National Cancer Center Japan

Towards high-quality clinical trials and
implementation of genomic medicine

ATLAS Training Program

Cancer Genome-based Medicine Course
Lecture Title : Medical genetics and Hereditary tumors
Speaker : Makoto Hirata

National Cancer Center Japan

Makoto HIRATA

Department of Genetic Medicine and Services,
National Cancer Center Hospital (Tokyo, Japan)

Education

MD (University of Tokyo 2001)
PhD (University of Tokyo 2010)

Research Fellow, Hospital for Sick Children, Toronto 2012–2014

Specialty and Research Field of Interest

Medical Genetics, Molecular Genomics, Orthopedic Surgery

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Brief Overview

1. Hereditary tumors
2. Actual response to germline findings in oncogene panel testing
   - 2-1. Points to note when discussing genetic test results with the patient
   - 2-2. Evaluation by an expert panel
   - 2-3. Points to note when disclosing the germline findings and actual response in outpatient genetic counseling

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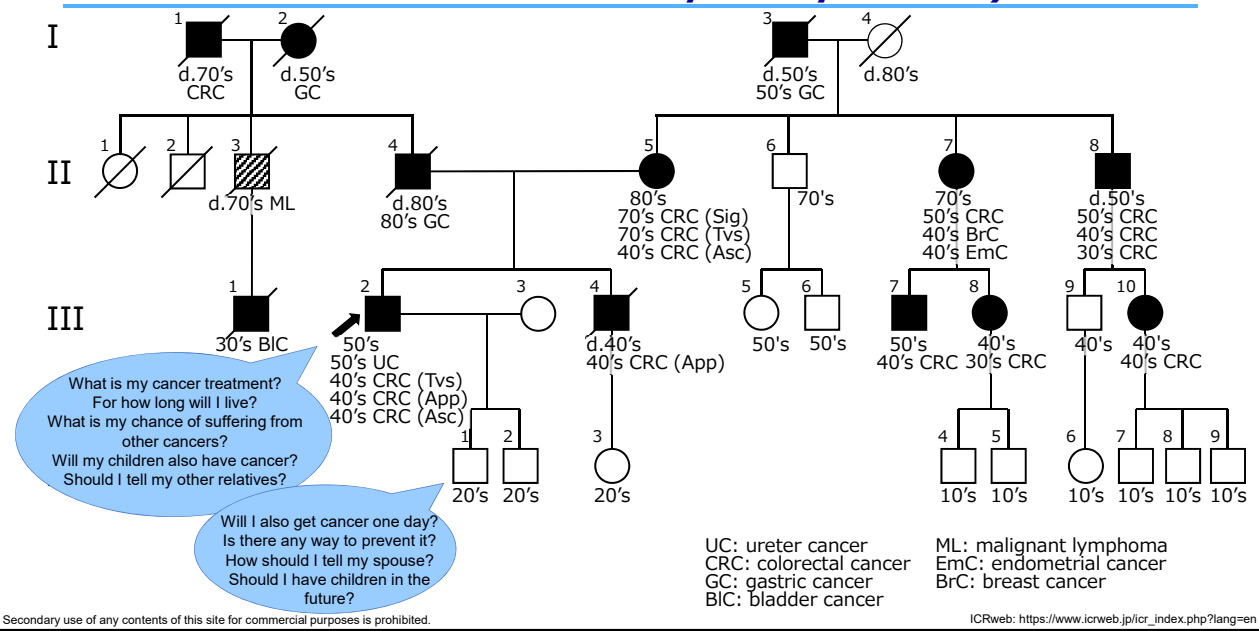
Case Presentation (Lynch Syndrome)



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Special Characteristics of Genetic Information

- **Immutability** : Genetic information remains unchanged throughout life
- **Predictability** : Ability to predict future health condition (onset of disease)
- **Commonality** : Genetic information is shared by family members (relatives)

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Genetic Counseling

Genetic counseling is a process to help people understand the medical, psychological, and family impact of genetic involvement in disease, and help them to adapt their lives to the condition.

This process includes:

1) Analysis of the individual's family and medical histories to assess the likelihood of disease onset and recurrence
2) Education regarding genetic profile, testing, management, prevention, resources, and research
3) Informed choice (informed autonomous choice) and counseling to promote dealing with risks and the situation

The Japanese Association of Medical Sciences – Guidelines for Genetic Tests and Diagnoses in Medical Practice (February 2011)

- ! It is not simply an outpatient visit for genetic testing.
- ! It is important to have accurate knowledge of the genetic background.

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Characteristics of Hereditary Tumors

There are many actionable genetic variants

There are many carriers of the mutation, and many of these mutations are inherited in a dominant manner (The frequency of BRCA1/2 mutations is approximately 1 in 500 people)	**The main advantage of identifying genetic predisposition is that it enables early detection and treatment**	**Onset timing and phenotype (type of cancer, etc.) vary depending on the individual**	**Results of genetic testing/testing related to germline information may lead to treatment**

It is good to know if the disease is hereditary

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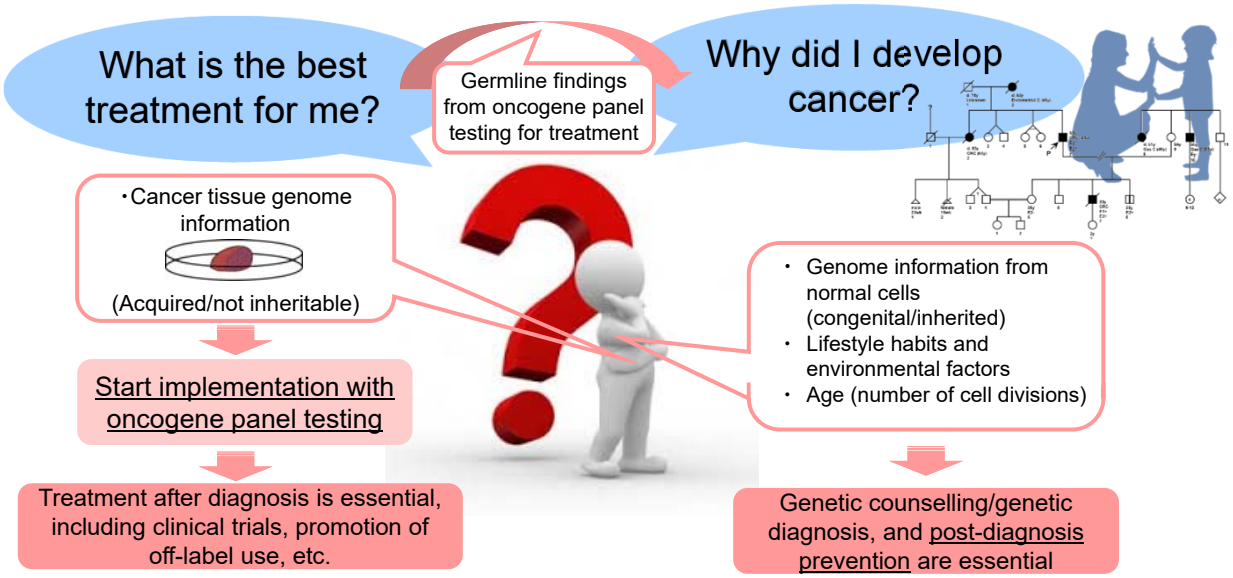
Two Genomic Medicines for Cancer



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Role of Medical Personnel Involved in Oncogene Panel Testing

Attending physician

Patient explanation (tests) → Sample preparation → Implement sequencing → Create report → Specialist meeting → Patient explanation (results) → Treatment / Prevention → Preventative/ pre-emptive medical care for relatives

Cancer Genomic Medicine Coordinator

Duties

[Before Oncogene Panel Testing]

- Explain panel testing, explain about possibility of discovering secondary findings (incidental findings discovered through the course of genome testing)

[After Oncogene Panel Testing]

- Arrange referral of patients with secondary findings to genetic counsellor
- Arrange referral of patients with treatable genetic abnormalities to treatment
- Assumed person
- A person who has completed the training on the Ministry of Health, Labour and Welfare program – Cancer Genomic Medicine Personnel Training Program (from 2017) (Cancer Genomic Medicine Coordinator)

Genetic Counselor

Duties

- Provide specialist genetic counseling, including support for ethical, legal and social (ELSI) issues
- Build and maintain a collaborative relationship with attending physician and other medical departments
- Envisioned person
- A certified genetic counselor, certified by the Japanese Society for Genetic Counseling and The Japan Society of Human Genetics (a person with a Master's Degree in Genetic Counselor Training)

Specialist Doctor In Genetic Medicine

Duties

- Implement appropriate genetic medical care in response to consultation from all clinical departments
- Analyze problems related to genetics expected to occur in medical institutions
- Assumed person
- A clinical geneticist certified by The Japan Society of Human Genetics

Partially modified from p.27 of 2017 Advisory Board Report on Promoting Facilitation of Genomic Medicine

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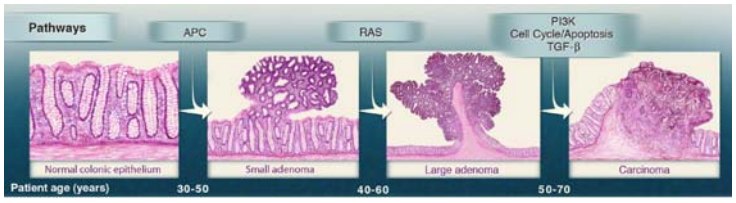
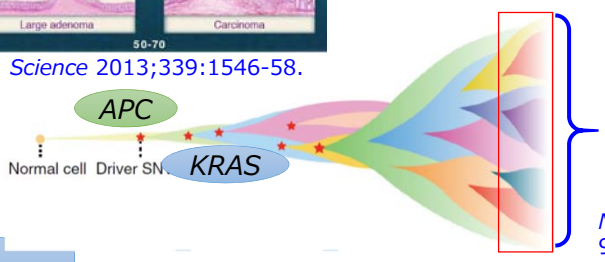
Oncogene Panel Testing



Science 2013;339:1546-58.



Many genetic abnormalities exist at diagnosis

Nat Commun 2018; 9:2884.

Biopsy/resection

Oncogene panel testing

- Collection of information regarding gene mutations in tumors
- Simultaneous analysis of a large number of genes (100 or more)
- Enabling the selection of therapeutic agents based on the mutation identified

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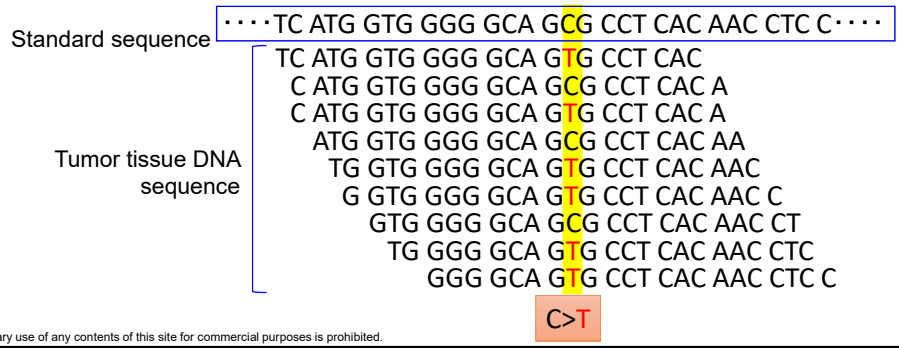
Gene Panel Testing using Next-generation Sequencers (NGS)

What Is Done In Oncogene Panel Analysis?

- **Important regions** (exons and promoters) in genes related to cancer development are selected as regions of the genome
- DNA **sequences** of the selected genome regions are analyzed
- Genetic abnormalities are detected by analyzing the **differences in the DNA sequence** obtained after sequencing
- Differences in the DNA sequence are determined by **comparing** the tumor tissue sequence with the standard sequence or tumor tissue sequence with the normal tissue sequence



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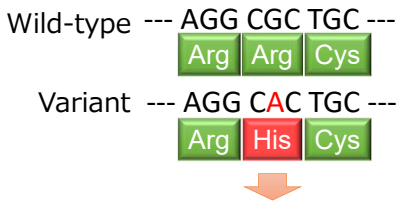
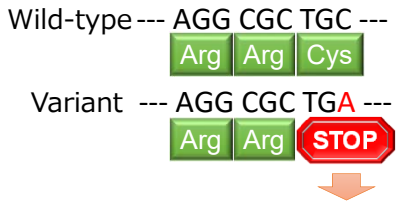
Various Gene Mutations

Base substitution / Missense mutation



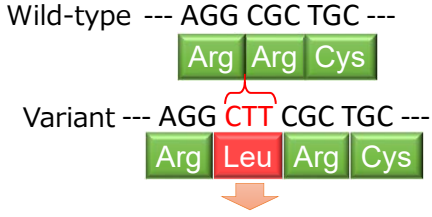
Higher-order structural changes

Base substitution / Nonsense mutation



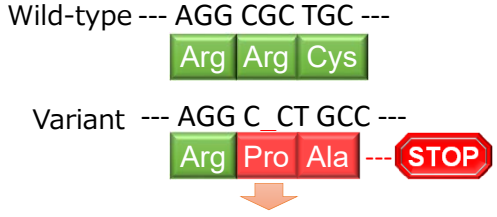
Truncation (disruption of translation)

Base insertion/deletion / In-frame mutation



Higher-order structural changes

Base insertion/deletion / Frameshift mutation



Unusual amino acid sequence + Truncation

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Various Gene Mutations

Base substitution / Missense mutation

Base substitution / Nonsense mutation

- There are various patterns of gene mutation
- Mutations that cause truncation often cause loss of function
- Mutations that cause higher-order structure changes can range from causing no effect to causing loss of function and gain of function
- **Effect on genetic function caused by various mutations is often difficult to predict**
- **Some gene mutations are difficult to detect with next-generation sequencers**

on)

Ba

Variant --- AGG CTT CGC TGC ---

Arg Leu Arg Cys

Higher-order structural changes

Variant --- AGG C_CT GCC ---

Arg Pro Ala --- STOP

Unusual amino acid sequence + Truncation

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Differences in Analysis using Tumor Tissue Only, and Analysis using Tumor and Normal Tissue

Standard sequence

····TC ATG GTG GGG GCA GCG CCT CAC AAC CTC C····

Tumor tissue DNA sequence

TC ATG GTG GCG GCA GTG CCT CAC
C ATG GTG GGG GCA GCG CCT CAC A
C ATG GTG GCG GCA GTG CCT CAC A
ATG GTG GGG GCA GCG CCT CAC AA
TG GTG GCG GCA GTG CCT CAC AAC
G GTG GGG GCA GCG CCT CAC AAC C
GTG GCG GCA GCG CCT CAC AAC CT
TG GGG GCA GCG CCT CAC AAC CTC
GCG GCA GCG CCT CAC AAC CTC C

G>C C>T

Analysis using only tumor tissue excludes high-frequency SNPs cataloged in databases

Standard sequence

····TC ATG GTG GGG GCA GCG CCT CAC AAC CTC C····

Normal tissue DNA sequence

TC ATG GTG GCG GCA GCG CCT CAC
C ATG GTG GGG GCA GCG CCT CAC A
C ATG GTG GCG GCA GCG CCT CAC A
ATG GTG GGG GCA GCG CCT CAC AA
TG GTG GCG GCA GCG CCT CAC AAC
G GTG GGG GCA GCG CCT CAC AAC C
GTG GCG GCA GCG CCT CAC AAC CT
TG GGG GCA GCG CCT CAC AAC CTC
GGG GCA GCG CCT CAC AAC CTC C

G>C

Analysis using tumor tissue and normal tissue excludes SNPs based on the normal tissue analysis results

SNP (Single-nucleotide polymorphism)

SNV (Single-nucleotide variant)

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Differences in Analysis using Tumor Tissue Only, and Analysis using Tumor and Normal Tissue

Standard sequence

····TC ATG GTG GGG GCA GCG CCT CAC AAC CTC C····

Rare SNPs and somatic mutations cannot be differentiated by tumor tissue analysis alone.
Rare SNPs include pathogenic germline variants that induce the development of hereditary tumors

G>C C>T

Standard sequence

Tumor-specific somatic mutations can be detected by analysing the genomic DNA of the tumor tissue and normal tissue.
Germline variants can also be detected by separately comparing the tumor tissue with normal tissue and the standard sequence

GGG GCA GCG CCT CAC AAC CTC C

G>C

SNP (Single-nucleotide polymorphism)

SNV (Single-nucleotide variant)

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Oncogene Panel Testing (Oncogene Profiling Test)/Covered by Insurance

Purpose
- To investigate genetic changes in cancer cells and examine treatment methods suited to the patient's cancer characteristics

Feature
- Comprehensively investigates multiple gene sequences at once
- Currently two types of profiling tests are covered by insurance

	sysmex OncoGuide™ NCC Oncopanel System	FoundationOne® CDx Cancer Genome Profile
Number of genes	114	324
Target sample	Tumor tissue-derived DNA + peripheral blood-derived DNA	Tumor tissue-derived DNA
Germline mutations	Differentiates (reference information)	Does not differentiate
Polymorphisms	Excluded	It may not be possible to exclude some items
Companion diagnosis	No	Applicable
TMB measurement	Detected	Detected
MSI	Not detected	Detected

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2-1. Points to Note When Discussing Genetic Test Results with the Patient

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Explaining Germline Findings in the Informed Consent Form

sysmex OncoGuide™ NCC Oncopanel system

4. Possibility of discovering cancer-related genetic information (hereditary tumor)

This test examines a diverse range of genes to investigate the characteristics of your cancer cells. During this process, in addition to finding information useful for your cancer treatment, there is also about a 3% chance that your cancer is related to your innate constitution (hereditary tumor). We would like to inform you of results that may be beneficial to managing your health and the health of your relatives, such as preventative measures and treatment methods, but we will respect your wishes. If you do not wish to be informed at the present time, please let us know. If you would like more detailed information, you will need to have separate genetic counseling and genetic testing, which may incur additional costs.

FoundationOne® CDx Cancer Genome Profile

4. Possibility of discovering cancer-related genetic information (hereditary tumor)

This test examines a diverse range of genes to investigate the characteristics of your cancer cells. During this process, in addition to finding information useful for your cancer treatment, your cancer may also be suspected to be related to your innate constitution (hereditary tumor). We would like to inform you of results that may be beneficial to managing your health and the health of your relatives, such as preventative measures and treatment methods, but we will respect your wishes. If you do not wish to be informed at the present time, please let us know.

However, please note that the information related to your constitution revealed in this test is only reference information that indicates a possibility, and does not constitute a definitive diagnosis. If you would like more detailed information, you will need to have separate genetic counseling and genetic testing, which may incur additional costs.

ICRweb: https://www.icrweb.jp/icr_index.php?lang=en

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Explaining Germline Findings in the Informed Consent Form

- Both informed consent forms describe the results as “beneficial”, but it is up to the recipient to decide if the information is beneficial.
- Among hereditary diseases, many hereditary tumors are actionable, and there are important advantages to knowing about hereditary tumors compared to other hereditary diseases.
- It is also possible that the recipients do not wish to know this information because of the effect on their family (how do you tell them?) and anxiety about the risk of developing multiple cancers.

It is quite difficult to explain the disadvantages of knowing this information, within the allocated busy consultation time, and so explaining this information using an informed consent form is typically adequate. However, it must be remembered that some people may consider that knowing about the germline findings is a disadvantage.

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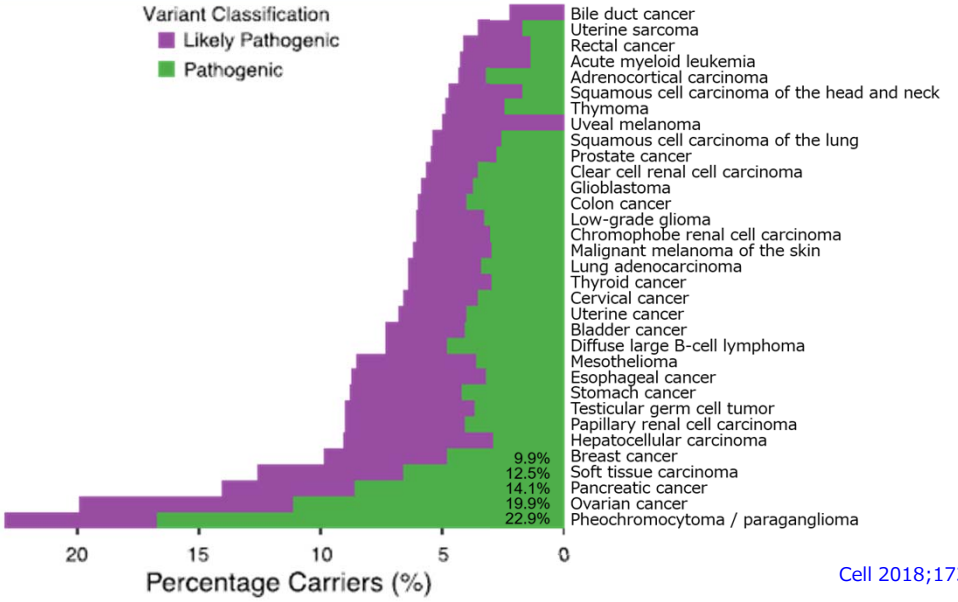
Detection Frequency of Germline Findings by Cancer Type



TCGA

Cell 2018;173:355–370

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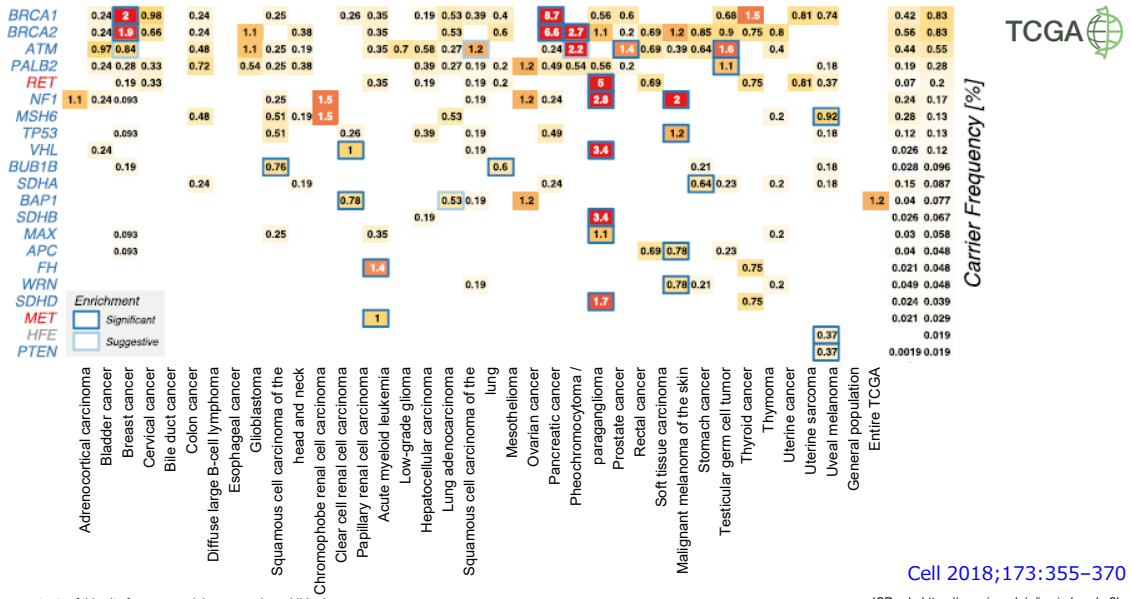
Frequency of Detectable Genetic Pathogenic Variants Related to Hereditary Tumors by Cancer Type



TCGA

Cell 2018;173:355–370

Summary 2-1

- NCC Oncopanel: Obtains findings that reveal the presence of germline gene mutations
- FoundationOneCDx: Obtains findings that indicate the presence of germline gene mutations
- From the outset when explaining the tests, it is vital to consider to a certain extent how the patients and their families will receive the news upon disclosing the germline findings.
- It is important to know which genes (hereditary tumors) can be detected as germline findings for the types of cancers covered in your practice.

2-2. Assessment by an Expert Panel

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Oncogene Panel Testing (Oncogene Profiling Test)/Covered by Insurance

	sysmex OncoGuide™ NCC Oncopanel system	FoundationOne® CDx Cancer Genome Profile
Number of genes	114	324
Target sample	Tumor tissue-derived DNA + peripheral blood-derived DNA	Tumor tissue-derived DNA
Germline mutations	Differentiates (reference information)	Does not differentiate
Polymorphisms	Excluded	May not be possible to exclude some items
Companion diagnosis	No	Applicable
TMB measurement	Detected	Detected
MSI	Not detected	Detected

- Only tumor cells are analyzed. That said, if genetic information regarding normal tissue is also stored, that information is also included.
  - → Not a definitive diagnosis of hereditary tumors
  - → Requires a confirmation test with normal tissue
- The degree of agreement between “variants found in tumor tissue” and “variants found in normal tissue” varies depending on factors such as the gene, cancer type, and age.

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Potentially Actionable SF Gene List

Gene	Major Phenotype	Recommendation grade of disclosure	Proposal grade of germline test
BRCA1	HBOC	AAA	◎
BRCA2	HBOC	AAA	◎
MLH1	Lynch	AAA	◎
MSH2	Lynch	AAA	◎
MSH6	Lynch	AAA	◎
PMS2	Lynch	AAA	◎
RET	MEN2	AAA	◎
VHL	VHL	AAA	◎
MUTYH	MAP	AA	◎
PALB2	Breast Ca	AA	◎
SDHAF2	HPPS	AA	◎
SDHB	HPPS	AA	◎
SDHC	HPPS	AA	◎
SDHD	HPPS	AA	◎
TSC2	Tuberous Sclerosis	AA	◎
ATM	Breast Ca	A	◎
BRIP1	Ovarian Ca	A	◎
RAD51C	Ovarian Ca	A	◎
RAD51D	Ovarian Ca	A	◎
MEN1	MEN1	AAA	○
CDH1	Diffuse Gastric Ca	AA	○
NF2	NF2	AA	○
TSC1	Tuberous Sclerosis	AA	○
WT1	WT1-related Wilms	AA	○
SMAD4	Juvenile Polyposis	AA	○
CDKN2A	Melanoma/Pancreatic Ca	A	○
CHEK2	Breast Ca	A	○
CDK4	Melanoma	B	○
FH	Hereditary Leiomyomatosis and Renal Cell Ca	B	○
FLCN	Birt-Hogg-Dube syndrome	B	○
POLE	Colon Ca	B	○

Gene	Major Phenotype	Recommendation grade of disclosure	Proposal grade of germline test
NBN	Breast Ca	A	
SDHA	HPPS	A	
TGFBR2	Loeys-Dietz	A	
BAP1	Malignant Mesothelioma etc	B	
MET	HPRC	B	
POLD1	Colon Ca	B	
TERT	Acute Myeloid Leukemia	B	
APC	FAP	AAA	△
RB1	Retinoblastoma	AAA	△
STK11	Peutz-Jeghers	AA	△
TP53	Li-Fraumeni Syndrome	AA	△
PTEN	PTEN hamartoma	AA	△
NF1	NF1	A	△

Proposal grade of germline test

- Grade 1: definitely
- Grade 2: as much as possible
- Grade 3: if possible
- Grade 4: if clinically suspected

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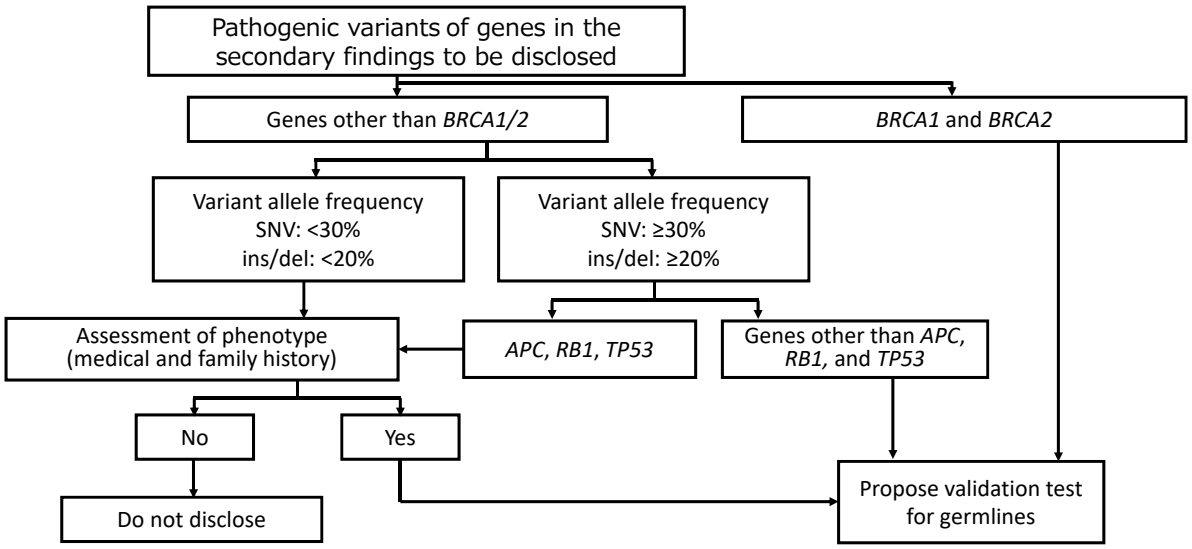
Operation Guidelines for Germline Tests to Confirm Secondary Findings from Tumor Profiling Test of Tumor Cells



Proposal concerning the information transmission process in genomic medicine [Revised 2nd edition]

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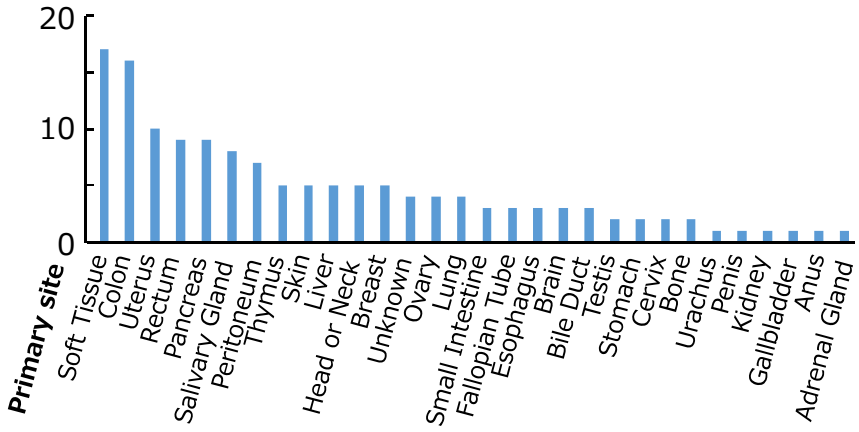
Frequency of Putative Germline Findings in NCC Hospital

Subjects

142 patients (male 64, female 78)
_ Patients underwent FoundationOneCDx tests from July 1, 2019 to June 30, 2020
_ Their detected genomic alterations were evaluated by expert panels



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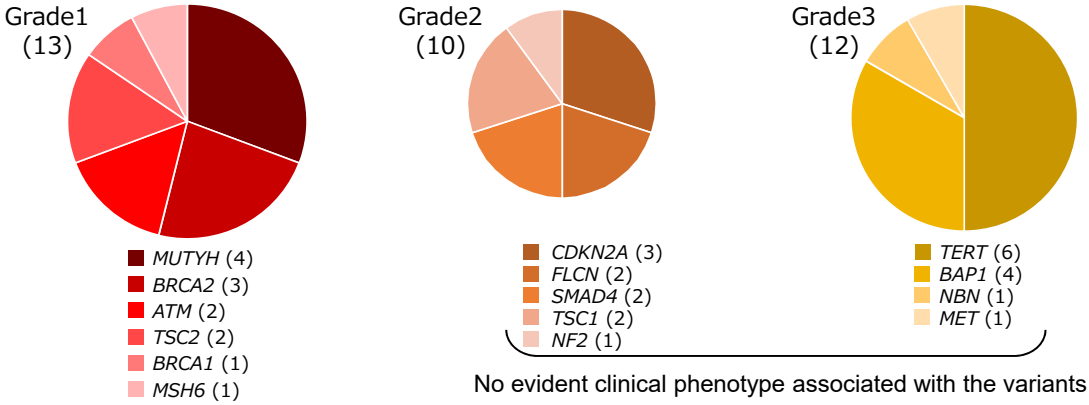
Suspected Pathogenic/Likely Pathogenic Genomic Alterations

	Known	Likely
Grade 1	5 (0.035)	8 (0.056)
Grade 2	4 (0.028)	6 (0.042)
Grade 3	7 (0.049)	5 (0.035)

(variants/case)



No evident clinical phenotype associated with the variants

ICRweb: https://www.icrweb.jp/icr_index.php?lang=en

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Suspected Pathogenic/Likely Pathogenic Genomic Alterations

	Known	Likely
Grade 1	5 (0.035)	8 (0.056)
Grade 2	4 (0.028)	6 (0.042)

When cases up to grade 3 are included, the number of variants subject to confirmation tests is around 2.5 variants per 10 cases.

⇒ Many confirmation tests are not covered by insurance, and when the cost burden is considered in circumstances in which there is insufficient evidence for conducting the test, it becomes difficult to recommend confirmation tests.

⇒ The expert panel is considering recommending genetic counseling support (confirmation test) while considering clinical information, other than for Grade 1 genes.

ATM (2)
TSC2 (2)
BRCA1 (1)
MSH6 (1)
TSC1 (2)
NF2 (1)
MET (1)

No evident clinical phenotype associated with the variants

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Companion diagnosis	No	Applicable
TMB measurement	Detected	Detected
MSI	Not detected	Detected

- Analysis of normal tissue (peripheral blood). A germline pathogenic variant has been reported and this equates to a diagnosis of hereditary tumor
  - → A definitive diagnosis of hereditary tumor
  - → Not necessary to conduct a confirmation test using normal tissue
- Not all germline pathogenic variants are reported, but **16 genes with high actionability are reported.**

ICRweb: https://www.icrweb.jp/icr_index.php?lang=en

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Genes Analyzed in the NCC Oncopanel Test

114 genes examine for mutation and amplification					12 genes examined for fusion
ABL1	CRKL	IDH2	NF1	RAC2	ALK
ACTN4	CREBBP	IGF1R	NFE2L2	RAD51C	AKT2
AKT1	CTNNB1	IGF2	NOTCH1	RAF1	BRAF
AKT2	CUL3	IL7R	NOTCH2	RB1	ERBB4
AKT3	DDR2	JAK1	NOTCH3	RET	FGFR2
ALK	EGFR	JAK2	NRAS	RHOA	FGFR3
APC	ENO1	JAK3	NRG1	ROS1	NRG1
ARAF	EP300	KND6/UTX	NTRK1	SETBP1	NTRK1
ARID1A	ERBB2/HER2	KEAP1	NTRK2	SETD2	NTRK2
ARID2	ERBB3	KIT	NTRK3	SMAD4	PDGFRA
ATM	ERBB4	KRAS	NT5C2	SMARCA4	RET
AXIN1	ESR1/ER	MAP2K1/MEK1	PALB2	SMARCB1	ROS1
AXL	EZH2	MAP2K2/MEK2	PBRM1	SMO	
BAP1	FBXW7	MAP2K4	PDGFRA	STAT3	
BARD1	FGFR1	MAP3K1	PDGFRB	STK11/LKB1	
BCL2L11	FGFR2	MAP3K4	PIK3CA	TP53	
BRAF	FGFR3	MDM2	PIK3R1	TSC1	
BRCA1	FGFR4	MDM4	PIK3R2	VHL	
BRCA2	FLT3	MET	POLD1		
CCND1	GNA11	MLH1	POLE		
CD274/PD-L1	GNAQ	MTOR	PRKCI		
CDK4	GNAS	MSH2	PTCH1		
CDKN2A	HRAS	MYC	PTEN		
CHEK2	IDH1	MYCN	RAC1		

Red font: Returned genes when germline variants are detected

ICRweb: https://www.icrweb.jp/icr_index.php?lang=en

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Genes Analyzed in the NCC Oncopanel Test

114 genes examine for mutation and amplification

ABL1	CRKL	IDH2	NF1	RAC2	12 genes examined for fusion
ACTN4	CREBBP	IGF1R	NFE2L2	RAD51C	ALK
AKT1	CTNNB1	IGF2	NOTCH1	RAF1	AKT2
AKT2	CUL3	IL7R	NOTCH2	RB1	BRAF
AKT3	DDR2	JAK1	NOTCH3	RET	ERBB4
ALK	EGFR	JAK2	NRAS	RHOA	FGFR2
APC	ENO1	JAK3	NRG1	ROS1	FGFR3
ARAF	EP300	KND6/UTX	NTRK1	SETBP1	NRG1
ARID1A	ERBB2/HER2	KEAP1	NTRK2	SETD2	NTRK1
ARID2	ERBB3	KIT	NTRK3	SMAD4	NTRK2
ATM	ERBB4	KRAS	NT5C2	SMARCA4	PDGFRA
AXIN1	ESR1/ER	MAP2K1/MEK1	PALB2	SMARCB1	RET
AXL	EZH2	MAP2K2/MEK2	PBRM1	SMO	ROS1
BAP1	FBXW7	MAP2K4	PDGFRA	STAT3	
BARD1	FGFR1	MAP3K1	PDGFRB	STK11/LKB1	
BCL2L11	FGFR2	MAP3K4	PIK3CA	TP53	
BRAF	FGFR3	MDM2	PIK3R1	TSC1	
BRCA1	FGFR4	MDM4	PIK3R2	VHL	
BRCA2	FLT3	MET	POLD1		
CCND1	GNA11	MLH1	POLE		
CD274/PD-L1	GNAQ	MTOR	PRKCI		
CDK4	GNAS	MSH2	PTCH1		
CDKN2A	HRAS	MYC	PTEN		
CHEK2	IDH1	MYCN	RAC1		

Red font: Returned genes when germline variants are detected

List of secondary findings in oncogene panel testing for patient disclosure by level of recommendation (Ver2.0_20191210)

Recommendation grade of disclosure (Grade)
AAA
AA
A
B

ICRweb: https://www.icrweb.jp/icr_index.php?lang=en

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Germline Variant Report in NCC Oncopanel


RG summary draft

Test information

Test name	NCC Oncopanel Test
System name	OncoGuide TM NCC Oncopanel System

Sample information

Anonymization code (Patient ID, etc.)	RGXT-0001
C-CAT patient ID	1234567890
C-CAT registration ID	XX99123450

Gene mutation

Gene name	Variant allele frequency	CDS variant	Amino acid variant	COSMIC ID (No. of registrations)
BRAF	17.9 (312/1,741)	exon15:c.1799T>A	V600E	476 (21,855)
KRAS	5.1 (72/1,407)	exon2:c.38G>A	G13D	532 (3,974)
BRCA2	47.7 (96/199)	exon15:c.7601C>T	A2534V	4152749 (2)

Gene amplification/deletion information

Gene name	Number of gene copies (corrected read number ratio)
MYCN	amplification x 4.54 (Control Depth: 2,637)

Gene rearrangement (fusion) information

Gene name	Physical position
-	-

*1 When there are variant detection results, the read number shows the variants that fall below the threshold

Number of somatic cell mutations

Region division		SNV Number of mutation	SNV Mutation appearanc	InDel Number of mutation	InDel Mutation appearanc	Total Number of mutation appearances	Total Mutation appearanc
Exon	nonsyn	5	14.1/Mb	0	0.0/Mb	150	422.3/Mb
	syn	145	408.3/Mb				
Non-exon		1262	1350.0/Mb	143	153.0/Mb	1405	1502.9/Mb
Entire region		1412	1094.6/Mb	143	110.9/Mb	1555	1205.4/Mb

Additional information on germline variants is reported as supplementary information in the summary report

RG summary draft + supplement: Germline mutation information

RG summary report draft Supplement: Germline mutation information

Test information

Test name	NCC Oncopanel Test
System name	OncoGuide TM NCC Oncopanel System

Sample information

nonymization code (Patient ID, etc	RGXT-0001
C-CAT patient ID	1234567890
C-CAT registration ID	XX99123450

Germline genetic mutation (SNV, short INDEL only)

Gene name	Variant allele frequency	CDS variant	Amino acid variant	COSMIC ID (No. of registrations)	ClinVar ID (No. of registrations)
BRCA2	47.7 (96/199)	exon15:c.7601C>T	A2534V	4152749 (2)	RCV000045256.6 (4)

*1 When there are variant detection results, the read number shows the variants that fall below the threshold

Analysis report

BRCA2: A2534V: Registered in COSMIC database, and it may be loss-of-function mutation.

Matters that may affect the test results

None

A germline variant report on all 114 genes and addition of analyzed genes is being prepared

ICRweb: https://www.icrweb.jp/icr_index.php?lang=en

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Germline Variant Assessment by ACMG/AMP Classification

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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

Sue Richards, PhD[1], Nazneen Aziz, PhD[2,16], Sherri Bale, PhD[3], David Bick, MD[4], Soma Das, PhD[5], Julie Gastier-Foster, PhD[6,7,8], Wayne W. Grody, MD, PhD[9,10,11], Madhuri Hegde, PhD[12], Elaine Lyon, PhD[13], Elaine Spector, PhD[14], Karl Voelkerding, MD[13] and Heidi L. Rehm, PhD[15]; on behalf of the ACMG Laboratory Quality Assurance Committee

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Table 5 Rules for combining criteria to classify sequence variants

Pathogenic	(i) 1 Very strong (PVS1) *AND* (a) ≥1 Strong (PS1–PS4) *OR* (b) ≥2 Moderate (PM1–PM6) *OR* (c) 1 Moderate (PM1–PM6) and 1 supporting (PP1–PP5) *OR* (d) ≥2 Supporting (PP1–PP5) (ii) ≥2 Strong (PS1–PS4) *OR* (iii) 1 Strong (PS1–PS4) *AND* (a)≥3 Moderate (PM1–PM6) *OR* (b)2 Moderate (PM1–PM6) *AND* ≥2 Supporting (PP1–PP5) *OR* (c)1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
Likely pathogenic	(i) 1 Very strong (PVS1) *AND* 1 moderate (PM1–PM6) *OR* (ii) 1 Strong (PS1–PS4) *AND* 1–2 moderate (PM1–PM6) *OR* (iii) 1 Strong (PS1–PS4) *AND* ≥2 supporting (PP1–PP5) *OR* (iv) ≥3 Moderate (PM1–PM6) *OR* (v) 2 Moderate (PM1–PM6) *AND* ≥2 supporting (PP1–PP5) *OR* (vi) 1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
Benign	(i) 1 Stand-alone (BA1) *OR* (ii) ≥2 Strong (BS1–BS4)
Likely benign	(i) 1 Strong (BS1–BS4) and 1 supporting (BP1–BP7) *OR* (ii) ≥2 Supporting (BP1–BP7)
Uncertain significance	(i) Other criteria shown above are not met OR (ii) the criteria for benign and pathogenic are contradictory

Pathogenic

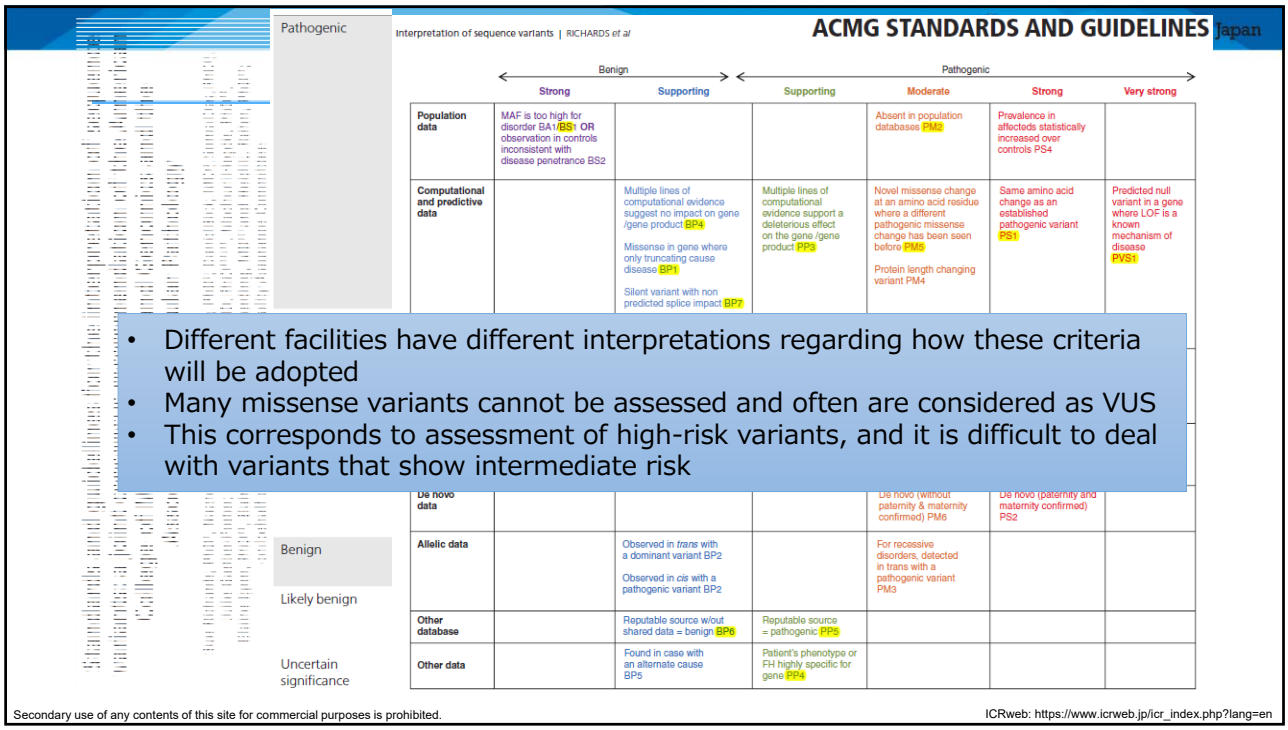
Likely pathogenic

Benign

Likely benign

Uncertain significance

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
Population data	MAF is too high for disorder BA1/BS1 OR observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
Computational and predictive data		Multiple lines of computational evidence suggest no impact on gene /gene product BP4 Missense in gene where only truncating cause disease BP1 Silent variant with non predicted splice impact BP7 In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5 Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
Functional data	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
Segregation data	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation data →		
De novo data				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
Allelic data		Observed in *trans* with a dominant variant BP2 Observed in *cis* with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
Other database		Reputable source w/out shared data = benign BP6	Reputable source = pathogenic PP5			
Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			



- Different facilities have different interpretations regarding how these criteria will be adopted
- Many missense variants cannot be assessed and often are considered as VUS
- This corresponds to assessment of high-risk variants, and it is difficult to deal with variants that show intermediate risk

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Summary 2-2

- FoundationOneCDx is investigating the possibility of germline findings based on previous database and research, and associated information regarding the obtained variants.

  (Clinical information input into C-CAT will become important!!)
- It is important that germline variants obtained using the NCC Oncopanel be reevaluated by expert panels to determine the significance of the variants.
- Currently only 16 highly actionable genes are analyzed for germline variants in NCC Oncopanel.

  (In the future, germline variants for all genes will be included in the sequencing report)

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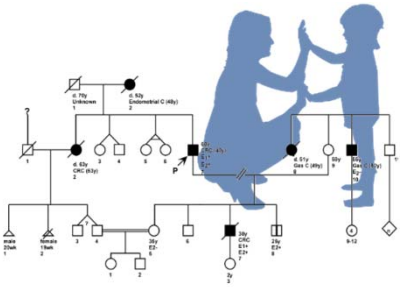
2-3. Points to Note When Disclosing the Results of Germline Findings and the Actual Response in Outpatient Genetic Counseling



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Points to Note When Disclosing Germline Findings

Preliminary checks before outpatient consultation

- Check which pathogenic variant of the gene was found and in which disease
  (For example, if the *BRCA1* pathogenic variant is found in breast cancer or ovarian cancer, it may be perceived differently if found in lung cancer)
- Check diseases related to the detected gene (not limited to cancer)

At disclosure of results

- First, reconfirm the person's wishes
- Explain the detected variant
- Explain the diseases related to the gene
- Reconfirm the person's family history (related diseases: not limited to cancer)
- FoundationOne CDx: Explain that retesting is required to confirm the results

Referring to outpatient genetic consultations

- First, assess the wishes of the patient and their family
- When the patient is concerned about future surveillance and diagnosis of relatives
- When genetic testing is required to confirm the results of FoundationOne CDx

Outpatient Genetic Consultations

1) Assess the possibility of onset or recurrence of the disease
   - Check medical history
   - Check family history
   - Reconfirm and reassess the variant
   - Assess risk
2) Educate the patient on genetic phenomena, testing, management, prevention, resources, and research
   - Possibility of inheritance by relatives
   - Discuss whether the patient will proceed to further genetic testing if they had the FoundationOne CDx test
     ⇒ If the patient wishes, perform genetic testing to confirm the results
   - Diagnosis of relatives
   - Formulate and implement strategies for surveillance of pathogenic variant carriers, pre-emptive medicine, and prevention
   - Follow-up for VUS (confirm variant carriers in the family and respond to significant changes in the future)
3) Counseling to promote informed choice (informed and autonomous choice) and facilitate responses to risks and situations

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Summary 2-3

- Confirm the disease related to the detected variant before disclosing the test results
- Reconfirm the wishes of the patient and their family when disclosing the test results
- Reconfirm the family history with the patient, focusing on the related disease/s when disclosing the test results
- (When the results are disclosed by the attending physician in each clinical department), refer the patient to outpatient genetic consultation if the patient wishes, after confirming the wishes of the patient and his or her family

Summary

- Now that oncogene panel testing is covered by insurance, knowledge on inheritance and genes is required in clinical practice
- When ordering oncogene panel testing, collect information in advance to explain the results to patients, considering that the test may also reveal germline findings
- Hereditary tumors require a cross-disciplinary response and interprofessional collaboration depending on the disease risk, and a system must be established in advance based on the possibility of obtaining germline findings