



UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
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Prevalence of the germline *TP53* p.R337H mutation in families with multiple cases of cancer

Kelvin César de Andrade

Relatório Científico apresentado ao
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Prevalence of the germline *TP53* p.R337H mutation in families with multiple cases of cancer

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ABSTRACT

Germline mutations in *TP53* gene are associated with Li-Fraumeni syndrome (LFS) and its variants Li-Fraumeni-like (LFL). They predispose carriers to a wide variety of early onset tumors. In Brazil, there is a high frequency of a germline mutation in this gene (NC_000017.9: c.1010G>A; p.R337H) in Southern and Southeastern regions, due to a founder effect. It is estimated to be present in 0,3% of the local population, but only few families have been detected. Due to this significant divergence, the purpose of this study was to verify the effectiveness of wider criteria for detection of these individuals. Herein, clinical criteria were established, DNA samples were collected, analyzed by Restriction Fragment Length Polymorphism (RFLP) and sequenced. Thus, assessing the prevalence of this mutation in families with multiple cases of cancer. Based on our proposed criteria, one out of 31 patients (3,22%) was found to carry p.R337H mutation. The patient developed ductal invasive breast cancer at age 47, invasive adenocarcinoma of the lung at age 48 and soft-tissue sarcoma at age 49. In addition, an extensive cancer family history was referred, atypical for LFS, including a case of Ewing's sarcoma. These outcomes indicate that the proposed criteria may detect probable carriers who did not fit previous LFS criteria. Nevertheless, additional studies, which might include a larger number of families and more stringent parameters, will be useful to improve screening sensibility.

1. BACKGROUND

Li-Fraumeni syndrome (LFS, OMIM #151623) and its variants *Li-Fraumeni-like* (LFL) are rare autosomal dominant genetic disorders inherited by germline *TP53* mutations [1]. Carriers are predisposed to develop a wide variety of early onset tumors, including (but not restricted to): premenopausal breast cancer, soft-tissue sarcoma (STS), central nervous system tumors (CNS) and adrenocortical carcinomas (ADR) [2, 3]. In order to identify at-risk families which carry these mutations, several criteria for clinical diagnosis have been established [2, 4-7].

Interestingly, a specific germline *TP53* mutation (NC_000017.9: c.1010G>A; p.R337H) was reported as highly associated with LFS/LFL families in Brazil [8]. It is estimated to be present in 0,3% of the local population from Southern and Southeastern regions of the country [9, 10], due to a founder effect [11, 12]. One of the hypothesis to explain why this deleterious mutation has persisted is based on its relatively reduced penetrance, which confers to its carriers tumor risk of 30% before the age of 30 [12]. Thus, most young adults who are carriers may have their offspring before developing cancer, spreading the mutation throughout generations. Also, tumor profile among Brazilian carriers is similar to regular DNA-binding domain mutations found elsewhere in the world but at different ages of onset. Moreover, a higher risk for other types of tumors may exist. However, in spite of its elevated prevalence, appropriate criteria to identify carriers, as well as, guidelines to facilitate and direct its genetic testing are still missing. Hence, the aim of this research was to determine the efficacy of wider criteria for detection of germline *TP53* p.R337H mutation carriers in families with multiple cases of cancer.

2. SUBJECTS AND METHODS

2.1 Approval

This research was approved by the Institutional Review Board (IRB) from A.C. Camargo Cancer Center (number 1725/12 – February 5th 2013).

2.2 Study design

Thirty-one patients from Oncogenetics Department at A.C. Camargo Cancer Center, Sao Paulo - Brazil were selected and tested for the p.R337H mutation. All the participants accepted to sign informed consent after genetic counseling consultation. Biological samples were collected only from probands who had developed any malignancy and fulfilled the following inclusion criteria:

Inclusion Criteria	
1	More than three family members with cancer AND;
2	at least one of them under age 50 AND;
3	two of them being first- or second-degree relatives.

Patients who had developed basal cell carcinomas or presented other germline *TP53* mutations already detected were not eligible for the study and considered as exclusion criteria.

2.3 Molecular analysis

Germline DNA was extracted from peripheral blood leukocytes using *Puregene* DNA isolation kit. After DNA extraction, primers 5'–CAA TTG TAA CTT GAA CCA TC–3' and 3'–GGA TGA GAA TGG AAT CCT AT–5' were used for exon 10 amplification. This process consisted of 35 cycles of denaturation at 94°C; annealing at 57°C; extension at 68°C and 1µl of the *Tth* DNA polymerase (*Biotoools*). The reaction product was digested by 5µl of *HhaI* enzyme (*Fermentas*) during 16 hours at 37°C and the electrophoresis running was conducted in a 2% agarose gel. This gel was colored with 3µl of DNA double-strand intercalating *GelRed* and photographed under UV light. 1Kb Plus ladder (*Invitrogen*) was used as molecular weight marker. The mutation presence resulted in the loss of restriction site. Therefore, observed banding patterns were: 1) normal homozygous samples compound by fragments with 168bp and 92bp and 2) p.R337H heterozygous samples with 260bp, 168bp

and 92bp. Confirmation of positive sample was done by direct sequencing of exon 10 in the Applied BioSystem 3130xl equipment according to manufacturer's instructions.

3. RESULTS

After RFLP reaction and subsequent confirmation by sequencing, one out of 31 patients (3,22%) was found to carry the germline *TP53* p.R337H mutation in heterozygous (Figure 1.A). The confirmation of base change G>A at codon 337 was verified by direct sequencing of exon 10 (Figure 1.B).

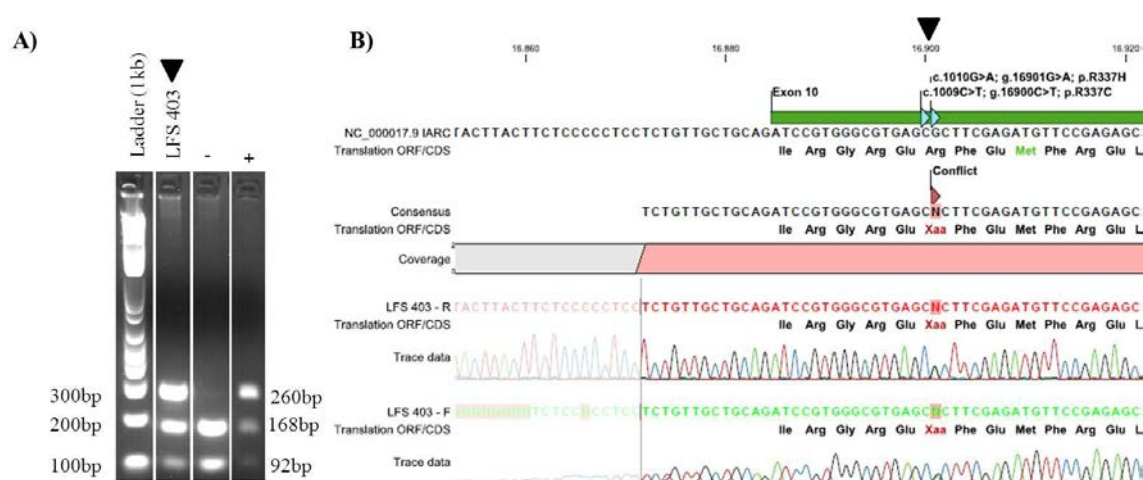


Figure 1. A) Agarose gel picture. Positive sample (arrow); - negative control; + positive control. **B) Sequencing image.** Confirmation of base change G>A at codon 337 (arrow).

The patient's personal clinical history is compound by: 1) ductal invasive breast cancer at age 47, 2) invasive adenocarcinoma of the lung at age 48 and 3) soft-tissue sarcoma at age 49. In addition, an extensive familial history of cancer occurrence was also referred, especially regarding her paternal side (Figure 2).

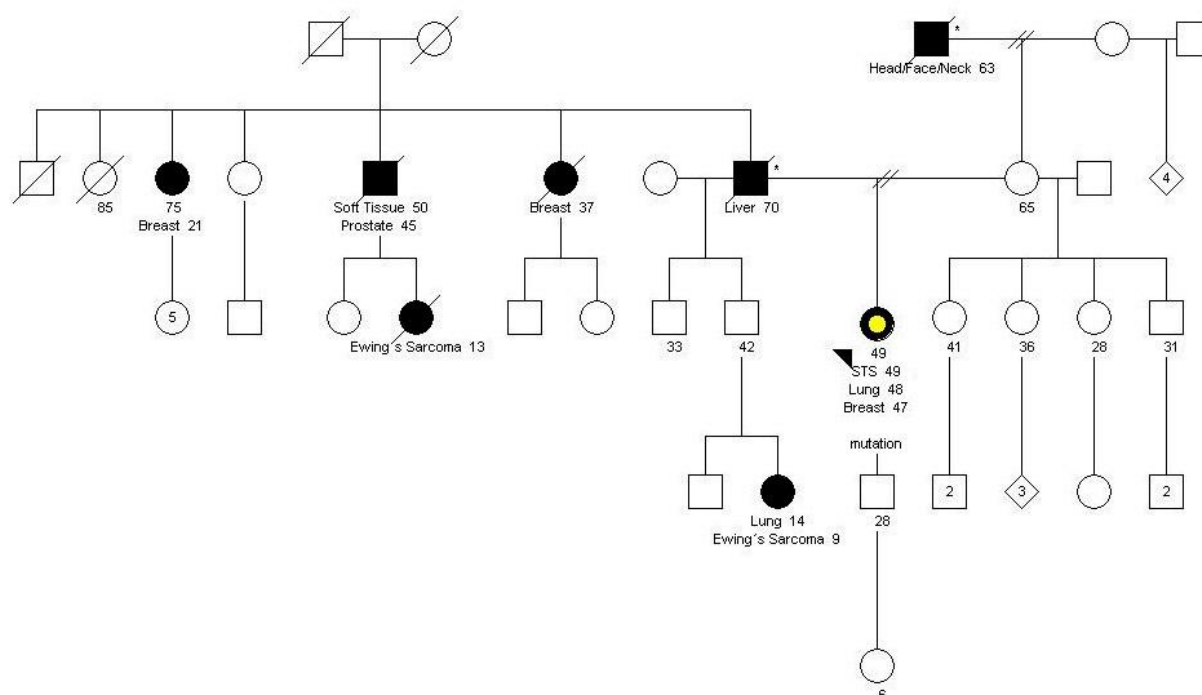


Figure 2. Pedigree of the family affected by the germline *TP53*p.R337H mutation (LFS 403). Proband is indicated by an arrow; black symbol: patient affected by malignant tumor; yellow symbol: patient carrier of the p.R337H mutation; first information below the symbol: current age; second information: type of tumor and age at onset.

4. DISCUSSION

Through this pilot study and based on our proposed inclusion criteria, it was identified, as carrier, one patient out of 31 (3,22%) selected participants. Proband's personal clinical history, especially the cases of breast cancer and soft-tissue sarcoma in a relatively older age than those presented in carriers of other *TP53* mutations, supports both the tumor profile described by Achatz *et al*, as well as, the trend to a later onset proposed by Garritano *et al*. Furthermore, one of the tumors manifested in the family history is of great interest: the development of an Ewing's Sarcoma (ES) in her third degree relative at age 9. Current data indicate that annual incidence of ES has had an average of 2.93 cases per million, corresponding to 34% of all bone cancers. Overall, 27% of them occur in the first decade of life [13]. In addition, somatic mutations in the *TP53* gene are only observed in approximately 10% of this kind of cancer [14]. Therefore, it is one of the few reports of a very early onset childhood ES in a LFS family. Proband received genetic counseling and was included in follow-up protocol for germline *TP53* mutation carriers. Also, she was counseled to contact her family members to be tested.

5. CONCLUSION

Our findings show that the established criteria may detect families with the germline *TP53* p.R337H mutation. However, further studies including a larger group of families will be useful to define their effectiveness. Also, we suggest the inclusion of more stringent parameters in order to improve screening sensibility.

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