Early Genetic Diagnosis of Neurofibromatosis Type 2 From Skin Plaque Plexiform Schwannomas in Childhood

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IMPORTANCE Neurofibromatosis type 2 (NF2) is a devastating genetic condition characterized by the development of multiple tumors of the nervous system. An early diagnosis of individuals with NF2 would facilitate treatment and reduction of disease impact because most severe effects of the disease do not usually develop before adolescence. Little attention has traditionally been paid to dermatological signs in NF2. However, skin plaques are commonly seen in patients with NF2, normally appearing either at birth or early childhood, providing an opportunity for early NF2 detection and testing.

OBJECTIVE To determine the clinical utility of skin plaque identification and characterization in children for reaching an early diagnosis of patients with NF2 and to evaluate their molecular pathogenesis and their use in the genetic diagnostics of NF2.

DESIGN, SETTING, AND PARTICIPANTS Diagnostic test study by the histological and genetic characterization of skin plaques from patients with NF2. Patients were 7 individuals with NF2 or clinical suspicion of NF2 treated at the Spanish Reference Center on Phakomatoses.

MAIN OUTCOMES AND MEASURES Histological evaluation of all skin plaques was performed. Fresh skin plaques were cultured to obtain Schwann cells and the NF2 gene was genetically analyzed. For all 7 patients, NF2 clinical history was reviewed.

RESULTS In all 7 patients (4 male and 3 female), all skin plaques analyzed were histologically characterized as plexiform schwannomas. Genetic analysis of primary Schwann cell cultures derived from them allowed the identification of a constitutional and a somatic NF2 mutation. Genetic testing allowed the early diagnosis of NF2 in a child only exhibiting the presence of skin plaques. Most of the patients with NF2 analyzed had an early presentation of skin plaques and a severe NF2 phenotype.

CONCLUSIONS AND RELEVANCE This work emphasizes the clinical utility of a careful dermatological inspection and the correct identification of skin plaques in children for an early diagnosis of NF2. We show for the first time that Schwann cells derived from skin plaque plexiform schwannomas bear the double inactivation of the NF2 gene and thus constitute an excellent source of tissue for genetic testing, especially in the context of mosaicism.
neurofibromatosis type 2 (NF2) is an autosomal dominant genetic condition caused by mutations in the NF2 gene (OMIM 607379). It is characterized by the development of multiple tumors of the nervous system and meninges, as well as lesions of the eyes and skin, causing high morbidity and reduced life expectancy.1-3 Bilateral vestibular schwannomas (BVSs) occur in more than 90% of adults with NF2, constituting a hallmark of this disorder.1 The type and timing of BVS intervention(s) are key factors to preserve hearing and neurological functions, with early NF2 diagnosis being crucial for their correct management.4-6 Because BVSs do not usually develop before adolescence,4,5,7 there is a window of opportunity to treat the patient early before BVS development.1,2,4,5,7 Thus, for an early NF2 diagnosis it is important to take into account other clinical features that can have onset at a younger age. One example is skin schwannomas, which can take 2 clinical forms: subcutaneous tumors of variable size, elastic on palpation and covered by skin with a normal appearance; or otherwise, well-circumscribed brownish plaques, soft on palpation and sometimes exhibiting hypertrichosis. Clinical and genetic diagnostics of NF2 is complicated by the fact that approximately 30% of sporadic NF2 cases are mosaic.1,7

Methods

The 7 participants in this study were treated at the Spanish Reference Center on Phakomatoses at the Germans Trias i Pujol Hospital (HUGTIP). The HUGTIP institutional review board approved the study, and all patients gave written informed consent. Histological and genetic analyses were performed as previously described.8,9 For cell culture, the epidermis was carefully separated and the remaining tissue was cut and dissociated. Pure cultures of Schwann-like cells and fibroblasts were obtained as described elsewhere.10 For immunocytochemical analysis, fixed cells were stained with polyclonal rabbit anti-S100 (Dako) and mouse monoclonal anti-MelanA (Ventana Medical Systems) primary antibodies and secondary antibodies conjugated to Alexa Fluor dyes (Invitrogen). DNA was visualized by staining with 4′,6-diamidino-2-phenylindole.

Results

Report of a Case

A preschool-aged boy presenting with 3 congenital skin lesions was seen in our dermatology department. These lesions consisted of well-circumscribed, slightly pigmented plaques that were soft on palpation, measuring between 0.5 and 2 cm in diameter (Figure 1A and B), similar to the NF2-associated skin plaques often seen in adults with NF2. Histological evaluation of a biopsy sample taken from 1 of these plaques revealed a dermal plexiform schwannoma (Figure 1C). Skin plaques have been described to correspond to plexiform schwannomas11-13 and are present in approximately 40% to 78% of children with NF2.1,4 Although cutaneous signs are not conspicuous in NF2, these lesions have been suggested to be pathognomonic of NF2.4,7,12-14 In the absence of any other evident NF2 clinical symptom, the medical record of the boy was reviewed, revealing a Bell-like palsy 2 years before that did not fully resolve, consistent with the NF2 disease. After written informed consent was obtained, the complete skin plaque was removed for both further pathologic examination and genetic testing. On hematoxylin-eosin stain, a dermal multinodular neural proliferation was observed. Closely packed spindle cells with elongated wavy nuclei formed well-circumscribed interconnected masses of different sizes, which occupied superficial and deep dermis. Immunohistochemical analysis revealed strong and diffuse S100 positivity in tumoral cells (Figure 1D) and epithelial membrane antigen positivity in perineural cells surrounding nodules. Occasional neurofilament-positive axons were evident (data not shown).

Skin Plaque–Derived Schwann Cells: NF2 Homozygous Mutated Cells Suitable for Genetic Testing

Fibroblasts and Schwann-like cells were obtained from the same skin plaque. Schwann-like cells that spread out from the nodules stained positive for S100 and negative for the melanocyte-specific marker Melan-A (Figure IE and F) (data not shown), confirming their Schwann cell (SC) nature. RNA and DNA were obtained. NF2 mutation analysis starting from SC mRNA uncovered a mutation in the acceptor site of exon 3 (g.35526A>G; c.241-9A>G) that was causing the insertion of 8 bp of intron 2 (r.240_241insNG_009057.1:g.35526_35534) and resulting in a truncating merlin protein (p.Val81Phefs*44) (Table). The same pathogenic variant was identified in blood and fibroblast DNA in heterozygosity, confirming the constitutional nature of the mutation, and the presence of NF2 in the boy.

Furthermore, microsatellite multiplex polymerase chain reaction analysis of chromosome 22q8 in SCs revealed loss of heterozygosity involving NF2 (patient 139 in the Table), indicating the double inactivation of NF2 in skin plaque SCs. We identified 6 additional patients with NF2 who presented with skin plaques (Table) and confirmed their plexiform schwannoma nature by histological examination of 1 skin plaque from each individual (Figure 1G and H). From 2 of them (patients 403 and 416) we were able to obtain fresh tissue and cultured SCs. Genetic analysis of SCs identified in each case a different deleterious NF2 mutation accompanied by loss of heterozygosity involving NF2 at different
Figure 1. Clinical, Histopathological, and Cell Culture Characterization of Skin Plaques

A and B. Macroscopic appearance of a skin plaque from patient 139. C. Histological low-power field view of skin plaque plexiform schwannoma depicted in (A) showing multiple nodules interconnected with each other on hematoxylin-eosin (H&E) staining (original magnification ×40). D. Tumoral nodules within skin plaque showed strong and diffuse S100 positivity by immunostaining (original magnification ×40). E. Microscopic bright-field image of a cell culture from skin plaque plexiform schwannoma depicted in (A) showing fragments of skin plaque nodules (original magnification ×200). F. Microscopic fluorescence image showing S100 immunostaining of the cell culture in (E) exhibiting Schwann cell nature (original magnification ×200). G. Biopsy of skin plaque plexiform schwannoma of patient 403 (original magnification ×40). H. Biopsy of skin plaque plexiform schwannoma of patient 3 (original magnification ×200).
chromosome 22q regions (Table). A third formalin-fixed paraffin-embedded skin plaque sample was genetically analyzed after microdissection of 1 SC nodule (patient 3) (Figure 1H and Table), also revealing a double inactivation of NF2. The genetic analysis of these 4 skin plaques confirmed that SCs contained in their nodules bear a double inactivation of the NF2 gene, like other NF2 tumors, explaining their molecular pathogenesis.

**Early Presentation of Skin Plaques**

Finally, we reviewed the clinical history of all patients with NF2 whose skin plaques were analyzed (Table). In all of them, these lesions appeared to be congenital or had developed in early childhood, being in most cases the first clinical manifestation of NF2. Furthermore, we also noticed that all 7 patients developed a severe NF2 phenotype despite their young age, suggesting that the development of skin plaques could be an indicator of bad prognosis in NF2. In most cases, patients bore truncating mutations.

**Discussion**

An early diagnosis of NF2 has the potential to reduce the disease’s effect on patient health through early surveillance

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex (Age)</th>
<th>Skin Lesions (Age of Detection)</th>
<th>NF2-Related Phenotype (Age of BVS Detection)</th>
<th>Germline Mutation</th>
<th>Second Hit in Plaque-Derived Schwann Cells</th>
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<tr>
<td>1</td>
<td>Female (adult)</td>
<td>1 CAL, 2 skin plaques (childhood)</td>
<td>BVS (&lt;20 y), corneal reflex loss</td>
<td>NF2 deletion (in mosaicism)</td>
<td>Not analyzed</td>
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<tr>
<td>3</td>
<td>Female (adult)</td>
<td>4 CALs, 3 skin plaques (childhood)</td>
<td>BVS (&lt;25 y)</td>
<td>c.241_363del (p.Met39_Gln121 del)</td>
<td>LOH chr22q* unknown extension</td>
</tr>
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<td>139</td>
<td>Male (preschooler)</td>
<td>3 Skin plaques (congenital) and subcutaneous tumors</td>
<td>BVS (&lt;10 y), recurrent bilateral facial nerve palsy, epiretinal membranes</td>
<td>c.241-9A&gt;G (p.Val81Phefs*44)</td>
<td>LOH chr22q (from D22S420 to D22S268)</td>
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<td>267</td>
<td>Male (teen)</td>
<td>1 Skin plaque (congenital)</td>
<td>BVS (&lt;15 y), retinal hamartomas</td>
<td>c.784C&gt;T (p.Arg262*)</td>
<td>Not analyzed</td>
</tr>
<tr>
<td>965</td>
<td>Male (adult)</td>
<td>1 CAL and 4 skin plaques</td>
<td>BVS (unknown), ependymoma, meningioma</td>
<td>c.517-2A&gt;G (p.Val173Glyfs*2)</td>
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<td>403</td>
<td>Male (adult)</td>
<td>&gt;10 Skin plaques (toddler) and subcutaneous tumors</td>
<td>BVS (&lt;15 y), multiples meningiomas, spinal tumor, multiple schwannomas, muscular atrophy of left hand</td>
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<td>416</td>
<td>Female (teen)</td>
<td>5 CALs and 3 skin plaques (congenital)</td>
<td>BVS (&lt;15 y), congenital peripheral right facial nerve palsy, ependymoma</td>
<td>c.520dupA (p.Ile174Asnfs*29)</td>
<td>LOH chr22q (from D22S420 to D22S274)</td>
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**Table. Patients With Neurofibromatosis Type 2 (NF2) With Skin Plaques**

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Abbreviations: BVS, bilateral vestibular schwannoma; CAL, café au lait spot; LOH, loss of heterozygosity.
and management of the disease. The presentation of multiple skin plaques in children has been suggested to be pathognomonic of NF2. Here we confirm the double inactivation of NF2 in their SCs and show their utility for genetic testing, especially in the context of mosaicism. We provide a flow diagram proposing clinical guidelines derived from the present work (Figure 2). According to the results presented in the Table, in most cases NF2 mutation could be identified in blood, but mosaicism is also compatible with this clinical presentation. Because only a fraction of the plexiform schwannoma is composed of NF2 homozygous mutated cells, we recommend deep sequencing for the presentation. Because only a fraction of the plexiform schwannoma is composed of NF2 homozygous mutated cells, we recommend deep sequencing for the NF2 gene if skin plaque samples are used directly for testing, to deal with the presence of mosaicism. If SC culturing is available, standard NF2 testing is sufficient. In mosaic cases, the identification of a common NF2 mutation in at least 2 tissues is mandatory to differentiate mosaicism from mutations confined only to the skin plaque.

**Limitations**

There exist individuals presenting with sporadic plexiform schwannoma tumors, normally adults with solitary lesions. It would be interesting to understand the molecular pathogenesis of these lesions in relation to the NF2 status and clarify whether they represent signs of a mosaic NF2 or just solitary lesions.

**Conclusions**

The results presented herein emphasize the clinical utility of a careful dermatological inspection and correct identification of skin plaques in children for an early diagnosis of NF2. Schwann cells derived from skin plaques bear the double inactivation of the NF2 gene. In the context of mosaicism, they could constitute an excellent source of tissue for genetic testing.

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REFERENCES


