 248 (R248C), in 11 of 33 (33%) patients with nonorganoid, nonepidermolytic epidermal nevi. In 4 of these cases, samples from adjacent histologically normal skin could be analyzed, and FGFR3 mutations were found to be absent. Our results suggest that a large proportion of epidermal nevi are caused by a mosaicism of activating FGFR3 mutations in the human epidermis, secondary to a postzygotic mutation in early embryonic development. The R248C mutation appears to be a hot spot for FGFR3 mutations in epidermal nevi.

Introduction

Epidermal nevi show a prevalence of about 1 in 1,000 people and can be divided into either nonorganoid (keratinocytic) types or organoid types characterized by hyperplasia of adnexal structures such as sebaceous glands, sweat glands, and hair follicles. Epidermal nevi of the common, nonorganoid and nonepidermolytic type are benign skin lesions and may vary in their extent from a single (usually linear) lesion to widespread and systematized involvement (Figure 1). They may be present at birth or develop early during childhood as localized epidermal thickening with hyperpigmentation, frequently following the lines of Blaschko. This suggests that epidermal nevi may be due to mosaicism resulting from postzygotic mutations in keratinocytes. Mutations of keratins 1 and 10 were shown to be responsible for a rare subgroup of epidermal nevi, the linear epidermolytic hyperkeratosis (1, 2). Another variant of epidermal nevi, the congenital hemidysplasia with ichthyosiform nevus and limb defects (CHILD) nevus, is caused by NADPH steroid dehydrogenase-like protein (NSDHL) mutations (Xq28) and represents a functional X chromosomal mosaicism (3). However, the genetic basis of the much more common nonorganoid, nonepidermolytic keratinocytic epidermal nevi remains elusive.

The FGF receptor (FGFR) family comprises 4 major transmembrane receptor tyrosine kinases (FGFR1–4) and is involved in embryogenesis, angiogenesis, and tissue homeostasis (4). The FGFR3 gene contains 19 exons encoding an extracellular region for ligand binding composed of 3 Ig-like domains, a hydrophobic transmembrane domain and 2 cytoplasmic tyrosine kinase domains (Figure 2). Alternative splicing of the second half of the IgIII-like domain (exon 8 versus exon 9) results in the isoforms IIIb and IIIc. The isoforms show different ligand specificity and tissue expression. FGFR3 IIIb is mainly expressed in epithelial cells while FGFR3 IIIc is predominantly found in mesenchymal cells (5). This paper assigns all codon numbers to the open reading frame of the FGFR3 IIIb isoform. More than 20 FGFRs are known as ligands now (4). The interaction of the ligand with the receptor requires the presence of sulfated glycosaminoglycans such as heparin and leads to the dimerization of the receptor with consequent phosphorylation of intracellular tyrosine residues in the kinase domain and activation of intracellular signaling pathways. The autophosphorylation sites of FGFR3 represent potential binding sites for signaling proteins, for example, with phosphotyrosine binding (PTB) and Src homology 2 (SH2) domains. Activation of the cytoplasmic region causes phosphorylation of Shp2, PLCγ, ERK1/2, and STAT3 and also PI3K activation (4). FGFRs can also be activated by interaction with Epha4, another receptor tyrosine kinase, which demonstrates the complexity of FGF signaling (6).

Activating germline mutations of the FGFR3 gene result in dwarfism, severe skeletal dysplasia, and craniosynostosis syndromes such as achondroplasia (ACH), hypochondroplasia (HCH), thanatophoric dysplasia (TD), Crouzon syndrome (CS), Muenke syndrome (MS), and SADDAN (severe achondroplasia with developmental delay and acanthosis nigricans) syndrome (7–10). These activating mutations result in negative chondrocytic growth regulation of the epiphyseal plates of long bones, causing dwarfism (11). Identical mutations are found in different cancer entities and probably provide proliferative signals (4). FGFR3 signaling in mutated cells is poorly understood, but previous studies provide some insights. All known missense mutations causing TD I create an unpaired cysteine residue (12–14), such as the R248C and S249C mutations. These mutations are localized in the extracellular domain at the linker region between the Ig-like domains II and III while other
to the formation of hydrogen bonds between 2 FGFRs, resulting in constitutive receptor activation (20). The A393E mutation in CS also affects the transmembrane domain and results in FGFR3 dimer stabilization, measured by the change in the free energy of the dimerization, thus largely increasing the fraction of dimers (21). A third class of mutations affect the tyrosine kinase domain, such as the K652E mutation in TD II or the N542K mutation in HCH (11). These mutations likely cause conformational changes in the activation loop that activate the receptor tyrosine kinase activity and downstream ERK1/2 (22). Other studies suggest that FGFR3 mutations may delay the downregulation and ligand-mediated internalization of the receptor (23). The phosphorylated immature form of the mutant receptor accumulates in the endoplasmic reticulum and fails to be degraded (24). The different degree of receptor activation seems to correlate with the severity of the phenotype. Stronger activation of the receptor by ligand-independent dimerization via disulfide bonds in TD patients determines the more severe phenotype compared with other skeletal dysplasia syndromes such as ACH and HCH (15, 25).

Somatic activating FGFR3 mutations have been identified in 40% of human seborrheic keratoses (26) and in several human cancers (4), including multiple myeloma (27), urothelial carcinoma (28), cervix carcinoma (29), and colorectal carcinoma (30). Some of the skeletal dysplasia syndromes (TD, CS, SADDAN) caused by FGFR3 mutations are also characterized by marked thickening of the epidermis. This skin lesion, termed acanthosis nigricans, and epidermal nevi share similar histological features, including acanthosis and papillomatosis (31, 32). Herein we investigate the role of FGFR3 mutations in common nonorganoid, nonepidermolytic keratinocytic epidermal nevi.

Results
We analyzed 39 common nonepidermolytic, nonorganoid keratinocytic epidermal nevi of 33 patients using a SNaPshot multiplex assay that covered 11 FGFR3 point mutations described in skeletal dysplasia syndromes and cancer entities (Figure 2). The following subtypes of keratinocytic epidermal nevi were

Figure 2
FGFR3 gene. The position of the mutations covered by the SNaPshot multiplex assay is indicated. Codons are numbered according to the FGFR3 IIIb isoform; potential mutations of the stop codon 809 in exon 19 associated with TD I were analyzed by direct sequencing. C, C-terminus; Ig I, Ig II, Ig III, Ig-like domains I–III; N, N-terminus; TM, transmembrane domain; TKI, TKII, tyrosine kinase domains I–II.

Figure 1
Patient 29 displayed a systematized epidermal nevus of the common soft type with involvement of the face (bilateral), the right scapular region, the right arm, the right hip, and the right thigh. Abnormalities of the skeletal or nervous system were not present. A biopsy was taken from the epidermal nevus of the right forearm. This epidermal nevus revealed an R248C FGFR3 gene mutation. DNA isolated from the blood of this patient revealed WT status at codon 248, excluding a germline mutation.
FGFR3 mutations were found in common-type epidermal nevi (14 common soft type, and 2 common hard type).

In 4 patients (patients 3, 17, 21, and 32) with an FGFR3 mutation (R248C) in the epidermal nevus, clinically and histologically normal epidermis adjacent to the nevus showed a WT codon 248, suggesting an epidermal mosaicism of the FGFR3 mutation and a strong genotype-phenotype correlation (Figure 3). In 1 patient (patient 29) with a systematized epidermal nevus displaying the R248C mutation (biopsy was taken from the right forearm), additional genomic DNA was isolated from blood. The DNA revealed the WT codon 248, thus excluding a germline mutation. Multiple intradividual epidermal nevi biopsies could be analyzed in 2 patients. One patient (patient 11a, b) did not show any FGFR3 mutations. The other patient (patient 33a–e) underwent ablative laser treatment of the common soft-type epidermal nevus at the right side of the neck (Figure 4). Before treatment, 6 of the scattered brownish papules were curetted for FGFR3 mutation analysis. All 6 samples, which were spatially distant from each other, revealed the R248C mutation in the SNaPshot analysis.

All FGFR3 mutations detected so far in skin lesions (seborrheic keratoses and epidermal nevi) are associated with TD, CS, and SADDAN syndrome in the germline. The SNaPshot multiplex assay covered all mutations responsible for these syndromes except for the stop codon mutation X809L/G/R/C/W in exon 19 causing TD 1 (Figure 2). For 20 epidermal nevi (nevi of patients 1, 2, 5, 6, 7, 8, 9, 10, 11 [a, b], 14, 16, 17, 20, 23, 27, 28, 30, 31, and 32), DNA was available for further analysis. We additionally sequenced exon 19 of these samples. Seventeen of the 20 epidermal nevi had not shown any FGFR3 mutations in the SNaPshot analysis. However, no further FGFR3 mutations were detected in exon 19.

Since epidermal nevi represent a heterogeneous skin disorder, we also studied an organoid type of epidermal nevus, the sebaceous nevus. This nevus is found almost exclusively on the scalp or face and is usually present at birth. Sebaceous nevi are histopathologically characterized by the presence of large numbers of mature sebaceous glands and papillomatous hyperplasia of the epidermis. We analyzed 13 sebaceous nevi for FGFR3 point mutations using the SNaPshot multiplex assay (Table 2). In contrast to the common nonorganoid keratinocytic nevi, this organoid type of epidermal nevus revealed no FGFR3 mutations. Thus, activating FGFR3 mutations, especially the frequently detected R248C mutation, appear to be specific for common nonorganoid keratinocytic nevi.

**Discussion**

Our results indicate that a significant number of epidermal nevi of the common, nonorganoid, and nonepidermolytic keratinocytic type are caused by postzygotic mutations in the FGFR3 gene, which

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**Table 1**

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*Age, age at time of biopsy; site, site of biopsy; control, DNA from clinically and histologically normal epidermis adjacent to the epidermal nevus; syst., systematized epidermal nevus; M, male; F, female; acro-verruc, acrokeratosis verruciformis-like; R248C, exon 7, codon 248, GGC to TGC with Arg to Cys; G372C, exon 10, codon 372, GGC to TGC with Gly to Cys; G382R, exon 10, codon 382, GGG to AGG with Gly to Arg. Codon numbers refer to the open reading frame of the FGFR3 IIIb isoform.*
mosaicism (34). The R248C mutation results in strong constitutive
individuals are often stillborn or die as neonates. It has previously
been postulated that such lethal mutations may only survive by
mutations in this lethal skeletal dysplasia syndrome. Affected
along the lines of Blaschko, resulting in the linear or whirled cuta-
nous patterns usually observed in epidermal nevi (33).

Several findings support the notion that the detected FGFR3
mutations are causative for the development of epidermal nevi:
(a) The detected mutations R248C and, in one case, G372C are
known to occur in TD I patients and act as dominant germline
mutations are causative for the development of epidermal nevi:
neous patterns usually observed in epidermal nevi (33).

The epidermal nevus histologically showed the typical acanthosis and papil-
lar changes, and bladder cancer. (e) In one patient of
our series (patient 33), multiple intraindividual samples of
the epidermal nevus localized on the neck could be analyzed.  
All 6 samples revealed the same R248C mutation, suggest-
ing the presence of a scattered FGFR3 mutation mosaicism in the
skin of this patient following the lines of Blaschko. (f) The stron-
gest support that the detected mutations are indeed causative for
epidermal nevi is that 4 patients with an R248C mutation in the
epidermal nevus showed the WT allele in clinically and histologi-
cally normal epidermis adjacent to the epidermal nevus. This also
suggests mosaicism of an FGFR3 mutation in the epidermis and a
strong genotype-phenotype correlation.

In our series, 15 of 16 epidermal nevi with an FGFR3 mutation
displayed the R248C mutation, resulting in a cytokine-thymine
substitution (CGC to TGC). This C to T transition is a typical
example of deamination of methylated cytosines leading to CpG
dinucleotide depletion. The reason for this mutational hot spot is
unknown. One could speculate that the development of epi-
show acanthosis, papillomatosis, hyperkeratosis, and basal
hyperpigmentation (31, 32). A potential overlap between
the 2 skin disorders is acknowledged since both acanthosis
nigricans type of epidermal nevus and nevoid acanthosis
nigricans have been described (32, 35, 36). In our series of
epidermal nevi, an acanthosis nigricans-like type was not
included. A female patient with a mosaicism of R248C (25% of
the blood lymphocytes were affected by the mutation) developed disseminated thickening and hyperpigmenta-
tion of the skin consistent with acanthosis nigricans (37).
(d) Some patients with epidermal nevus syndrome showed
typical keratinocytic epidermal nevi, skeletal abnormalities,
and the occurrence of urothelial carcinoma at an early age
(38–40). This correlation of epidermal nevus and urothelial
carcinoma is thought to be nonstochastic. FGFR3 mutations
are frequent events in papillary urothelial carcinoma
(29, 41). These findings and the skeletal abnormalities rem-
inscent of skeletal dysplasia syndromes strongly suggest
that such patients feature a mosaicism of activating FGFR3
mutations, which in turn cause epidermal nevi of the skin,
skeletal changes, and bladder cancer. (e) In one patient of
our series (patient 33), multiple intraindividual samples of
the epidermal nevus localized on the neck could be analyzed.
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Figure 3
Patient 32 had a common soft-type epidermal nevus on his back. Two biop-
sies were taken from the epidermal nevus and the adjacent normal skin.  
The epidermal nevus histologically showed the typical acanthosis and papil-
lar changes, and bladder cancer. (e) In one patient of
our series (patient 33), multiple intraindividual samples of
the epidermal nevus localized on the neck could be analyzed.  
All 6 samples revealed the same R248C mutation, suggest-
ing the presence of a scattered FGFR3 mutation mosaicism in the
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unknown. One could speculate that the development of epi-

Figure 4
Patient 33 revealed a common soft-type epidermal nevus at the right
side of the neck consisting of scattered brownish papules following
the lines of Blaschko. Before ablative laser treatment, 6 papules dis-
tant from each other were curetted for FGFR3 mutation analysis. The
green peaks represent WT codon 375, the black peaks WT codon 248.
The R248C mutation is characterized by a red peak. All 6 samples
displayed the R248C mutation. In the bottom right corner, control DNA
with WT codon 248 is shown.
Epidermal nevi with acanthosis and papillomatosis of the epidermis requires strong activation of the receptor and not all activating FGFR3 mutations are capable of this strong activation. Indeed, Naski et al. found that the R248C mutation activates the receptor more strongly than the G382R mutation responsible for ACH (15). Stronger activation of the receptor is thought to result in a more severe phenotype in TD compared with other skeletal dysplasia syndromes (14). According to this theory, mosaicism of FGFR3 mutations other than R248C may also be present in the skin, but they may be insufficient to induce epidermal nevi. This hypothesis is supported by Logie et al. (26), who found that seborrheic keratoses sharing some histological characteristics with epidermal nevi are caused by acquired somatic FGFR3 mutations. All somatic mutations detected so far in seborrheic keratoses (R248C, S249C, G372C, S373C, Y375C, K652E, and K652M) are associated with TD and SADDAN syndrome in germline. Remarkably, none of the mutations associated with ACH or HCH were found in this series of 62 seborrheic keratoses. Patients with TD and SADDAN syndrome show acanthosis nigricans whereas ACH and HCH are usually not associated with this skin lesion except for in 1 reported case (42). ACH is the most common cause of dwarfism in humans, with an incidence of 1:15,000 to 1:40,000 of live births, and the mutated nucleotide 1138 in ACH is thought to be the most mutable nucleotide in the human genome described so far (9, 43). The R248C mutation is the most common mutation in TD (44), but it remains elusive why other mutations causing TD and SADDAN syndrome (S249C, S373C, Y375C, K652E, and K652M) were not detected in our series of epidermal nevi.

Epidermal nevus syndromes are characterized by epidermal nevi, abnormalities of the skeletal and nervous system, and rarely, some associated cancer entities (45, 46). The occurrence of papillary bladder cancer and skeletal abnormalities in epidermal nevus syndromes (38–40) suggests that FGFR3 is a promising candidate gene for epidermal nevus syndromes that may be caused by a more widespread mosaicism of FGFR3 mutations. If the FGFR3 mosaicism involves the germ cells in those patients, the offspring should show a TD phenotype. However, we did not find any reports of patients with systematized epidermal nevi or epidermal nevus syndrome and one offspring with TD in the literature. Another interesting fact is that acanthosis nigricans is predominantly observed in intertriginous areas in skeletal dysplasia syndromes. The reason for this preferential localization remains unclear since the entire skin carries the mutation. There seems to be a preponderance of intertriginous localization (neck, axilla, groin) also in the epidermal nevi with FGFR3 mutations in our series (Table 1). Additional cofactors in intertriginous areas may favor the development of acanthosis and papillomatosis on the basis of activating FGFR3 mutations.

One epidermal nevus in our series displayed a double mutation, the G372C mutation known from TD I and the typical G382R mutation known from ACH. It remains elusive whether the G372C mutation alone, which causes a stronger activation of the FGFR3 receptor according to the associated skeletal dysplasia syndromes (13–15), was able to cause the epidermal nevus or whether the combination of both mutations was necessary for the induction of the nevus. The possibility that the nevus was mainly caused by the G382R mutation seems unlikely since ACH patients, in contrast to TD patients, usually do not develop acanthosis nigricans (10, 42). Double mutations in the FGFR3 gene have been reported in urothelial carcinoma (28, 47, 48). To our knowledge, germline compound heterozygosity for mutations associated with TD and ACH has not been described. The epidermal nevus carrying the double mutation displayed no histopathological abnormalities.

Each of the major 3 cell types present in our microdissected samples (keratinocytes, dendritic cells, and melanocytes) may theoretically be the carrier of the detected mutations, but keratinocytes represent the likely host cell type. Logie et al. studied a mouse model in which the S249C mutation (causing TD I) was targeted to the basal layer of the epidermis using the keratin 5 promoter (26). Keratin 5 is a marker for basal keratinocytes and not expressed in melanocytes or dendritic cells. The transgenic mice developed thickening of the skin and verrucous skin tumors with histological features similar to epidermal nevi. The basal hyperpigmentation mediated by melanocytes would be a secondary phenomenon if the mutations affect keratinocytes. However, further studies are needed to prove this hypothesis.

It is unclear why the same mutation (R248C) can cause both seborrheic keratoses and epidermal nevi. FGFR3 mutations exhibit pleiotropic effects ranging from inherited skeletal dysplasia syndromes and benign skin tumors to cancer. The cell type–specific involvement of different signaling pathways such as Ras/MAPK and STAT as well as the FGFR3-dependent recruitment of cell-specific second receptors such as EphA4 (6) may determine the effect of activating FGFR3 mutations in each cell type (4).

Mutations of FGFR3 in the urothelium are significantly associated with benign urethelial papillomas (49) and with low-grade and low-stage pTa G1/2 tumors, which rarely progress (41, 48, 50). These findings support the concept that mutations of the FGFR3 IIIb isoform can induce proliferation and tumor formation but are associated with a low malignant potential. This would be consistent with our findings of FGFR3 mutations in epidermal nevi that represent a benign skin disorder and show signs of hyperproliferation (namely acanthosis and papillomatosis) but bear no malignant potential.

Several small molecule tyrosine kinase inhibitors of FGFR3, such as PKC412, PD173074, and SU5402, are already available. They have been used in vitro and in animal models to inhibit the growth of multiple myeloma cell lines with activating FGFR3 mutations (51–54). PKC412 is currently being evaluated in phase II trials for acute myeloid leukemia patients (55). This molecule effectively inhibits the tyrosine kinase activity of FGFR3, as shown by the

<table>
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Table 2  FGFR3 mutation analysis in sebaceous nevi
inhibition of proliferation in hematopoietic cells transformed by a mutant FGFR3 gene (55). A broader use of this drug in other disorders associated with an increased activity of FGFR3 has been suggested. This may also include benign skin lesions such as seborrheic keratoses (26) and epidermal nevi. The current standard therapy for epidermal nevi and seborrheic keratoses is surgery or ablative laser treatment, which is often associated with irreversible scar formation. Topical application of tyrosine kinase inhibitors of FGFR3 may obviate the need for surgical intervention.

In summary, a large proportion of human epidermal nevi are caused by mosaicism of postzygotic activating FGFR3 mutations in the human epidermis. The R248C mutation appears to be a hot spot for FGFR3 mutations in epidermal nevi. Additional studies are needed to investigate other regions of FGFR3 and different receptor tyrosine kinases as possible mutational targets in epidermal nevi and elucidate functional aspects of enhanced FGFR3 signaling in the skin.

Methods
Microdissection. Thirty-nine histologically confirmed nonepidermolytic epidermal nevi of the common nonorganoid type of 33 patients with varying degrees of skin involvement were retrieved from the histology files of the Department of Dermatology, University of Regensburg. Informed consent for the scientific use of the material and photographs had been obtained from all patients according to the guidelines of the ethics committee of the University of Regensburg and the Declaration of Helsinki. Classical symmetrical acanthosis nigricans in addition to the epidermal nevi was not seen in any of the study patients, and no signs of skeletal dysplasia or associated cancer were observed. We classified the different subtypes of epidermal nevi according to a previous study (32). The common keratinocytic type was subdivided into soft and hard types according to the degree of hyperkeratosis. The characteristics of the patients and their nevi are shown in Table 1. We also investigated an organoid epidermal nevus type, the sebaceous nevus, because epidermal nevi represent a heterogeneous group of lesions. Thirteen sebaceous nevi were retrieved from the histology files of the Department of Dermatology of the University of Regensburg (Table 2).

Sections of 10-μm thickness were microdissected manually from paraffin-embedded epidermal nevi tissues with a needle under an inverted microscope. We dissected the anatomic epicrism of the nevi containing mainly keratinocytes but also small numbers of melanocytes and dendritic cells. In sebaceous nevi, both the hypertrophic sebaceous glands and the epidermal nevi were dissected. Clinically and histologically normal epidermis adjacent to the nevus was microdissected in 4 patients with common keratinocytic nevi to serve as a source of control DNA.

DNA isolation. DNA was isolated following standard protocols. In brief, about 25–50 mg formalin-fixed paraffin-embedded tissue was microdissected for each sample. The microdissected tissue was digested with proteinase K overnight in lysis buffer, and DNA isolation was performed with the High Pure PCR Template Preparation Kit (Roche Diagnostics) according to the manufacturer’s protocol. The DNA of each tissue was eluted in a volume of 200 μl elution buffer. The amount of isolated DNA ranged from 5 μg to 30 μg for each sample.

SNAPshot assay. A previously described SNAPshot multiplex assay, based on the SNAPshot Multiplex System assay (Applied Biosystems), was used to screen for activating FGFR3 point mutations (56). We used 2 μl template DNA for the multiplex PCR. The SNAPshot multiplex PCR assay can detect mutations with an input DNA amount of only 1 ng genomic DNA (56). Three regions of interest in exons 7, 10, and 15 comprising 11 FGFR3 mutations were amplified in 1 multiplex PCR, followed by extension of mutation-specific primers with a labeled dideoxynucleotide. Two new antisense primers were added to the original assay to screen for mutations S373C (5′-T19GAGGATGCCCTGCATACACAC-3′) and G382R (5′-T56GAAACAG-GAAAGGCCACCCACC-3′). Concentrations for those primers used in the multiplex assay were 1.0 and 0.6 pmol/μl, respectively. Thus, screening could be performed for 11 known mutations found in bladder tumors and other noncutaneous epithelial malignancies (R248C, S249C, G372C, S373C, Y375C, G382R, A393E, K652E, K652M, K652Q, and K652T; codons are numbered according to the open reading frame of the FGFR3 IIIB isoform, which is predominantly present in epithelial cells). This mutation spectrum also covers the most frequently found FGFR3 mutations in skeletal dysplasia syndromes. Extended primers were separated by capillary electrophoresis in an automatic sequencer, and the presence or absence of a mutation was indicated by the incorporated WT or mutant labeled dideoxynucleotide. When a mutation is present, a second peak from the mutated nucleotide will appear next to the WT peak in the electrophogram (see Figures 3 and 4). However, the assay is not quantitative due to the different emission efficiencies of the labels. Mutations were confirmed by a second independent reaction.

FGFR3 sequence analysis. In addition to the SNAPshot analysis, exon 19 of the FGFR3 gene was directly sequenced. This exon contains the potential mutation at stop codon 809 associated with TD I. We were able to analyze 20 epidermal nevi. The other samples failed due to limited DNA amounts. We used the forward primer 5′-CCTGTGCCGGCCTCTTGAGCAG-3′ and the reverse primer 5′-CAGACCAAAGCTCTGTAGCT-3′ to generate a 235 bp PCR product of exon 19 containing the stop codon 809. Sequence analysis was performed following standard protocols.

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