Short Report

Ring chromosome 22 and neurofibromatosis type II: proof of two-hit model for the loss of the \(NF2\) gene in the development of meningioma


Carriers of a ring chromosome 22 are mentally retarded and show variable facial dysmorphism. They may also present with features of neurofibromatosis type II (NF2) such as vestibular schwannomas and multiple meningiomas. In these cases, tumourigenesis has been suspected to be caused by the loss of both alleles of the \(NF2\) gene, a tumour suppressor localized in 22q12.2. Here, we describe an 18-year-old patient with constitutional ring chromosome 22 and mental retardation who developed rapid-onset spastic paraparesis at the age of 15 years. The causative spinal meningioma at the level of T3, which compressed the spinal cord, was surgically removed, and the patient regained ambulation. Array comparative genomic hybridization (array CGH) and multiplex ligation-dependent probe amplification (MLPA) analyses in blood revealed a terminal deletion in 22q13.32, not comprising the \(NF2\) gene. In tumour tissue, loss of the whole ring chromosome 22 including one \(NF2\) gene due to mitotic instability constituted the likely first hit, while a point mutation in the other allele of the \(NF2\) gene (c.784C>T, p.R262X) was shown as second hit. We review all cases from the literature and suggest clinical guidelines for surveillance of patients with ring chromosome 22.

Conflict of interest
None of the authors has financial or personal relationships that bias the submitted manuscript.

Ring chromosome 22 is a rare constitutional cytogenetic aberration, in which the formation of a ring with breakage in both chromosome arms, loss of distal fragments and fusion of the points of fracture leads to a terminal deletion in 22q. Therefore, carriers of a ring chromosome 22 present with most of the features of the 22q13.3 deletion syndrome such as mental retardation, absent expressive speech and variable facial dysmorphism (1). Moreover, clinical features of neurofibromatosis type II (NF2) are reported in some patients with constitutional ring chromosome 22 (2–8).

NF2 is characterized by the occurrence of different tumours such as vestibular schwannomas, multiple meningiomas, and neurofibromas (9). The \(NF2\) gene, localized on chromosome 22q12.2, acts as tumour suppressor (10). According to the two-hit model (11) the loss of both \(NF2\) alleles initiates
tumourigenesis. NF2 patients with two normal chromosomes 22 usually carry a germline NF2 mutation in one allele and acquire a second somatic mutation in the other allele, which then gives rise to uncontrolled cell proliferation.

In patients with ring chromosome 22, the NF2 gene is usually present and intact within the ring (7). However, the ring itself is prone to loss during mitotic cell division (12). For that reason, carriers of a ring chromosome 22 may become mosaic for monosomy 22 and lose one NF2 allele. An NF2 gene mutation on the second allele has been postulated as trigger for tumourigenesis in somatic cells, having lost the entire chromosome 22. Here, we present a patient with ring chromosome 22 and meningioma due to somatic loss of the ring chromosome in combination with a point mutation in the NF2 gene in tumour tissue.

The patient is the second child of healthy non-consanguineous parents. An older brother and all family members in three generations are healthy. The boy was born spontaneously at term after normal pregnancy. Birth weight was 3240 g (25th percentile), length 50 cm (25th percentile), and head circumference 34 cm (25th percentile). He was able to sit independently at age 11 months and started walking at age 24 months. The boy’s further motor milestones were also delayed, and he did not acquire active speech. A diagnosis of severe global retardation was made at age 5 years, and he was enrolled to a school for mentally handicapped children. His character is described as friendly, but sometimes he shows hyperactive and aggressive behaviour.

At age 14.5 years, the boy developed gait problems. On examination, he walked clumsily, while his neurological status was otherwise normal. Apart from deep-set ears, a rather long face, and a low frontal hairline, no dysmorphic features were observed (Fig. 1a–c). Examination of the skin yielded two café au lait spots measuring 0.5 × 1 cm (right cheek) and 1 × 2.5 cm (thorax). Neurofibromas were not present. Weight and height were within the normal range, while head circumference was 1.5 cm below the third percentile (51.5 cm). His motor problems were explained by a pubertal growth spurt and his global retardation. However, within the next months, the boy’s motor functions worsened and his walking distance steadily decreased. At age 15 years, neurological examination revealed a spastic paraparesis of the legs with exaggerated tendon reflexes and a bilaterally positive Babinski sign. Spinal magnetic resonance imaging (MRI) disclosed a larger intramedullary contrast-enhancing tumour at the level of T3, compressing the spinal cord, and a smaller intramedullary tumour at the level of C7 (Fig. 1d,e). Cranial MRI, ophthalmological examination, audiometry, and auditory evoked potentials gave normal results. The compressing tumour could be completely removed and histopathological examination displayed a grade I meningioma. The patient was discharged to a rehabilitation unit and regained ambulation within 2 months. MRI after 6, 12, and 24 months showed no recurrence of the extramedullary meningioma, while the upper intramedullary tumour remained unchanged. Actually, at age 18 years, the young man’s neurological status is normal. Annual follow-up examinations have neither revealed a cataract nor a vestibular schwannoma.

Cytogenetic analysis of peripheral blood lymphocytes revealed a constitutional ring chromosome 22 in 25 analysed cells with identical size (Fig. 2b). Fluorescence in situ hybridization (FISH) with subtelomeric probe 22qter (D22S1056, Kreatech, Amsterdam, the Netherlands) and 22q11.2 (BCR, Abbott, Wiesbaden, Germany) as a control revealed absence of subtelomeric sequences on the ring chromosome [karyotype 46,XY,r(22)(p11q13).ish r(22)(qter-,BCR+)]. The karyotypes of both parents were normal. Array CGH (Agilent, 180K array, Santa Clara, CA) was performed for further delineation of the size and break point on the distal part of chromosome 22 and revealed a deletion of approximately 1.57 Mb with break point in band 22q13.32 [Arr cgh chr22: g.(47866712_49565816) (NCBI build 36.3)]. The NF2 gene on 22q12.2 lies proximal of the break point and is not deleted. The deletion was confirmed by MLPA (Fig. 2a).

NF2 gene sequencing and deletion analysis by MLPA on DNA from peripheral lymphocytes did not reveal any abnormality. In DNA extracted from the spinal meningioma, both an NF2 gene point mutation (c.784 C>T in exon 8, nonsense mutation R262X, Fig. 2c) and a complete NF2 gene deletion were identified (heterozygous loss of all NF2 exons and two probes located 21.9 kb 5‘ of the promoter and 26 kb downstream of the NF2 gene, Fig. 2d). Moreover, a probe on 22q11 was heterozygously deleted indicating complete loss of one chromosome 22 in tumour cells (Fig. 2d). To exclude a low-grade mosaicism for the NF2 gene point mutation in somatic tissue, we reanalysed a further blood sample as well as oral mucosa cells of the patient and did not find the mutation c.784 C>T.

Thus, our combined approach of cytogenetic and molecular genetic methods indicated a loss of the ring chromosome 22 as probably first hit and a somatic NF2 gene mutation (c.784C>T, p.R262X)
as second hit in tumour tissue. These analyses prove that the two-hit model is the pathogenetic mechanism leading to the development of meningioma.

In 90% of patients with NF2 due to germline mutations, bilateral vestibular schwannomas are detected on MRI and more than 90% suffer eye lesions, most commonly juvenile subcapsular cataract. About 50% of affected subjects develop spinal lesions, but only 40% of these tumours are symptomatic (9). As in our case, intramedullary processes, usually spinal astrocytomas or ependymomas, and extramedullary tumours such as schwannomas or meningiomas can occur. In our patient, a symptomatic spinal meningioma manifested at the relatively young age of 15 years, while no vestibular schwannoma or cataract could be detected until the current age of 18 years. Although our patient does not meet the criteria for the clinical diagnosis of NF2 (9) yet, it is most probable that he will develop further features of NF2 in future.

In addition to our patient, nine patients with constitutional ring chromosome 22 and features of NF2 have been described to date (Table 1). Four of these presented with multiple meningiomas without vestibular schwannomas, whereas two had vestibular schwannoma and no meningioma. This inconsistent clinical picture and the variability of age at manifestation most probably result from

Fig. 1. (a–c) Photographs of the patient at age 16 years. Note the two café au lait spots (indicated by arrows). Sagittal T2-weighted (d, e) and T1-weighted post-contrast (f) magnetic resonance images. Notice a larger, extramedullary contrast-enhancing tumour at the level of T3 (long arrow), and a smaller intramedullary with cystic widening of the central canal at the level of C7 (small arrows).
Fig. 2. (a) The patient’s terminal deletion in 22q13.32 was confirmed in a blood sample by MLPA analysis using the SALSA P356 kit (MRC-Holland, Amsterdam, the Netherlands) which contains 17 probes in the terminal 4.5 Mb of chromosome arm 22q. The heights of the columns represent the dosage of the respective segments in genomic DNA [level ‘1’ (0.8–1.2) corresponds to two alleles, ‘0.5’ (0.4–0.6) corresponds to deletion of one allele]. Note the deletion of several genes (indicated by black columns, e.g. deletion of the \textit{SHANK3} gene). (b) Cytogenetic analysis in peripheral blood lymphocytes revealed a constitutional ring chromosome 22 of identical size in all metaphases analysed. (c) Sequence analysis in tumour tissue identified the mutation c.784C>T in exon 8, whereas this mutation was not found in the corresponding wild type DNA in blood. Note the small signal peak that represents the second normal allele present in contaminated non-tumour tissue. (d) MLPA analysis in tumour tissue (SALSA P044 NF2 kit, MRC-Holland) revealed loss of one complete chromosome 22. The black columns correspond to reduced allele dosages for all 17 coding exons of the \textit{NF2} gene and \textit{NF2} promoter regions as well as reduced allele dosage for a fragment in 22q11. The dosage level ranges about 0.6 as non-tumour tissue is present in a small fraction of the probe (compare Fig. 2c). Grey columns indicate control fragments on other chromosomes without deletion.

dynamic mosaicism. This means that the \textit{NF2} gene, primarily present within the ring 22 (5, 7, 8), is lost as a result of mitotic instability of the whole ring chromosome 22 during cell division in a variable percentage of cells. The frequency of monosomic cells may differ between cell types.
Table 1. Synopsis of patients with ring chromosome 22 and features of NF2

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Age at tumour manifestation</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Multiple meningiomas, MR, two café au lait spots</td>
<td>15 Years</td>
<td>This manuscript</td>
</tr>
<tr>
<td>Multiple meningiomas, MR</td>
<td>25 Years, death at age 27</td>
<td>(2)</td>
</tr>
<tr>
<td>Neurofibromas</td>
<td>Not reported</td>
<td>(3)</td>
</tr>
<tr>
<td>Multiple meningiomas, MR, seizures</td>
<td>Death at age 16 years</td>
<td>(4)</td>
</tr>
<tr>
<td>Neurofibromas, peripheral neurofibromas, optic atrophy, MR</td>
<td>Not reported</td>
<td>(5)</td>
</tr>
<tr>
<td>Bilateral vestibular schwannomas, multiple meningiomas, MR, goitre, MR</td>
<td>38 Years</td>
<td>(6)</td>
</tr>
<tr>
<td>Bilateral vestibular schwannomas, multiple meningiomas, multiple neurinomas, MR, paranoid psychosis</td>
<td>52 Years</td>
<td>(7)</td>
</tr>
<tr>
<td>Vestibular schwannoma, multiple meningiomas, goitre, MR</td>
<td>Puberty</td>
<td>(7)</td>
</tr>
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MR, mental retardation; NF2, neurofibromatosis type II.

as well as individuals and may rise with age. Carriers of a ring chromosome 22 are thus affected by a continually evolving, i.e. dynamic process. Absence of vestibular schwannomas and an overall milder NF2 phenotype in subjects with ring chromosome 22 compared to carriers of germline mutations may be explained by the fact that only a fraction of cells harbour monosomy 22. In parallel, a rather mild phenotype of neurofibromatosis type I has also been described in a girl with ring chromosome 17 (13).

Kehrer-Sawatzki et al. (6) investigated the occurrence of multiple tumours (meningiomas and bilateral vestibular schwannomas) in a patient with ring chromosome 22 by applying molecular genetic techniques. Using a meningioma cell line lacking the ring 22, they searched for mutations and deletions on the remaining NF2 allele, but detected no alterations. By contrast, Tsilchorozidou et al. (7) found two truncating NF2 gene mutations (c.531_560del29 and c.1188dupA), the first at low level, in meningioma tissue, and confirmed the loss of the ring chromosome 22 by heterozygosity analysis. The affected patient was a 39-year-old woman with mental retardation as well as multiple meningiomas and unilateral vestibular schwannoma. In comparison to our patient, mental retardation in this woman appears to be less severe, as she was able to read and write at the age of 9 years which our patient is not.

Prior to these molecular analyses, it was speculated that other tumour suppressor genes such as CHEK2 in 22q12.1 and SMARCB1/INI in 22q11.23 may have a pathogenetic impact in the development of multiple meningiomas and vestibular schwannomas in patients with ring chromosome 22 (3, 5, 7). However, the molecular genetic findings in our patient and the patient of Tsilchorozidou et al. (7) indicate that the NF2 gene is responsible for the development of meningiomas. In addition, the biallelic loss of the NF2 gene was recently also proven in the development of vestibular schwannoma in a patient with ring chromosome 22 (8).

In our patient, the presence of the NF2 gene on the ring chromosome was determined by array CGH showing break points in 22q13.32 [chr22: g.(47866712_49565816)del], confirmed by MLPA. Only one other patient with ring chromosome 22 has been analysed by array CGH to date, and a similar telomeric deletion of 22q13.32-qter was found [chr22: g.(47248304_49453810)del (8)]. As chromosome 22 is an acrocentric chromosome, it contains redundant information on the short arm that is not relevant for the phenotype. The deleted region on the long chromosome arm 22 comprises 47 genes. Of these, the SHANK3 gene is the most probable candidate gene for mental retardation, speech impairment and mild facial dysmorphism in patients with ring chromosome 22. Similar clinical features occur in patients with the deletion syndrome 22q13 (14). Moreover, SHANK3 point mutations have been reported in
patients with neurodevelopmental disorders such as autism and schizophrenia (15–17). The application of array CGH in additional patients with ring chromosome 22 is crucial to confirm a possible recurrent break point in 22q13.32.

The transmission of ring chromosome 22 from parent to child has not yet been reported. However, detailed clinical description of additional patients will help to further delineate the clinical spectrum in ring chromosome 22 and learn about its variability, especially with regard to the severity of mental retardation and the occurrence of tumours. This will improve genetic counselling for prenatal diagnosis when a de novo ring chromosome 22 is detected in the foetus.

A tumour predisposition is also known for carriers of other ring chromosomes, e.g. ring 11 [Wilms tumour (18)], and ring 17 [NF1 (13)]. We conclude that early screening for specific tumours should be performed in all children and adults with ring chromosomes that harbour tumour suppressor genes. Carriers of a ring chromosome 22 should undergo regular neurological examination and audiometry (e.g. twice a year) for recognition of early clinical signs of meningiomas (e.g. gait anomalies) and vestibular schwannomas (hearing loss). In addition, all patients with ring chromosome 22 should undergo cranial and spinal MRI at the age of 15–20 years, as this is the critical age of first tumour manifestation (compare Table 1). The interval of following MRIs should be adjusted according to previous findings and the current neurological status.

References