Next-generation sequencing reveals germline mutations in an infant with synchronous occurrence of nephro- and neuroblastoma

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ABSTRACT

Although neuro- and nephroblastoma are common solid tumors in children, the simultaneous occurrence is very rare and is often associated with syndromes. Here, we present a unique case of synchronous occurrence of neuro- and nephroblastoma in an infant with no signs of congenital anomalies or a syndrome. We performed genetic testing for possible candidate genes as underlying mutation using the next-generation sequencing (NGS) approach to target 94 genes and 284 single-nucleotide polymorphisms (SNPs) involved in cancer. We uncovered a novel heterozygous germline missense mutation p.F58L (c.172T→C) in the anaplastic lymphoma kinase (ALK) gene and one novel heterozygous rearrangement Q418Hfs*11 (c.1254_1264delins TTACTAGTACAAGAACTG) in the Fanconi anemia gene FANCD2 leading to a truncated protein. Besides, several SNPs associated with the occurrence of neuroblastoma and/or nephroblastoma or multiple primary tumors were identified. The next-generation sequencing approach might in the future be useful not only in understanding tumor etiology but also in recognizing new genetic markers and targets for future personalized therapy.

Introduction

Neuroblastoma (NB) is the most common extracranial solid tumor in childhood and the most frequently diagnosed neoplasm during infancy. NB accounts for 7%–10% of all pediatric malignancies [1, 2]. The incidence rates of NB in infants and young children support the hypothesis of a major role of hereditary factors [3, 4]. Survival rates vary considerably between
Figure 1. ALK and FANCD2 protein structures (adapted from references 12, 31). (a) The structure of ALK consists of a long ECD (residues 19–1038) and a TK domain within the ICD. A novel germline ALK mutation (c.172T→C) at codon 58 was identified. SP, signal peptide; ECD, extracellular domain; TM, transmembrane; TK, tyrosine kinase; ICD, intracellular domain. (b) FANCD2 consists of 4 domains; a novel FANCD2 rearrangement composed of a deletion of 11 bp and a concurrent insertion of 19 bp at the same site of the gene was identified. NLS, nuclear localization signal; CUE, coupling of ubiquitin conjugation to endoplasmic reticulum degradation; PIP-box, PCNA-interacting protein motif; EDGE, C-terminal motif.

low-, intermediate-, and high-risk disease. Despite intensive multimodal therapy, overall survival rates remain low for the high-risk group [1].

Wilms tumor (WT), also known as nephroblastoma, is the fifth most common pediatric malignancy and accounts for approximately 6% of all pediatric cancers. Approximately 75% of cases occur in children younger than 5 years of age [5, 6]. With adequate therapy, outcome for children with WT results in a survival rate greater than 90% [7].

Advances in the field of genomics have led to multiple recent discoveries in the understanding of genetic predisposition and molecular pathogenesis of childhood cancers. Next-generation sequencing (NGS) technologies are helpful in understanding genetic alterations in a timely and comprehensive manner. As part of these advances, genomic alterations in neuroblastoma are now much better understood. Only a few single-gene alterations have been shown to be driver mutations in NB oncogenesis [8]. Activating mutations of the anaplastic lymphoma kinase (ALK) gene have been shown to occur in more than half of rare familial neuroblastoma and in approximately 8%–10% of sporadic cases at diagnosis [9–11]. ALK is a receptor tyrosine kinase (RTK) and consists of several domains (Figure 1 [12]). Until now, over 50 different ALK mutations affecting 12 different amino acid residues have been described, with most mutations affecting the tyrosine kinase domain (TKD) and leading to constant activation [9–11, 13]. Apart from NB, the receptor ALK was found to be mutated in many types of cancers (i.e., T-cell non-Hodgkin lymphoma [T-NHL], inflammatory myofibroblastic tumor, renal medullary carcinoma) [12, 14]. Just recently, ALK expression was also described in nephroblastoma [15]. Other, less-frequent predisposing genes in NB include protein tyrosine phosphatase, non-receptor type 11 (PTPN11), Alpha thalassemia/mental retardation syndrome X-linked (ATRX), and Na+/K+ transporting ATPase interacting 2 (NKAIN2) [16, 17]. Besides, single-nucleotide polymorphisms (SNPs) that are believed to confer susceptibility to neuroblastoma have been discovered within multiple genes, such as BRCA1-associated RING domain protein 1 (BARD1), Cancer susceptibility candidate 15 (LINC00340), Lin-28 homolog B (LIN28B), HECT domain and ankyrin repeat containing E3 ubiquitin protein ligase 1 (HACE1) and LIM domain only 1 (LMO1) [18–20].

Genetic alterations are firmly believed to contribute to WT, especially in young patients [21]. However, the underlying basis of most Wilms tumor cases is unknown [22]. The most studied gene of Wilms tumor is WT1 [5]. Germline mutation in WT1 may be associated with syndromic conditions such as WAGR (Wilms tumor-aniridia-genitourinary malformation-retardation) and Denys-Drash syndromes. Genetic or epigenetic defects at chromosome 11p15.5 (WT2) leading to Beckwith-Wiedemann syndrome also predispose
to embryonal tumors, especially WT but also NB [23]. The majority of WT, however, are sporadic and only have somatic mutations. Other than the described defects of WT1 and WT2, other genes such as Dicer1, (Cadherin-Associated Protein) Beta 1 (CTNNB1), and tumor protein (TP53) have also been implicated in tumorigenesis of WT [24, 25]. Apart from nephroblastoma, TP53 is involved in tumorigenesis across numerous tumor entities. Likewise, DNA repair genes have been linked in oncogenesis of multiple cancers [26]. DNA repair mechanisms are important to maintain genomic stability. Hence, accumulation of DNA damage due to genetic deficiencies of DNA repair genes lead to tumor development [26–28]. Particularly, germline mutations in the Fanconi anemia (FA) genes Breast Cancer 2 (BRCA2) and Partner And Localizer Of BRCA2 (PALB2) have been associated with the development of embryonal tumors, including Wilms tumors and neuroblastoma [29]. Fanconi anemia is a rare recessive disease resulting from mutations in 1 of at least 15 different genes and characterized by chromosome instability, several congenital defects, a predisposition to develop early-onset progressive bone marrow failure, and an increased risk of developing cancer [30, 31]. The most severe phenotype is caused by mutations in Fanconi Anemia Complementation Group D2 (FANCD2). Affected individuals show a high prevalence of congenital defects and an earlier onset of FA-associated disorders [31]. Moreover, Fancd2−/− mice show an increased incidence of tumors [31]. FANCD2 consists of 1451 amino acids, and until now, only 4 domains have been described (Figure 1 [31]). Just as in NB, there are several tumor susceptibility SNPs identified predisposing to WT [21].

Here, we report a next-generation sequencing–based analysis strategy to capture common genetic germline information in an infant diagnosed with the simultaneous occurrence of neuro- and nephroblastoma.

Materials and methods

Saliva and tumor samples from the patient and informed consent from the patient’s parents were obtained before genetic analysis. Formal approval of the local ethics committee for this study was not required, as this was a single-case investigation. Genetic analysis was performed by next-generation sequencing to target 94 genes and 284 single-nucleotide polymorphisms (SNPs) previously identified to be involved in cancer predisposition and oncogenesis (TruSight Cancer Panel, Illumina, FC-121-0202; Eindhoven, Netherlands). Genomic DNA was extracted from salivary using Oragene DNA (OG-575) from DNA Genotek (Ottawa, Canada). Tumor DNA was extracted from paraffin using QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. Fifty nanograms of DNA of patient’s saliva was used for the library construction according to the manufacturer’s instructions (Nextera Rapid Capture; Eindhoven, Netherlands). Paired-end sequencing was performed on an Illumina NextSeq 500 platform (2 × 150 cycles). Data were processed using BWA Enrichment version 1.0 (Eindhoven, Netherlands) for the generation of BAM files and Genome Analysis Toolkit (GATK) for variant calling. Analysis of variants was performed with the VariantStudio software (Illumina), which applies the PolyPhen algorithm for the identification of potential deleterious variants. For Sanger sequencing, the primers (available on request) were designed encompassing each identified alteration. Fifty nanograms of the DNA was used for a conventional polymerase chain reaction (PCR) using 0.1 U of Taq polymerase (Axon Labortechnik, Kaiserslautern, Germany), 0.4 mM of each primer, 200 mM dNTP mix, 1.5 mM MgCl2, as well as 0.2 M betaine and the following PCR conditions: an initial denaturation step at 94°C for 5 minutes, 36 cycles at 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 60 seconds, and a final extension step at 72°C for 10 minutes. PCR products were purified
by an enzymatic method using 10 U exonuclease I (Biolabs, Ipswich, USA) and 2 U shrimp alkaline phosphatase (SAP) for 30 minutes at 37°C and 15 minutes at 80°C and sequenced by using ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, California) and the BigDye v3 Terminator Kit (ABI). The sequences were compared with the reference sequence using Sequencher program (Gene Codes, Ann Arbor, Michigan, USA). To separate the alleles of FANCD2, the PCR product was inserted into pGEM-T easy vector (Promega, Sunnyvale, California). Colonies were picked and used directly for the subsequent PCR reaction with M13 primers; the PCR products were sequenced as described above.

**Results**

**Clinical description**

An 11-month-old male infant was referred to our center in suspicion of a nephroblastoma for further diagnostic evaluation. Clinical examination showed no symptoms or pathological abnormalities. We did not observe any apparent congenital anomalies or a syndromal appearance, especially no macrosomia, macroglossia, aniridia, visceromegaly, or retardation. Pregnancy and birth history revealed no abnormalities. Until this date, medical history was unremarkable and the patient's growth chart showed normal development. The patient was the youngest child of a family with 5 children. All other siblings (female, 7 years old; female, 6 years old; female, 4 years old; male, 2½ years old) were healthy at the time. Other than breast cancer (diagnosed at the age of 43) of the mother's mother, there was no cancer in the family history.

**Diagnostics**

Blood tests showed a 2-fold increase of neuron-specific enolase as well as increased nor- and metanephrines. Urine samples revealed increased levels of catecholamines and catecholamine metabolites. Magnetic resonance imaging (MRI) of the abdomen revealed 2 tumors, a large tumor (6 × 5.5 × 4.8 cm) inside the left kidney and a smaller mass (3 × 2.6 × 1.8 cm) inside the suprarenal space (Figure 2). Subsequent 123I-meta-iodobenzylguanidine (MIBG) scan revealed significant MIBG uptake in the suprarenal space and diffuse MIBG affine tissue in the skeleton, indicating bone marrow involvement. However, there was no MIBG uptake in the left kidney tumor (Figure 2). In order to complete diagnostic investigation, bone marrow analysis was performed, which revealed a very small infiltration (<1%) of neuroblastoma cells. Based on these results, 2 different tumor entities—neuroblastoma (International Neuroblastoma Staging System [INSS] stage IV) and nephroblastoma—were expected.

**Therapy**

In agreement with the German Nephroblastoma Study Group as well as the German Neuroblastoma Study Group, the initial therapeutic approach aimed to treat the cancer of higher malignancy, hence the suspected neuroblastoma. Therefore, neoadjuvant therapy according to the current neuroblastoma, high risk protocol at that time (NB 2004) was initiated. Response evaluation after 2 cycles of chemotherapy (N5, N6) showed good response: The tumor in the left suprarenal space decreased to 9 mm in size. The size of the kidney tumor size was also reduced to less than 40% of the initial volume. Subsequently, the left suprarenal gland with local lymph nodes was removed with organ-preserving resection of the renal mass.
Pathological analysis revealed a poorly differentiated NB of the suprarenal region with a low mitosis-karyorrhexis index according to the International Neuroblastoma Pathology Classification. In addition, 2 lymph node metastases (paraadrenal and parahiliar) of the neuroblastoma were found. 1p deletion or MYCN amplification was not detected. The kidney tumor was indeed a completely resected regressive nephroblastoma. According to the International Society of Paediatric Oncology (SIOP) staging, stage I was diagnosed. After recovering from surgery, adjuvant chemotherapy according to the neuroblastoma, high risk protocol was completed. The patient is currently more than 2 years after cessation of therapy and is in complete remission.

**Genetic analysis**

Due to the unique nature of the simultaneous occurrence of 2 different tumor entities and the young age of the patient, we hypothesized that a common underlying genetic alteration might be present. In order to understand the etiology of the tumors, an NGS approach was used to target 94 genes and 284 SNPs previously identified to be involved in cancer predisposition and/or oncogenesis (see Supplemental Table 1 for complete list of analyzed loci). The sequencing quality score (Q30) was 84.7%. A total of 9,018,618 pass-filtered reads were produced; 95.8% were aligned using BWA. The mean region coverage depth was of 1885×. Target coverage at 50× was 99.6%. Initially, 289 variants were identified, 141 of them in coding regions (Supplemental Table 2); 98.6% were found in NCBI database of
Figure 3. Rationale for further genetic evaluation. The depicted approach was used for further genetic validation via Sanger sequencing (SS). The list of all investigated genes and SNPs through NGS is provided in Supplemental Table 1, and the 289 identified alterations are listed in Supplemental Table 2.

genetic variation (dbSNP). The rationale for the selection of alterations for further analysis is depicted in Figure 3. Four alterations have global allele frequency (from all populations of 1000 genomes data in April 2012) <0.1% (Supplemental Table 2). The threshold corresponds to the validation criteria by Zhang et al., where only rare variants with a global allele frequency below 0.1% were retained [32]. These 4 alterations were selected for validation by Sanger sequencing (SS) in DNA extracted from patient’s saliva and additionally in formaldehyde-fixed, paraffin-embedded (FFPE) tumor tissues. Furthermore, literature research of all 289 alterations revealed 6 tumor susceptibility SNPs predisposing to WT
Table 1. Validated genetic alterations and their effects.

| Gene / SNP         | Variant (HGVSc)                                                                 | Chromosome | Genotype saliva | Genotype tumor (WT) | Genotype tumor (NB) | Protein consequence |
|--------------------|--------------------------------------------------------------------------------|------------|-----------------|---------------------|---------------------|---------------------|
| ALK                | NM_004304.4: c.172T→C                                                        | 2          | Heterologous    | Heterologous        | Heterologous        | F58L               |
| FANCD2             | NM_033084.3: c.1254_1264delins TTACTTATGACAA-GACTG                           | 3          | Heterologous    | Heterologous        | Heterologous        | Q418Hfs*11          |
| BARD1/rs6435862    | NM_000465.2: c.158+1590C→A                                                   | 2          | Homologous      | Homologous          | Homologous          | Intrinsic           |
| DLG2/rs790356       | NM_00142699.1: c.1496+20584T→C                                               | 11         | Heterologous    | Heterologous        | Heterologous        | Intrinsic           |
| EMBPI/rs1249433     | NR_003955.1: n.349-176955A→G                                                 | 1          | Heterologous    | Heterologous        | Heterologous        | Intrinsic           |
| LINC00340/rs4712653 | NR_015410.1: n.1391+1481ST→C                                                  | 6          | Homologous      | Homologous          | Homologous          | Intrinsic           |
| NSD1/rs807624       | g.15782471 (coordinate)                                                      | 2          | Heterologous    | Heterologous        | Heterologous        | Intrinsic L2277 (synonymous) |
|                    | NM_022455.4: c.6829T→C                                                       | 5          | Homologous      | Homologous          | Homologous          | Intrinsic L2277 (synonymous) |

Note. Alterations identified via NGS and selected according to the approach depicted in Figure 3 were validated by Sanger sequencing. Nomenclature is according to the Human Genome Variation Society (HGVS).

and/or NB or multiple primary tumors (Supplemental Table 2). These tumor susceptibility SNPs were also validated by Sanger sequencing. Validated alterations are listed in Table 1. All Sanger sequencing analysis comparing saliva and both tumor FFPE tissues revealed concordant results. Hence, all validated variants were either heterozygous or homozygous in all 3 samples. We validated a novel point mutation p.F58L A→G in the ALK gene (c.172T→C) leading to a change in the amino acid chain (Figure 4). This found missense mutation affects the extracellular domain of the receptor tyrosine kinase (Figure 1) and is predicted to be deleterious according to PolyPhen (xx, xx). NGS detected several mutations in exon 15 of the FANCD2 gene. By Sanger sequencing with primers able to discriminate between the FANCD2 pseudogenes, this locus showed a complex rearrangement (c.1254_1264delins TTACTTATGACAA-GACTG) composed of a deletion of 11 bp (GAGTACATTCT) and a concurrent insertion of 19 bp at the same site of the gene (Figure 4). The presence of the

Figure 4. Novel ALK and FANCD2 mutations. (a) Heterozygous single-nucleotide variant in exon 1 of the ALK gene encoding leucine (L) instead of phenylalanine (F) at codon 58 (A→G at codon 58, c.172T→C). This heterozygous point mutation was found in DNA of patient’s saliva, WT and NB FFPE tissues. (b) Heterozygous 11-bp deletion (red) with concurrent 19-bp insertion in exon 15 of the FANCD2 gene. To discriminate the WT and the mutant alleles, the PCR product of exon 15 was cloned and sequenced.
wild-type and mutated allele was confirmed by cloning and sequencing of exon 15 (Figure 4). This rearrangement leads to a frameshift and a premature stop codon. Both FANCD2 alleles were present in the WT and NB tissues. This links specific FANCD2 mutations to embryonal tumors without evidence of allelic loss in the tumor tissue. Review of current available literature on predisposing genomic alterations to NB and/or WT revealed 6 tumor susceptibility loci (rs807624, BRCA1 associated RING domain 1 (BARD1) rs6435862, NSD1 rs28580074, LINC00340 rs4712653, Discs, Large Homolog 2 (DLG2) rs790356, Embigin Pseudogene 1 (EMBP1) rs11249433). The rs807624 variant was recently described to predispose to both tumors [21]. All of these SNPs are considered to predispose to neuroblastoma and/or nephroblastoma. We identified all those variants in our patient through NGS and validated them via Sanger sequencing.

Discussion

Here, we present a unique case of synchronous neuro- and nephroblastoma in an infant without apparent syndrome association or congenital anomalies. Our initial therapeutic approach was to treat the cancer of higher malignancy, hence the suspected neuroblastoma. As we assumed a high likelihood of correct diagnosis based on the performed noninvasive techniques and to prevent tumor spillage and potential upstaging to stage III of the nephroblastoma, the option of an open biopsy or a fine-needle aspiration cytopathology (FNAC) with intrinsic diagnostic limitations was discarded. The disadvantage was to start treatment without definite histopathological confirmation of tumor origin and no information about MYCN or 1p deletion status. The advantage of neoadjuvant chemotherapy prior to definite surgery was the reduction of tumor mass and therefore the possibility of organ-preserving resection of the nephroblastoma. Especially in our patient, a partial nephrectomy, resulting in more remaining renal tissue and renal functional capacity, seemed to be advantageous due to the high toxicity of the anticipated subsequent neuroblastoma, high risk protocol to reduce therapy-related side effects. Even though standardized therapies are necessary guidelines for adequate cancer treatment, individualized therapy strategies are needed for exceptional cases like the one presented here.

Synchronous occurrence of 2 different malignancies in childhood is extremely rare and is usually associated with underlying genetic syndromes. A recent report from Sarin et al. described a case of a 10-month-old girl with synchronous occurrence of neuroblastoma and nephroblastoma. However, neither information on underlying congenital anomalies nor the genetic background was provided [33]. Moreover, review of current literature revealed single cases of syndrome associated patients with synchronous neuro- and nephroblastoma and Beckwith-Wiedemann syndrome or Fanconi anemia [34, 35]. Our patient had no evidence of a syndrome-associated disorder. Abbaszadeh et al. reported 9 families with 2 or more individuals with WT or neuroblastoma without syndromic features. Three individuals presented with WT and ganglioneuroma; a benign tumor of the sympathetic nervous system but no concomitance of malignant neuroblastoma and WT was identified. In the WT/ganglioneuroma group, genetic abnormalities of WT1 were excluded as underlying mutations [36].

We also excluded WT1 as underlying mutation in our patient. Apart from WT1, we did not detect any pathogenic abnormalities in DICOR1, paired like homeobox 2b (PHOX2B), PALB2, Nuclear Receptor Binding SET Domain Protein 1 (NSD1), NKAIN2, TP53, and BRAC2, which are known predisposing genes for NB and/or WT [9, 16, 37–40]. We did not exclude epigenetic and copy-number abnormalities at 11p15, which are known to predispose to embryonal tumors, including Wilms tumor and nephroblastoma. However, we did not find any clinical
features of Beckwith-Wiedemann syndrome in this patient; hence, 11p15 abnormalities are unlikely as underlying cause.

We identified a novel heterozygous germline mutation p.F58L in the ALK gene. This point mutation leads to a change in the amino sequence from phenylalanine to leucine and therefore might change the protein structure. The position of the mutation falls into the extracellular domain of the protein (Figure 1). This segment is known to be important for ligand binding and is involved in cell-to-cell adhesion [12, 41]. Until now, there are several ALK mutations identified in NB, mostly in the TKD of the protein, that lead to constant activation of ALK. Activating mutations and overexpression of ALK promote proliferation of NB cells. Okubo et al. showed a different mechanism of ALK activation via deletion of exon 2 and 3 in the ALK gene leading to high-level expression of ALK protein variant with a truncated extracellular domain and constitutive kinase activity [42]. However, we do not know if the described unique missense mutation in this region may have a similar effect on ALK stability or activation. The presence of constitutional ALK mutations constitute key events in NB oncogenesis in affected individuals, but with variable penetrance [43]. It is estimated that approximately only 50% of carriers of constitutional ALK mutations are affected by NB [44, 45]. It is believed that variants in the nonmutated ALK gene or other genes affect penetrance of constitutional ALK mutations and hence development of NB [45]. Besides, a precise penetrance of ALK mutation is difficult to state, since many apparently unaffected carriers might in fact have had spontaneously regressive tumors [44]. Finally, the detected germline mutation might have occurred spontaneously. Even though many studies have focused on ALK mutations and NB, little is known about how ALK mutations contribute to the development of WT. Just recently, ALK expression was also described in nephroblastoma, although no functional analysis were included in this study [15]. The significance of p.F58L in NB and especially its role in WT has to be evaluated in further functional studies.

Defects in DNA repair lead to genomic instability and an increased mutation rate, which can be found in pediatric cancers [46]. We identified a deletion/insertion in the FANCD2 gene leading to a truncated protein. FANCD2 belongs to the family of FANC genes, which encode proteins that interact in a common DNA damage response pathway [47]. Biallelic mutations in 1 of the 15 FANC genes lead to the rare cancer susceptibility syndrome Fanconi anemia (FA). FA is an autosomal recessive disorder characterized by chromosome instability, hematological abnormalities, several congenital defects, and strong cancer predisposition [30]. Even though approximately 25%–40% of FA patients lack phenotypical manifestation of Fanconi anemia, Kalb et al. performed a comprehensive mutation analysis in affected individuals and found not a single case with FANCD2 mutation without anomalies or malformations [48]. This demonstrates the critical role of FANCD2 [48]. Indeed, a murine Fancd2−/− model revealed a more severe role in carcinogenesis compared with other FANC deficiencies, most likely due to the more downstream role of FANCD2 [49]. Nevertheless, Borriello et al. reported on an individual without clinical features typical of FA but with FANCD2 protein dysfunctions contributing to chromosomal instability, toxic response to therapy, and development of cancers [50]. Heterozygous mutation carrier status of several FANC genes has been firmly established to be associated with an increased risk of breast and/or ovarian cancer [51–54]. FANCD2 heterozygous mutations have been associated with childhood T-cell acute lymphoblastic leukemia (ALL) and testicular seminoma [47]. Our finding associates FANCD2 heterozygous mutations also with embryonal cancer, as described so far for FANCD1/BRCA2 and FANCN/PALB2 homozygous mutations [29]. Intriguingly, the possibility of sex-determined modulators of the oncogenic potential of FANCD2 mutations has been discussed, with male having a higher risk to develop neoplasm [47]. Our analysis revealed a heterozygous frameshift mutation in
exon 15, which leads to a premature stop of the protein. We did not detect any concomitant pathological mutations in any of the other 14 FA genes, including FANCD1 (BRACA2). Even though, no specific association of FANCD2 and development of WT and NB has been proven yet, it is plausible that impairment of DNA repair mechanisms contribute to tumorigenesis of those tumors. Even though several reports elucidate the critical role of FANCD2 in tumorigenesis and illustrate that even heterozygous mutations lead to cancer, so far no association between heterozygous FANCD2 mutations and the occurrence of embryonal tumors has been reported. We establish the first possible association between heterozygous FANCD2 dysfunction and the occurrence of embryonal tumors.

The vast majority of WT and NB occur sporadically and common, low-penetration polymorphisms lead to individual predisposition. Rs807624 has been associated with NB and WT [21]. Additionally, we identified 4 tumor susceptibility SNPs with association to WT or NB (BARD1 rs6435862, NSD1 rs28580074, LINC00340 rs4712653, DLG2 rs790356) and 1 associated with multiple primary tumors (EMBP1 rs11249433) [18–20, 55]. However, whether those cancer-predisposing variants confer synergistic effects is still fully unknown, and can only be assumed.

To get a clearer picture, it would have been of great interest to extend genetic testing to the rest of the family members, including the grandmother with breast cancer. However, the family did not agree with further genetic evaluation of extended family members. Even though it cannot be completely answered to what extent the detected genomic alterations contribute to the development of both tumors, our analysis revealed important insights into possible disease driving mutations.

In conclusion, we present a very unique case of synchronous neuro- and nephroblastoma in an otherwise healthy infant. Using the NGS approach, we identified a novel heterozygous germline mutation in ALK and a novel FANCD2 rearrangement. NGS is a valid approach in detecting common genomic alterations in rare synchronous tumors. With functional analysis combined, this information will be helpful in recognizing new genetic markers and targets for future personalized therapy.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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