Is p53 intronic variant G13964C associated with predisposition to cancer?

Lucja FISZER-MALISZEWSKA¹, Bernarda KAZANOWSKA², Piotr KUŚNIERCZYK¹, Maria MAŃCZAK³, Wanda NIEPIEKŁO¹, Bogusława POCHRON-ZEMAN³, Beata NOWAKOWSKA¹

¹Laboratory of Tissue Immunology, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland
²Department of Pediatric Hematology and Oncology, Wrocław Medical University, Wrocław, Poland
³Laboratory of Immunogenetics, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland

Abstract. Germline mutations of the p53 gene confer a high risk of diverse malignancies. The highest frequency of inherited p53 defects was noted in Li-Fraumeni syndrome (LFS), but almost half of the mutations were found in families with incomplete Li-Fraumeni-like syndrome (LFL), including familial breast cancer cases. Recently, a germline intronic G13964C base change of the p53 was reported as a high-risk mutation associated with familial breast cancer (LEHMAN et al. 2000). We genotyped Polish cancer patients and healthy control individuals for the G13964C variant. Patients were chosen from cancer families with phenotypes typical for germline mutations of p53 (LFS, LFL), BRCA1 [hereditary breast (ovarian) cancer, HB(O)C] or a complex consistent with both LFL and HB(O)C. Children with leukemia were included in the study as another high risk group (FELIX et al. 1992). The G13964C variant was detected in six of 87 (6.9%) cancer patients (including two ALL children), but also in eight of 96 (8.3%) control individuals (p > 0.4). Thus we found no evidence of the variant’s association with a high risk of cancer.

Key words: germline mutation, hereditary breast (ovarian) cancer, Li-Fraumeni syndrome, polymorphism, p53.
Germline mutations of the \( p53 \) gene are associated with Li-Fraumeni syndrome (LFS) and Li-Fraumeni-like syndrome (LFL) (KLEIHUES et al. 1997, VARLEY 2003). The frequency of germline \( p53 \) mutations in LFS and LFL families is estimated to be, respectively, 70% and 30%. Patients with these syndromes (having the wild \( p53 \) or a heterozygous germline mutation) have strong familial histories of diverse tumor types, mostly bone and soft tissue sarcomas, breast cancers, leukemias and brain tumors. Due to a high frequency of breast cancers in LFS/LFL, there is an overlap between these syndromes and hereditary breast (ovarian) cancer syndrome [HB(O)C] and, in fact, several cancer families conform to the criteria for both LFL and HB(O)C (HUUSKU et al. 1999, LEHMAN et al. 2000). Nevertheless, germline mutations of the \( BRCA1 \) gene are not detected in LFS/LFL and are rare in families of a complex cancer phenotype consistent with both LFL and HB(O)C [LFL/HB(O)C]. The search for another predisposing defect in LFS/LFL has led to the identification of germline mutations of the \( CHK2 \) gene (BELL et al. 1999, VAHTERISTO et al. 2001). However, in a significant proportion of LFS/LFL families and those of mixed phenotypes, LFL/HB(O)C, the predisposing genetic defect is yet to be discovered.

The spectrum of pathogenic germline \( p53 \) mutations corresponds to that of somatic mutations, that is, they are distributed over a large region of the molecule, mostly in the highly conserved domains with the most frequent mutations at the hot-spot codons: 248 and 273 (BEROUD, SOUSSI 2003).

It is of note that some alterations considered to be neutral, according to BEROUD and SOUSSI (2003), actually result in creation of putative acceptor or donor splice sites, leading to protein inactivation. An example of this is a germline base substitution at codon 125 ACG > ACA (Thr > Thr), thought to be a silent mutation, which only in RNA-based analysis was demonstrated to lead to abnormal splicing (VARLEY et al. 1999). A matter of controversy is the constitutional \( p53 \) intronic G to C base change at nucleotide 13964 (GenBank accession number X54156/U94788), found by BULLER et al. (1995) in patients with ovarian and breast cancer. Recently, LEHMAN and co-workers (2000) detected the rare C allele in 3 of 42 American LFL/HB(O)C families, but not in 171 control sporadic breast cancer patients. Moreover, they showed that in the lymphoblastoid cell lines derived from the heterozygous GC patients, the variant functioned as a germline mutation of comprised apoptotic response to cisplatinum. The authors stated that the G13964C base change functioned as a dominant mutation similar to the more common missense, nonsense and splice-site mutations, and as such conferred a high risk of cancer in LFL/HB(O)C families. However, contradictory data were reported by MARSH et al. (2001), who found the G13964C variant at the same frequency in Australian HB(O)C patients (3/71) and in healthy control individuals (5/143). VARLEY et al. (2001) also examined the G13964C variant in respect to its association with a high risk of cancer, unfortunately, in a fairly small group of nineteen LFS/LFL patients, none of whom appeared to be its carrier. The contradictory data prompted us to examine the incidence of
the G13964C variant in a series of Polish cancer families previously screened for germline mutations of p53 and found not to carry any known mutation (FISZER-MALISZEWSKA et al. 2000, 2002).

The group of 87 cancer patients comprised 64 probands of families classified as LFS/LFL (LI et al. 1988, BIRCH et al. 1994, BRUGIÈRES et al. 1993), HB(O)C (three or more breast and/or ovarian cancers in first- or second-degree relatives) or LFL/HB(O)C (complex phenotype) and 23 children with leukemia not selected for family history. Detailed family histories were obtained by interview of the probands or their parents. Informed consent was obtained from all patients (or parents). The control group consisted of 96 anonymous blood donors representing the general population. DNA was isolated from fresh or frozen blood samples according to GUSTINCICH et al. (1991) or by a salting-out procedure (MAŃCZAK et al. 1998). To detect the G13964C variant, PCR-RFLP assay was used (LEHMAN et al. 2000). A 207 bp product containing a fragment of intron 6 and exon 7 was amplified by using 25 pM of each primer: 5’-CTCCCCCTGTGTTGCCACAGGT-3’ (forward) and 5’-CAGTGTGCAGGGTGCCAAGT-3’ (reverse), 1 × PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM MgCl₂), 40 μM of each nucleotide, 0.4 U Taq polymerase (Gibco), and 100 ng genomic DNA. Cycling conditions were:

94°C for 5 min, followed by 35 cycles of 94°C for 35 s, 60°C for 40 s and 72°C for 40 s, with a final extension step at 72°C for 5 min PCR products were digested with HhaI restriction enzyme (New England Biolabs) and separated on a 7% polyacrylamide gel (Figure 1). Homozygous GG and heterozygous GC genotypes were determined by distinct banding patterns (G > C substitution destroys HhaI site) and confirmed by sequencing.

Figure 1. PCR-RFLP genotyping for the p53 G13964C variant. PCR products (207 bp) were digested with HhaI restrictase (G > C substitution destroys the HhaI site) and analysed on 7% polyacrylamide gels. Products amplified from the homozygous GG genotype were digested to 175 and 32 bp fragments (the 32 bp fragment is not visible on the gel); the heterozygous GC genotype corresponded to three bands of 207, 175 and 32 bp. GC genotype, lanes: 1, 3, 6, 7; GG genotype, lanes: 2, 4, 5, 10; uncut PCR product, lane 8; size marker, pUC18/HaeIII, lane 9.
As shown in Table 1, in cancer patients the G13964C variant was detected in four of 64 cancer families and in two of 23 children with leukemia, giving an overall frequency of 6.9% (6 of 87). One of the HB(O)C patients with the rare C allele also had a germline BRCA1 5382insC mutation, the most frequent BRCA1 mutation in Poland (GÓRSKI et al. 2000, FISZER-MALISZEWSKA et al. 2002). In the control group, the G13964C variant was detected in eight out of 96 individuals, resulting in a frequency of 8.3%. This is not significantly different from the value of 6.9% observed in cancer patients (p > 0.4; FISCHER’S exact test). We verified the health status of the four “C” allele carriers found in the control group, checking the Lower Silesia Cancer Registry; as expected, none was registered. These data do not provide any evidence to support LEHMAN’S et al. (2000) suggestion that the G13964C variant functions as a germline mutation associated with a high-risk of cancer. It is of note, however, that the overall 7.65% (both in the control and patients) incidence of the variant found in the Polish population, corresponded with its highest frequency of 7.1% (3 of 42), so far reported only for American LFL/HBOC patients. Interestingly, of the three American patients with the rare C allele, two were of Ashkenazi ancestry and the third one was of German-French origin (LEHMAN 2000). The lack of the G13964C variant in a relatively large control group of 171 sporadic breast cancer patients (matched for Ashkenazi ancestry and tumor histopathology) in the Lehman’s study may reflect age-related changes in its frequency.

In conclusion, our data indicate that the p53 G13964C variant is not a germline mutation associated with a high risk of cancer. Screening altogether 183 DNA samples has revealed the 7.65% frequency of the G13964C variant in Poland. Whether the G13964C variant functions as a rare polymorphism conferring a modest increase in cancer risk is to be established.

### Table 1. Incidence of the p53 G13964C variant in cancer patients and healthy control individuals

<table>
<thead>
<tr>
<th>Data</th>
<th>Group</th>
<th>LFS/LFL</th>
<th>LFL/HB(O)C</th>
<th>HB(O)C</th>
<th>Children with leukemia</th>
<th>Total no. of patients</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total tested</td>
<td></td>
<td>10</td>
<td>26</td>
<td>28</td>
<td>23</td>
<td>87</td>
<td>96</td>
</tr>
<tr>
<td>G13964C carriers (%)</td>
<td></td>
<td>0 (0)</td>
<td>1 b (3.8)</td>
<td>3 b (10.7)</td>
<td>2 b (8.7)</td>
<td>6 (6.9)</td>
<td>8 (8.3)</td>
</tr>
<tr>
<td>C allele frequency a</td>
<td></td>
<td>0</td>
<td>0.0192</td>
<td>0.0536</td>
<td>0.0435</td>
<td>0.0345</td>
<td>0.0417</td>
</tr>
</tbody>
</table>

a In all cases, p > 0.4
b Breast cancer patient/s
c ALL patients
REFERENCES


