Null phenotype of neurofibromatosis type 1 in a carrier of a heterozygous atypical NF1 deletion due to mosaicism

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Abstract
We coincidently detected an atypical deletion of at least 1.3-Mb, encompassing the NF1 tumor suppressor gene and several adjacent genes at an apparent heterozygous level in the blood of a 65-year-old female patient. She had multiple subcutaneous tumors that appeared with a certain similarity of subcutaneous neurofibromas, which, however, was revealed as lipomas by histological examination. Comprehensive and exhaustive clinical and radiological examinations did not detect any neurofibromatosis type 1-related clinical symptoms in the patient. Multiplex ligation-dependent probe amplification detected no or only very low level of the 1.3-Mb NF1 deletion in six lipomas and two skin biopsies. Digital polymerase chain reaction estimated the proportion of cells carrying a heterozygous NF1 deletion at 87% in the blood, and 8%, 10%, 13%, 17%, and 20%, respectively, in the five lipomas investigated by this method, confirming our hypothesis of mosaicism. Our findings suggest that de novo cases of genetic disease are potentially mosaic regardless of finding the mutation at an apparently heterozygous level in the blood and that the possibility of mosaicism should be considered in genotype–phenotype studies and genetic counseling.

KEYWORDS
lipoma, mosaicism, neurofibromatosis type 1, NF1 deletion

1 | INTRODUCTION

Mosaicism in a genetic disease describes a condition of coexistence of cell populations carrying the causal mutation and cell populations not carrying that mutation in a single individual. Postzygotic mutations, reverse mutation, and recombination may all lead to somatic mosaicism (Campbell, Shaw, Stankiewicz, & Lupski, 2015). Our studies on neurofibromatosis type 1 (NF1; MIM# 162200) and type 2 (NF2; MIM# 10100), which are two distinct tumor suppressor gene disorders, have revealed frequent mosaicism among de novo patients (Kehrer-Sawatzki et al., 2004; Kluwe et al., 2003). A recent study estimated a 60% frequency for mosaicism in de novo NF2 patients (Evans et al., 2019).

By contrast, a constitutional mutation can be found in up to 95% of NF1 patients (Messiaen et al., 2000) and consequently, mosaicism may be considered as less frequent in NF1 than in NF2. However, for a subgroup of NF1 patients, mosaicism was found at a frequency of >40% (Kehrer-Sawatzki et al., 2004). This subgroup of 4.7–11% of all NF1 patients carries a distinctive causal genetic alteration, which is heterozygous deletions of 1.2–1.4 Mb covering the entire NF1 gene and 12–13 adjacent genes on chromosome 17. Especially, among the 1.2 Mb NF1 deletions, which have breakpoints within the SUZ12 gene.
and its pseudogene SUZ12P1, the frequency of mosaicism is as high as 63% (Vogt et al., 2012).

The large (1.2–1.4 Mb) NF1 deletions are frequently associated with additional clinical phenotypes including facial dysmorphic features, tall-for-age status, large hands and feet, skeletal anomalies, and hyperflexibility of joints. In addition, some NF1-related manifestations are more frequent and severe in patients with large NF1 deletion, for example, low intelligence (8 → 38%), plexiform neurofibromas (50 → 76%), malignant peripheral nerve sheath tumors (5 → 21%), skeletal anomalies (31 → 76%), and congenital heart defect (2 → 29%). Large numbers of cutaneous neurofibromas and a high burden of internal tumors are also frequently found in patients with large NF1 deletions (reviewed by Kehrer-Sawatzki, Mautner, & Cooper, 2017).

Therefore, severe phenotypes would be expected for large NF1 deletions detected in the patients’ blood at an apparently normal heterozygous level. However, we report a case where not a single NF1-related manifestation was found in a carrier with such an NF1 deletion.

2 | PATIENT AND METHODS

A 65-year-old female patient with multiple tumors was suspected by her primary physician as having NF1 and subsequently sent to our NF Clinic, Hamburg. However, clinical examination excluded NF1 and suggested a form of lipomatosis. A hematological proliferation disorder such as MDS was not observed in the patient. As the patient suggested a form of lipomatosis, a hematological proliferation (MLPA) for the dosage of each exon was performed using the two kits P081 and P082 (MRC, Holland) for the NF1 exons. As a deletion covering the entire NF1 gene was found by this MLPA analysis, a second MLPA with another kit covering multiple genes flanking the NF1 gene (P122, MRC) was carried out. To exclude the mix-up of the patient’s blood sample, another blood was taken from the patient and MLPA was repeated. In addition, six lipomas were resected from the left hip, chest, left and right costal arches, left and right upper arms, suprapubic nerve, and left heel. Two skin biopsies were taken from the left and right axillae. The lipomas were histologically examined. DNA was isolated from these fresh tumors and skin samples (two skin samples and six lipomas) and subjected to MLPA analysis for the NF1 gene (kits P081 and P082) and the NF1 gene area (kit P122). To analyze the MLPA results, we used the Coffalyser MLPA analysis software made and supported by MRC.

Dual-probe digital polymerase chain reaction (PCR) was carried out using the validated assays for the NF1 (FAM-labeled probe) and the gene RPP30 (HEX-labeled probe) as the reference in the same reaction on a QX100 BioRad system (Kluwe, 2016). In addition to the DNA from the blood and five lipomas of the patient, we included DNA from a healthy control with no NF1 deletion and DNA from two NF1 patients with NF1 deletions as a comparison. The two NF1 deletions have been studied previously by fluorescence in situ hybridization, which detected the deletion in 100% and 91% of the leukocytes, respectively (Kehrer-Sawatzki et al., 2004). The ratio of the NF1 gene to the reference gene RPP30 (NF1/RPP30) is calculated automatically by the QX100 program. The proportion of the cells carrying a heterozygous NF1 deletion in each sample was calculated as $2 \times (1 - \text{NF1/RPP30})$. For replicated digital PCR reactions, the mean values were taken.

To detect possible asymptomatic deeper-located plexiform neurofibromas and other NF1-related tumors, magnetic resonance tomography for the brain, thorax, abdomen, and lower extremities including both feet was carried out using a 3 Tesla device in six sections, each with four sequences. Due to a known allergy, no contrast agent was given to the patient. The patient had one adult daughter who was clinically examined by V-F. M.

The study was approved by the local authority and the patient gave a written consent.

3 | RESULTS

3.1 | Lack of NF1-phenotypes

The patient had multiple subcutaneous tumors in various regions including hip, chest, costal arches, upper arms, suprapubic nerve, and heel.

![FIGURE 1](image) A lipoma (arrow) on the left arm exhibiting similarity with a subcutaneous neurofibroma (a). However, histology clearly revealed the feature of a lipoma (b)
These tumors exhibit some similarities with subcutaneous neurofibromas (Figure 1a). However, general examination and close inspection of these tumors at the NF clinic Hamburg suggested a diagnosis of multiple lipomas and excluded NF1. Histological examination of the resected tumors confirmed the initial diagnosis of lipomas (Figure 1b). Distribution and appearance of the lipomas do not meet the diagnosis of multiple symmetric lipomatosis (Enzi et al., 2002). No excessive alcohol consumption is reported by the patient.

No other NF1-related skin or eye manifestations were evident. Pigmentary lesions such as axillary freckling and Café-au-lait spots were not observed. Lumbar scoliosis of <15° was detected. The patient’s body height of 160 cm is within the normal range. Also, her cognitive capabilities were deemed to be normal. The patient had finished secondary school and her profession was to design the interior of windows in retail stores.

Magnetic resonance tomography for the brain, thorax, abdomen, and lower extremities including feet did not detect any NF1-related alterations nor any indication for optical gliomas and plexiform neurofibroma. Gliosis was seen in the brain parenchyma and brain stem.

According to the patient’s report, none of her parents was diagnosed with NF1. The patient had one daughter who did not present any NF1-related symptom as revealed by clinical examination by one of the authors (V.-F. M.).

3.2 | Characterization of the 1.3-Mb NF1 deletion

As the patient insisted on genetic analysis, mutation analysis was carried out which did not detect any intragenic NF1 mutation in the leukocytes of the patient derived from a sample of venous blood. However, to our surprise, MLPA revealed a heterozygous deletion covering the entire NF1 gene (Figure 2a, left). To exclude a mix-up of her blood sample, another blood sample was taken and MLPA was repeated, which confirmed the deletion. Further analysis using probes in the NF1 gene area revealed that these deletions span at least 1.3 Mb up to 1.9 Mb with the centromeric breakpoint in the SUZ12P gene or between the SUZ12P pseudogenes and the LRRC37B-P gene. The telomeric breakpoint is either within or telomeric to the LRRC37B-1 gene (Figure 2b). We consider this deletion as “atypical” as the breakpoints are unlikely to be located within homologous regions. Similar atypical deletions have been described in our previous study (Vogt et al., 2014). According to the HGVS nomenclature standards, this deletion based on the MLPA finding can be described as:

\[
\text{chr17:g.(28,789,443_29,058,391)_(30,348,592_30,693,753)del.}
\]

The deletion detected by MLPA in the patient’s blood appeared to be present at a heterozygous level and there was no indication for mosaicism using this technique. By contrast, the NF1 deletion was not detected by MLPA in DNA isolated from nonfrozen, fresh tissue samples of the six lipomas and two skin biopsies (an example is in Figure 2a, right).

3.3 | Proportion of the NF1-deletion-carrying cells

To investigate the proportion of cells with the atypical NF1 deletion and normal cells in the patient, we performed digital PCR, which has a much higher sensitivity to detect mosaicism compared with MLPA (Kluwe, 2016). On the basis of the results of digital PCR, the proportion of cells carrying the heterozygous NF1 deletion in the blood of the patient was 87 ± 1.2%. In the five lipomas investigated by digital PCR, the proportion of the NF1-deletion-carrying cells was estimated at 8%, 10%, 13%, 17%, and 20%, respectively (Figure 2c).

4 | DISCUSSION

The finding of a heterozygous atypical NF1 deletion of at least 1.3-Mb in two different blood samples of the patient who did not exhibit any NF1-related clinical symptom was a coincidence because her lipomas were first assumed to represent subcutaneous neurofibromas, the hallmark of NF1. However, histological analyses revealed that these tumors were lipomas and not neurofibromas. Large NF1 deletions are known to be associated with more severe NF1-related phenotypes and with additional distinctive features (reviewed by Kehrer-Sawatzki et al., 2017). It is, therefore, surprising that this patient with an NF1 deletion present at a nearly heterozygous level in venous blood completely lacked any symptom of NF1 at the age of 65 years. Not even asymptomatic tumors or other NF1-associated
clinical manifestations were detected by comprehensive clinical and radiological examination.

The most plausible explanation for the unusual phenotype characterized by the absence of NF1-associated symptoms is mosaicism with normal cells not harboring the NF1 deletion, which was confirmed by digital PCR. By this means, the proportion of blood cells with the deletion was estimated to be 87%. By contrast, the number of cells with deletion was very low in the lipomas (<20%).

In previous studies, we have observed that 1.2-Mb-spanning type 2 NF1 deletions are frequently of postzygotic origin and high proportions of cells carrying the deletion were detected in the blood of the affected patients. By contrast, much lower proportions of cells harboring the deletion were detected in other tissues (Roehl et al., 2012; Steinmann et al., 2007). Our findings suggested that hematopoietic stem cells with the deletion may have a selective growth advantage over normal cells lacking the deletion (Roehl et al., 2012). However, functional studies supportive of this hypothesis have not been performed as yet. The high proportion of cells with the atypical NF1 deletion of the patient described in this study may also be explained by a selective growth advantage of hematopoietic stem cells with the deletion. In other cell types of mesodermal origin, such as lipocytes, a selective growth advantage may have been absent leading to a much lower number of cells with the NF1 deletion. However, we cannot exclude that the deletion had occurred rather late during embryonic development causing fewer numbers of cells harboring the deletion in certain tissues.

A recent study reported an atypical 1.14-Mb NF1 deletion in a 73-year-old patient with a single isolated pheochromocytoma (Parisien-La Salle, Dumas, Rondeau, Latour, & Bourdeau, 2019). Interestingly, another study also reported splicing and nonsense NF1 mutations in two patients with pheochromocytomas who had macrocephaly and short stature, respectively (Gieldon et al., 2018). Pheochromocytoma, macrocephaly, and short stature are manifestations that are not exclusively associated with NF1 but have increased frequency in NF1 patients than in the general population. Compared with these three reported cases, the case in the present study is more extreme, as classical NF1-associated clinical symptoms were not observed even though the patient was already 65 years old. Though she had gliosis and mild scoliosis of <15°, these findings are also frequent in the general population and are, therefore, not specific signs for NF1.

Theoretically, mosaicism is possible for any patient with a de novo genetic disease. However, conventionally, defining mosaicism is based on a lack or clearly reduced level of mutation in the leukocytes. For example, in the case of NF2, mosaicism is often defined by finding an identical mutation in two different tumors and not finding this mutation or finding it at a reduced level in the blood. By contrast, if a mutation is detected in the blood with an apparent normal heterozygous signal intensity, mosaicism would not be considered. As exhaustive screening can find causative NF1 alterations in up to 95% of the patients (Messiaen et al., 2000), mosaicism appeared to be less frequent in NF1 than in NF2. However, the apparent lower frequency of mosaicism in NF1 may partially be explained by the less severe and less obvious manifestation of mosaic NF1 as compared with that of mosaic NF2. As mosaicism shifts the clinical spectrum toward the mild end, some mosaic NF1 patients may have very mild or and subclinical phenotypes and consequently may not be recognized as patients at all. By contrast, NF2 patients have cerebral and spinal tumors, which more frequently lead to severe deficits. Consequently, even after the modification by mosaicism, the phenotypes are still sufficiently severe and the patients with mosaic NF2 will be identified as such.

In addition, as demonstrated in the present study, mosaicism is possible also in cases where mutations/deletions are detected in the blood at an apparent normal level. This finding has important implications in genotype-phenotype correlation and in genetic consulting because mosaicism shifts the clinical spectrum of a genetic disease toward the mild end. Therefore, mosaicism may need to be considered for all de novo patients, especially those with mild phenotypes, regardless of finding mutation/deletion in the blood.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the (Global Variome shared LOVD) at https://databases.lovd.nl/shared/variants/0000602980. Reference number: 0000602980.

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REFERENCES

Campbell, I. M., Shaw, C. A., Stankiewicz, P., & Lupski, J. R. (2015). Somatic mosaicism: Implications for disease and transmission genetics. Trends in Endocrinology and Metabolism, 26, 53–59. https://doi.org/10.1016/j.tem.2014.12.006

Enzi, G., Busetto, L., Ceschin, E., Coin, A., Digito, M., & Piggozzo, S. (2002). Multiple symmetric lipomatosis: Clinical aspects and outcome in a long-term longitudinal study. International Journal of Obesity and Related Metabolic Disorders, 26, 253–261.

Evans, D. G., Hartley, C. L., Smith, P. T., King, A. T., Bowers, N. L., Tobi, S., Obholzer, R., English Specialist NF research group. (2019). Incidence of mosaicism in 1055 de novo NF2 cases: Much higher than previous estimates with high utility of next-generation sequencing. Genetics in Medicine, 22, 53–59. https://doi.org/10.1016/j.gim.2019.05.019

Gieldon, L., Masjkur, J. R., Richter, S., Därr, R., Lahera, M., Aust, D., Obholzer, R., English Specialist NF research group. (2018). The data that support the findings of this study are openly available in the (Global Variome shared LOVD) at https://databases.lovd.nl/shared/variants/0000602980. Reference number: 0000602980.

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REFERENCES

Campbell, I. M., Shaw, C. A., Stankiewicz, P., & Lupski, J. R. (2015). Somatic mosaicism: Implications for disease and transmission genetics. Trends in Endocrinology and Metabolism, 26, 53–59. https://doi.org/10.1016/j.tem.2014.12.006

Enzi, G., Busetto, L., Ceschin, E., Coin, A., Digito, M., & Piggozzo, S. (2002). Multiple symmetric lipomatosis: Clinical aspects and outcome in a long-term longitudinal study. International Journal of Obesity and Related Metabolic Disorders, 26, 253–261.

Evans, D. G., Hartley, C. L., Smith, P. T., King, A. T., Bowers, N. L., Tobi, S., Obholzer, R., English Specialist NF research group. (2019). Incidence of mosaicism in 1055 de novo NF2 cases: Much higher than previous estimates with high utility of next-generation sequencing. Genetics in Medicine, 22, 53–59. https://doi.org/10.1016/j.gim.2019.05.019

Gieldon, L., Masjkur, J. R., Richter, S., Därr, R., Lahera, M., Aust, D., Obholzer, R., English Specialist NF research group. (2018). The data that support the findings of this study are openly available in the (Global Variome shared LOVD) at https://databases.lovd.nl/shared/variants/0000602980. Reference number: 0000602980.
deletions. Human Genetics, 136, 349–376. https://doi.org/10.1007/s00439-017-1766-y

Kluwe, L. (2016). Digital PCR for discriminating mosaic deletions and for determining proportion of tumor cells in specimen. European Journal of Human Genetics, 24, 1644–1648. https://doi.org/10.1038/ejhg.2016.56

Kluwe, L., Mautner, V., Heinrich, B., Dezube, R., Jacoby, L. B., Friedrich, R. E., & MacCollin, M. (2003). Molecular study of frequency of mosaicism in neurofibromatosis 2 patients with bilateral vestibular schwannomas. Journal of Medical Genetics, 40, 109–114.

Messiaen, L. M., Callens, T., Mortier, G., Beysen, D., Vandenbroucke, I., Van Roy, N., ... Paepe, A. D. (2000). Exhaustive mutation analysis of the NF1 gene allows identification of 95% of mutations and reveals a high frequency of unusual splicing defects. Human Mutation, 15, 541–555.

Parisien-La Salle, S., Dumas, N., Rondeau, G., Latour, M., & Bourdeau, I. (2019). Isolated pheochromocytoma in a 73-year-old man with no clinical manifestations of type 1 neurofibromatosis carrying an unsuspected deletion of the entire NF1 gene. Frontiers in Endocrinology, 10, 546. https://doi.org/10.3389/fendo.2019.00546

Roehl, A. C., Mussotter, T., Cooper, D. N., Kluwe, L., Wimmer, K., Högel, J., ... Kehrer-Sawatzki, H. (2012). Tissue-specific differences in the proportion of mosaic large NF1 deletions are suggestive of a selective growth advantage of hematopoietic del(+/−) stem cells. Human Mutation, 33, 541–550.

Steinmann, K., Cooper, D. N., Kluwe, L., Chuzhanova, N. A., Senger, C., Serra, E., ... Kehrer-Sawatzki, H. (2007). Type 2 NF1 deletions are highly unusual by virtue of the absence of nonallelic homologous recombination hotspots and an apparent preference for female mitotic recombination. American Journal of Human Genetics, 81, 1201–1220.

Vogt, J., Bengesser, K., Claes, K., Wimmer, K., Mautner, V. F., van Minkelen, R., ... Kehrer-Sawatzki, H. (2014). SVA retrotransposon insertion-associated deletion represents a novel mutational mechanism underlying large genomic copy number changes with non-recurrent breakpoints. Genome Biology, 15, R80. https://doi.org/10.1186/gb-2014-15-6-r80

Vogt, J., Mussotter, T., Bengesser, K., Claes, K., Högel, J., Chuzhanova, N., ... Kehrer-Sawatzki, H. (2012). Identification of recurrent type-2 NF1 microdeletions reveals a mitotic nonallelic homologous recombination hotspot underlying a human genomic disorder. Human Mutation, 33, 1599–1609. https://doi.org/10.1002/humu.22171

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