Short Report

Modification of risk for cancer as a coincidental finding in DNA array investigation


The high resolution of modern DNA arrays has the implication of unintended coincidental detection of gene deletions predisposing to late-onset neurological and oncological disorders. Here, we report the case of an 18-year-old girl with mild intellectual disability, facial dysmorphisms, and a microdeletion of approximately 6.3 Mb on 22q12.1q12.3 including NF2, the gene for neurofibromatosis type 2, and CHEK2, a modifier gene for breast cancer. Subsequent magnetic resonance imaging of the brain showed she had already developed bilateral vestibular schwannomas. The challenge of DNA arrays and the consequences for genetic counselling and informed consent will be discussed in the light of this unique case with a microdeletion including both a high risk and a moderate risk cancer predisposition gene.

Conflict of interest
Nothing to declare.

High-resolution DNA arrays are a new and powerful tool to detect submicroscopic deletions and duplications in patients with multiple congenital anomalies (MCA) and/or intellectual disability (ID). Dependent on the localization and gene content such a deletion or duplication might be a non-pathological copy number variant or causative for MCA and/or ID as well as other disorders. Much more frequently than conventional karyotyping, DNA-array analysis may render information which was not expected but has major harmful implications later in life.

Here, we discuss the clinical impact of a coincidental array result showing a microdeletion of approximately 6.3 Mb located on 22q12.1q12.3 including NF2 and CHEK2, two genes relevant for tumourigenesis in an 18-year-old girl who was tested because of minor dysmorphisms and mild ID.

Clinical report
The girl is the third child of healthy, non-consanguineous parents, who were 28 (mother) and 31 (father) years old at her birth. Pregnancy and birth at term were unremarkable. Length (52 cm), weight (3270 g), and occipitofrontal head circumference (36 cm) were in the upper normal ranges. Apgar was 8/9/10. In the first days, a talus verticalis, which later on was repeatedly operated, was identified. Developmental milestones were delayed in all major areas. She was able to walk at the age of 2 years and 6 months. Toilet training was achieved at 11 years. She attended a special school for children with developmental delay. Neuropsychological testing performed at the age of 18 years confirmed the impairment in all major cognitive areas with an IQ of 55. Menstrual bleeding started late and is irregular.
Modification of risk for cancer as a coincidental finding

Clinical examination at the age of 18 years showed dysmorphic features including broad eye brows, short palpebral fissures, telecanthus, deep-set eyes, strabismus, microgenia but no cleft palate or submucosal cleft, posteriorly rotated ears, and tapering fingers (Fig. 1). Length (170 cm), weight (65 kg), occipitofrontal head circumference (57 cm), total hand length (19 cm) and middle finger length (9 cm) were in the normal range.

Detailed neurological investigation performed subsequently to the array result revealed an intention tremor more pronounced on the left side and a mild truncal and gait ataxia. Hearing examination showed a moderate sensorineural hearing loss affecting both ears. Magnetic resonance imaging of the brain and spine revealed two large contrast enhancing lesions in the cerebellar-pontine angle highly suggestive of bilateral vestibular schwannomas (Fig. 1). Both lesions were hypointense on axial T1-weighted sequences and already showed impingement on cerebellar and brainstem structures. No other tumours were present on cranio-spinal imaging.

Methods and results

Routine cytogenetic studies revealed a normal karyotype in the patient. By array-based copy number analysis using the Illumina CytoSNP12v2.1 (Illumina, San Diego, CA), which has an average resolution of about 10 kb, a heterozygous deletion of 6.26 Mb located on the long arm of chromosome 22 was found. Breakpoints were localized proximal to single-nucleotide polymorphism (SNP) rs5752726 (28,757,950 bp) and distal to SNP rs5749872 (34,930,216 bp) on 22q12.2 (NCBI built 36.1) (Fig. 2). The deletion contains a minimum of 76 genes, among them are the NF2, the CHEK2, and the EWSR1 genes. The deletion was confirmed by fluorescence in situ hybridization (FISH) with the Vysis LSI EWSR1 dual colour break apart rearrangement probe performed according to standard protocols as well as by multiplex ligation dependent probe amplification (MLPA) analysis with NF2-specific probes [SALSA MLPA-Kit P044 (MRC Holland, Amsterdam, The Netherlands; Lot. Nr. 0307)]. A balanced insertion was ruled out in both parents by FISH. Polymerase chain reaction of a panel of highly polymorphic chromosome 22 microsatellite markers indicated maternal origin of the deletion.

Discussion

Neurofibromatosis type 2 (NF2) is a tumour predisposition syndrome resulting from mutations in NF2, a tumour suppressor gene, located on 22q12.2 (1). The gene codes for merlin (moesin–ezrin–radixin-like protein), a protein which is localized to the cell...
Fig. 2. Molecular karyotyping using a Human CytoSNP-12v2 array (Illumina) revealed a 6.2-Mb deletion on chromosome 22q12.1-q12.3. Copy number information is given as log R ratio (LRR), the logged ratio of observed probe intensity to expected intensity (Norm = 0) (upper panel). Genotype information is given as B-allele frequency (BAF), with homozygosity for the arbitrary 'B' allele indicated by BAF = 1, homozygosity for the arbitrary 'A' allele indicated by BAF = 0, and heterozygosity for A/B indicated by BAF = 0.5 (lower panel). The upper diagram (LRR) shows a clear loss of signal intensity in the deleted region (red bar), while the lower diagram shows loss of heterozygosity in the same region (absence of the BAF = 0.5 dots). Panels were extracted from NEXUS® (BioDiscovery, El Segundo, CA).

membrane–cytoskeletal interface and plays a role in cell-to-cell adhesion, cytoskeletal architecture, and by interaction with cytosolic proteins. The incidence of NF2 has been calculated to be up to one in 25,000–33,000 live births (1, 2). Bilateral vestibular schwannomas are the hallmark of NF2. The average age of onset is 18–24 years and almost all patients carrying a germline mutation develop symptoms by the age of 30 years (1). In addition to vestibular schwannomas, patients can also develop schwannomas of spinal roots and peripheral nerves as well as meningo- mas and ependymomas. Investigating 116 NF2 patients Bruder et al. found deletions associated with a mild, moderate, and severe phenotype (3). However, genotype–phenotype correlation indicates a later age at diagnosis and a lower prevalence/proportion of all features of neurofibromatosis type II in patients with an intragenic NF2 deletion than with nonsense/frameshift mutations (4). As in a case reported by Bruder et al. in 1999 most patients with very large deletions including neighbouring genes show a severe phenotype (5).

Inheritance of NF2 is autosomal dominant and so – according to Knudson’s two-hit hypothesis of tumourigenesis – haploinsufficiency through a microdeletion as in our patient can be the first step towards formation of multiple tumours. Molecular NF2 testing is usually initiated in patients with a positive family history and/or the presence of one or more typical tumours at an earlier age than in the average population. In contrast to this usual diagnostic situation, the mutation in our case was found by chance as part of a diagnostic work-up for MCA/ID. The situation is different from regular gene-specific and most clinical diagnostic situations, where a specific disorder discussed beforehand is investigated. According to international recommendations, the patient and her parents were generally advised beforehand that unexpected results might be possible but not specifically of the impact of tumour predisposition genes like the NF2 gene or the CHEK2 gene, and particularly not on the different risks of these genes. Nevertheless, the finding of haploinsufficiency of NF2 allows appropriate follow-up evaluations according to international guidelines (6).

Completely different is the situation for CHEK2 (cell cycle checkpoint kinase 2) haploinsufficiency. The CHEK2 gene product is a signalling component in DNA repair. Heterozygosity for the recurrent CHEK2 mutations, the most well-studied one being c.1100delC, is assumed to be associated with a 2–4.8 relative risk and a lifetime risk of approximately 25% for breast cancer in those women with a first degree relative with breast cancer (7, 8). Relative risk figures are not known for deletions of the entire CHEK2 gene. However, in contrast to, for example, mutations in the tumour suppressor genes BRCA1 and BRCA2, which confer a high tumour risk to any mutation carrier, the CHEK2 gene appears to function as a modifier of risk of other susceptibility genes (9, 10). Therefore, it is not recommended to test for the known CHEK2 mutations in individuals without a personal
or family history of breast cancer (10). The same is true for surveillance or even prophylactic therapy. On the basis of these considerations as well as on the recommendations of Offit and Garber (11), who do not recommend 1100delC-dependent adaption of therapy or surveillance, at the moment we did not propose a specific breast cancer surveillance program to our patient.

This report adds another and unique case to the so far only limited number of patients where an increased tumour risk was identified in a similar situation – i.e. by array analysis that was initiated to uncover the aetiology of unexplained MCA/ID. Schwarzbraun et al. discussed the challenges for counselling and clinical management in a patient with MCA/ID in whom a deletion on 17p13.1 including the TP53 gene causing the cancer prone Li-Fraumeni syndrome was identified (12). Subsequently, several other cases with MCA/ID and a deletion including TP53 have been reported (13–15). Chabchoub et al. described a 17-year-old male with MCA/ID, epilepsy, and a duplication of 251 kb including IRAK2 and VHL, the gene for Von Hippel-Lindau syndrome (16). Adam et al. reported on an 8-year-old boy with MCA/ID and a deletion of 1.1 Mb on 19p13.3 including STK11, the gene for Peutz-Jeghers syndrome (14). Using a targeted CGH array Adams et al. found 30 microdeletions and 4 microduplications implying cancer predisposition in 18,437 patients with developmental disabilities and congenital anomalies (0.18%) (17). Most recently Pichert et al. reported on chromosomal imbalances (i.e. 14 deletions, 14 duplications, and 1 mosaic duplication) including different cancer predisposition genes in 29 out of 4805 patients (0.6%) referred for routine diagnostic array investigation with the Agilent oligonucleotide arrays 44 K platform due to MCA/ID (18). The authors recommend pre- and post-testing discussion of this possibility. The results of both studies are in agreement with general epidemiological studies indicating a slightly increased overall cancer risk in children with birth defects (19). In daily pre-testing genetic counselling, however, it might be difficult to find the balance between relevant information and scaring the patients and their families, the latter particularly in a clinical setting not familiar with oncological genetic counselling.

In conclusion, the patient reported here is another and unique example for the power of high-resolution DNA-array investigations. Coincidental and unintended results can outweigh the initial diagnostic goal. Genetic counselling should keep in mind that successful diagnostic work-up might imply new issues and uncertainties not truly realized beforehand.

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References