Germline Variants of Prostate Cancer in Japanese Families

Takahide Hayano¹, Hiroshi Matsui², Hirofumi Nakaoka¹, Nobuaki Ohtake², Kazuyoshi Hosomichi³, Kazuhiro Suzuki², Ituro Inoue¹*

¹ Division of Human Genetics, National Institute of Genetics, Mishima, Japan, ² Department of Urology, Gunma University Graduate School of Medicine, Maebashi, Japan, ³ Department of Bioinformatics and Genomics, Graduate School of Medical Sciences, Kanazawa University, Ishikawa, Japan

* itinoue@nig.ac.jp

Abstract

Prostate cancer (PC) is the second most common cancer in men. Family history is the major risk factor for PC. Only two susceptibility genes were identified in PC, BRCA2 and HOXB13. A comprehensive search of germline variants for patients with PC has not been reported in Japanese families. In this study, we conducted exome sequencing followed by Sanger sequencing to explore responsible germline variants in 140 Japanese patients with PC from 66 families. In addition to known susceptibility genes, BRCA2 and HOXB13, we identified TRRAP variants in a mutually exclusive manner in seven large PC families (three or four patients per family). We also found shared variants of BRCA2, HOXB13, and TRRAP from 59 additional small PC families (two patients per family). We identified two deleterious HOXB13 variants (F127C and G132E). Further exploration of the shared variants in the rest of the families revealed deleterious variants of the so-called cancer genes (ATP1A1, BRIP1, FANCA, FGFR3, FLT3, HOXD11, MUTF1, PDGFRα, SMARCA4, and TCF3). The germline variant profile provides a new insight to clarify the genetic etiology and heterogeneity of PC among Japanese men.

Introduction

Prostate cancer (PC) is the second most common cancer (1.1 million new cases in 2012) in men worldwide and had the highest incidence rate in developed countries [1]. Family history is one of the established risk factors in addition to age and population [2]. A meta-analysis showed that first-degree relatives of an affected patient are 2.48 times more likely to develop PC [3]. The study of the Nordic Twin Study of Cancer cohort indicated that heritable factors may be attributable to 58% of the PC risk and liability [4]. In addition to family and twin studies, differences of the PC incidence in different populations suggest that genetic components and lifestyle are important factors in PC risk [1, 2, 5].

Genome-wide association studies and meta-analyses identified 99 variants associated with PC risk [2, 6, 7]. These variants explain 33% of the familial risk of PC in European descendants [7]. Only two susceptibility genes for PC, BRCA2 and HOXB13, were identified [2]. Germline
variants of BRCA2 are established risk factors of PC [8]. HOXB13 is a key transcription factor for PC and the association between a rare variant of HOXB13 G84E and PC risk was confirmed in a recent large-scale study [9]. To date, the HOXB13 G84E variant has been found only among European descendants. Identification of different HOXB13 variants in individuals with non-European descent, including HOXB13 G135E in Chinese men, indicates allelic heterogeneity of HOXB13 variants depending on populations [10, 11, 12].

The age-standardized incidence rate (ASIR) among Japanese men was relatively low compared with that among European men [1, 5]. However, ASIR has been increasing in Japan probably owing to lifestyle changes [1, 13]. Genetic exploration of PC among Japanese men is important to understand the development of PC at the population level. In our previous study, we identified PC susceptibility loci of chromosomes 8p23 and 1p36 in Japanese patients with affected siblings by genome-wide linkage analysis. However, the confirmative linkage results were not obtained [14]. Comprehensive study for germline variants of Japanese patients with PC has not been reported.

To find novel responsible genes for PC, exome sequencing (exome-seq) of 81 patients with PC from seven large families (three or four patients per family) and 59 small families (two patients per family) was conducted.

**Materials and Methods**

**Ethics Statement**

The study protocols were approved by the Institutional Review Boards of Gunma University (No. 5, 2013.12.2) and National Institute of Genetics (No. 26–6, 2014.8.8). Each participant provided written informed consent for the collection of samples and subsequent analyses.

**PC families and study design**

Sixty-six families were categorized as large PC families and small PC families: The large PC families consisted of three or four patients with PC (22 patients in seven families). The small PC families are pairs of patients with PC (118 patients in 59 families). Only patients were recruited. All of the 22 patients of the large families and probands of small families were analyzed by exome-seq. Shared variants in the patients of small families were confirmed by Sanger-seq using the counterpart of PC pairs in each family.

**Clinical information**

All 140 patients with PC were histologically diagnosed at Gunma university hospital and its affiliated hospitals. Patients had a mean age at diagnosis of 69.0 (range, 40–88 years). Gleason scores [15] were lower than 7 in 42 patients and equal to 7 or higher in 97 patients (unknown in one patient).

**DNA preparation and exome-seq**

Genomic DNA was isolated from peripheral blood using a GENOMIX kit (Talent srl. Treisete, Italy). Fragmentation and adaptor tagmentation of the genomic DNA followed by hybridization for capturing probes were performed using a SureSelect Human All Exon V5+IncRNA (Agilent) for preparing capture libraries. The libraries were sequenced using the Illumina HiSeq 2500 (Illumina) with 150 base-paired end modules (for the large PC families) or 100 base-paired end modules (for the small PC families).
Exome-seq data analyses
Sequencing reads were mapped to a reference genome (hg19) using BWA-mem [16] and SAMtools [17]. Picard MarkDuplicatesWithMateCigar module (http://broadinstitute.github.io/picard/) was used for removing duplicate reads. Local realignment of reads around known indels and recalibration of base quality were performed using Genome Analysis Toolkit (GATK) IndelRealigner and BaseRecalibrator module, respectively [18, 19, 20]. Variant call and genotyping were performed using GATK HaplotypeCaller. Vcf files were decomposed and normalized by vt program [21]. For the large families, variant calls in the same family were combined with GATK CombineGVCF module. Shared variants in same family members were extracted using snpEff and SnpSift [22].

Filtering and prioritizing of variants
The variants_reduction.pl script of ANNOVAR was used for filtering [23]. We focused on exonic and splicing variants. The synonymous variants were filtered out. Variants in the genomic super duplicated regions were removed. Database-registered single nucleotide polymorphisms (SNPs) were removed, except for clinically reported variants using dbSNP Flagged information. Rare variants with minor allele frequency (MAF) of <0.001 were filtered in from information from the NHLBI GO Exome Sequencing Project [24] and the 1000 Genomes Project (1KGP) [25]. The remaining variants were annotated using table_annovar.pl script of ANNOVAR. For prioritization, the hiPIVE module in Exomiser was used [26, 27], which compiled information from phenotype (human, mouse, and zebra fish), protein–protein interaction, and in silico predictions (SIFT, Polyphen2, and MutationTaster). The OMIM term (OMIM: 176807) and HPO term (HPO: 0012125) for “Prostate Cancer” were used for the phenotype input for the hiPIVE module. Combined score greater than or equal to 0.80 of Exomiser was set as the threshold. Overlapped genes from ANNOVAR and Exomiser results were extracted as candidate genes.

Allele frequency databases
Allele frequency data from more than 60,000 individuals of Exome Aggregation Consortium (ExAC) was used as reference (http://exac.broadinstitute.org/). For allele frequencies in Japanese, we referred to two databases of the integrative Japanese Genome Variation Database (iJGVD) [28, 29] and the Human Genetic Variation Database (HGVD) [30]. The iJGVD was derived from more than 1,000 healthy Japanese individuals using whole genome sequencing. Data were downloaded on 26 April 2016. The HGVD collected exome variants information from more than 1,000 Japanese individuals. Data release version 1.42 was used.

The CGC genes
To further extract cancer-related genes, information from the CGC database [31] was used. CGC is a census of cancer genes with variants obtained from literature searches. A gene list of CGC was downloaded on 29 October 2015 from the web site (http://cancer.sanger.ac.uk/census/).

Functional prediction of variants and selection of genes for Sanger-seq
For the functional prediction of variants, we referred to two in silico prediction scores of LR and RadialSVM, which are ensemble scores from nine prediction methods (FATHMM, LRT, MutationAssessor, MutationTaster, PolyPhen-2, SIFT, GERP++, PhyloP, and SiPhy) and allele frequency [32]. These two ensemble scores are implemented in the ANNOVAR [23].
Variants of the CGC genes predicted from both of the LR and RadialSVM were analyzed by Sanger-seq.

**Results**

**Results of exome-seq**

Germline variants were identified in 81 patients with PC from 66 families through exome-seq data analyses. Twenty-two and fifty-nine patients were from 7 large PC families (Fig 1) and 59 small PC families, respectively. Mapping results achieved the average read depth of 97 and 72, and the coverage of 95.2% and 80.7% at read depth of 20 for the large families and small families, respectively (S1 and S2 Tables). A total of 1,082,617 variants (154,659 per family) were detected and an average of 83,197 variants (from 79,146 to 87,027) was shared in each large family (S3 Table). In the small families, an average of 116,536 variants (from 113,178 to 119,238) was detected (S4 Table). After filtering and prioritizing variants (Materials and Methods), 564 genes with variants remained. In these genes with variants, none of the genes from the 66 families had a frequency greater than 12%, except for MUC6 (66 of 66, 100%), TBP (44 of 66, 66.7%), and MUC5B (32 of 66, 48.5%). We excluded mucin genes (MUC6 and MUC5B).

**Fig 1. Pedigrees of the seven large PC families.** Solid black rectangles represent affected patients with PC. Patients with PC analyzed by exome-seq were numbered (from 01 to 22). PC, prostate cancer.

doi:10.1371/journal.pone.0164233.g001
because variants of these genes were frequently detected in public exome data sets and less likely to be associated with diseases [33]. After removing common variants of TBP observed in HGVD (MAF > 0.3), the frequency of TBP was lower than 12% (3 of 66, 4.5%).

To find shared genes with variants, we first focused on the seven large PC families with more than three patients. In the large families, TRRAP was identified in two families (Fam04 and Fam07) together with known PC susceptibility genes of BRCA2 (Fam01) and HOXB13 (Fam03 and Fam06) in a mutually exclusive manner (Fig 2A). The BRCA2 variant (V2109I) was found in a patient with breast cancer (BC) with family history of pancreas cancer [34] and a patient with esophageal cancer with family history of gastric cardia cancer [35]. For two HOXB13 variants (F127C and G132E), the allele frequency information was not reported in the ExAC database. Although we found allele frequency information for HOXB13 G132E in Japanese databases (MAF = 0.00048 in iJGVD and MAF = 0.0012 in HGVD), this might not reject the pathogenicity of the variants considering the frequency of PC. The TRRAP R2195H variant was a novel indicating pathogenic and the TRRAP T2738P variant was found in the ExAC (MAF = 0.028) and iJGVD (MAF = 0.027) databases; therefore, the pathogenicity of the variant would be reserved (Fig 2B). All variants were heterozygous in the families.

### Shared variants in the small PC families

We further examined shared variants of BRCA2, HOXB13, and TRRAP in the 59 small families of PC pairs. A variant of BRCA2 R18H was shared in a family (GFPC043/044). However, this variant is reported as "Benign" in the ClinVar database [36]; thus, it was excluded from candidate variants. Five BRCA2 variants (L61P, H1458R, G2508S, H3056Y, and R3384X) detected in

---

**Fig 2. Shared genes with variants in the large PC families.** (A) Twenty-two genes in the seven large families remained after filtering and prioritizing. Known susceptibility genes (BRCA2 and HOXB13) and one novel gene (TRRAP) are shown by green rectangles. The combined scores of Exomiser are shown on the right side of the gene names. (B) Variant status of BRCA2, HOXB13, and TRRAP. ExAC_all, MAF of all subjects in the ExAC; iJGVD, MAF in the iJGVD; HGVD, MAF in the HGVD; NA, Not applicable. PC, prostate cancer.

doi:10.1371/journal.pone.0164233.g002
five probands were not shared in the counterpart of each family. The same variant of \( \text{HOXB13} \) G132E, which was found in a large family (Fam06), was also shared in two small families (GFPC023/024 and GFPC079/080). We also identified a shared novel variant of \( \text{TRRAP} \) C1217R indicating pathogenicity in a family (GFPC071/072) (Fig 3). All shared variants were heterozygous except for \( \text{HOXB13} \) G132E in GFPC079 (homozygous).

In the remaining 56 small families without shared variants of \( \text{HOXB13} \) and \( \text{TRRAP} \), patients with PC from 44 families had at least one variant of cancer genes of the CGC (Materials and Methods). In a total of 74 variants in 60 CGC genes, we performed Sanger-seq for 50 variants in 46 genes and confirmed shared variants of 18 genes (Fig 4 and S5 Table). Ten variants in 10 genes (\( \text{ATP1A1}, \text{BRIP1}, \text{FANCA}, \text{FGFR3}, \text{FLT3}, \text{HOXD11}, \text{MUTYH}, \text{PDGFRA}, \text{SMARCA4}, \) and \( \text{TCF3} \)) were predicted to be deleterious by two prediction methods, RadialSVM and LR. Notably, the same variant of \( \text{FLT3} \) T820N was found in four probands (GFPC039, GFPC095, GFPC098, and GFPC111) and shared in three families (GFPC039/040, GFPC095/096, and GFPC111/112).

**Clinical relevance**

In total, 58 patients from 26 families shared variants of \( \text{BRCA2}, \text{HOXB13}, \text{TRRAP}, \) or CGC genes (Fig 4A). We assessed associations between clinical features (Gleason score and age at diagnosis) and shared variant status. We compared the families with shared variants (shared families) and without shared variants (unshared families). We found that the shared families showed high Gleason scores (averaged scores in each family) compared with unshared families.
**Fig 4. Shared genes with variants.** (A) Heat map of the shared genes with variants. Each column shows the family identification of large PC families or PC pairs of the small PC families. Each row shows the gene names and shared variants are filled with red (deleterious) or orange (nondeleterious) color. (B) Deleterious variants of the Cancer Gene Census genes. The variant status is shown. ExAC_all, MAF of all subjects in the ExAC; iJGVD, MAF in the iJGVD; HGVD, MAF in the HGVD; NA, Not applicable; PC, prostate cancer.

doi:10.1371/journal.pone.0164233.g004
The mean age at diagnosis of the shared families and unshared families was 68.7 (range from 53.7 to 80.5 years) and 69.6 (range from 57.5 to 85.0 years), respectively, showing no statistical difference (Wilcoxon rank sum test p value = 0.99) (Fig 5B).

Discussion

In this study, we profiled germline variants in a large numbers of patients with PC in Japanese families. In the total of 140 patients with PC from 66 families, TRRAP was identified as a novel candidate gene for PC together with known susceptibility genes of BRCA2 and HOXB13.

TRRAP is a common component of many histone acetyltransferase (HAT) complexes, and it is involved in transcriptional regulation and DNA repair [37]. Protein expression of TRRAP was low in BC compared with matched normal breast tissues, indicating a tumor suppressive role of TRRAP for BC [38]. The association between germline variants of TRRAP and PC remains unclear, and further studies are needed to clarify this association.

Two variants of HOXB13 (F127C and G132E) were identified. Both of the variants were considered to be deleterious and were located at near the homeobox protein Hox1A3 N-terminal domain (residues 21–123) [12]. We could not find the specific European HOXB13 G84E variant among Japanese samples, which was consistent with previous reports [10, 11, 12]. A large-scale study for HOXB13 variants in a Chinese population (96 patients with PC) failed to detect the two HOXB13 variants that were detected in the present study, indicating the allelic heterogeneity of HOXB13 variants in Asian populations.
The variant of \textit{BRCA2} V2019I was found in patients with family history of cancer \cite{34, 35}. This variant was also found in a hereditary breast and ovarian cancer study in Japanese women. Further, this variant was reported as a variant of uncertain significance \cite{39}. Further evidence may be required to determine the pathogenicity of the variant.

We further identified shared variants of the CGC genes in the remaining small families, and 10 variants were considered to be deleterious (Fig 4 and S5 Table). Three genes (\textit{BRIP1}, \textit{FANCA}, and \textit{MUTYH}) were DNA repair genes. Additionally, rare germline variants of \textit{BRIP1} and \textit{MUTYH} were previously identified in PC \cite{40}. A deleterious germline mutation of \textit{FANCA} was found in familial BC \cite{41}. Three receptor tyrosine kinase (RTK) genes (\textit{FGFR3}, \textit{FLT3}, and \textit{PDGFRA}) were identified. Somatic variants of RTK have been reported to confer oncogenic function in various cancers and inhibitors of RTK are targeted drugs for the anticancer therapy \cite{42}. Germline variants of \textit{FGFR3} and \textit{PDGFRA} were also found in medulloblastoma and gastrointestinal stromal tumors, respectively \cite{43, 44}. The deleterious variant of \textit{FLT3} T820N in three families located on the C lobe of the kinase domain \cite{45} could affect kinase activity. The patient (GFPC097) who did not share the \textit{FLT3} T820N variant could be a sporadic case. \textit{HOXD11} and \textit{TCF3} are coding transcription factors. Somatic fusion genes of these genes such as \textit{NUP98-HOXD11} and \textit{TCF3-HLF} were found in leukemia \cite{46, 47}. Frequent germline and somatic mutations of \textit{SMARCA4} in small cell carcinoma of the ovary of hyper calcemic type (SCCOHT) suggested a tumor suppressor role \cite{48, 49}. Somatic variants of \textit{ATP1A1} were reported to cause aldosterone producing adenomas in primary aldosteronism \cite{50}. An association between germline mutations of four genes (\textit{FLT3}, \textit{HOXD11}, \textit{TCF3}, and \textit{ATP1A1}) and cancer has not been reported.

We consider that the main limitation of this study was that our study design lacked the inclusion of healthy controls. Additionally, the heterogeneity of PC causality, which is shown here, limits the current causal variant detection in the families studied. Further association studies using case and control samples and functional studies are required to evaluate the contribution of these variants in the development of PC.

In this study, we re-evaluated a collection of familial patients with PC in our previous genome-wide linkage study \cite{14} using exome analysis. High Gleason scores in the shared families compared with unshared families suggested that germline variants of the candidate genes could contribute to the malignancy of PC. Our results provide a resource for further understanding of PC development at population level.

**Supporting Information**

\textbf{S1 Table.} Depth and coverage results of exome-seq in the large prostate cancer families. Family, ID of the large prostate cancer families; ID, ID of each individual; Depth, mean depth of each sample; Coverage, coverage at depth of 20. (XLSX)

\textbf{S2 Table.} Depth and coverage results of exome-seq in the small prostate cancer families. ID, ID of each individual; Depth, mean depth of each sample; Coverage, coverage at depth of 20. (XLSX)

\textbf{S3 Table.} Numbers of variant in the seven large prostate cancer families. Family, ID of the large prostate cancer families; Variants; total number of variants detected in each family; Shared variants, number of shared variants in each family. (XLSX)
S4 Table. Numbers of variant in the 59 small prostate cancer families. ID, ID of each individual; Variants, number of variants detected in each individual. (XLSX)

S5 Table. Variants of the Cancer Gene Census genes. This table is an output of ANNOVAR. Additional columns of “Sanger-seq” and “SangerResults” were added. Done, Sanger-seq was performed; Undone, Sanger-seq was not performed; Shared, shared variant was confirmed; WT, wild type; Not confirmed, variant status was not confirmed. (XLSX)

Acknowledgments
We are grateful to Junko Kitayama, Junko Kajiwara, and Yumiko Sato (Division of Human Genetics, National Institute of Genetics) for their technical supports. The authors would like to thank Enago (www.enago.jp) for the English language review.

Author Contributions
Conceptualization: HM KS II.
Data curation: TH.
Formal analysis: TH.
Funding acquisition: HM KS II.
Investigation: HM NO KH KS.
Methodology: TH II.
Resources: HM NO KH KS.
Supervision: II.
Validation: TH.
Visualization: TH.
Writing – original draft: TH.
Writing – review & editing: HM HN II.

References
1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. (2015) Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 136: E359–386. doi: 10.1002/ijc.29210 PMID: 25220842
2. Attard G, Parker C, Eeles RA, Schröder F, Tomlins SA, Tannock I, et al. (2016) Prostate cancer. Lancet 387: 70–82. doi: 10.1016/S0140-6736(14)61947-4 PMID: 26074382
3. Kiciński M, Vangronsveld J, Nawrot TS. (2011) An epidemiological reappraisal of the familial aggregation of prostate cancer: a meta-analysis. PLoS One 6: e27130. doi: 10.1371/journal.pone.0027130 PMID: 22073129
4. Hjelmborg JB, Scheike T, Holst K, Skytte A, Penney KL, Graff RE, et al. (2014) The heritability of prostate cancer in the Nordic Twin Study of Cancer. Cancer Epidemiol Biomarkers Prev. 23: 2303–2310. doi: 10.1158/1055-9965.EPI-13-0568 PMID: 24812039
5. Marugame T, Katanoda K. (2006) International comparisons of cumulative risk of breast and prostate cancer, from cancer incidence in five continents Vol. VIII. Jpn J Clin Oncol 36: 399–400. doi: https://dx.doi.org/10.1093/jjco/hyl049 PMID: 16818481
6. Eeles RA, Olama AA, Benlloch S, Saunders EJ, Leongamornlert DA, Tymskiewicz M, et al. (2013) Identification of 23 new prostate cancer susceptibility loci using the iCOGS custom genotyping array. Nat Genet 45: 385–391. doi: 10.1038/ng.2560 PMID: 23535732

7. Al Olama AA, Kote-Jarai Z, Berndt SI, Conti DV, Schumacher F, Han Y, et al. (2014) A meta-analysis of 87,040 individuals identifies 23 new susceptibility loci for prostate cancer. Nat Genet 46: 1103–1109. doi: 10.1038/ng.3094 PMID: 25217961

8. Cavanagh H, Rogers KM. (2015) The role of BRCA1 and BRCA2 variants in prostate, pancreatic and stomach cancers. Hered Cancer Clin Pract 13: 16. doi: 10.1186/s13053-015-0038-x PMID: 26236408

9. Hoffman TJ, Sakoda LC, Shen L, Jorgenson E, Habel LA, Liu J, et al. (2015) Imputation of the rare HOXB13 G84E variant and cancer risk in a large population-based cohort. PLoS Genet 11: e1004930. doi: 10.1371/journal.pgen.1004930 PMID: 25629170

10. Ewing CM, Ray AM, Lange EM, Zulike KA, Robbins CM, Tembe WD, et al. (2012) Germline variants in HOXB13 and prostate-cancer risk. N Engl J Med 366: 141–149. doi: 10.1056/NEJMoa1110000 PMID: 22718278

11. Lin X, Qu L, Chen Z, Xu C, Ye D, Shao Q, et al. (2013) A novel germline variant in HOXB13 is associated with prostate cancer risk in Chinese men. Prostate 73: 169–175. doi: 10.1002/pros.22552 PMID: 22718278

12. Maia S, Cardoso M, Pinto P, Pinheiro M, Santos C, Peixoto A, et al. (2015) Identification of Two Novel HOXB13 Germline Variants in Portuguese Prostate Cancer Patients. PLoS One 10: e0132728. doi: 10.1371/journal.pone.0132728 PMID: 26176944

13. Katanoda K, Hori M, Matsuda T, Shibata A, Nishino Y, Hattori M, et al. (2015) An updated report on the trends in cancer incidence and mortality in Japan, 1958–2013. Jpn J Clin Oncol 45: 390–401. doi: 10.1093/jco/hvy002 PMID: 25637502

14. Matsui H, Suzuki K, Ohtake N, Nakata S, Takeuchi T, Yamanaka H, et al. (2004) Genome wide linkage analysis of familial prostate cancer in the Japanese population. J Hum Genet 49: 9–15. doi: https://doi.org/10.1007/s10038-003-0099-y PMID: 1466403

15. Gleason DF. (1992) Histologic grading of prostate cancer: a perspective. Hum Pathol 23: 273–279. doi: 10.1016/0046-8177(92)90108-f PMID: 1555838

16. Li H. (2014) Toward better understanding of artifacts in variant calling from high-coverage samples. Bioinformatics 30: 2843–2851. doi: 10.1093/bioinformatics/btu356 PMID: 24974202

17. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, et al. (2009) The Sequence Alignment/Map format and SAMtools. Bioinformatics 25: 2078–2079. doi: 10.1093/bioinformatics/btp352 PMID: 19505943

18. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. (2010) The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20: 1297–303. doi: 10.1101/gr.107524.110 PMID: 20644199

19. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, et al. (2011) A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nature 493: 491–498. doi: 10.1038/ng.806 PMID: 21478889

20. Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, Del Angel G, Levy-Moonshine A, et al. (2013) From fastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. Curr Protoc Bioinformatics 11: 11.10.1–11.10.33.

21. Tan A, Abecasis GR, Kang HM. (2015) Unified representation of genetic variants. Bioinformatics 31: 2202–2204. doi: 10.1093/bioinformatics/btv112 PMID: 25701572

22. Cingolani P, Platts A, Wang le L, Coon M, Nguyen T, Wang L, et al. (2012) A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly (Austin) 6: 80–92. doi: https://dx.doi.org/10.4161/fly.19695 PMID: 22728627

23. Wang K, Li M, Hakonarson H. (2010) ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 38: e164. doi: 10.1093/nar/gkq603 PMID: 20601685

24. Fu W, O’Connor TD, Jun G, Kang HM, Abecasis G, Leal SM, et al. (2015) Analysis of 6,515 exomes reveals the recent origin of most human protein-coding variants. Nature 493: 216–220.

25. The 1000 Genomes Project Consortium. (2015) A global reference for human genetic variation. Nature 526: 68–74. doi: 10.1038/nature15393 PMID: 26432245

26. Robinson PN, Köhler S, Oellrich A, Sanger Mouse Genetics Project, Wang K, Mungall CJ, et al. (2014) Improved exome prioritization of disease genes through cross-species phenotype comparison. Genome Res 24: 340–348. doi: 10.1101/gr.160325.113 PMID: 24182188

27. Smedley D, Jacobsean JO, Jäger M, Köhler S, Holtgrewe M, Schubach M, et al. (2015) Next-generation diagnostics and disease-gene discovery with the Exomiser. Nat Protoc 10: 2004–2015. doi: 10.1038/nprot.2015.124 PMID: 26562621
28. Nagasaki M, Yasuda J, Katsuoka F, Narai N, Kojima K, Kawai Y, et al. (2015) Rare variant discovery by deep whole-genome sequencing of 1,070 Japanese individuals. Nat Commun 6: 8018. doi: 10.1038/ncomms9018 PMID: 26292667

29. Yamaguchi-Kabata Y, Narai N, Kawai Y, Sato Y, Kojima K, Tateno M, et al. (2015) iJGVD: an integrative Japanese genome variation database based on whole-genome sequencing. Hum Genome Var 2: 15050. doi: 10.1038/hgv.2015.50 PMID: 26552646

30. Higasa K, Miyake N, Yoshimura J, Okamura K, Niihori T, Saitsu H, et al. (2016) Human genetic variation database, a reference database of genetic variations in the Japanese population. J Hum Genet [Epub ahead of print]. doi: https://dx.doi.org/10.1038/jhg.2016.12 PMID: 26911352

31. Futreal PA, Coin L, Marshall M, Down T, Hubbard T, Wooster R, et al. (2004) A census of human cancer genes. Nat Rev Cancer 4: 177–183. doi: 10.1038/nrc1299 PMID: 14993899

32. Dong C, Wei P, Jian X, Gibbs R, Boerwinkle E, Wang K, et al. (2015) Comparison and integration of deleteriousness prediction methods for nonsynonymous SNVs in whole exome sequencing studies. Hum Mol Genet 24: 2125–2137. doi: 10.1093/hmg/ddu733 PMID: 25552646

33. Shyr C, Tarailo-Graovac M, Gottlieb M, Lee JJ, van Karnebeek C, Wasserman WW. (2014) FLAGS, frequently mutated genes in public exomes. BMC Med Genomics 7: 64. doi: 10.1186/s12920-014-0064-y PMID: 25466818

34. Choi DH, Lee MH, Bale AE, Carter D, Haftfy BG. (2004) Incidence of BRCA1 and BRCA2 mutations in young Korean breast cancer patients. J Clin Oncol 22: 1638–1645. doi: https://dx.doi.org/10.1200/JCO.2004.04.179 PMID: 15117986

35. Hu N, Wang C, Han XY, He LJ, Tang ZZ, Giffen C et al. (2004) Evaluation of BRCA2 in the genetic susceptibility of familial esophageal cancer. Oncogene 23: 852–858. doi: https://dx.doi.org/10.1038/sj.onc.1207150 PMID: 14647438

36. Landrum MJ, Lee JM, Benson M, Brown G, Chao C, Chitipiralla S, et al. (2016) ClinVar: public archive of interpretations of clinically relevant variants. Nucleic Acids Res 44: D862–868. doi: 10.1093/nar/gkv1222 PMID: 26582918

37. Mori R, Vaissière T, Sawan C, Shukla V, Hercog Z. (2007) Orchestrating control of TRRAP mediated processes: mind the TRRAP. Oncogene 26: 5358–5372. doi: https://dx.doi.org/10.1038/sj.onc.1210605 PMID: 17694078

38. Wang J, Shan M, Liu T, Shi Q, Zhong Z, Wei W, et al. (2016) Analysis of TRRAP as a Potential Molecular Marker and Therapeutic Target for Breast Cancer. J Breast Cancer 19: 61–67. doi: 10.4048/jbc.2016.19.1.61 PMID: 27066097

39. Hirotsu Y, Nakagomi H, Sakamoto I, Amemiya K, Mochizuki H, Omata M. (2015) Detection of BRCA1 and BRCA2 germline mutations in Japanese population using next-generation sequencing. Mol Genet Genomic Med 3: 121–129. doi: 10.1002/mgg3.120 PMID: 25802882

40. Leongamoniet D, Saunders E, Dadavet T, Tymrakiewicz M, Goh C, Jugernaut-Little S, et al. (2014) Frequent germline deleterious variants in DNA repair genes in familial prostate cancer cases are associated with advanced disease. Br J Cancer 110: 1663–1672. doi: 10.1038/bjc.2014.30 PMID: 24556621

41. Ellingson MS, Hart SN, Kalari KR, Sumam V, Schahl KA, Dockter TJ, et al. (2015) Exome sequencing reveals frequent deleterious germline variants in cancer susceptibility genes in women with invasive breast cancer undergoing neoadjuvant chemotherapy. Breast Cancer Res Treat 153: 435–443. doi: 10.1007/s10549-015-3545-6 PMID: 26296701

42. Raval SH, Singh RD, Joshi DV, Patel HB, Mody SK. (2016) Recent developments in receptor tyrosine kinases targeted anticancer therapy. Vet World 9: 80–90. doi: 10.14202/vetworld.2016.80-90 PMID: 27051190

43. Bourdeaut F, Miquel C, Di Rocco F, Grison C, Richer W, Brugieres L, et al. (2013) Germline mutations in FGFR receptors and medulloblastomas. Am J Med Genet A 161A: 382–385. doi: 10.1002/ajmg.a.35719 PMID: 23325524

44. Postow MA, Robson ME. (2012) Inherited gastrointestinal stromal tumor syndromes: mutations, clinical features, and therapeutic implications. Clin Sarcoma Res 2: 16. doi: 10.1186/2045-3329-2-16 PMID: 23036227

45. Griffith J, Black J, Faerman C, Swenson L, Wynn M, Lu F, et al. (2004) The structural basis for autoinhibition of FLT3 by the juxtamembrane domain. Mol Cell 13: 169–178. doi: 10.1016/s1097-2765(03)00505-7 PMID: 14753933

46. Takedani T, Taki T, Shibuya N, Ito E, Kitazawa J, Terui K, et al. (2002) The HOXD11 gene is fused to the NUP98 gene in acute myeloid leukemia with t(2;11)(q31;p15). Cancer Res 62: 33–37. PMID: 11782354
47. Moorman AV. (2016) New and emerging prognostic and predictive genetic biomarkers in B-cell precursor acute lymphoblastic leukemia. Haematologica 101: 407–416. doi: 10.3324/haematol.2015.141101 PMID: 27033238

48. Ramos P, Karnezis AN, Craig DW, Sekulic A, Russell ML, Hendricks WP, et al. (2014) Small cell carcinoma of the ovary, hypercalcemic type, displays frequent inactivating germline and somatic mutations in SMARCA4. Nat Genet 46: 427–429. doi: 10.1038/ng.2928 PMID: 24658001

49. Witkowski L, Carrot-Zhang J, Albrecht S, Fahiminiya S, Hamel N, Tomiak E, et al. (2014) Germline and somatic SMARCA4 mutations characterize small cell carcinoma of the ovary, hypercalcemic type. Nat Genet 46: 438–443. doi: 10.1038/ng.2931 PMID: 24658002

50. Beuschlein F, Boulkroun S, Osswald A, Wieland T, Nielsen HN, Lichtenauer UD, et al. (2013) Somatic variants in ATP1A1 and ATP2B3 lead to aldosterone-producing adenomas and secondary hypertension. Nat Genet 45: 440–444. doi: 10.1038/ng.2550 PMID: 23416519