Original article

Follow-up System for Childhood Cancer Survivors Via Germline Clinical Sequencing

小児がん経験者に対する生殖細胞系列のクリニカルシークエンスを用いたフォローアップシステム

Tatsuo Watanabe1), **Koichi Hirabayashi**1) *, **Daisuke Morita**1) 2), **Tomomi Yamaguchi**3) 4) 5), **Eri Okura**1), **Hirokazu Morokawa**1), **Shoji Saito**1), **Miyuki Tanaka**1), **Yozo Nakazawa**1), **and Tomoki Kosho**3) 4) 5) 6)

1) Department of Pediatrics, Shinshu University School of Medicine
2) Institute for Biomedical Sciences, Shinshu University Interdisciplinary Cluster for Cutting Edge Research
3) Department of Medical Genetics, Shinshu University School of Medicine
4) Center for Medical Genetics, Shinshu University Hospital
5) Research Center for Supports to Advanced Science, Shinshu University
6) Division of Clinical Sequencing, Shinshu University School of Medicine

Key words: cancer predisposition genes, childhood cancer survivor, clinical sequencing, follow-up system, second malignant neoplasm

Running title: CCSs follow-up using clinical sequencing

Number of text pages: 10

Figures: 1

Tables: 4

Required number of reprints: 5

*** Corresponding author:** Koichi Hirabayashi

Department of Pediatrics, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto,

Nagano 390-8621, Japan

E-mail: kohira@shinshu-u.ac.jp

Abstract

Background: A second malignant neoplasm (SMN) has the greatest impact on the prognosis of childhood cancer survivors (CCSs). Although germline abnormalities in cancer predisposition genes have been reported as a cause of SMN in CCSs, genetic background is not considered for SMN surveillance in the follow-up guidelines. The study aimed to present an SMN surveillance system for CCSs using germline cancer predisposition genes and evaluate their efficacy. We also aimed to elucidate the psychological impact of surveillance system on CCSs and their guardians.

Methods: CCSs who visited the long-term follow-up clinic at Shinshu University Hospital were recruited. They underwent next-generation sequencing-based germline genetic investigation using a custom panel including 165 cancer predisposition genes and a multiplex ligation-dependent probe amplification method for *TP53*. Based on the molecular findings, appropriate SMN surveillance was proposed. A questionnaire-based survey was conducted to comprehend the thoughts of CCSs and/or their guardians regarding SMN, clinical sequencing, and SMN surveillance.

Results: As of March 2021, 16 CCSs, mostly with leukemia as a primary cancer, participated in this study. No pathogenic or likely pathogenic variants were detected in any of the participants. Variants of uncertain significance were found in four CCSs showing increased anxiety.

Conclusions: This study could not show the efficacy of SMN surveillance system for CCSs, because no pathogenic or likely pathogenic variants were detected. Further evaluation, including more CCSs with a wider spectrum of cancers, would be necessary to evaluate this system. Genetic counseling might require careful anticipatory guidance for clinical sequencing and follow-up services.

Abstract

背景: 二次がんは小児がん経験者の予後に対して最も大きな影響を与えるため、早期発見がきわめて重要である。がん素因遺伝子の生殖細胞系列異常は、小児がん経験者の二次がんの原因になると報告されているにもかかわらず、フォローアップガイドラインにおける二次がんのサーベイランスの中には遺伝的背景が考慮されていない。本研究では、生殖細胞系列のがん素因遺伝子を用いた小児がん経験者に対する二次がんサーベイランスシステムを示し、それらの有効性に関して評価することを目的とした。我々はまた、サーベイランスシステムの小児がん経験者および保護者に対する心理的影響を明らかにすることを目的とした。

方法：信州大学医学部附属病院の長期フォローアップ外来を受診している小児がん経験者から参加者を募った。参加者は、次世代シークエンシングに基づく生殖細胞系列の遺伝子解析を、我々が独自に作成した165種類のがん素因遺伝子パネルを用いて実施した。また、*TP53*に対しては、multiplex ligation-dependent probe amplification法で解析した。解析結果に基づき、適切な二次がんサーベイランスを提案した。小児がん経験者および保護者の二次がん、クリニカルシークエンス、および二次がんサーベイランスに対する考えを理解するため質問紙法に基づいた調査も実施した。

結果:2021年3月までに、16名の小児がん経験者が本研究に参加した。白血病が多くの参加者の初発時腫瘍だった。病的または病的らしいバリアントはすべての参加者で検出されなかった。病的意義不明のバリアントが検出された4名の小児がん経験者では、二次がんの不安が上昇していた。

結論：本研究では、病的または病的らしいバリアントが検出されなかったため、小

児がん経験者に対する二次がんのサーベイランスシステムの有効性を示すことができなかった。本システムを評価するためには、より多岐にわたる小児がん経験者を対象としたさらなる評価が必要を考えられた。慎重で先を見越した説明が遺伝カウンセリングでは必要を考えられた。

I Introduction

The improvement in the cure rate of pediatric cancer in recent years has led to the increase in childhood cancer survivors (CCSs) 1) 2). Consequently, late effects in CCSs have become a serious problem 3). The second malignant neoplasm (SMN) has the greatest impact on long-term life prognosis among the late effects 4). Therefore, early detection and early stage treatment of CCSs with SMN is very important to improve their long-term life prognosis 5).

Anticancer drugs and/or radiation therapy are well-known causes of SMN. Additionally, germline abnormalities in cancer predisposition genes in CCSs have also been reported as a cause of SMN in recent years 6). CCSs have been shown to have a higher probability of carrying germline abnormalities in cancer predisposition genes than the general population 6) 7). Hence, the assessment of germline abnormalities in cancer predisposition genes for CCSs could be useful for the stratification of the risk for development of SMN. However, in the current follow-up guidelines for CCSs, the genetic background is considered only in some SMN screening systems, such as breast cancer (BRCA 1/2), and the stratification of SMN risk is mainly based on the history of anticancer therapies 8).

Therefore, we introduced a surveillance system using clinical sequencing of germline cancer predisposition genes for CCSs to assess the risk of developing SMN in a long-term follow-up (LTFU) clinic at Shinshu University Hospital. The purpose of this study was to present the clinical and molecular findings in CCSs, application of SMN surveillance, and discuss the thoughts of the participants obtained through this follow-up system. The study also aimed evaluate the efficacy of the follow-up system.

II Methods

A Participants

An outline of this study is presented in Fig. 1. The participants were CCSs who were recruited from the LTFU clinic at Shinshu University Hospital. The LTFU clinic was established in 2014 and included approximately 140 CCSs as of October 2020, some of whom were introduced from other hospitals after the completion of their cancer therapy. This study was approved by the Ethics Committee of Shinshu University School of Medicine in January 2019 (approval number: 633) and was initiated in August 2019.

We informed the CCSs and/or their guardians of this study at the LTFU Clinic of the Department of Pediatrics in Shinshu University Hospital. Upon agreeing to participate in the study, CCSs and/or their guardians were referred to the Center for Medical Genetics for genetic counseling for clinical sequencing provided by a clinical geneticist (TK), a pediatric oncologist (TW), and certified genetic counselors. Informed consent was obtained from adult CCSs and guardians of minor CCSs (younger than 20 years old). As of March, 2021, 16 CCSs participated in the study.

B Clinical sequencing

After obtaining consent from the participants and/or their guardians, 5 mL of peripheral blood was collected from CCSs with no history of allogeneic hematopoietic stem cell transplantation (allo-HSCT). In contrast, both nails and hair were collected from CCSs with a history of allo-HSCT. To extract genomic DNA from the samples, QIAcube (Qiagen, Hilden, Germany) was used for peripheral blood, and ISOHAIR (NIPPON GENE, Tokyo, Japan) was used for nails and hair. All exons and the flanking sequences of 165 cancer predisposition genes (Table 1) were analyzed using a next-generation sequencer, Ion GeneStudio S5 (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer’s protocol. These 165 cancer predisposition genes, selected from literature reviews and National Comprehensive Cancer Network (NCCN) guidelines, have been reported to cause cancers associated with germline abnormalities [6) 9)-16)].

Sequencing data were mapped to human genome hg19 using Torrent Suite software (Thermo Fisher Scientific), and single nucleotide variants and small insertions/deletions were detected from the mapped data using the Torrent Variant Caller plug-in. Detected variants were annotated using SnpEff[17)] and SnpSift using the processed vcf file of the Genome Aggregation Database (gnomAD) version 2.1.1 [18)] and Human Genetic Variation Database version 2.3 [19)]. Missense variants were analyzed with dbNSFP3.4c, and splice site alterations were analyzed using dbscSNV1.1. Integrative Genomics Viewer (Broad Institute, Cambridge, MA, USA) was used to visualize the read alignments and sequencing errors.

In addition, copy number variants were analyzed using the multiplex ligation-dependent probe amplification method for *TP53*, the most common gene related to leukemia and pediatric cancers, using Applied Biosystems VRTi Dx and Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA), according to the manufacturer's protocol.

Detected variants were assessed through Human Gene Mutation Database professional 2020.1 (Qiagen) and ClinVar [20)]. If not registered, they were interpreted according to the 2015 American College of Medical Genetics and Genomics or the Association for Molecular Pathology guidelines [21)] by a clinical geneticist (TK), pediatric oncologist (TW), and molecular geneticist (TY). Genetic counseling was provided to CCSs and/or their guardians by presenting the results of clinical sequencing and relevant surveillance plans.

C Surveillance for SMN

When pathogenic or likely pathogenic variants were detected in *TP53*, surveillance for SMN was proposed according to the Toronto protocol [5)] in combination with the guidelines of the Children's Oncology Group (COG) LTFU program [8)].

When pathogenic or likely pathogenic variants in other genes, for which surveillance methods were specified in the NCCN guidelines [22)], American Association for Cancer

Research–Childhood Cancer Predisposition Workshop review articles 23)-39), and/or GeneReviews 40), SMN surveillance was proposed according to the COG LTFU guidelines. In contrast, when pathogenic or likely pathogenic variants were detected in genes for which no guidelines were established, SMN surveillance was proposed based on previous reports in combination with COG LTFU guidelines.

D Questionnaire-based survey

To comprehend the thoughts of CCSs and/or their guardians regarding SMN, clinical sequencing, and SMN surveillance, a questionnaire-based survey was conducted. The questionnaire consisted of six questions, each of which had five choices (strongly disagree, disagree a little, neither agree nor disagree, agree a little, and strongly agree) (Table 2). The same survey was conducted before and after clinical sequencing. To assess differences in their thoughts between the two surveys, a paired *t*-test was performed using the GraphPad Prism software package (version 9.2; GraphPad Software, San Diego, CA, USA).

III Results

As of March 2021, 37 CCSs were informed of this study, and 16 of them wished to participate. The clinical and molecular findings of the participants are presented in Table 3. Types of childhood cancer included acute lymphoblastic leukemia (ALL, n = 9), acute myeloid leukemia (AML, n = 2), neuroblastoma (n = 2), myeloid/NK cell precursor acute leukemia (n = 1), juvenile myelomonocytic leukemia (n = 1), and Wilms tumor (n = 1). The median age of the CCSs undergoing clinical sequencing was 22 years (range, 15–42 years), six of whom were minors. The male-to-female sex ratio was 1:1. Four CCSs had a history of SMN. No pathogenic variants corresponding to likely pathogenic or pathogenic variants were detected in any of the participants. Twelve variants of uncertain significance (VUSs) were detected in 10 participants.

Most VUSs (eight of 12) were missense variants.

Since Patient 1 had four cancers (ALL, rectal cancer, ovarian cancer, and breast cancer) and a family history of gastric cancer, she was supposed to have a germline pathogenic variant of some cancer predisposition genes. Contrary to our expectations, only the VUSs of *SAMD9L* and *POLD1* genes were detected. No specific SMN surveillance has been proposed to date. Patient 10 developed three tumors (neuroblastoma, cervical cancer, and meningioma), but only a VUS of the *MPL* gene was detected. No specific SMN surveillance has been proposed to date. Patient 13 developed ALL and meningioma, but no pathogenic variants were detected. In Patient 16, who developed AML, a heterozygous variant was detected in the *MUTYH* gene. The variant was interpreted as a VUS, according to ClinVar® 20), in which the variant was registered as likely benign in three cases, VUS in five cases, likely pathogenic in three cases, and pathogenic in one case. *MUTYH*-related polyposis is an autosomal recessive disease associated with an increased risk of colorectal cancer (CRC); however, the risk of CRC and extraintestinal cancer in individuals with pathogenic variants in *MUTYH* is unclear 41). Since Patient 16 was treated with anticancer agents and underwent allo-HSCT using total body irradiation, he was considered to have a relatively high risk of SMN. Therefore, we proposed the option of SMN surveillance in this case. No specific or further SMN surveillance was proposed for other patients with VUSs.

We also conducted a questionnaire-based survey before and after clinical sequencing of the CCSs or guardians who participated in this study (Table 2). Table 4 summarizes the results of the questionnaire-based survey. No significant change in scores was observed in any of the questions before and after clinical sequencing. For the first question, which focused on anxiety for SMN, the results were unchanged before and after clinical sequencing in seven CCSs. However, anxiety increased in four CCSs, and decreased in five CCSs. All four patients with increased anxiety were CCSs who were found to have VUS.

IV Discussion

In this study, we described the preliminary data of an originally established SMN surveillance system for CCSs, considering the results of clinical sequencing for germline cancer predisposition genes. The purpose of this system is to detect SMNs in CCSs at an early stage and consequently improve their long-term prognosis. A distinctive feature of this system is the introduction of a germline genetic investigation, as a clinical basis, into a previously established LTFU clinic in the Department of Pediatrics in our hospital. There have been several reports regarding the detection of pathological variants of cancer predisposition genes in CCSs using the gene panel 6) 42) 43), but there has been no report of an SMN follow-up system routinely linking clinical sequencing for germline cancer predisposition genes.

No pathogenic or likely pathogenic variants in the 165 germline cancer predisposition genes were detected in this study. The prevalence of pathogenic variants ranged from 5.8% to 11.5% 6) 42) 43) according to previous reports on germline abnormalities in cancer predisposition genes in CCSs. Possible causes for detecting no pathogenic or likely pathogenic variants in this study are estimated as follows: First, the number of CCSs who underwent clinical sequencing was small. Second, the types of primary cancer were biased, 13 of 16 participants in the current study developed acute leukemias as primary cancer; CCSs who had Li-Fraumeni syndrome-related solid tumors (e.g., adrenocortical cancer, osteosarcoma, rhabdomyosarcoma) as primary cancer and were likely to have pathogenic variants in *TP53* 6) were not included in this study.

Four participants (25%) developed secondary tumors at the time of participation in this study. Patient 1 had four malignant tumors by the age of 42 and met the Chompret criteria 44) because her relative developed young-onset cancer. However, no pathogenic variants, including copy number abnormalities in *TP53*, were detected. She received both chemotherapy and allo-HSCT after a 12-Gy total body irradiation-based conditioning regimen for ALL. Chemotherapy at onset consisted of cyclophosphamide, etoposide, pirarubicin, and mitoxantrone, which are at high risk for SMN. In addition, allo-HSCT at the time of relapse may be associated with SMN.

The risk of CRC in individuals heterozygous for a germline *MUTYH* pathogenic variant was slightly increased in large population-based and family-based studies, while the frequency of colonic and upper gastrointestinal polyps did not increase in 62 heterozygotes for *MUTYH* 41). Although a slightly increased cumulative risk for *MUTYH* heterozygotes for gastric, hepatobiliary, endometrial, and breast cancers has been reported, other case-control studies did not find an association between *MUTYH* heterozygosity and risk for breast cancer or hepatocellular carcinoma 41). Based on the history of treatment and pathogenicity of the variant, he was considered to have a relatively high risk of SMN and was proposed an SMN surveillance as a precautionary measure.

A questionnaire-based survey was conducted to comprehend the thoughts of CCSs and/or their guardians regarding SMN, clinical sequencing, and SMN surveillance. Anxiety about SMN did not change significantly before and after clinical sequencing, but all four CCSs who were found to have VUS showed increased anxiety. This result suggests that the presence or uncertainty of variants in cancer predisposition genes could increase anxiety about SMN for CCSs and their families, even after genetic counseling. Genetic counseling in the current system would require more careful anticipatory guidance for this clinical sequencing (e.g., low detection rates, possibilities of VUS) as well as follow-up services, including variant interpretation and psychological aspects.

During the study period, the current clinical sequencing-based surveillance system was proposed to 37 CCSs and their families in the LTFU clinic, and 16 of them wished to participate in this study and were recruited. In this system, the clinical sequencing and relevant genetic counseling costs approximately 53,000 JPY (about $ 485), which are not covered by the health insurance system in Japan. We were concerned that CCSs and their parents might be reluctant to receive genetic services at their own expenses because most of the costs of treatment for specific pediatric chronic diseases, including cancer, are covered by the health insurance system. Low estimated detection rates for pathogenic or likely pathogenic variants and possible anxieties through the risk of SMN and uncovering hereditary cancer predisposing syndromes could have been negative factors for participation in this study. However, the fact that 16 CCSs (43%) participated in this study suggests that a certain proportion of CCSs and their guardians would potentially like to know the risk of developing SMN. Further questionnaire-based survey regarding participation in this study to the remaining CCSs who did not accept the proposal might clarify the causes of their nonparticipation.

This study has some limitations. First, the number of participants is small, and experiences of actionable pathogenic variants have been lacking, which would be required for discussing the efficacy of SMN surveillance in this system. Second, the types of primary cancer were biased, 13 of 16 participants in the current study developed acute leukemias as primary cancer. Third, selection of the target genes (165 cancer predisposition genes) might not have been sufficient. It is presumed that more cancer predisposition genes will be newly discovered in the future, and hence, it would be necessary to update the surveillance guidelines, as appropriate. Fourth, the estimated pathogenicity of the detected variants in these cancer predisposition genes could change. Therefore, it would be useful to estimate the pathogenicity of detected variants on a regular basis (e.g., searching the ClinVar® website at the time of the annual LTFU clinic). Fifth, specific interpretation of the detected variants may be difficult (e.g., a heterozygous variant in *MUTYH* could or could not be a risk for CRC or extraintestinal cancer depending on the relevant population or the study design).

In summary, our novel follow-up system for CCSs is presented, comprising germline clinical sequencing of 165 cancer predisposition genes and relevant SMN surveillance. No pathogenic or likely pathogenic variants were detected among the 16 participants, mainly developing leukemia as a primary cancer; and currently the efficacy of SMN surveillance system for CCSs could not be shown. The presence or uncertainty of variants in cancer predisposition genes could increase anxiety about SMN in CCSs and their families. Further evaluation, including more CCSs with a wider spectrum of cancers, would be necessary to evaluate this system. Genetic counseling might require careful anticipatory guidance for clinical sequencing and follow-up services, including variant interpretation and psychological aspects.

Acknowledgments

We are grateful to the participants in this study. We are also thankful to Dr. A. Fujikawa, MD, and Ms. Y. Takiguchi for their technical support, and Editage (www.editage.com) for English language editing. This study was supported by Project Mirai Cancer Research Grants in 2018 (Japan Cancer Society; DM) and Childhood cancer survivor follow-up research in 2020 (Heart Link Working Project; TW).

Disclosure

YT and TK are members of an endowed chair named as “Division of Clinical Sequencing, Shinshu University School of Medicine” sponsored by BML, Inc. and Life Technologies Japan Ltd. of Thermo Fisher Scientific Inc.

Author contribution

TW, DM, YN, and TK designed the study; TY performed molecular investigation and interpreted the molecular data with TW and TK; KH, DM, EO, HM, SS, MT, and YN recruited participants and collected clinical information; TW and KH combined and interpreted the whole data (molecular and clinical investigation; questionnaire-based survey) and wrote the manuscript; all authors read and approved the final manuscript.

References

1） Linabery AM, Ross JA: Childhood and adolescent cancer survival in the US by race and ethnicity for the diagnostic period 1975–1999. Cancer 113: 2575-2596, 2008

2） Linabery AM, Ross JA: Trends in childhood cancer incidence in the US (1992–2004). Cancer 112: 416-432, 2008

3） Bhakta N, Liu Q, Ness KK, et al: The cumulative burden of surviving childhood cancer: An initial report from the St Jude Lifetime Cohort Study (SJLIFE). Lancet 390: 2569-2582, 2017

4） Lawless SC, Verma P, Green DM, Mahoney MC: Mortality experiences among 15+ year survivors of childhood and adolescent cancers. Pediatr Blood Cancer 48: 333-8, 2007

5） Villani A, Shore A, Wasserman JD, et al: Biochemical and imaging surveillance in germline TP53 mutation carriers with Li–Fraumeni syndrome: 11-year follow-up of a prospective observational study. Lancet Oncol 17: 1295-1305, 2016

6) Zhang J, Walsh MF, Wu G, et al: Germline mutations in predisposition genes in pediatric cancer. N Engl J Med 373: 2336-2346, 2015

7) Gröbner SN, Worst BC, Weischenfeldt J, et al: The landscape of genomic alterations across childhood cancers. Nature 555: 321-327, 2018

8) COG Long-Term Follow-Up Guidelines, version 5.0. Children’s Oncology Group. 2018. (Accessed on November 1, 2021. at http://www.survivorshipguidelines.org/).

9) Rahman N: Realizing the promise of cancer predisposition genes. Nature 505: 302-308, 2014

10) Cheah JJC, Hahn CN, Hiwase DK, Scott HS, Brown AL: Myeloid neoplasms with germline DDX41 mutation. Int J Hematol 106: 163-174, 2017

11) Drazer MW, Kadri S, Sukhanova M, et al: Prognostic tumor sequencing panels frequently identify germ line variants associated with hereditary hematopoietic malignancies. Blood Adv 2: 146-150, 2018

12) Zhang MY, Churpek JE, Keel SB, et al: Germline ETV6 mutations in familial thrombocytopenia and hematologic malignancy. Nat Genet 47: 180-185, 2015

13) Churchman ML, Qian M, Te Kronnie G, et al: Germline genetic IKZF1 variation and predisposition to childhood acute lymphoblastic leukemia. Cancer Cell 33: 937-948, 2018

14) Harutyunyan AS, Giambruno R, Krendl C, et al: Germline RBBP6 mutations in familial myeloproliferative neoplasms. Blood 127: 362-365, 2016

15) Narumi S, Amano N, Ishii T, et al: SAMD9 mutations cause a novel multisystem disorder, Mirage syndrome, and are associated with loss of chromosome 7. Nat Genet 48: 792-797, 2016

16) Tesi B, Davidsson J, Voss M, et al: Gain-of-function SAMD9L mutations cause a syndrome of cytopenia, immunodeficiency, MDS, and neurological symptoms. Blood 129: 2266-2279, 2017

17) Genomic variant annotations and functional effect prediction toolbox. SnpEff & SnpSift. 2021. (Accessed on November 1, 2021. at http://snpeff.sourceforge.net).

18) Genome Aggregation Database. gnomAD. 2018. (Accessed on November 1,2021. at https://gnomad.broadinstitute.org).

19) Human Genetic Variation Database. Kyoto University. 2017. (Accessed on November 1, 2021. At https://www.hgvd.genome.med.kyoto-u.ac.jp).

20) ClinVar. 2013. National Center for Biotechnology Information. (Accessed on November 1, 2021. at https://www.ncbi.nlm.nih.gov/clinvar/).

21) Richards S, Aziz N, Bale S, et al: Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17: 405-424, 2015

22) NCCN Guidelines. National Comprehensive Cancer Network®. (Accessed on November

1, 2021. at https://www.nccn.org/professionals/physician_gls/).

23) Brodeur GM, Nichols KE, Plon SE, Schiffman JD, Malkin D: Pediatric cancer predisposition and surveillance: An overview, and a tribute to Alfred G. Knudson Jr. Clin Cancer Res 23: e1-e5, 2017

24) Greer MC, Voss SD, States LJ: Pediatric cancer predisposition imaging: Focus on whole-body MRI. Clin Cancer Res 23: e6-e13, 2017

25) Porter CC, Druley TE, Erez A, et al: Recommendations for surveillance for children with leukemia-predisposing conditions. Clin Cancer Res 23: e14-e22, 2017

26) Walsh MF, Chang VY, Kohlmann WK, et al: Recommendations for childhood cancer screening and surveillance in DNA repair disorders. Clin Cancer Res 23: e23-e31, 2017

27) Tabori U, Hansford JR, Achatz MI, et al: Clinical management and tumor surveillance recommendations of inherited mismatch repair deficiency in childhood. Clin Cancer Res 23: e32-37, 2017

28) Kratz CP, Achatz MI, Brugières L, et al: Cancer screening recommendations for individuals with Li–Fraumeni syndrome. Clin Cancer Res 23: e38-e45, 2017

29) Evans DGR, Salvador H, Chang VY, et al: Cancer and central nervous system tumor surveillance in pediatric Neurofibromatosis 1. Clin Cancer Res 23: e46-e53, 2017

30) Evans DGR, Salvador H, Chang VY, et al: Cancer and central nervous system tumor surveillance in pediatric Neurofibromatosis 2 and Related Disorders. Clin Cancer Res 23: e54-e61, 2017

31) Foulkes WD, Kamihara J, Evans DGR, et al: Cancer surveillance in Gorlin syndrome and rhabdoid tumor predisposition syndrome. Clin Cancer Res 23: e62-67, 2017

32) Rednam SP, Erez A, Druker H, et al: Von Hippel–Lindau and hereditary pheochromocytoma/paraganglioma syndromes: Clinical features, genetics, and surveillance recommendations in childhood. Clin Cancer Res 23: e68-e75, 2017

33) Schultz KAP, Rednam SP, Kamihara J, et al: PTEN, DICER1, FH, and their associated tumor susceptibility syndromes: Clinical features, genetics, and surveillance recommendations in childhood. Clin Cancer Res 23: e76-e82, 2017

34) Villani A, Greer MC, Kalish JM, et al: Recommendations for cancer surveillance in individuals with RASopathies and other rare genetic conditions with increased cancer risk. Clin Cancer Res 23: e83-e90, 2017

35) Druker H, Zelley K, McGee RB, et al: Genetic counselor recommendations for cancer predisposition evaluation and surveillance in the pediatric oncology patient. Clin Cancer Res 23: e91-7, 2017

36) Kamihara J, Bourdeaut F, Foulkes WD, et al: Retinoblastoma and neuroblastoma predisposition and surveillance. Clin Cancer Res 23: e98-e106, 2017

37) Achatz MI, Porter CC, Brugières L, et al: Cancer screening recommendations and clinical management of inherited gastrointestinal cancer syndromes in childhood. Clin Cancer Res 23: e107-e114, 2017

38) Kalish JM, Doros L, Helman LJ, et al: Surveillance recommendations for children with overgrowth syndromes and predisposition to Wilms tumors and hepatoblastoma. Clin Cancer Res 23: e115-e122, 2017

39) Wasserman JD, Tomlinson GE, Druker H, et al: Multiple endocrine neoplasia and hyperparathyroid-jaw tumor syndromes: Clinical features, genetics, and surveillance recommendations in childhood. Clin Cancer Res 23: e123-e132, 2017

40) GeneReviews®. University of Washington. (Accessed on November 1, 2021. at https://www.ncbi.nlm.nih.gov/books/NBK1116/).

41) GeneReviews®. MUTYH Polyposis. University of Washington. 2021. (Accessed on November 1, 2021. at https://www.ncbi.nlm.nih.gov/books/NBK107219/).

42) Wang Z, Wilson CL, Easton J, et al: Genetic risk for subsequent neoplasms among long-

term survivors of childhood cancer. J Clin Oncol 36: 2078-2087, 2018

43) Qin N, Wang Z, Liu Q, et al: Pathogenic germline mutations in DNA repair genes in combination with cancer treatment exposures and risk of subsequent neoplasms among long-term survivors of childhood cancer. J Clin Oncol 38: 2728-2740, 2020

44) Bougeard G, Renaux-Petel M, Flaman JM, et al: Revisiting Li–Fraumeni syndrome from TP53 mutation carriers. J Clin Oncol 33: 2345-2352, 2015

Table 1 A list of 165 cancer predisposition genes analyzed in this study

ABCB11, ACD, ALK, ANKRD26, APC, ATM, AXIN2, BAP1, BLM, BMPR1A, BRAF, BRCA1, BRCA2, BRIP1, BUB1B, CBL, CDC73, CDH1, CDK4, CDKN1B, CDKN1C, CDKN2A, CEBPA, CHEK2, COL7A1, CTC1, CYLD, DDB2, DDX41, DICER1, DIS3L2, DKC1, DOCK8, EGFR, ELANE, EPCAM, ERCC1, ERCC2, ERCC3, ERCC5, ETV6, EXT1, EXT2, FAH, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FANCQ, FANCR, FANCT, FH, FLCN, GATA2, GBA, GFI1, GJB2, GPC3, GREM1, HAX1, HFE, HMBS, HRAS, IKZF1, ITK, KIT, KRAS, MAP2K1, MAP2K2, MAX, MEN1, MET, MLH1, MPL, MSH2, MSH3, MSH6, MTAP, MUTYH, NBN, NF1, NF2, NHP2, NOP10, NRAS, NTHL1, PALB2, PAX5, PDGFRA, PHOX2B, PMS2, POLD1, POLE, POLH, PRKAR1A, PRSS1, PTCH1, PTEN, PTPN11, RAD51C, RAD51D, RAF1, RB1, RBBP6, RECQL4, RET, RHBDF2, RMRP, RPL5, RPL11, RPL35A, RPS10, RPS17, RPS19, RPS24, RPS26, RTEL1, RUNX1, SAMD9, SAMD9L, SBDS, SDHA, SDHAF2, SDHB, SDHC, SDHD, SERPINA1, SH2B3, SH2D1A, SHOC2, SLC25A13, SLX4, SMAD4, SMARCA4, SMARCB1, SMARCE1, SOS1, SRY, STAT3, STK11, SUFU, TERC, TERT, TGFBR1, TINF2, TMEM127, TNFRSF6, TP53, TRIM37, TSC1, TSC2, UROD, VHL, WAS, WRAP53, WRN, WT1, XPA, XPC

Table 2 The questionnaire used in this study

Q1. Are you worried about your child developing a second malignant neoplasm (SMN) in the future?

Strongly disagree	Disagree a little	Neither agree nor disagree	Agree a little	Strongly agree
1	2	3	4	5

Q2. Do you think that this clinical sequencing of cancer predisposition genes is useful for yourself (or your child)?

Strongly disagree	Disagree a little	Neither agree nor disagree	Agree a little	Strongly agree
1	2	3	4	5

Q3. Do you want to tell the results of this clinical sequencing of cancer predisposition genes to your family?

Strongly disagree	Disagree a little	Neither agree nor disagree	Agree a little	Strongly agree
1	2	3	4	5

Q4. Do you want to receive regular follow-up examinations of late effects (including SMN) in the future?

Strongly disagree	Disagree a little	Neither agree nor disagree	Agree a little	Strongly agree
1	2	3	4	5

Q5. Do you want to receive periodic follow-up by a clinical geneticist and/or certified genetic counselor?

Strongly disagree	Disagree a little	Neither agree nor disagree	Agree a little	Strongly agree
1	2	3	4	5

Q6. Do you want to know about late effects apart from the SMN?

Strongly disagree	Disagree a little	Neither agree nor disagree	Agree a little	Strongly agree
1	2	3	4	5

Table 3 Clinical and molecular findings in 16 participants

Patient no.	Age at clinical sequencing (years)	Sex	Primary disease	Age at onset of primary disease (years)	Second tumor (age, years)	Family history of malignancy	Detected variant(s)		
							P	LP	VUS
1	42	F	ALL	15	Rectal cancer (30) Ovarian cancer (30) Breast cancer (42)	Father: gastric cancer Paternal grandfather: gastric cancer	-	-	*SAMD9L* (NM_152703.3:c.2256G>A): missense variant *POLD1* (NM_002691.3:c.1138-7C>A): splice-site variant
2	22	M	ALL	14	Colorectal polyps (20, 22)	Maternal grandfather: colorectal cancer	-	-	*ATM* (NM_000051.3:c.323C>G): missense variant *BRIP1* (NM_032043.2:c.2830C>G): missense variant
3	27	F	Myeloid/NK cell precursor acute leukemia	10	-	Paternal grandmother: cancer	-	-	*POLE* (NM_006231.3:c.76A>G): missense variant
4	37	M	ALL	12	-	Maternal grandmother: cancer	-	-	-
5	16	M	ALL	4	-	-	-	-	*CDK4* (NM_000075.3:c.887A>G): missense variant
6	24	M	ALL	13	-	-	-	-	-
7	15	F	ALL	13	-	Maternal grandfather: cholangiocarcinoma Maternal grandmother: breast cancer	-	-	*BRCA1* (NM_007294.3:c.626C>T): missense variant
8	16	F	JMML	15	-	Maternal grandmother: uterine cancer	-	-	-
9	19	M	ALL	14	-	Mother: bilateral breast cancer	-	-	*MSH6* (NM_000179.2:c.3772C>G): missense variant
10	32	F	NB	0	Cervical cancer (20) Meningioma (31)	Maternal grandfather: prostate cancer	-	-	*MPL* (NM_005373.2:c.853G>T): missense and/or splice-site variant
11	22	M	AML	0	-	Paternal grandmother: malignant lymphoma	-	-	*SAMD9L* (NM_152703.3:c.2357C>G): missense variant
12	34	F	NB	6	-	Mother: breast cancer Paternal grandmother: kidney cancer	-	-	-
13	30	M	ALL	5	Meningioma (22)	Paternal grandfather: gastric cancer, lung cancer Maternal grandfather: gastric cancer	-	-	-
14	20	F	WT	5	-	-	-	-	-
15	16	F	ALL	3	-	-	-	-	*GJB2*(NM_004004.5:c.508_511dup): frameshift variant
16	16	M	AML	9	-	Maternal grandfather: lung cancer	-	-	*MUTYH* (NM_001128425.2:c.934-2A>G): splice-site variant

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; F, female; JMML, juvenile myelomonocytic leukemia; LP, likely pathogenic; M, male; NK, natural killer; P, pathogenic; VUS, variant of uncertain significant; WT, Wilms tumor

Table 4 Responses of childhood cancer survivors or their guardians the questionnaires regarding a novel follow-up system

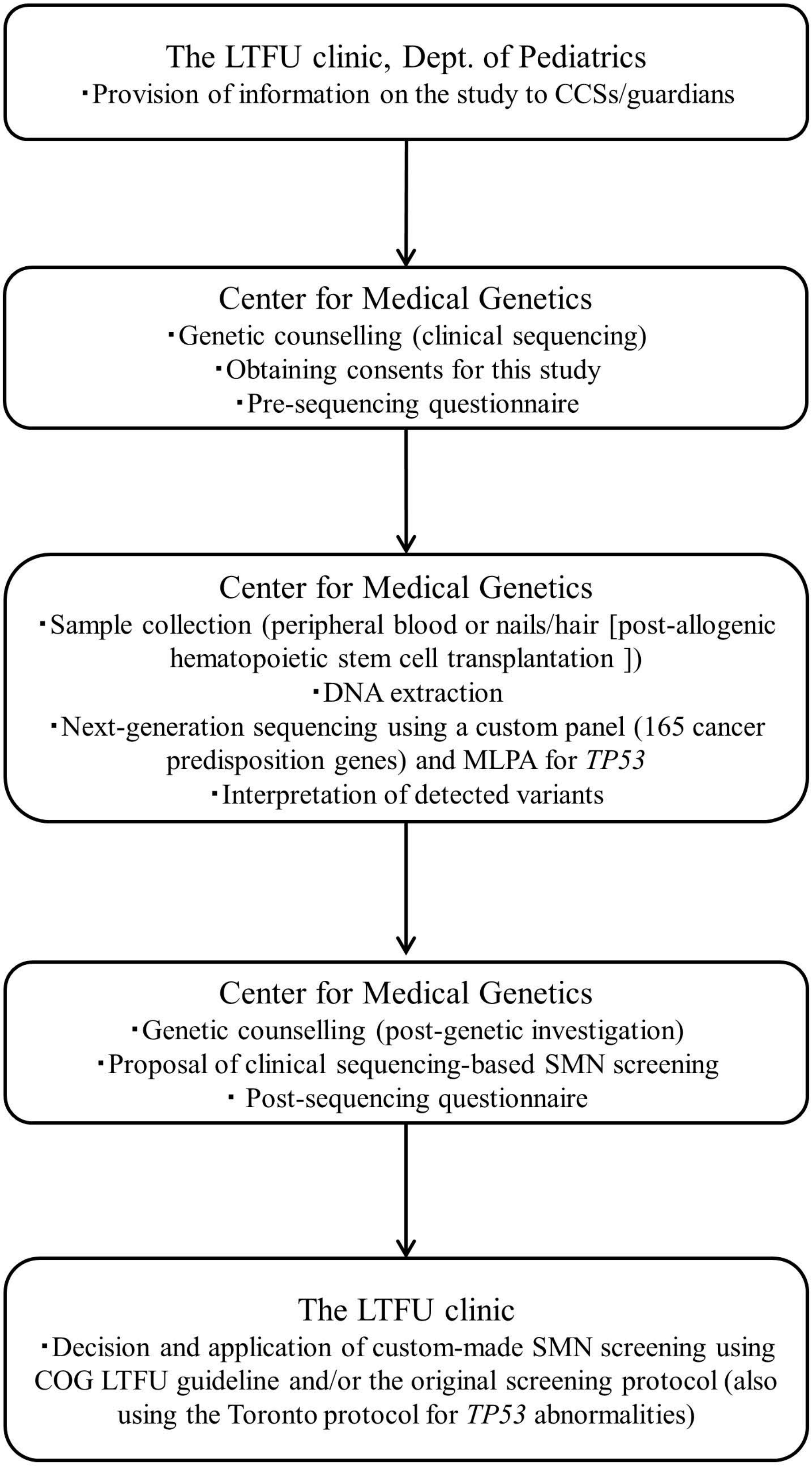
Patient no.	Q1		Q2		Q3		Q4		Q5		Q6	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	5	5	5	5	4	4	5	5	5	5	5	5
2	2	2	5	5	5	5	5	5	5	5	4	5
3	3	4	5	4	4	5	5	5	4	5	5	5
4	5	4	4	5	5	5	5	5	4	5	4	4
5	4	5	5	5	5	5	4	4	3	4	4	4
6	4	4	5	5	4	4	5	5	3	3	4	4
7	1	1	5	5	5	5	5	5	5	5	5	4
8	4	2	5	5	5	5	5	5	5	4	5	5
9	3	4	5	5	4	5	4	5	4	5	4	4
10	5	3	5	5	5	5	5	5	5	5	5	5
11	3	4	4	5	4	4	4	5	4	4	4	4
12	4	3	4	4	4	4	4	4	2	4	4	4
13	5	3	5	4	5	5	5	5	5	5	5	5
14	2	2	4	4	4	4	5	5	3	3	4	4
15	4	4	4	4	4	4	4	4	4	3	4	4
16	4	4	5	5	5	5	5	5	3	3	4	4
Mean	3.6	3.4	4.7	4.7	4.5	4.6	4.7	4.8	4.0	4.3	4.4	4.4
p-value	0.362		1.000		0.164		0.164		0.216		1.000	

Figure Legends

Fig. 1 An outline of this study

COG, Children’s Oncology Group; LTFU, long-term follow-up; SMN, second malignant neoplasm

Fig. 1


The LTFU clinic, Dept. of Pediatrics
▪Provision of information on the study to CCSs/guardians
Center for Medical Genetics
▪Genetic counselling (clinical sequencing)
▪Obtaining consents for this study
▪Pre-sequencing questionnaire
Center for Medical Genetics
▪Sample collection (peripheral blood or nails/hair [post-allogenic hematopoietic stem cell transplantation])
▪DNA extraction
▪Next-generation sequencing using a custom panel (165 cancer predisposition genes) and MLPA for *TP53*
▪Interpretation of detected variants
Center for Medical Genetics
▪Genetic counselling (post-genetic investigation)
▪Proposal of clinical sequencing-based SMN screening
▪ Post-sequencing questionnaire
The LTFU clinic
▪Decision and application of custom-made SMN screening using COG LTFU guideline and/or the original screening protocol (also using the Toronto protocol for *TP53* abnormalities)