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Psychosocial and info-medical genetic studies of the ambiguous results of genetic testing for hereditary cancer predisposition syndrome

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"Removing the fear of uncertainty from the future" is an important motive for genetic testing. If a pathogenic mutation is detected in the genetic testing for hereditary cancer predisposition syndrome, appropriate treatments, surveillance programs and preventive therapy are available for patients. The genetic testing for hereditary cancer syndrome is, therefore, useful before and after cancer development.

Most of the results of genetic testing are definitive, but in some cases ambiguous results, *e.g.* variants of uncertain significance (VUS) and uninformative negative (UN), are obtained. Clinical geneticists and genetic counselors face the task of disclosing the ambiguous results to counselees and of interpretation of VUS, such as missense variants. Ambiguous results are often obtained in the genetic testing for hereditary cancer syndrome. Hence, this study was focused on the field of hereditary cancer. This study includes the following three components: 1) investigation of the psychosocial impact of ambiguous results in the genetic testing by literature search, 2) development of an *in silico* tool in order to predict the impact of missense variants of uncertain significance, and 3) interpretation of the VUS detected by mutation analysis, using the clinical and molecular data and the *in silico* prediction results. A series of the following studies proposed a method of the appropriate genetic counseling and of the accurate interpretation of missense variants in hereditary cancer predisposition syndrome.

In the first study, I initially hypothesized that VUS and UN have different psychosocial effects, because VUS and UN differ in the presence and absence of the genetic changes. To underline the hypothesis above, I investigated psychosocial impact of VUS and UN reported in the literature. By the systematic literature search, twenty-six papers were found, all of which were on hereditary breast and ovarian cancer (HBOC). In these literatures, I found that the consistency between a result of genetic testing and the interpretation of the result was worse in VUS than those in UN, pathogenic mutation and true negative. I also found that the perceived risk of cancer helped to predict the psychological outcome and health behaviors after the genetic testing in counselees. Hence understanding the risk perceptions of the counselees will be helpful for the practice of genetic counseling. Counselees who received a UN result were reported to have reduced level of distress, anxiety and risk perceptions compared with those received VUS, pathogenic mutation and true negative results. The intention to have mammography of UN counselees was, however, not reduced. These results suggested that the appropriate risk communication prevented counselees' "false reassurance." In addition, previous findings have suggested that the anticipatory guidance of ambiguous results and the presentation with the simple formats should have prevented the counselee's confusion and have led to the

accurate understanding. I, therefore, proposed an appropriate method for risk communication in disclosure of the ambiguous testing results by this study.

It was inferred from the literature review above that providing the information individually and concretely would promote appropriate health behaviors of counselees. It is, however, difficult to provide concrete interpretation for VUS, particularly in the case of missense variants in the coding region, and hence disclosure of the VUS results is problematic. Accurate interpretation of missense variants is indispensable to appropriate risk communication. I, therefore, developed a bioinformatics tool to predict the impact of missense variants in MSH6 for Lynch syndrome as a test case. Lynch syndrome is the most common hereditary colon cancer syndrome that accounts for 1-5% of all colorectal cancer patients and medical interventions for Lynch syndrome has the benefits of preventing cancer development. Lynch syndrome is associated with germline mutations in one of the DNA mismatch repair (MMR) genes, such as *MLH1*, *MSH2*, *MSH6* or *PMS2*, and the frequency of missense variants in *MSH6* is the highest among these MMR genes. MSH6 missense variants and their associated clinical and molecular data were collected from the various databases in order to use for adjustment of prediction parameters and for performance test. MAPP program was optimized for MSH6 and then integrated with SIFT, PolyPhen-2 and two properties from protein structure, namely solvent accessibility and the volume change in amino acid residues, by the logistic regression model. The newly developed CoDP, Combination of Different Properties (http://cib.cf.ocha.ac.jp/CoDP/), had the highest performance compared with the conventional methods: positive predictive value (PPV) was 93.3% (14/15), negative predictive value (NPV) was 94.7% (18/19), sensitivity was 93.3% (14/15), specificity was 94.7% (18/19) and accuracy was 94.1% (32/34).

About 20% of the detectable mutations in Lynch syndrome were found in *MSH6* and the distribution of *MSH6* mutation carriers differ geographically. The majority of the previous studies, however, have been conducted only on Caucasian European populations and there were few reports on Japanese Lynch syndrome patients with *MSH6* mutations. I, therefore, investigated the clinical and molecular features of *MSH6* mutation carriers in Japan. Surgically resected 1,720 colorectal carcinoma specimens were screened by microsatellite instability (MSI) testing and the MSI-high cases were subjected to a germline mutation analysis of mismatch repair genes including *MLH1*, *MSH2* and *MSH*6. I investigated the clinical and molecular features of the *MSH6* variants, such as the family cancer history, pathological findings, immunohistochemistry, methylation status of the *MLH1* promoter, and *BRAF* mutation in the colorectal tumor. In addition, the impact of the missense variants on MSH6 protein was predicted by *in silico* tools. Nine novel pathogenic mutations and eight unclassified missense variants were identified. The two missense variants were suspected pathogenic by the comprehensive analysis. I also found that most colorectal cancers in the *MSH6* mutation carriers were localized distally and that the mean age at the diagnosis of endometrial cancer in Japanese *MSH6* mutation carriers was significantly younger than that in the previous report from Western countries. These results should improve the surveillance program for the Japanese *MSH*6 mutation carriers.