Prevalence of low-penetrant germline TP53 D49H mutation in Japanese cancer patients

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ABSTRACT

Using whole exome sequencing data obtained from 1,685 Japanese cancer patients, we examined genetic variations of germline TP53 and found 10 types of non-synonymous single nucleotide variants. In the present study, we focused on 6 patients with germline D49H mutation located in the transactivation domain 2 of p53 protein, since the mutation seemed to be prevalent in cancer patients and to be pathogenic. According to the initial survey for family history of the proband with the germline TP53 D49H mutation, one osteosarcoma patient and his pedigree fulfill the criteria for Li-Fraumeni-like syndrome and the 2009 Chompret criteria for germline TP53 mutation screening. Since this patient possesses double germline mutations of TP53 D49H and A159D, further studies are required to evaluate contribution of the D49H mutation in this morbidity. The remaining 5 patients had family histories of cancer, but none fulfills the criteria either for the Li-Fraumeni/Li-Fraumeni-like syndromes or the 2009 Chompret criteria for germline TP53 mutation screening. It is possible to postulate that the germline TP53 D49H mutation is likely to be low-penetrant in some pedigrees. The present study also indicates that the survey for the germline TP53 mutation plays
an important role in clinical practice as it will prevent mistaking cancer patients with unusual hereditities for sporadic cases.

The study to elucidate the relationship between cancer and heredity is a major topic in cancer research and clinical practice. Although the hereditary cancer syndromes are rare clinical entities, they play a very important role for developing cancer genomics. Clinically, medical staff always carries out genetic testing when the patients fulfill the established diagnostic criteria for the syndromes, and approximately 5–10% of cancer patients are estimated to belong to the hereditary cancer syndromes, in which cancers and/or benign tumors develop in the pedigrees according to Mendel’s law of genetic inheritance with high penetrance (13).

Recent development in genetic testing device, especially the next-generation DNA sequencer, seems to make changes in clinical practice; in cancer genomics, a survey for cancer and normal tissues frequently demonstrates hereditary diseases as incidental findings (6). Also, many clinicians and researchers may believe that, in comparison with a previous view, a large number of cancer patients have genetic predispositions to cancer, and that a part of these patients could be associated with germline mutations of hereditary cancer genes with low penetrance. However, recent studies were not ready yet to form a comprehensive view on the role of hereditary cancer genes with low penetrance.

Since January 2014, we have conducted the Project HOPE (High-tech Omics-based Patient Evaluation) with an aim to evaluate biological characteristics of cancer and diathesis of each patient by multiomics-based analyses (25). The project is characterized by several distinctive features. First, the project is launched in the single institution under the crosstalk between clinicians and researchers, who are able to refer to all clinical information for evaluating genetic data of patients under the strict ethical rules. Secondly, the results of blood cell analysis by the whole exome sequencing reveal germline mutations of genes causing hereditary cancer syndromes or non-cancerous inheritable diseases. And thirdly, the samples can be reevaluated by different methods, since all the samples used are fresh surgical specimens.

As of May 2016, 2,683 cancer patients with various histological types have been enrolled, and to date, 2,211 sets of fresh tumor specimens and blood cells have been analyzed by the next-generation sequencer, Ion Proton platform (Thermo Fisher Scientific Inc., Waltham, MA, USA) for the whole exome sequencing (25). In addition, a piece of DNA from fresh tumor specimens were reevaluated by the Ion AmpliSeq™ Comprehensive Cancer Panel Kit for 409 genes (Thermo Fisher Scientific Inc.) (25) in order to confirm the results of the whole exome sequencing. For analyzing fusion genes, each RNA specimen of tumor tissue was evaluated by the newly-developed comprehensive panel for 491 fusion genes using next-generation sequencer (22). The research plan was designed according to the revised Ethical Guidelines for Human Genome/Gene Analysis Research in Japan (20) and was approved by the Institutional Review Board of the Shizuoka Cancer Center (approval number #25-33) (25). Only patients giving written informed consents participate in the study. For protecting anonymities of the patients, collaboration among surgeons, anesthesiologists, pathologists and the staff at the Patient’s Information Protection Office plays an important role.

In the present study, we analyzed the validated data from 1,685 patients belonging to the Project HOPE cohort for the presence of germline TP53 mutations. TP53 is a well-established driver gene for various types of sporadic cancers, and the germline TP53 mutations cause one of the most famous hereditary cancer syndromes, Li-Fraumeni syndrome (15). This morbidity is inherited by autosomal dominant trait, and blood relatives in the pedigree develop frequently several types of cancer including sarcoma, adrenocortical cancer, breast cancer, lung cancer, etc. To date, TP53 database of the International Agency for Research on Cancer (IARC) collected several hundred pedigrees having Li-Fraumeni/Li-Fraumeni-like syndromes, with the genetic status of germline TP53 mutations (8, 16). We also found that a large number of publications was dealing with cancer patients with the germline TP53 mutations showing no typical features of Li-Fraumeni/Li-Fraumeni-like syndromes; a part of these cases could be explained by the low penetrance of the germline TP53 mutations (2, 23).

We investigated the germline TP53 variations in cancer patients belonging to the Project HOPE cohort through several steps. At the initial step, using whole exome sequencing data of blood samples obtained from 1,685 cancer patients, we extracted germline TP53 non-synonymous single nucleotide
variants by collation with reference human genome (UCSC hg19) (9). In total, 14 amplicons covering all 11 exons of TP53 gene were amplified and analyzed by the next-generation sequencer with an average coverage of around 140-fold. At the next step, genetic changes detected were confirmed by the whole exome sequencing data of the respective tumor tissue obtained from each patient. At the third step, the germline TP53 variations detected by whole exome sequencing were confirmed by the data obtained from tumor tissue analyses for TP53 mutations which had been evaluated by the Ion AmpliSeq™ Comprehensive Cancer Panel Kit. Nineteen amplicons targeting all 11 exons of TP53 gene were amplified and analyzed by the next-generation sequencer with an average coverage of around 900-fold. And at the final step, the detected mutations further analyzed in this study were confirmed by conventional Sanger sequencing in blood cells and tumor tissues; the data were also used to evaluate the heterozygosity of the D49H mutation in normal and tumor tissues. Annotations for detected germline TP53 variations were performed by using various public databases including dbSNP (19), COSMIC (4), ClinVar (10) and IARC TP53 database (16).

As a result, we found 10 types of non-synonymous single nucleotide variants in the exons of TP53 gene in blood cells of the patients (Table 1). Although it is sometimes difficult to decide whether the variation is pathogenic or non-pathogenic, we regard following several points as important; 1) functional abnormalities in in vitro studies, 2) uncommon polymorphisms in general population, 3) previous reports describing the relationship between the mutation and cancer hereditary syndromes, and 4) clinical evidences supporting pathogenicity. In the present study, we focused on 6 patients with the D49H mutation based on following reasons. The mutation was judged to be “non-functional (lower activity than wild type)” by in vitro studies; the incidence of the mutation in the Project HOPE cohort is extremely high when compared to that of polymorphisms previously reported in general population; all patients had family histories of cancer; and one patient fulfilled the criteria of the Li-Fraumeni-like syndrome.

Table 2 shows the genetic and clinical features of 6 patients with the germline TP53 D49H mutation. The clinical features and the family history of case 1 fulfill the criteria for the Li-Fraumeni-like syndrome (15) and the 2009 Chompret criteria for germline TP53 mutation screening (21). In terms of germline TP53 genetics, this patient is a rare case with double germline mutations of TP53 D49H and A159D. Since there is no report indicating that each mutation is a defective genetic change responsible for the Li-Fraumeni/Li-Fraumeni-like syndromes (8), further studies are required to evaluate contribution of the germline TP53 D49H mutation in this morbidity. The remaining 5 patients had family histories of cancer, but none fulfills the criteria for either the Li-Fraumeni/Li-Fraumeni-like syndromes (15) or the 2009 Chompret criteria for germline TP53 mutation screening (21). From the standpoint of TP53 genetics, none of the germline TP53 mutations except for D49H and A159D was detected in all cases, and blood cells were heterozygous with a wild type allele and a D49H/A159D allele. With regard to loss of heterozygosity in tumor tissues, 3 out of 6 were judged to be heterozygous with regard to D49H, but the remaining 3 were difficult to judge due to contamination of normal tissues. The data of tumor-specific somatic mutations in 138 cancer driver genes proposed by Vogelstein et al. (24) indicated that one case possessed a somatic TP53 mutation at the different position. There were not any specific patterns of somatic mutations of cancer driver genes in tumor tissues obtained from 6 patients with the germline TP53 D49H mutation (Table 2). Moreover, none of somatic fusion genes with activities of cancer driver gene was detected in these cases. According to these observations, it is reasonable to postulate that the germline TP53 D49H mutation may be pathogenic, at least in some pedigrees, to explain genetic predisposition to cancer.

The germline TP53 D49H mutation is unique in several ways. First, the position of amino acid replacement is located in the transactivation domain 2 of the p53 protein translated from TP53 gene (16). Two transactivation domains of p53 protein bind co-factors which modify the activities of this protein as a transcription factor (17). In in vitro experiments, the D49H mutation in TP53 severely compromised the transcriptional activities of p53 protein (8). Lee et al. demonstrated that the transactivation domain 2 of p53 protein forms a complex with CREB binding protein, and that a salt bridge between D49 of p53 protein and R2105 of nuclear coactivator binding domain of CREB binding protein may contribute to the binding specificity (11). Okuda and Nishimura suggested that the transactivation domain 2 of p53 protein binds the human TFIID subunit p62 in an extended string-like conformation; they claimed that two phosphorylation sites, S46 and T55, play an important role (14). It is worth noting that D49 locates between these amino acids. Furthermore, animal experiments also revealed that p53 transactivation do-
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Table 1  Germline TP53 variations and their characteristic features detected in 1,685 cancer patients belonging to the Project HOPE Cohort

<table>
<thead>
<tr>
<th>Variation</th>
<th>No. patients&lt;sup&gt;a&lt;/sup&gt; with variation in the Project HOPE Cohort</th>
<th>dbSNP (build 144)</th>
<th>COSMIC (V73)</th>
<th>iJGVD&lt;sup&gt;b&lt;/sup&gt;</th>
<th>HGVD&lt;sup&gt;c&lt;/sup&gt;</th>
<th>ExAC&lt;sup&gt;d&lt;/sup&gt;</th>
<th>in vitro function&lt;sup&gt;e&lt;/sup&gt;</th>
<th>No. patients&lt;sup&gt;f&lt;/sup&gt; with somatic mutation</th>
<th>No. pedigrees&lt;sup&gt;g&lt;/sup&gt; with germline mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>V10F</td>
<td>1</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>functional</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E11Q</td>
<td>17 rs201382018</td>
<td>N.A.</td>
<td>N.A.</td>
<td>0.0043</td>
<td>0.000034</td>
<td>functional</td>
<td>10</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>V31I</td>
<td>29 rs201753350</td>
<td>COSM111935</td>
<td>N.A.</td>
<td>N.A.</td>
<td>0.000008</td>
<td>functional</td>
<td>7</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>D49H</td>
<td>6 rs587780728</td>
<td>COSM250061</td>
<td>N.A.</td>
<td>N.A.</td>
<td>non-functional</td>
<td>8</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P72R</td>
<td>484 rs1042522</td>
<td>N.A.</td>
<td>N.A.</td>
<td>0.6604</td>
<td>0.66</td>
<td>functional</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>P82S</td>
<td>1</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>functional</td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q144R</td>
<td>1</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>partially functional</td>
<td>8</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A159D</td>
<td>1</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>non-functional</td>
<td>14</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L201F</td>
<td>1 rs730882024</td>
<td>COSM45489</td>
<td>N.A.</td>
<td>N.A.</td>
<td>functional</td>
<td>4</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E339K</td>
<td>2 rs17882252</td>
<td>COSM46153</td>
<td>N.A.</td>
<td>0.0012</td>
<td>0.000068</td>
<td>functional</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>No. in 1,685 patients
<sup>b</sup>Data from Integrative Japanese Genome Variation Database (iJGVD), Tohoku Medical Megabank Organization. N.A., data not available
<sup>c</sup>Data from Human Genetic Variation Database (HGVD), Kyoto University. N.A., data not available
<sup>d</sup>Data from Exome Aggregation Consortium (ExAC), Cambridge, MA [3 June 2016 accessed]. N.A., data not available
<sup>e</sup>Functional properties of p53 mutant proteins evaluated by overall transcriptional activities based on IARC TP53 database R.17 (November 2013)
- non-functional, lower than wild type;
- partially functional, positive but lower than wild type;
- functional, equal to wild type
<sup>f</sup>No. of sporadic cancer patients with TP53 somatic mutations based on IARC TP53 database R.18 (April 2016)
<sup>g</sup>No. of hereditary cancer pedigrees with TP53 germline mutation based on IARC TP53 database R.18 (April 2016)

main 1 and 2 contribute to tumor suppression (1). Taken together these findings, it is reasonable to postulate that transcriptional activation domains of p53 protein are critical for tumor suppression. However, it is worth noting that, in the IARC TP53 database, germline TP53 mutations in the transactivation
domains are extremely rare in the pedigrees with the Li-Fraumeni/Li-Fraumeni-like syndromes (8). Moreover, somatic mutations in this domain in various types of cancer are infrequent in comparison with those in the DNA binding domain and the tetramerization domain; only 8 out of 22,968 cancer tissues possessed somatic TP53 D49H mutation (8). Clinically, the present study revealed that only one patient with double germline mutations of TP53 D49H and A159D had typical Li-Fraumeni-like syndrome, and the remaining 5 cancer patients had family histories of cancer. Considering the several reports demonstrating that 92–95% of individuals who had germline TP53 pathogenic variants met the revised Chompret criteria for germline TP53 mutation screening (5, 18, 21), the incidence developing typical clinical features in patients with germline TP53 D49H mutation is lower than expected. According to these information, it is possible to postulate that germline TP53 D49H mutation may have lower pathogenic activities, and that the mutation is likely to be low-penetrant. Secondly, previous reports indicated that the germline TP53 D49H mutation is extremely uncommon in general population; in the population surveys, the D49H mutation was not detected in two Japanese cohorts (7, 12), and was reported in only one out of 121,058 alleles in the ExAC database (3). On the contrary, in the Project HOPE cohort, strong accumulation of the germline TP53 D49H mutation in Japanese cancer patients was observed; 6 out of 1,685 patients had this mutation. Further survey for family histories of these 6 patients will be required to know whether these patients belong to one large pedigree or not.

In conclusion, the present study indicates that the survey for the germline TP53 mutation plays an important role in clinical practice as it will prevent mistaking cancer patients with unusual heredities for sporadic cases. The study also suggests that the research to elucidate pathogenic roles of hereditary cancer genes with low penetrance is important to clarify genetic predisposition to cancer.

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2. Exome Aggregation Consortium (ExAC) (URL: http://exac.broadinstitute.org) (Version 0.3.1).