Chapter 6
Fanconi anemia gene mutations are not involved in sporadic Wilms tumor

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Abstract

Bi-allelic germline mutations of the Fanconi anemia (FA) genes, *PALB2/FANCN* and *BRCA2/FANCD1*, have been reported in a few Wilms tumor (WT) patients with an atypical FA phenotype. Therefore, we screened a random cohort of 47 Dutch WT cases for germline mutations in these two FA-genes by DNA sequencing and Multiplex Ligation-dependent Probe Amplification (MLPA). Although several cases appeared to carry missense variants, no bi-allelic pathogenic mutations were identified, indicating that bi-allelic mutations in these FA-genes do not contribute significantly to the occurrence of WT.
Introduction

Wilms tumors (WT), representing 7% of childhood malignancies are known to be associated with congenital malformations as in Beckwith-Wiedemann syndrome (BWS), Denys-Drash syndrome, Frasier syndrome, and WAGR syndrome. Fanconi anemia (FA) is a heterogeneous autosomal recessive and X-linked disease with variable clinical features, including short stature, radial hypoplasia, thumb anomalies, hyper-and hypo-pigmentation, bone marrow failure, and predisposition to cancers, most commonly acute myeloid leukemia and squamous cell carcinoma. FA has a high phenotypic variability, ranging from no dysmorphic features to multiple congenital malformations.

Bi-allelic (homozygous or compound heterozygous) germline mutations of the FA genes PALB2/FANCN and BRCA2/FANCD1 give rise to a severe FA phenotype with childhood solid tumors. Twenty-two families have been described with bi-allelic BRCA2/FANCD1 mutations including 26 cases with 35 childhood malignancies, including 7 WT. Most patients had pigmentation anomalies, such as café-au-lait spots and short stature. Bi-allelic mutations in PALB2/FANCN have been described in eight children, of which three developed a WT. All PALB2/FANCN patients suffered from growth retardation and a diversity of congenital malformations. In addition, PALB2/FANCN and BRCA2/FANCD1 are known breast cancer susceptibility genes, in which mono-allelic (heterozygous) mutations are associated with a 2-fold increased risk of breast cancer in PALB2/FANCN and a 10- to 20-fold risk in BRCA2/FANCD1 carriers.

As FA is phenotypically high variable, we hypothesized that sporadic WT patients might carry germline bi-allelic PALB2/FANCN or BRCA2/FANCD1 mutations. The clinical implications of missing the diagnosis of FA in WT patients may be significant because of the hypersensitivity of these patients to chemotherapy and radiotherapy. We therefore decided to study the occurrence of bi-allelic germline PALB2/FANCN and BRCA2/FANCD1 mutations in an unselected cohort of WT patients.
Methods

Patients
Whole peripheral blood DNA of a prospective cohort of 47 unselected WT cases from two Dutch pediatric oncology centers (Emma Children’s Hospital-AMC and Erasmus MC-Sophia Children’s Hospital), 27 females, 20 males, median age 45 months (range 6-146 months). The patients were staged I (n=24), II (n=7), III (n=9), IV (n=5) and V (n=2). Patients were categorized as low risk (n=2), intermediated risk (n=40) and high risk (n=5) according to stage and histology. None had an apparent FA phenotype. Informed consent was obtained from all parents.

Sequencing and MLPA
The presence of germline mutations in $\text{PALB2/FANCN}$ and $\text{BRCA2/FANCD1}$ was evaluated by direct sequencing. Sequencing was successful in all samples except for three cases in which one, four and six exons of $\text{BRCA2/FANCD1}$ repeatedly did not show a result. Silent mutations which were not present in splice acceptor/donor sites are unlikely to be clinical relevant and therefore left out of the discussion. Pre-amplified DNA was analyzed for the presence of large rearrangements in the $\text{PALB2/FANCN}$ gene using Multiplex Ligation-dependent Probe Amplification (MLPA), as previously described. MLPA is a rapid quantitative method for the detection of deletion/amplification of up to 40 specific DNA fragments in a single PCR reaction.

Results
No truncating mutations of the $\text{PALB2/FANCN}$ gene and $\text{BRCA2/FANCD1}$ were identified in any of the 47 patients. In $\text{PALB2/FANCN}$, nine different amino acid substitutions were identified of which seven are known SNP’s and were previously described in a large familial breast cancer study. Only three (Leu337Ser, Leu939Trp, Gly989Glu) amino acid substitutions were predicted to possibly affect the protein function by two or more programs (Table 1).

In $\text{BRCA2/FANCD1}$, three missense variants were identified, of which the clinical importance of two is classified as “unknown” in the international Breast Cancer Information Core (BIC) database (http://research.nhgri.nih.gov/bic/), whereas the third one has not been reported (Table 2).

No alterations in the $\text{PALB2/FANCN}$ gene were identified by MLPA in 45 samples with available pre-amplified DNA.
Table 1  Missense variants identified in PALB2/FANCN

<table>
<thead>
<tr>
<th>PALB2/FANCN gDNA change</th>
<th>Protein change</th>
<th>SNP ID</th>
<th>Prediction programs</th>
<th>Clinical characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Polyphen</td>
<td>SIFT</td>
</tr>
<tr>
<td>c.629C&gt;T</td>
<td>p.Pro210Leu</td>
<td>rs57605909</td>
<td>Benign</td>
<td>Affect protein function</td>
</tr>
<tr>
<td>c.721A&gt;G</td>
<td>p.Asn241Asp</td>
<td>Unknown</td>
<td>Benign</td>
<td>Tolerated</td>
</tr>
<tr>
<td>c.1010T&gt;C</td>
<td>p.Leu337Ser</td>
<td>rs45494092</td>
<td>Possibly damaging</td>
<td>Affect protein function</td>
</tr>
<tr>
<td>c.2014G&gt;C</td>
<td>p.Glu672Gln</td>
<td>rs45532440</td>
<td>Benign</td>
<td>Affect protein function</td>
</tr>
<tr>
<td>c.2130C&gt;T</td>
<td>p.Ala712Val</td>
<td>Unknown</td>
<td>Benign</td>
<td>Tolerated</td>
</tr>
<tr>
<td>c.2590C&gt;T</td>
<td>p.Pro864Ser</td>
<td>rs45683539</td>
<td>Probably damaging</td>
<td>Tolerated</td>
</tr>
<tr>
<td>c.2794G&gt;A</td>
<td>p.Val932Met</td>
<td>rs45624036</td>
<td>Benign</td>
<td>Affect protein function</td>
</tr>
<tr>
<td>c.2816T&gt;G</td>
<td>p.Leu939Trp</td>
<td>rs45478192</td>
<td>Possibly damaging</td>
<td>Affect protein function</td>
</tr>
<tr>
<td>c.2903G&gt;A</td>
<td>p.Gly963Glu</td>
<td>rs45515636</td>
<td>Probably damaging</td>
<td>Affect protein function</td>
</tr>
</tbody>
</table>

Nomenclature according to the human genome variation society (HGVS) recommendations (www.hgvs.org). Single Nucleotide Polymorphism (SNP) identification (ID) reference numbers (rs) are included if known. Pathogenicity of variants was predicted using four databases: Polymorphism Phenotyping (PolyPhen) prediction (http://genetics.bwh.harvard.edu/pph); Sorting Intolerant From Tolerant (SIFT) (http://blocks.fhcrc.org/sift/SIFT.html); Align GVGD (http://agvgd.iarc.fr) and Mutation Taster http://www.cse.psu.edu/MutationTaster/index.html. The sample Rwt62 in italics carries more than one variant.
Table 2: Missense variants identified in BRCA2/FANCD1

<table>
<thead>
<tr>
<th>BRCA2/FANCD1 gDNA change</th>
<th>Protein change</th>
<th>Prediction programs</th>
<th>BIC</th>
<th>Clinical characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Polyphen</td>
<td>SIFT</td>
<td>GVGD</td>
</tr>
<tr>
<td>c.3499A&gt;G p.Ile1167Val</td>
<td>Benign</td>
<td>Tolerated</td>
<td>Less likely (C0)</td>
<td>Presumably harmless</td>
</tr>
<tr>
<td>c.5455C&gt;T p.Pro1819Ser</td>
<td>Probably damaging</td>
<td>Tolerated</td>
<td>Less likely (C0)</td>
<td>Presumably harmless</td>
</tr>
<tr>
<td>c.8187G&gt;T p.Lys2729Asn</td>
<td>Benign</td>
<td>Affect protein function</td>
<td>Less likely (C0)</td>
<td>Presumably harmless</td>
</tr>
</tbody>
</table>

Nomenclature according to the human genome variation society (HGVS) recommendations (www.hgvs.org). Pathogeneity of variants was predicted using four databases; Polymorphism Phenotyping (PolyPhen) prediction (http://genetics.bwh.harvard.edu/pph); Sorting Intolerant From Tolerant (SIFT) (http://blocks.fhcrc.org/sift/SIFT.html); Align GVGD (http://agvgd.iarc.fr) and Mutation Taster (http://www.mutation.taster.org/index.html). No variant is a known Single Nucleotide Polymorphism (SNP), but two of the three variants are recurrently reported in the international Breast Cancer Information Core (BIC) database (http://research.nhgri.nih.gov/bic/).
Discussion

Although the importance of FA genes in cancer predisposition is well described, the involvement of germline FA-gene mutations in solid childhood cancers has not been systematically investigated in a prospective study. Several reports have shown that bi-allelic mutations in two FA genes, BRCA2/FANCD1 and PALB2/FANCN, may play a role in the etiology of WT in patients with FA with an apparent FA phenotype.\textsuperscript{5,7} As PALB2/FANCN and BRCA2/FANCD1 affected FA patients might not necessarily show an abnormal phenotype, the FA diagnosis can easily be missed in children with a seemingly sporadic WT. Due to the hypersensitivity for cancer treatment causing higher morbidity and mortality in patients with FA it is important to investigate a cohort of patients with sporadic WT for BRCA2/FANCD1 and PALB2/FANCN gene mutations. Moreover, since the occurrence of breast cancer in a child's family representing mono-allelic mutations might be unnoticed or unknown, we searched for subclinical FA cases in patients with sporadic WT. The results of our unselected cohort revealed no bi-allelic pathogenic mutated cases, indicating that these mutations do not seem to play a major role in sporadic WT. The chance of finding a bi-allelic mutated WT patient was likely low due to the low prevalence of mutations in BRCA2/FANCD1 and PALB2/FANCN in the population and our relatively small cohort.

Nevertheless, we did find several missense variants which may affect the function of the protein (Table 1). The three missense variants in PALB2/FANCN that could affect the function of the protein have been previously described to occur with equal frequencies within cases and controls in a familial breast cancer study in which more than 4,000 alleles were screened.\textsuperscript{12} In addition, these three variants have a low confidence prediction and are therefore unlikely to be pathogenic. In our study, only one case carried two of these missense variants. Clinically, this child was not different from the other children in terms of the type of tumor, further the child had no congenital malformations. Interestingly, this child was only 6 months old at diagnosis of the WT, an age at which germline mutations tend to occur more often than in older children with WT.

BRCA2/FANCD1 mono-allelic missense variants were found in three cases. These variants did not result in major protein changes and are therefore unlikely to have any pathogenic effect. We did not observe any patient with more than one variant implicating that no bi-allelic mutation carrier was present in this cohort (Table 2).

Although a higher incidence of WT has not been reported in breast cancer families with mono-allelic BRCA2/FANCD1 and PALB2/FANCN mutations, we cannot fully exclude a modifying effect of pathogenic mono-allelic mutations in the etiology of WT.\textsuperscript{13}
In conclusion, germline bi-allelic mutations in both the \textit{PALB2/FANCN} and \textit{BRCA2/FANCD1} genes do not appear to play a major role in Wilms tumor development in Dutch patients. The role of mono-allelic missense \textit{PALB2/FANCN} and \textit{BRCA2/FANCD1} mutations remains to be determined.

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References


