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JCS GUIDELINES

JCS/JCC/JSPCCS 2024 Guideline on Genetic Testing and Counseling in Cardiovascular Disease

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Refer to **Appendix 2** for the details of members.

JCS Joint Working Groups: The Japanese Circulation Society; Japanese College of Cardiology; Japanese Society of Pediatric Cardiology and Cardiac Surgery; The Japan Society of Human Genetics; Japanese Association of Cardiovascular Intervention and Therapeutics; The Japanese Heart Failure Society; Japanese Heart Rhythm Society; The Japanese Association of Cardiac Rehabilitation; The Japanese Society for Cardiovascular Surgery; The Japanese Society for Vascular Surgery

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Abbreviations

ACM	arrhythmogenic cardiomyopathy
AD	autosomal dominant
AF	atrial fibrillation
AR	autosomal recessive
ARH	autosomal recessive hypercholesterolemia
ARVC	arrhythmogenic right ventricular cardiomyopathy
AS	aortic stenosis
AVB	atrioventricular block
BAV	bicuspid aortic valve
BrS	Brugada syndrome
CAD	coronary artery disease
CALJA	calcification of joints and arteries
CGH	comparative genomic hybridization
CHCC	Chapel Hill Consensus Conference
CHIP	monoclonal hematopoiesis of indeterminate potential
CNV	copy number variation
CPVT	catecholaminergic polymorphic ventricular tachycardia
CSA	coronary spastic angina
CVD	cardiovascular disease
CVS	chorionic villi sampling
DCM	dilated cardiomyopathy
DTC	direct-to-consumer genetic testing
EDS	Ehlers-Danlos syndrome
FH	familial hypercholesterolemia
FISH	fluorescence in situ hybridization
FTAAD	familial aortic aneurysm and dissection
GACI	generalized arterial calcification of infancy
GRS	genetic risk score
GWAS	genome-wide association study
HCM	hypertrophic cardiomyopathy
HHT	hereditary hemorrhagic telangiectasia
HLHS	hypoplastic left heart syndrome
IAA	interruption of aortic arch
IC	informed consent

IHD	ischemic heart disease
JLNS	Jarvell and Lange-Nielsen syndrome
LDL	low-density lipoprotein
LOH	loss of heterozygosity
LQTS	long QT syndrome
MAPCA	major aortopulmonary collateral arteries
MLPA	multiplex ligation-dependent probe amplification
MVA	microvascular angina
NGS	next-generation sequencing
NIPT	non-invasive prenatal genetic test
NO	nitric oxide
PAH	pulmonary arterial hypertension
PAVSD	pulmonary atresia with ventricular septal defect
PCCD	progressive cardiac conduction disease
PCR	polymerase chain reaction
PGT	preimplantation genetic testing
PH	pulmonary hypertension
P/LP	pathogenic/likely pathogenic
PPS	peripheral pulmonary stenosis
PRS	polygenic risk score
PVC	premature ventricular contraction
PVOD/PCH	pulmonary veno-occlusive disease/pulmonary capillary hemangiomatosis
RWS	Romano-Ward syndrome
SNP	single-nucleotide polymorphism
SQTS	short QT syndrome
SVAS	supravalvular-aortic stenosis
TA	truncus arteriosus
TdP	torsade de pointes
TGA	transposition of the great arteries
TOF	tetralogy of Fallot
UPD	uniparental disomy
VF	ventricular fibrillation
VSA	vasospastic angina
VSD	ventricular septal defect
VUS	variant of uncertain significance

Preamble

The 1st edition of the "Guidelines for genetic testing and genetic counseling in cardiovascular disease" was published in 2006, the revised edition was published in 2011, and the 2nd edition is now being published in 2024. During this time, there have been enormous developments in cardiovascular care, and genetic testing has become more familiar in daily practice due to the rapid progress in genetic analysis guided by advanced technology and the remarkable increase in insurance coverage of genetic testing.

This revised 2nd edition was prepared jointly by the Japanese Circulation Society, the Japanese College of Cardiology, and the Japanese Society of Pediatric Cardiology and Pediatric Surgery. In addition, the Japanese Society of Human Genetics, the Japanese Association of Cardiovascular Intervention and Therapeutics, the Japanese Society of Heart Failure, the Japanese Heart Rhythm Society, the Japanese Society of Cardiac Rehabilitation, the Japanese Society of Cardiovascular Surgery, the Japanese Society of Thoracic Surgery, and the Japanese Society of Vascular Surgery participated in this project, and the group members, including representatives from these participating societies, were organized to revise the guidelines.

This version, as with the 1st and 2nd editions, is intended for clinical cardiologists and healthcare professionals who treat patients with cardiac and vascular diseases and summarizes basic information on genetic testing and genetic counseling in those diseases, to serve as a guide for deciding whether or not to recommend or perform genetic testing, according to the Japanese Society of Medical Science Guidelines for genetic testing and diagnosis in medical care (https://jams.med.or.jp/guideline/genetics-diagnosis_2022.pdf) and Ethical Guidelines for Life Sciences and Medical Research Involving Human Subjects (Revised 2022) (https://www.meti.go.jp/press/2021/03/20220310006/20220310006.html)

The information obtained from genetic testing is the ultimate privacy and therefore requires particular care and attention, we should observe the Act on the Protection of Personal Information.

Each section has a detailed description of what genetic testing should be used for cardiovascular clinicians, both medical and surgical, can better understand the diseases and conditions, current genetic testing, and the genetic research that may be used in clinical practice in the future; that is, the genetic tests that have not yet reached the point of routine practice as of the time of this guideline (2022–2023). In the field of oncology, somatic mutations in cancer cells are analyzed together with germline cells, and the information is used not only to elucidate pathophysiological and pathological mechanisms but also to develop therapeutic strategies such as drug treatment. In the field of cardiovascular medicine, the relationship between somatic mutations, such as monoclonal hematopoiesis of indeterminate potential (CHIP), and the development of cardiovascular diseases has attracted attention in recent years, but describing somatic mutations has been left to other research guidelines, and in this revised 2nd edition of the guideline, the discussion is again limited to germline variants. Pharmacogenetics related to drug therapy is thought to be beneficial without demerits, although we do not describe precisely in this guideline.

In these guidelines of the Japanese Circulation Society, the following terms are defined.

Genetic testing refers to testing for genetic mutations or chromosomal abnormalities in the human germ line and related tests. Genetic testing in the medical setting includes not only testing for the diagnosis of patients who have already developed the disease, but also testing for carriers, pre-onset testing, susceptibility testing, pharmacogenetic testing, prenatal testing, and newborn mass screening for inborn errors of metabolism and other disorders.

Genetic counseling is a medical practice and interactive process that provides genetic information such as genetic test results, clinical findings, family history, and all related information that addresses the needs of the patient and family, and helps the patient and family to make decisions based on an understanding of their needs, values, and expectations.

Privacy and confidentiality are concepts that arise in a relationship. Privacy of information (i.e., what will be shared) is usually controlled by the individual, whereas confidentiality refers to the obligation imposed on those who retain an individual's personal information (medical professionals, institutions, etc.) to manage that information to maintain privacy.

Minors, children, neonates, and fetuses are respectively defined, in principle, as <18 years of age, <16 years of age, <28 days of age, and unborn, including intrauterine embryos.

This guideline also presents recommendations and levels of evidence as shown in **Tables 1** and **2**.

Table 1. Class of Recommendation

Class I	Evidence and/or general agreement that a given procedure or treatment is useful and effective
Class II	Conflicting evidence and/or a divergence of opinion about the usefulness/efficacy of the given procedure or treatment
Class IIa	Weight of evidence/opinion is in favor of usefulness/efficacy
Class IIb	Usefulness/efficacy is less well established by evidence/opinion
Class III	Evidence or general agreement that the given procedure or treatment is not useful/effective, and in some cases may be harmful

Table 2. Level of Evidence

Level A	Data derived from multiple randomized clinical trials or meta-analyses
Level B	Data derived from a single randomized clinical trial or large-scale nonrandomized studies
Level C	Consensus of opinion of the experts and/or small-sized clinical studies, retrospective studies, and registries

I. Basic Stance of the Guidelines

In the implementation of genetic testing for cardiac and vascular diseases, the rights of human subjects, including the right to self-determination and refusal, the right to know or remain ignorant, and the right to be free from discrimination, must be given priority over scientific and social interests. In addition, the benefits to the patient and family should be respected to the greatest extent possible, and disadvantages or risks should be minimized. The genetic information obtained must be used for the medical treatment of the patient or his/her relatives, and sufficient care must be taken to ensure that the patient and his/her relatives do not suffer any social or economic disadvantage such as genetic discrimination.

1. Genetic Testing and Counseling

Genetic testing includes DNA testing as well as chromosomal testing and genetic and biochemical testing. In addition to genetic, chromosomal, and biochemical genetic testing for specific cardiovascular diseases (CVD), tests for definitive diagnosis, carrier testing, prenatal testing, susceptibility testing, and prenatal testing are also included. In genetic testing, (1) the test results should remain unchanged throughout life ("invariance"), (2) the test results are personal genetic information that is also shared by blood relatives ("covariance"), (3) it may be possible to predict future disease onset ("predictability"), but it should be difficult or impossible to predict the timing and symptoms of disease onset, and (4) there is a possibility of social disadvantage, such as discrimination of the patient and his/her family. When asking for informed consent (IC) from a subject or a surrogate, it is important to take sufficient time to explain the results in plain language and to obtain adequate assent for the underage, unlike for general examinations in routine medical care, because the results obtained are genetic information that may directly affect not only the subject but also his/her blood relatives. In addition, the advantages and disadvantages of genetic testing, information management systems, protection of personal information, procedures for disclosing results, and costs (whether or not the test is covered by insurance, and if not covered by insurance, whether or not the full cost is borne by the patient, or whether or not the test is free of charge and the hospital or educational/research facilities will cover the costs) should be explained.

Regarding the disclosure of results after genetic testing, the explanation must be guided by the subject's understanding of highly specialized genetic analysis. If the subject wishes, genetic counseling should be provided by a clinical geneticist or genetic counselor. Genetic testing should only be utilized in cardiovascular care after developing a system covering from IC to the explanation of the results.

2. Scope of Application of the Guideline

The scope of this guideline is genetic testing performed as part of medical treatment and is based on the Japanese Medical Association Guidelines for genetic testing and diagnosis in medical care (https://jams.med.or.jp/guideline/genetics-diagnosis_2022.pdf). The following is a summary of those guidelines.

With regard to genetic tests performed as part of medical care, some of them have already established their efficacy and uncontroversial use in clinical practice, but others have yet to demonstrate their efficacy The latter genetic tests should be thoroughly considered based on the premise of "minimizing the disadvantages and maximizing the benefits to the patient," and such tests should be fully explained to the patient and family members before implementation. If the medical significance and usefulness of a genetic test has not been established, it may be carried out under the category of research, in which case it will be carried out in accordance with the Ethical Guidelines for Life Sciences and Medical Research Involving Human Subjects (enacted in 2021 and revised in 2023) (https://www.meti.go.jp/press/2021/03/20220310006/20220310006.html), and must be evaluated and approved by an institutional or central ethical review committee.

II. Guidelines for Genetic Testing

Genetic information has the characteristics of "invariance", "predictability", "shareability", and "ambiguity". Because it is sensitive information, inappropriate handling could negatively affect the individual and his/her relatives.[1] Two guidelines for genetic testing and microarray chromosome testing exist in Japan,[2,3] which describe the basic principles and characteristics of tests for evaluating variants of various types. Conforming with those guidelines, this chapter is an overview of genetic testing and counseling in the cardiovascular setting.

Genetic information is defined as a personal identification code in the Order for Enforcement of the Act on the Protection of Personal Information (Cabinet Order No. 507 of 2003). Healthcare providers who handle genetic information are also required to comply with the following guidelines: Guidance for proper handling of personal information by medical and long-term care providers (No. 6, Medical Policy Bulletin No. 0414, April 14, 2009) and the Guidelines for provision of medical information (No. 0912001, Medical Policy Bulletin No. 0912001, September 12, 2003), and the Guideline for the provision of medical information (issued by the Ministry of Health, Labour and Welfare on September 12, 2003, No. 0912001).

1. Characteristics of Genetic Testing

The characteristics of germline genetic information that

should be considered when conducting genetic testing are listed in **Table 3**.[2] Genetic testing is performed to obtain information about the subject's constitution and the development of diseases based on changes in the genes and chromosomes of the individual. Of these characteristics, invariance means that genetic information does not change during the lifetime. Second, family members share it in part, and therefore it is predictive in that it can infer the genotype and phenotype of other members. Finally, ambiguities should be considered, because the pathological significance and clinical utility are often indeterminate at the time of testing, and the phenotype predicted from the pathogenic variant may vary among individuals.

Patients with genetic disorders often attend multiple departments, so healthcare providers must understand the characteristics of genetic information and when sharing it, they must always protect the privacy of the patient.

Table 3. Characteristics of Genetic Information (From the Guidelines of the Japan Medical Association[2])

- Invariance: Remain unchanged throughout the subject's lifetime
- Covariance: Partially shared among blood relatives; prediction of genotype and phenotype of blood relatives with relatively accurate probability
- Diagnosis of nondevelopmental carriers (persons who are unlikely to develop a disease caused by a pathogenic variant in the future, but who have the variant and may pass it on to the next generation) may be possible
- Possibility of future onset of disease can be predicted with near certainty in some cases before the onset of disease
- May be available for prenatal genetic testing and preimplantation genetic testing
- Possibility of social disadvantage to the examinee and the examinee's blood relatives if the examinee is treated inappropriately

(Ambiguity refers to the fact that the judgment of the pathological significance of a result can change, that there can be individual differences in the presence or absence of disease onset, timing of disease onset, symptoms, and severity predicted by pathological variants, and that clinical utility can change as medical and scientific advances are made.)

2. Disclosure of Genetic Information

Genetic testing is proposed when it is considered clinically and genetically useful, with due regard to its characteristics. The patient and family members often have significant concerns about how to deal with the predictability of the disease. When performing genetic testing, medical institution needs systems in place to provide psychological and social support through genetic counseling by a person with expertise in clinical genetics.

Genetic test results should be informed in a comprehensive manner based on the confirmation of the diagnosis, including information regarding the relationship between genotype and phenotype. Of the novel variants identified without established significance and high disease penetrance, disclosure should proceed with careful counseling.

When genetic testing is performed for children and adolescents, consent should be obtained from not only parents but children themselves. Performing genetic testing in childhood related to the pretesting for diseases that develop later in adulthood is controversial. In principle, testing should be postponed until the age of autonomous decision making.

Genetic testing can allow certain prediction of future disease onset before disease onset, providing patients with information regarding prevention and post-onset treatment in advance, as well as cases of low or unknown penetrance, where medical intervention can be clinically useful. It is essential that patients fully understand all information provided. Particularly in the case of genetic testing for diseases for which there is no established method, it is essential to provide psychological consideration and support for the examinee before and after testing.

This guideline does not cover genetic testing for multifactorial diseases or comprehensive genetic testing for the entire genome, but it is required that such testing be conducted with a clear scientific rationale. Secondary findings that are not the original purpose of testing are recognized, which include the case of healthy carriers with pathogenic variants. Results may provide useful information not only to the examinee but also to his/her family members, but the impact should be carefully considered in advance before disclosure.[4]

3. Protection of Personal Information

Healthcare providers must retain the results of genetic tests and genetic counseling for the purpose of diagnosing patients in medical records based on the above guidelines about protection of personal information. Therefore, healthcare providers who have access to genetic information are required to have sufficient education and training in basic knowledge of genetic medicine and appropriate handling of genetic information.[5,6]

Although genetic testing is performed with consideration for family members, test results must not be disclosed to third parties, including family members, without the consent of the test subject. However, if the test results are useful for their health care and there is no other equivalent method, disclosure may be considered under the approval of an ethics committee.

4. Guidelines for Genetic Research

A clear distinction should be made between medical practice and research in genetic analysis. This guideline describes genetic testing in clinical practice, not in research. However, genetic analysis that has not yet been established as a clinical practice may be examined within the scope of genetic analysis research. In such cases, the research should be conducted in accordance with the ethical guidelines for medical research, including the disclosure of the results.[7]

III. Purpose of Genetic Testing

1. Purpose of Genetic Testing

Genetic analysis performed as part of a medical examination is called clinical sequencing; some of the analyses are covered by the National Health Insurance and others are not. When germlines are analyzed, it is called genetic testing. The purpose of genetic testing is to contribute to the health of patients and their relatives by providing genetic information for their care.

Genetic testing is useful for clarifying the diagnosis, selecting and optimizing treatment, managing medical care, preventing disease progression after disease onset, and understanding the long-term prognosis for patients who have already developed the disease, as well as for preventing disease onset, early diagnosis, and early intervention for those who have not yet developed the disease. One of the most significant benefits of this test is that patients and their relatives can learn the cause of the disease and deepen their understanding of the disease.

Genetic testing for inherited cardiovascular diseases (CVD) is usually performed with the aim of making a definitive diagnosis for the individual presenting with symptoms, and **Figure 1** shows a representative list of the purposes of such testing.

As with other inherited or congenital diseases, the implementation of genetic testing for inherited CVD is based on the autonomy of the patient, with the patient's human rights taking precedence over scientific and social interests. Furthermore, because the genetic information obtained may be shared with the patient's family, genetic testing can contribute to the health not only of the patient but also of relatives, and it is important to respect their human rights in this regard as well. Genetic testing for inherited CVD is performed in compliance with the Guidelines for genetic testing and diagnosis in medical care of the Japanese Medical Association.[2]

2. Usefulness and Points to be kept in Mind for Genetic Testing

Genetic information has the characteristics of "invariance", "predictability", "shareability", and "ambiguity". With the identification of causative genes for numerous inherited or congenital diseases, including hereditary CVD, a framework has been developed to provide sophisticated and tailor-made medical care from generation to generation, using the characteristics of genetic information, in addition to its direct use in diagnosis and treatment. Against this backdrop, the utility of genetic testing is summarized in **Table 4**.

It is important to note that the method selected for genetic testing and the significance of the testing may vary depending on whether the examinee is a patient or a relative, symptomatic or asymptomatic, and so on. Genetic testing is classified as "patient analysis" for diagnosis of the patient and "cascade screening" for diagnosis of the relatives. Patient analysis refers to genetic testing in patients, such as Sanger sequencing for the entire region of the candidate gene, and panel-based analysis by next-generation sequencing. Sanger sequencing-based single-site analysis is

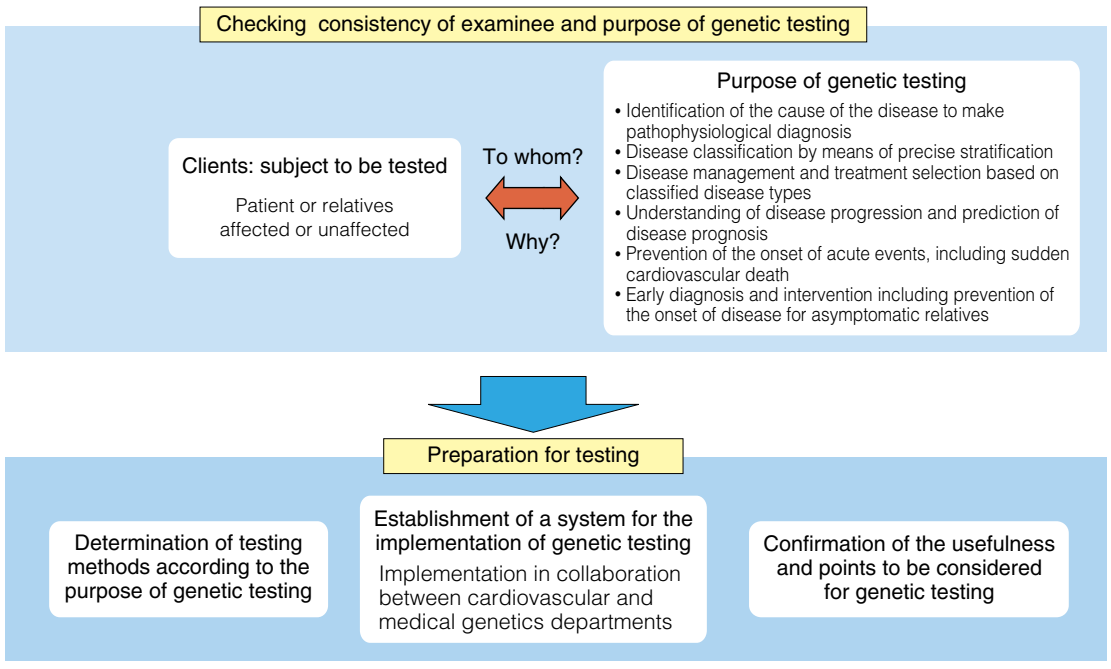

Figure 1. Items to be checked for the preparation of genetic testing.

typically performed for "cascade screening" when a pathogenic variant is identified in the family. The following points should be kept in mind when performing these 2 types of genetic testing (**Table 5**).

In recent years, pathogenic variants in the genes responsible for hereditary CVD have been identified as secondary findings through cancer gene panel tests (typically covered by the National Health Insurance) and research-based comprehensive genetic analyses (whole-exome and whole-genome sequencing) for rare undiagnosed diseases or cancers, and cardiology departments have been consulted on how to handle them.

Cancer gene panel tests covered by the National Health Insurance in Japan include genes for inherited cardiovascular diseases (e.g., *SMAD3*, *TGFBR1*, and *TGFBR2*, causative for Loeys-Dietz syndrome). The guidelines

Table 4. Usefulness of Genetic Testing

"Acceptance" of hereditary cardiovascular diseases by patients and their families will be promoted
Medical checkups (surveillance) and preventive measures will be presented
Treatment measures, including the prevention of the onset of acute events, will be presented
Social support will be provided through certification of designated intractable disease or specific chronic pediatric diseases
Healthcare of patients' relatives will be promoted through early diagnosis and intervention based on "cascade screening"

Table 5. Points to be kept in Mind for "Patient Analysis" or "Cascade Screening"

Notes	Patient analysis	Cascade screening
Minimal invasiveness (blood drawing)	Yes	Yes
Pathogenic variant may not be detected in the assumed gene • Possibility that a pathogenic variant is actually present in the analyzed gene but cannot be detected due to technical limitations • Possibility that a pathogenic variant is present in a gene that have not been analyzed • Possible mechanism unrelated to a genetic abnormality	Yes	No
Pathogenicity of the detected variant cannot be determined (variant of uncertain significance [VUS])	Yes	No
Accurate estimation for the age of onset and the severity will be impossible even when a pathogenic variant is identified	Yes	Yes
Radical treatment such as "gene correction or editing" is not available	Yes	Yes
Psychological burden regarding the patient's progression of disease and the morbidity of his/her relatives is likely	Yes	Yes
Psychosocial burden regarding early presymptomatic diagnosis is likely (e.g., a possibility of limitations in insurance coverage)	Yes	Yes

Table 6. Genes Listed by American College of Medical Genetics and Genomics Guidelines v3.2 as Secondary Findings (Presenting Hereditary Cardiovascular Diseases)[8]

Disease	Genes
Dilated cardiomyopathy	*MYH7, TNNT2, LMNA, FLNC, TTN*, BAG3, DES, DSP, RBM20, TNNC1, SCN5A*
Hypertrophic cardiomyopathy	*MYH7, MYBPC3, TNNI3, TNNT2, TPM1, MYL3, ACTC1, PRKAG2, MYL2, FLNC*
Arrhythmogenic right ventricular cardiomyopathy	*PKP2, DSP, DSC2, TMEM43, DSG2*
Congenital long QT syndrome	*KCNQ1, KCNH2, SCN5A, CALM1, CALM2, CALM3, TRDN*
Brugada syndrome	*SCN5A*
Catecholamine-induced polymorphic ventricular tachycardia	*RYR2, CASQ2, TRDN, CALM1, CALM2, CALM3*
Hereditary transthyretin amyloidosis	*TTR*
Fabry disease	*GLA*
Pompe disease	*GAA*
Aortic disease (including familial aortic aneurysm, Marfan syndrome, Loeys-Dietz syndrome)	*FBN1, TGFBR1, TGFBR2, SMAD3, ACTA2, MYH11*
Vascular Ehlers-Danlos syndrome	*COL3A1*
Familial hypercholesterolemia	*LDLR, APOB, PCSK9*
Hereditary hemorrhagic telangiectasia (Osler-Weber-Rendu syndrome)	*ENG, ACVRL1*

*For truncating variants that result in protein shortening. (Source: Prepared based on Miller DT, et al. 2023[8])

proposed by the American College of Medical Genetics and Genomics[8] (v3.2 is latest version as of 2023) (**Table 6**) present genes that should be considered for disclosure when conducting a comprehensive genetic analysis.

For inherited CVD, genetic testing of minors (<18 years of age) is often useful in the setting of both patient analysis and cascade screening,[9] including cardiovascular surveillance, early pharmacological intervention (e.g., β-blockers for congenital long QT syndrome, β-blockers and angiotensin-receptor blockers for Marfan syndrome), and lifestyle modifications (e.g., avoidance of contact and/or competitive sports, discussion about preferable activities[10]).

3. Collaborative Application of Genetic Testing by Cardiology and Medical Genetics Departments

In order to apply the results of genetic testing in the treatment of patients and their relatives with inherited CVD, collaboration of the two departments: cardiology and medical genetics is advisable.

According to the Guidelines on genetic testing and diagnosis in medical care by the Japan Medical Association,[2] "In principle, the examinee's attending doctor should perform the informed consent or assent before genetic testing, and if necessary should arrange genetic counseling of an expert so that the examinee can receive support for autonomous decision-making." In many facilities, specialists in the field of CVD (cardiologists, cardiovascular surgeons, pediatric cardiologists, pediatric cardiac surgeons, etc.) are expected to order genetic testing. However, the results report may not only include "Pathogenic" or "Likely pathogenic", which can be interpreted as the cause of the disease, but also VUS, which requires careful judgment in the interpretation and explanation to the patient based on clinical genetics expertise. In the explanation before testing, the usefulness of the testing should be explained sincerely, and the aforementioned points should be mentioned in detail, including handling these points responsibly (see: **Chapter V.1 "How to Order Genetic Testing by a Cardiologists"**). Medical genetics specialists (clinical geneticists and certified genetic counselors) should be in charge of pretest genetic counseling and involved in post-test genetic counseling, including assistance in interpreting results, as necessary.

In addition, many inherited CVD are autosomal dominant and "actionable", in which the usefulness of genetic testing outweighs the points listed in **Table 5**. The utility of early diagnosis and intervention before manifestation (e.g., from childhood) is internationally recognized. Genetic testing as cascade screening for asymptomatic relatives, including minors, should be conducted after genetic counseling in collaboration with a medical genetics department experienced in hereditary CVD (see: **Chapter V.2 "Genetic Counseling"**).

In institutions without a medical genetics department, facilities implementing genetic medicine are available on the website of the National Liaison Conference of Genetic Medicine Departments (URL: http://www.idenshiiryoubumon.org/) or there is the list of medical care delivery systems for intractable diseases at the Medical Center for Intractable Diseases (URL: https://www.nanbyou.or.jp/).

4. Classification of Genetic Testing

Genetic testing includes diagnosis of patients and their relatives, prenatal diagnosis for serious diseases, pharmacogenetic testing to estimate the effect and side effects of drugs, and susceptibility testing for multifactorial inherited disease.

4.1 Patient Analysis

The patient's diagnosis is made in collaboration with the cardiology and genetic medicine departments, based on an understanding of the purpose, usefulness, and considerations of the test.

4.2 Cascade Screening

If genetic testing is performed on a patient with an inherited CVD and a pathological variant is detected in the causative gene, genetic testing of relatives may be beneficial to their health care. The following points should be considered in such cases.

1. Penetrance: Typical inherited CVDs with high penetrance include inherited connective tissue diseases such as Marfan syndrome, catecholamine-induced polymorphic ventricular tachycardia, and *LMNA*-related dilated cardiomyopathy. Typical inherited CVDs with moderate penetrance include hypertrophic cardiomyopathy and congenital long QT syndrome. Typical inherited CVDs with low penetrance include *TTN*-related dilated cardiomyopathy.
2. Diagnosis: Considering penetrance, the diagnosis of relatives cannot be made solely due to the presence of inherited pathogenic variants. The diagnosis must be based on a combination of clinical symptoms and laboratory findings.
3. Fee: Genetic testing for asymptomatic relatives is not covered by the National Health Insurance in Japan under the revised reimbursement system as of 2023. However, early intervention (genetic testing, surveillance, prophylactic medication, etc.) for relatives is a fundamental part of genomic medicine, and cardiologists and genetic medicine specialists strongly hope that it will be covered by the National Health Insurance as soon as possible.

4.3 Prenatal Genetic Testing

Prenatal genetic testing/diagnosis (e.g., the subject of the testing, precautions for its implementation) is discussed in **Chapter VI.7 "Types of Prenatal Tests and Diagnoses"**. In Japan, pregnancy termination is not permitted under the Penal Code, and the Maternal Health Act provides for its limited application (a person for whom the continuation of pregnancy or delivery may significantly damage the person's physical health due to bodily or economic reasons; a person who was raped in a violent or threatening manner or at a time when the person could neither resist nor refuse and becomes pregnant). Pregnancy termination for the direct reason of fetal disease or disability is not permissible.

Preimplantation genetic testing (PGT), which has recently emerged, includes PGT for aneuploidy (PGT-A) for infertility/infertility, PGT for structural rearrangement (PGT-SR) for couples with balanced chromosome struc-

tural abnormalities, and PGT for monogenic (PGT-M) for couples with a high recurrence rate of certain single-gene disorders. Although abortion can be avoided because only unaffected embryos are transferred, it should be noted that the diseases and conditions covered are very limited. This testing is carefully reviewed by the institution where it is performed and by the Ethics Committee of the Japanese Society of Obstetrics and Gynecology.

4.4 Pharmacogenetic Testing

Pharmacogenetic testing is germline genetic evaluation that estimates the effect and possible side effects of a drug. The resulting genetic information is usually performed in the course of usual medical care unless it is relevant to the diagnosis or risk of developing a specific disease.

4.5 Susceptibility Testing

Susceptibility testing for CVD of multifactorial inheritance is currently being conducted as a research-base, and is still in the process of being validated clinically. Multifactorial CVD are associated with a number of genetic and environmental factors. From the perspective of preventive medicine, susceptibility genetic testing for multifactorial hereditary diseases is expected to be applied clinically in the future.

Direct-to-consumer genetic testing (DTC), which is provided directly to consumers without going through a medical institution, is not included in the susceptibility testing described here because it is not performed as medical management. At present, it may not be possible to confirm that analytical validity, clinical usefulness, scientific rationale, testing accuracy, quality assurance and protection of personal information are ensured, or that the content of test results is verified sufficiently. The Japanese Society of Human Genetics' position on DTC genetic testing also states, "To ensure that consumers are not disadvantaged, stakeholders should take every opportunity to educate and inform the public about the basics of genetics and DTC genetic testing".[11]

IV. Genetic Testing Practices

1. Genetic Testing Methods

Genetic testing methods are classified as (1) molecular, (2) biochemical, and (3) cytogenetic (**Table 7**). Molecular testing includes sequencing methods such as the Sanger method, and whole-exome and whole-genome analyses using next-generation sequencing (NGS). Biochemical testing includes enzyme activity measurements for inborn metabolic disorder with no genetic sequencing, and cytogenetic testing includes activity measurement for inborn metabolic disorders without sequencing, and chromosome testing (**Table 7**).

1.1 Molecular Genetic Diagnostics

1.1.1 PCR/Sequencing (Sanger Sequencing)

Sanger sequencing (SS) is a nucleotide sequencing method developed by Frederick Sanger, who was awarded the Nobel Prize in Chemistry in 1980. It is based on a reaction in which DNA polymerase uses single-stranded DNA as a template to elongate complementary bases (nucleotides) in the same way that genomic DNA is replicated in cells.

The principle of SS is briefly described. First, the DNA to be examined is single-stranded, and a short primer is bound near the region to be sequenced. Next, in the presence of 1 dideoxynucleotide (ddATP, ddTTP, ddGTP, or ddCTP) in addition to the 4 deoxynucleotides (dATP, dTTP, dGTP, dCTP), an elongation reaction is initiated by DNA polymerase. At that time, the ribose ring of the dideoxynucleotide does not have a hydroxy group at the 3' position for the next nucleotide to bind, so if a dideoxynucleotide is incorporated instead of a deoxynucleotide, strand elongation stops there. Because 4 types of deoxynucleotides are added here, multiple DNAs of various lengths are synthesized depending on the timing of dideoxynucleotide incorporation. In practice, the 4 different DNA synthesis reactions are performed simultaneously, with 1 dideoxynucleotide added to each of the 4 types of dideoxynucleotides. Each of the 4 dideoxynucleotides at this time should be labeled with a different colored fluorescent dye. Finally, reaction products of various lengths with a single base difference obtained in the sequencing reaction are separated by capillary electrophoresis. When the reaction products are electrically captured in a long glass capillary filled with gel polymer, the DNA fragments are negatively charged and flow toward the anode side by electrophoresis. The speed of movement through the gel is inversely proportional to the molecular weight of the DNA fragments, with shorter fragments flowing faster and longer fragments taking longer. By shining a laser beam from the end of the capillary tube, the dideoxynucleotides are incorporated and the DNA elongation is stopped, which is

Table 7. Methods Used in Genetic Testing

1. Molecular genetic diagnosis
(1) PCR/sequencing (Sanger sequencing)
(2) MLPA method
(3) Microarray chromosome testing
(4) Next-generation sequencing
Panel analysis
Whole-exome analysis
Whole-genome analysis
2. Biochemical genetic diagnosis
(1) Newborn mass screening
(2) Enzyme diagnosis
3. Cytogenetic diagnosis (chromosome examination by microscopic observation)
(1) Chromosome test (G staining)
(2) FISH

FISH, fluorescence in situ hybridization; MLPA, multiplex ligation-dependent probe amplification; PCR, polymerase chain reaction.

read as a fluorescent label and converted into nucleotide information.

This method is characterized by low read error and relatively long read lengths (~1,000 bases), and is frequently used to validate results from next-generation sequencing (NGS), as described later.

1.1.2 MLPA Method

The multiplex ligation-dependent probe amplification (MLPA) method is used to detect large-scale deletions and amplifications of genes that have been difficult to analyze using conventional gene amplification methods such as PCR and whole-gene sequencing.

The principle of this method is to first prepare 2 DNA probes that are paired next to a specific portion of the target gene, and then combine hybridization and linkage (ligation) of the DNA probes and PCR amplification techniques to examine dozens of minute internal gene regions. Each probe has a primer site bound to it to perform the PCR method. When 2 DNA probes are combined by ligase and then subjected to PCR reaction, a DNA fragment of the length of the 2 DNA probes is amplified only when the specific site is present. The amplified product is electrophoresed to determine gene deletion or amplification based on its pattern.[12]

The MLPA method is a multiple chain reaction PCR method for detecting genomic copy number abnormalities associated with specific genetic disorders and is considered the most reliable and cost-effective method for detecting deletions, duplications, and specific copy number variations (CNVs). There are 2 possible results.

(1) Positive (Pathogenic and likely pathogenic): indicates a confirmed deletion or duplication of a gene associated with the phenotype of the disease of interest. In this case, genetic counseling can be provided regarding treatment, disease progression, prevention strategies, and potential impact on family members.
(2) Negative: indicates that no disease-causing deletions or duplications were identified by the test, which does not guarantee that the subject is healthy or free of other genetic or medical conditions.

MLPA cannot detect (1) balanced reciprocal translocations, (2) telomere deletions and duplications, (3) deletions and duplications outside the target of the MLPA probe used, (4) point mutations, small insertions and deletions, or (5) repeated sequences and mutations in mitochondrial DNA.

Conventional genetic testing methods, such as SS, can detect small deletions and duplications of a few bases, but not deletions and duplications over larger regions. However, with the advent of the MLPA method, this has become possible, and genetic testing for Duchenne/Becker muscular dystrophy is now covered by insurance. In addition, the MLPA method can be used not only as a genetic test, but also as a convenient way to validate multiple samples of target regions narrowed down by exhaustive exploratory studies such as comparative genomic hybridization (CGH) arrays and DNA chips.

1.1.3 Microarray Chromosome Testing

The microarray method is a comprehensive analysis in which DNA fragments corresponding to the entire target region of the genome are densely arranged on a slide, the sample DNA to be examined is added to the slide, and the copy number of the entire genome is searched. The representative analysis methods are CGH and single-nucleotide polymorphism (SNP) arrays. Although they are similar analysis methods, their detection principles, test designs, and purposes of use are different.

CGH uses a test sample and a control sample, stained with different fluorescence, and quantitative comparisons are made at high density with reactions on the array. SNP arrays allow single nucleotide differences to be analyzed by means of probes designed on the array chip. Along with the number of copies of the chromosome, the genotype at the probe position can also be checked to see if it is specifically homozygous or heterozygous, which is detailed information that cannot be obtained by the chromosome segregation method. Changes of approximately 2 Mb in the amplified region and ≤10 Mb in the deleted region were previously undetectable, but CGH allows CNVs to be analyzed at various size levels, making it possible to detect minute undetectable abnormalities. It is also possible to simultaneously analyze a large number of genomic regions.[13] Genome-wide association studies (GWAS) using SNP array data also began in the early 2000s, and disease susceptibility regions in various diseases, including lifestyle-related diseases, have been identified. Drug discovery that targets genes regulated by these disease susceptibility regions has also been successful. Recently, the concept of polygenic risk score (PRS), which is calculated by weighting and adding the effects of variants of SNP regions one by one, has been established, and genetic risk scores can be calculated for a large number of traits including various diseases.

In addition to CNV search, trisomy, high-resolution loss of heterozygosity (LOH) region analysis, uniparental disomy (UPD), and low-frequency mosaicism can be detected. However, equilibrium translocations and inversions without copy number changes cannot be detected, and these must be confirmed by FISH or other methods.

In actual clinical practice, both types of array analysis are often used to detect minute quantitative changes in chromosomes. It has been suggested that in certain congenital diseases, microarray analysis should be preferred over chromosome sequencing.[14]

Catalogs of human CNVs include the Database of Genomic Variants (http://dgv.tcag.ca/dgv/app/home).[15,16] Another database that catalogs the relationship between chromosome imbalance and disease is DECIPHER (DatabasE of Chromosome Imbalance and Phenotype in Humans using Ensembl Resources),[17] in which 5 major functions (Genome Browser, Phenotype browser, Genes, CNV Syndromes, and GeneReviews) can be utilized by relating the exact genomic location to previously reported phenotypes to systematically understand information about rare variants, especially those that affect human development.

The high resolution of the array detects a variety of variants, including normal variants, but due to insufficient data maintenance of controls, CNVs of unknown pathological significance may be detected but difficult to interpret. Therefore, as with other genetic tests, care should be taken because the significance and purpose of the test will change when performed on affected and unaffected individuals.

The array CGH method, which measures chromosomal genomic DNA copy number changes and LOH using an in vitro diagnostic product that has been approved by pharmaceutical affairs agencies, can be supported as an insurance coverage when performed for diseases defined as

chromosomal structural variation analysis (D006-26).[18] However, for other diseases, it can only be performed at private expense or as a genomic analysis study.

1.1.4 Next-Generation Sequencing

In 2006, Solexa's genome analyzer, which utilized a technology that amplified DNA by performing bridge PCR on a flow cell, was introduced. Later, in 2007, Solexa merged with Illumina and the current NGS analysis became widely used. The principle of this technology is briefly introduced below.

a. Library Adjustment

First, a sequence library is prepared by randomly fragmenting the DNA to be examined, followed by ligation of adapters on the 5′ and 3′ sides. Next, DNA fragments are randomly fragmented and ligated with adapters on the 5′ and 3′ sides of the DNA. The DNA fragments to which adapters are added are then amplified by PCR and gel purified.

b. Cluster Formation

The sequence library is then loaded into a flow cell in which oligonuclerotides complementary to the adapters are placed to supplement the library. Each fragment is then amplified into individual clonal clusters by bridge amplification. This cluster formation completes the preparation of the template for sequencing.

c. Sequencing

Preparation of a reversible terminator for each of the 4 deoxynucleotides (dATP, dTTP, dGTP, dCTP) labeled with a different fluorescent dye so that each can be detected (only 1 base is elongated). For the above template, 4 different reversible terminators, DNA polymerase, and primers are added to perform a single-base synthesis reaction, and the bases of whichever color are incorporated are captured by imaging by excitation with a laser. The fluorescent label and terminator are then removed, and nucleotides are incorporated again in the next cycle and fluorescence detection is performed, sequencing 1 base per cluster.

This method is characterized by its ability to output a large number of reads, although each sequence read is short (100–250 bases). When detecting single-nucleotide variants, this method is advantageous because it uses the genome sequence as a reference sequence that has already been determined. On the other hand, the genome contains many repetitive sequences, and because this method can read only a part of such sequences, it cannot be used for analysis because it is not possible to determine where the reads correspond.

Using NGS technology, it is possible to analyze the entire human genome region. By enriching only DNA molecules derived from the desired region, such as the entire exome region or only the region of a specified gene, a comprehensive analysis can be performed for various ranges.

d. Panel Analysis

Targeted sequencing analysis selectively captures and sequences targeted genes or genomic regions out of the entire genomic region and by using a large number of reads it allows for targeted and intensive analysis of sequences of genomic regions associated with diseases. Generally, the region is set by selecting genes related to the disease, but intronic regions and regulatory regions related to the disease can also be incorporated into the analysis.

e. Whole-Exome Analysis

Whole-exome sequencing analysis selectively captures and sequences DNA molecules derived from exon regions that encode genes. Because the region to be analyzed is only about 2% of the whole genome region, the sequencing cost can be reduced. In addition, the analysis can be performed efficiently because disease-causing variants that exist on genes can be targeted for analysis. This method can detect variants on exons, but cannot detect base substitutions in other regions or structural changes in chromosomes.

f. Whole-Genome Analysis

Whole-genome sequencing, as the name implies, analyzes the sequence of all regions of the 3.2 billion human genomic DNA. In addition to gene-coding regions, all regions are analyzed, including introns and intergenic regions. As a result, structural changes such as deletions, insertions, and CNVs can be detected in addition to all base substitutions present in the genome. The rapid decline in sequencing costs and the ability to rapidly produce large amounts of data have made whole genome sequencing a powerful tool in genomic research.

1.1.5 Long-Read Sequence

As just described, NGS analysis, which analyzes short reads, cannot analyze repeat sequences or unannotated regions. To overcome this problem, Pacific Bioscience and Oxford Nanopore Technologies developed long-read sequencing technology. The previous issue of sequencing accuracy has been resolved and highly accurate analysis is now possible. In particular, the nanopore sequencing technology directly reads DNA molecules physically and chemically, enabling data analysis on the spot after sequencing, making genetic diagnosis possible at ultra-high speed. This technology has the potential to implement genetic diagnosis in clinical settings.

1.2 Genetic and Biochemical Diagnostics

1.2.1 Newborn Mass Screening

Local governments fund screening tests to detect substances such as amino acids using tandem mass spectrometry (tandem mass method) by collecting a small amount of blood (dried blood spot) from newborns on the day 4–6 after birth. The purpose of the test is to detect and treat children with treatable diseases that can cause disabilities if left untreated, and to prevent the occurrence of disabilities among newborns.

In addition to testing of 4 inherited metabolic disorders (hyperphenylalaninemia, maple syrup urine disease, homocystinuria type 1, and galactosemia) and 2 endocrine disorders (congenital hypothyroidism and congenital adrenal cortical hyperplasia) that has been performed since 1977, urea cycle disorders and organic acid metabolism and fatty acid metabolism disorders were added in 2013 and later. The total number of diseases covered is 25, including 20 primary and 5 secondary target diseases (**Table 8**).[19] In particular, fatty acid metabolism disorders are a group of diseases in which fatty acid β-oxidation in mitochondria is impaired, resulting in energy production failure, and the brain, heart, liver, and skeletal muscles with their high energy demands are easily affected. In addition, recently, expanded newborn mass screening tests have been imple-

Table 8. Newborn Mass Screening Target Diseases
1. Abnormal amino acid metabolism (6 diseases)
Phenylketonuria
Maple syrup urine
#Homocystinuria
Citrullinemia type 1
Argininosuccinosis
Hypertyrosinemia
2. Abnormal organic acid metabolism (8 diseases)
Methylmalonic acidemia
#Propionic acidemia
Isovaleric acidemia
Methylcrotonylglycinuria
Hydroxymethylglutaric acid (HMG) emia
#Complex carboxylase deficiency
Glutaric acidemia type 1
Beta-ketothiolase deficiency
3. Abnormal fatty acid metabolism (8 diseases)
#Medium-chain acyl CoA dehydrogenase (MCAD) deficiency
#Very long chain Acyl CoA dehydrogenase (VLCAD) Deficiency
#Triglycosyltransferase (TFP)/long-chain 3-hydroxyacyl CoA dehydrogenase (LCHAD) deficiency
#Carnitine palmitoyltransferase-1 (CPT1) deficiency
#Carnitine palmitoyltransferase-2 (CPT2) deficiency
#Carnitine acicarnitine translocase deficiency
#Systemic carnitine deficiency
#Glutaric acidemia type II
4. Abnormalities of carbohydrate metabolism (1 disease)
#Galactosemia
5. Endocrine disorders (2 diseases)
#Congenital hypothyroidism
#Congenital adrenal hyperplasia

#Cardiovascular phenotype is seen. (Source: Prepared based on Japanese Society for Inherited Metabolic Diseases. 2019[19])

mented for severe combined immunodeficiency (SCID), spinal muscular atrophy (SMA), and some lysosomal diseases from October 2021, although this will be at the expense of the patient rather than public funds.

The results of newborn mass screening are reported back by the 1-month checkup, and if the results cannot be determined with certainty to be normal, a retest is conducted. If a full examination is necessary, a visit to a specialized pediatric medical institution is required. In the case of inherited metabolic disorders, the definitive diagnosis of a positive mass screening case is the measurement of specific abnormal metabolites, specifically biochemical tests such as plasma and urinary amino acid analysis, urinary organic acid analysis, and dried blood spot and serum acylcarnitine analysis.

1.2.2 Enzyme Diagnostics

Enzymatic diagnosis is by biochemical tests that can be performed not only in children but also in adults. Fabry's disease and Pompe disease, which are known as lysosomal diseases, and glycogenic diseases, which are caused by abnormalities in enzymes involved in the pathways of sugar metabolism, are complicated by cardiac disease due to functional deletion (enzyme deficiency) of the causative gene, making enzyme diagnosis useful. Enzyme diagnosis is performed by measuring enzyme activity in dried blood spot, and is listed as a medical item in health insurance as a genetic test together with gene analysis. In practice, however, it is still difficult to keep the necessary costs within the scope of insurance reimbursement, so some tests are performed at research institutes specializing in each disease.

Ministry of Health, Labour and Welfare Research Group "Research Group on Establishment and Implementation of System for Realization of High Quality and Appropriate Medical Care in Lysosomal Disease and Peroxisomal Disease (including Adrenoleukodystrophy)".

The Japanese Society for Inherited Metabolic Diseases: http://jsimd.net/iof/iof_01.html

National Center for Child Health and Development website: https://www.ncchd.go.jp/hospital/about/section/clinical/senshin.html

1.3 Cytogenetic Diagnostics (Chromosome Testing Performed by Microscopic Observation)

1.3.1 Chromosome Banding Analysis (Especially G-Staining Method)

A typical chromosome examination is carried out by a culture to obtain cells in mitosis from which chromosomes can be observed, the addition of mitotic inhibitors to accumulate cells in mid-mitosis, hypotonic treatment to swell the cells and fixation for subsequent processing, development of stained specimens on glass slides, staining, microscopic observation, and imaging image analysis.

"Differential staining" is a generic term for a method of analyzing the characteristic bands of each chromosome. G-banding is the most common method used in cytogenetics to visualize karyotypes by staining aggregated chromosomes. Heterochromatin regions, which tend to have a high percentage of adenine and thymine and a low number of genes, are stained more intensely. In contrast, euchromatin regions with a high percentage of guanine and cytosine (GC content) and active transcription are less stained and appear as lighter bands. Each chromosome arm is numbered from centromere to telomere, which uniquely and accurately describes every band on the chromosome.

The G-staining method identifies 24 chromosomes by size, centromere position, and shading bands. The analysis is usually performed at the level of 550 bands per haploid (half-loop), and the position and sequence of the bands are used to determine the structural aberrations of the chromosomes. Theoretically, 1 band is approximately 5 Mb (≈50 genes), and structural abnormalities smaller than this are undetectable.

It is important to note that there are variants that do not affect phenotype or reproduction even if the chromosome karyotype is abnormal, and such variants that do not affect phenotype or reproduction are called normal variants or normal polymorphisms. Chromosome number abnormalities such as ploidy, autosomal trisomy, abnormal sex chromosome number, structural abnormalities (reciprocal translocation, Robertson translocation, deletion, duplication, insertion, intra-arm inversion, inter-arm inversion, homologous chromosome, ring chromosome, marker chromosome) are detected. Chromosome testing for congenital abnormalities and reproductive disorders uses aseptically collected peripheral blood lymphocytes and

includes 21, 13, and 18 trisomies, Turner syndrome, Kleinfelter syndrome, and other disorders. If necessary, the FISH method is used in combination to provide a comprehensive cytogenetic diagnosis.

1.3.2 FISH Method

The FISH method is used to analyze specific genomic regions of target genes, etc. It uses oligonucleotides or antibodies labeled with fluorescent dyes containing specific DNA sequences as probes to form a complementary complex (hybridization) with the target gene. If the chromosome has the same DNA sequence as the probe, the DNA sequence emits a fluorescent signal, which can be observed under a fluorescence microscope. The probe binding site is detected by fluorescence, and the presence, number, and site of the target gene can be identified. Depending on the probe used, it is possible to identify chromosomes of unknown origin that cannot be detected by the G-staining method, and to analyze minute structural abnormalities. The FISH method can visually detect microdeletions and microtranslocations, but it is difficult to determine microduplications of approximately 1Mb or less. FISH is superior to G-staining in detecting low-frequency mosaics; it is easier to analyze than G-staining and allows rapid reporting of results.

In clinical practice, the following should be considered when ordering tests and interpreting results.

(1) Some microstructural abnormalities are undetectable even by the FISH method in the diagnosis of microstructural abnormalities.
(2) It is necessary to examine the extent to which clinical symptoms can be inferred by FISH diagnosis and the clinical usefulness of this method.
(3) The resolution of the FISH method and the limits of diagnosable genomic deletions and duplications.

2. Genetic Testing System

2.1 Introduction

Genetic testing for intractable diseases, including hereditary CVD, has been mostly conducted as research in university laboratories, etc. Under the revised Medical Care Act, diagnostic testing on patients' specimens has to be performed as part of activities of the medical institution: in the building of the medical institution and under the authority of the administrator of the medical institution and the person responsible for quality control of laboratory testing.[20] In the case of gene-related and chromosome testing, it is mandatory to appoint a person responsible for the quality control of such testing in addition to the person responsible for the quality control of all laboratory testing, to conduct internal quality control, to provide appropriate training, to prepare various standard operating procedures, and to prepare and maintain various work logbooks and ledgers. In addition, it is obligatory to make efforts to undergo external quality control surveys, and third-party accreditation for laboratories, such as ISO 15189 and the College of American Pathologists,[21] is recommended.

ISO 15189 is an international standard developed by the International Organization for Standardization that specifies requirements for quality and competence in clinical laboratories.[22] The quality requirements stipulate that laboratories shall operate in accordance with a quality policy, quality objectives, quality manual, and other documents developed by the laboratory, and shall evaluate and continuously improve the appropriateness, validity, and effectiveness of their quality management system through periodic analysis of internal and external audit results and user feedback. On the other hand, the requirements for competence stipulate education and training of laboratory personnel, competency assessment, environmental maintenance, management of laboratory equipment, reagents, and consumables, validation of laboratory procedures, preparation of standard operating procedures, and internal/external accuracy control. In Japan, there are still few facilities that have obtained ISO 15189 accreditation for germline genetic testing using NGS. External quality control surveys include proficiency tests provided by the College of American Pathologists.

2.2 Genetic Testing Flow Using NGS

In genetic testing in the cardiovascular field, NGS panel testing, which simultaneously analyzes multiple genes, is extremely useful and widely used throughout the world. In Japan, a large number of genetic tests are performed in certified clinical laboratories such as Kazusa Genetic Testing Laboratory in the Kazusa DNA Research Institute.[23] When performed at a certified clinical laboratory, blood specimens are transported to the laboratory, DNA extraction and NGS testing are performed, and a report is returned to the submitting physician. Each laboratory has its own approach to verifying the pathological significance of variants. Kazusa Genetic Testing Laboratory offers a service to obtain comments from specialists in the disease concerned upon request.

As an example of implementation at a medical institution, the efforts of the Center for Medical Genetics, Shinshu University Hospital are shown (**Figure 2**).[24–26] In the case of in-hospital specimens, when a genetic testing order is issued from the electronic medical record by a physician at the Center for Medical Genetics, blood samples are collected and anonymized to allow for internal and external consultation outside the clinical team, DNA is extracted, and NGS analyses are performed. When pathogenic or likely pathogenic variants, and variants of uncertain significance (VUS), are detected, the NGS data are visualized using the Integrative Genomics Viewer[27] and confirmed with Sanger sequencing. The determination of pathological significance is made by a molecular geneticist and a clinical geneticist and verified through a conference of internal and external experts. After a report is generated, anonymization is removed and the report is returned to the electronic medical record. The entire process is barcode-controlled to prevent specimen mix-ups.

Additional analyses are then considered for VUS, including *de novo* confirmation, co-segregation analysis within families, and functional analysis.

2.3 Details of NGS-Based Genetic Testing

2.3.1 Basic Concept

When offering genetic testing, it is necessary to consider the test's analytical validity, clinical validity, and clinical utility. Analytical validity indicates that the test method is well established, with appropriate accuracy control and reproducible results. Clinical validity indicates that the clinical significance of the test results is well defined, including sensitivity, specificity, the positive-predictive

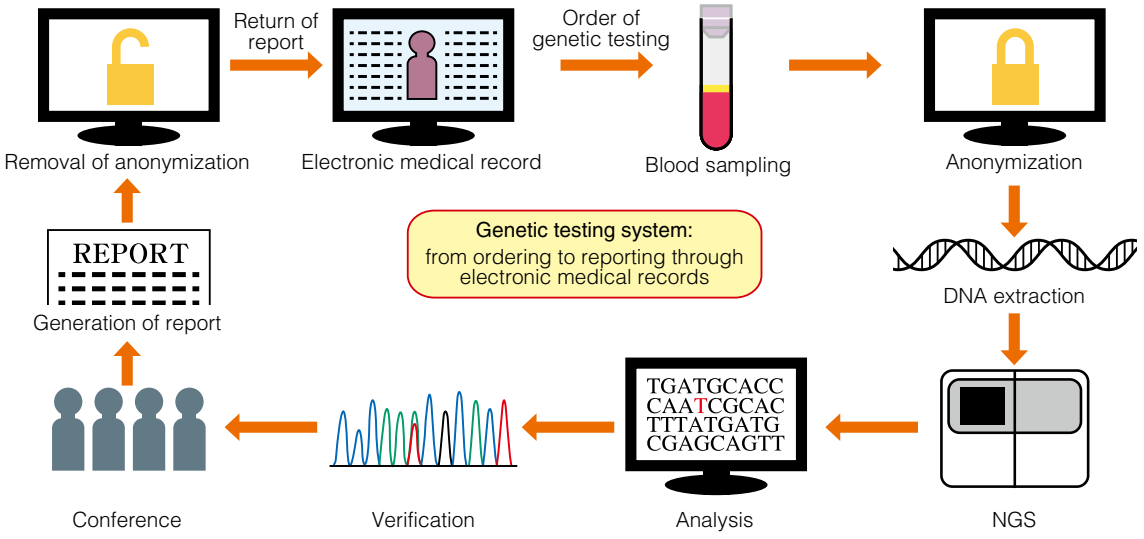

Figure 2. Genetic testing workflow at the Center for Medical Genetics, Shinshu University Hospital.[24,25]

Table 9. List of Hereditary Cardiovascular Diseases for Which Genetic Testing Is Covered by the National Health Insurance

Category	Names of hereditary cardiovascular disease (including hereditary diseases with cardiovascular manifestations)
Easy (3,880 points)	Fabry disease, Pompe disease, familial amyloidosis
Complex (5,000 points)	Hypertrophic cardiomyopathy, familial hypercholesterolemia, cardio-facio-cutaneous syndrome, Costello syndrome, Osler's disease
Extremely complex (8,000 points)	Congenital long QT syndrome, Noonan syndrome, Marfan syndrome, Loeys-Dietz syndrome, familial aortic aneurysm/dissection, Ehlers-Danlos syndrome (vascular type), Ehlers-Danlos syndrome (classical type), mitochondrial diseases

Table 10. Outline of Criteria for Evaluating Pathogenic Variants in the ACMG/AMP Guidelines 2015[36]

- The variant is detected in a gene in which loss of function is a known pathomechanism of disease, and is expected to result in nonsense-mediated mRNA decay
- Different types of base substitutions that produce the same amino acid substitution have been reported as pathogenic variants
- Variants with substitutions to different amino acids have been reported as pathogenic variants
- The variant is *de novo*
- Pathogenicity of the variant has been shown by a functional analysis
- The variant is located in a hotspot or in a functionally important domain of the gene
- The variant is located in a *trans* state to the other pathogenic variant in a gene (for autosomal recessive diseases)
- The variant is co-segregated with affected individuals in the family
- The variant is missense in a gene for which missense variants account for the majority of pathogenic variants
- Multiple *in silico* functional prediction programs support the pathogenicity of the variant
- A single gene is assumed to be the cause based on clinical symptoms or family history
- The variant is not registered in the database for general population

(Source: Prepared based on Richards S, et al. 2015[36])

value, and negative-predictive value. Clinical usefulness indicates that the test results have sufficient clinical relevance. Clinical utility indicates that the diagnosis of the disease for which the testing is performed has medical benefits, such as leading to appropriate surveillance, prevention, and treatment.

2.3.2 Panel Design

In a custom panel-based NGS, the rationale for the involvement of the genes in the panel in a particular disease is important for the design of the panel, and the ClinGen Gene-Disease Validity[28] (https://search.Clinicalgenome.org/kb/gene-validity) is usually referred.[29] The number of diseases for which genetic testing was covered by the National Health Insurance was 13 in the Revision of Medical Fee FY2008, increased in the revision every 2 years, and is 201 in the most recent Revision FY2022 including hereditary CVD (**Table 9**). The genes to be analyzed for each disease are selected with reference to the diagnostic criteria for the designated intractable diseases (Intractable Disease Information Center[30]).

2.3.3 NGS Analysis

Variant Detection: Short-read NGS are used to detect variants including a hybridization capture type and a multiplex PCR type.

Table 11. Variant Description According to the Nomenclature by the Human Genome Variation Society (Using *MYBPC3* as an Example)

Variant type	Coding DNA-level (NM_000256.3)#	Amino acid-level (NP_000247.2)♭	
Missense variant	c.772G>A	p.(Glu258Lys)	The G at the base 772 in the coding DNA is replaced by an A, and the glutamic acid at the amino acid 258 is expected to be replaced by lysine
Nonsense variant	c.2526C>G	p.(Tyr842*) or p.(Tyr842Ter)	The C at the base 2526 in the coding DNA is replaced by a G, and the tyrosine at the amino acid 842 is expected to generate a termination codon (* or termination; Ter)
Synonymous substitution	c.2274C>T	p.(Gly758=)	The C at the base 2274 in the coding DNA is replaced by a T, and the amino acid is expected to remain unchanged
Splice site variant	c.927-2A>G	–	The A is replaced by a G in an intron two bases away from the 5′ side of the exon starting at the base 927 of the coding DNA
Deletion	c.1800del	p.(Lys600fs) or p.(Lys600Asnfs*2) or p.(Lys600AsnfsTer2)	The base 1800 in the coding DNA is deleted, and a frameshift is expected to occur from lysine at the amino acid 600 and a termination codon is expected to be generated at the next codon

Reference sequences of coding DNA (#) and amino acid (♭) for *MYBPC3*. Variants are described with the reference sequences.

Annotation and Filtering: For a detected variant, the following information is annotated: (1) type of variant (e.g., missense variants), (2) frequency in databases of the general population (e.g., gnomAD[31] and jMorp[32]), (3) databases showing the association between variants and diseases (e.g., ClinVar,[33] Human Gene Mutation Database, Global Variome shared LOVD,[34] MGeND[35]), (4) *in silico* prediction program. Candidate variants are filtered by comparing their frequency in the general population with the frequency of the disease and by the form of inheritance. If two variants are detected in an autosomal recessive disease, the presence or absence of the variants on a different chromosome is examined by the analyses on the parents to confirm compound heterozygosity.

Evaluation: For a candidate variant, a check is made to see if the variant has been previously reported by ClinVar or other sources, and if so, a check is made of the literature content of the variant. The variant is also evaluated according to the ACMG/AMP Guidelines 2015[36] and the recommendations of the ClinGen Sequence Variant Interpretation Working Group[37] (**Table 10**), and is classified as pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, or benign. Following the ACMG/AMP guidelines 2015, several disease-specific guidelines have been proposed, including hereditary CVD: the ClinGen Cardiomyopathy Expert Panel Guidelines (*MYH7*), the ClinGen RASopathy Expert Panel Guidelines (*BRAF*, *CBL*, *HRAS*, *KRAS*, *LZTR1*, *MAP2K1*, *MAP2K2*, *MRAS*, *NRAS*, *PPP1CB*, *PTPN11*, *RAF1*, *RIT1*, *RRAS2*, *SHOC2*, *SOS1*, *SOS2*), the ClinGen Familial Hypercholesterolemia Expert Panel Guidelines (*LDLR*), and the ClinGen FBN1 Expert Panel Guidelines (Marfan syndrome).[38]

Although Sanger sequencing has been used to confirm variants detected by NGS, some institutions have recently adopted a policy without Sanger sequencing-based confirmation because the accuracy of NGS has improved.[39]

Report Preparation: The report describes the results including their interpretation, and associated information, together with related information (e.g., the type of specimen, method of testing, targeted genes, and limitations of the testing), according to the standard operating procedures. The information on the limitations of the testing should state that variant detection is unavailable in regions other than the translation region and splicing sites (e.g., transcriptional regulatory regions, deep introns) and also if there are regions within the targeted region that are difficult to analyze due to sequence characteristics. Variants are also designated according to the nomenclature[40] recommended by the Human Genome Variation Society (**Table 11**).

2.4 Conclusion

The required systems for providing genetic testing, as well as the overall and detailed process of genetic analyses, have been described. Genetic testing for inherited CVD is standardized in clinical laboratories and some medical institutions using NGS-based candidate gene panel analyses. Because of its highly clinical utility, further development and dissemination of genetic testing are expected.

V. Medical Treatment and Counseling Using Genetic Testing

1. How to Order Genetic Testing by a Cardiologist

1.1 Institutional Standards

1.1.1 Institution Where Tests Are Performed

According to the revised medical law of 2018, genetic testing for medical use, particularly for testing supported by insurance (D006-4; genetic testing) should be performed in certificated clinical laboratories or branch laboratories of medical institutions, which should be separate from those for the purpose of research in the laboratories of research institutions or university/college.

1.1.2 Entity Conducting the Inspection

When conducting genetic testing, it is advisable to comply with the "Guidelines for genetic testing and diagnosis in medical care" (2011 Japanese Medical Association, revised in 2022)[2] and conduct testing in a facility where comprehensive clinical genetic care including "genetic counseling" can be provided (details are described in **Chapter III.3** "**Collaborative Application of Genetic Testing by Cardiology and Medical Genetics Departments**").

1.1.3 Standards for the Protection of Personal Information

Medical personnel who have access to genetic information are required to fully understand the characteristics of that information and to handle it appropriately. In principle, the results of genetic tests performed for the purpose of diagnosing patients who have already developed the disease are recorded in the medical record as information that can be shared by medical personnel involved in the patient's care, just as for the results of other clinical tests.

This guideline covers genetic testing performed primarily for medical purposes, and thus anonymization of patient information is not mandatory. However, the testing companies or hospital laboratories are legally obligated to strongly protect personal information.

1.2 Determination of Adaptation

It is recommended to follow individual practice guidelines for determining the indications for genetic testing for each disease (see **Table 9** in the previous section for the names of diseases covered by insurance in the field of cardiovascular diseases [CVD]).

1.3 Informed Consent

Written informed consent (IC) is necessary, and only after fully understanding the characteristics of genetic information, should genetic testing and diagnosis be conducted, and the results recorded in the patient's medical record (the 2022 JMJ guidelines). In particular, when a familial inherited disease is suspected, it is necessary to consider that the result of a patient's genetic testing may have implications for other family members. In principle, diagnosis of presymptomatic gene carriers who will develop a disease after adulthood should be performed after when the subject has reached adulthood and is able to make decisions independently.[2]

However, in some cases of inherited arrhythmias, the initial onset can be fatal, and some of these such as congenital long QT syndrome are actionable (available of therapeutic strategies and prevention of sudden death), thus early genetic diagnosis and intervention from childhood is internationally accepted, even if the disease has not yet manifested. Genetic testing as cascade screening for unaffected individuals, including children and adolescents, should be conducted in conjunction with genetic counseling with a physician who has experience in hereditary cardiovascular diseases and a medical geneticist (see **Chapter III.3** "**Collaborative Application of Genetic Testing by Cardiology and Medical Genetics Departments**").

When comprehensive genetic testing using next-generation sequencing (NGS) is applied in medical practice, processes for the handling of secondary findings (e.g., when pathological variants of hereditary tumor syndromes are found in the search for the cause of hereditary arrhythmias) and for disclosure of test results should be established.

1.4 Disclosure and Explanation of Results

Written IC for genetic testing is necessary. In addition, the results should be presented by a physician who can explain the interpretation of the results, including whether the variant identified by the genetic test is pathogenic, its relationship to the disease, and its consistency within the family. When a physician who has sufficient knowledge of clinical genetics provides genetic counseling (see below) to the patient or his/her family members about the result of testing for some diseases covered by insurance, an additional fee for the genetic counseling (1,000 point [2023 year], one for each patient per month) can be charged. The reimbursement for genetic testing and genetic counseling can be applied only when they offered in institution that has been certified by the Regional Bureau of Health, Labour and Welfare.

1.5 Handling Results of Genetic Analysis Conducted as Medical Research

Genetic analysis conducted as research purpose is not covered by this guideline. When conducted as "research" rather than for medical purposes, it must be approved by an ethics committee[2] (see **Chapter I.2** "**Scope of Application of the Guideline**"). However, even if research analysis, when the results are disclosed to the subject for clinical purposes as having analytical validity, clinical validity, and clinical utility, disclosure must follow this guideline because the results are equivalent to those obtained from genetic testing as medical treatment. The subjects should be informed before genetic research analysis, should have the difference between genetic research and clinical genetic test explained and "the results were obtained as research". In addition, even if genetic testing performed as medical care has a research aspect, it must also be performed in accordance with research guidelines (e.g., "Ethical guidelines for life sciences and medical research involving human subjects").

2. Genetic Counseling

2.1 Purpose of Genetic Counseling

The characteristics of genetic diseases caused by both genetic and environmental factors will present the patient with a variety of medical, psychological, and familial effects. The various psychological effects such as denial, anger, threat of disease, and loss of sense of control that can occur in patients and their families in such situations can interfere with their ability to cope positively with the challenges that arise with their adaptation to the disease.

One of the characteristics is "heredity," which is the passing of disease from parent to child and from generation to generation. For example, if a person has a genetic disease that is inherited in the autosomal dominant form, there is a one-in-two chance that his or her children will also develop the same disease. Patients diagnosed with such genetic diseases may feel fear and guilt about the possibility of genetic occurrence in their children and other relatives. Patients whose parents are also patients, may feel anger toward their parents. Although these negative psychological feelings are natural reactions, it is necessary to prevent them from becoming excessive and interfering with the patient's and family's ability to adapt to the disease. Genetic counseling helps patients adapt to the effects of genetic disease by promoting risk awareness based on appropriate assessment of each family member's risk of recurrence, and by education and counseling on how to deal with the medical consequences of recurrence.

Another feature is "variation". For example, in Marfan syndrome, the presence of a pathological variant in *FBN1* alters the structural and regulatory properties of the binding weave of fibrillin-1, the main component of microfibrils, resulting in a variety of phenotypes in the cardiovascular and musculoskeletal systems. Those phenotypes can be thought as genetic variation, although they could cause serious event such as sudden death. The presence of a genetic factor such as the *FBN1* pathogenic variant is not in itself the responsibility of the patient or any member of the family. However, it is does exist and is not modifiable. Genetic counseling is provided to help patients and their families cope with the psychosocial challenges that may arise during the adjustment process by improving their sense of empowerment and self-efficacy. In the rapidly evolving field of inherited CVD, genetic counseling plays an important role in the process of adaptation of patients and their families to the disease.[41,42]

2.2 Situations in Which Genetic Counseling Should Be Provided

The Guidelines for genetic testing and diagnosis in medical care of the Japan Medical Association states that genetic counseling should be provided at the appropriate time during genetic testing and diagnosis. Genetic counseling is defined as "the process of helping people understand and adapt to the medical, psychological and familial implications of genetic contributions to disease".[2] The process of genetic testing and diagnosis of genetic disease cannot be separated from the patient's and family's adaptation to the disease. Patients and their families should make decisions about whether or not to undergo testing based on a thorough understanding of the significance of genetic testing and diagnosis, and can then manage their disease and risk based on the results (including cases in which they do not undergo testing). Therefore, it is desirable for all physicians to acquire fundamental knowledge and skills in genetic counseling.[2]

However, as indicated at the beginning of this section, genetic counseling is not limited to the time of genetic testing and diagnosis. The timing of when needs become apparent or when latent needs should be actively elicited may occur throughout the life of the patient or family member: when they have difficulty adapting to the disease, when they need follow-up to maintain and promote adaptation, or when new issues arise in response to a change of life stage (e.g., marriage, childbirth). Therefore, it is necessary to provide longitudinal genetic counseling to patients and their families living with a genetic disease across generations. Genetic counseling in this situation should be provided by specialists in genetic healthcare (clinical geneticist, certified genetic counselor [CGC], and genetic certified nursing specialist [CNS]). As of 2023, reimbursement for genetic counseling (1,000 points) is positioned as an additional fee to genetic testing. Therefore, genetic counseling without genetic testing is not covered by social health insurance. In addition, the number of CVD for which genetic testing is covered by insurance is limited.

2.3 Genetic Healthcare Professionals

The major certifications for genetic healthcare specialists recognized in Japan are clinical geneticist, certified genetic counselor, and certified nurse specialist in genetic nursing. When providing healthcare to patients with hereditary CVD and their families, referral to and collaboration with these specialists should be considered together with the provision of specialized cardiovascular care.

2.3.1 Clinical Geneticist

A clinical genetic specialist is a medical specialty jointly certified by the Japanese Society of Human Genetics and the Japanese Society for Genetic Counseling.[2] As of November 2023, there are currently 1,727 active clinical genetic specialists in Japan (for details, please refer to the website of the Committee of Clinical Genetic Specialists http://www.jbmg.jp/). The role of the clinical genetic specialist is to diagnose genetic disorders (and identify at-risk individuals in the family) based on clinical genetic examination and testing, develop a total management plan tailored to the characteristics of the genetic disorder, and provide medical treatment and care in cooperation with related departments and professions. It is recommended that cardiologists and clinical geneticists work closely together in the treatment and risk management of patients and at-risk individuals of hereditary CVD.

2.3.2 Certified Genetic Counselor[1]

CGCs are also jointly certified by the Japanese Society of Human Genetics and the Japanese Society for Genetic Counseling after completing a training course in a master's program (or doctoral program in some cases) and passing a certification examination. CGCs help patients and families to adapt to their condition, by providing appropriate information including genetic information and social support, assisting them in making autonomous decisions through psychological and social support, and counseling for adaptation. In the field of cardiovascular healthcare, where genetic medicine is closely related to prevention and

treatment, they also play a role in supporting behavioral change aimed at preventing the onset and progression of disease.

As of December 2023, 389 CGC are actively involved in genetic healthcare in Japan.

2.3.3 Certified Nursing Specialist in Genetic Nursing

A CNS has been certified by the Japan Nurses Association as having outstanding practical nursing skills in a specific nursing specialty. The certification is granted after completion of a master's degree in a specific nursing specialty and at least 5 years of practical experience (at least 3 years of which must be in the nursing specialty for which the certification is sought). Genetic nursing is a new field in which certification began in 2017, and as of 2023, 21 nurses have been certified as CNS in genetic nursing. CNSs are expected to be actively engaged in the cardiovascular field, where genetic healthcare is closely related to treatment.

2.3.4 Telemedicine in Genetic Healthcare

In the 2022 revision of the Medical Fee the additional fee for telegenetic counseling after specific genetic testing turned to be subject for reimbursement. To take advantage of this system, it is important to create nationwide networking among medical institutions that diagnose and treat genetic cardiovascular diseases and those providing highly specialized genetic counseling for genetic cardiovascular diseases.

2.4 Key Points of Genetic Counseling in Genetic Testing for Hereditary Cardiovascular Diseases

2.4.1 Adaptation to Uncertainty

The magnitude of genetic involvement in pathogenesis varies from disease to disease. Single-gene diseases are a group of diseases in which the presence of a pathogenic variant in a single gene (disease-causing gene) leads to the onset of the disease, and thus the genetic involvement is extremely large. However, phenotypes such as age of onset, symptoms, and severity vary among patients with the same single-gene disease. This is due to differences in pathogenicity among pathogenic variants, phenotypic differences even among blood relatives with the same pathogenic variant, modification of the risk of disease onset by environmental and unknown genetic or other uncertain factors.

Recent advances in genome research have revealed the involvement of many genes in a single phenotype and the complexity of the involvement of a single gene in multiple diseases. In the field of hereditary CVD, the involvement of multiple genes has been demonstrated in a variety of diseases, and the evidence is constantly being updated. For example, in congenital long QT syndrome, 17 genes have been shown to be associated with the disease, of which 7 have been classified as clearly or strongly associated.[43] In hypertrophic cardiomyopathy, not only genes identified by the conventional single-gene search approach, but also interactions among multiple genes and the influence of environmental factors have been suggested. Therefore, it may not be appropriate to consider hypertrophic cardiomyopathy as a single-gene disease, even when there is a strong familial clustering of the disease.[44]

For these reasons, it is important to note that the relationship between genotype and phenotype is variable, and that diagnosis and risk assessment of disease on the basis of genetic test results can always be subject to ambiguity. Appropriate evaluation of clinical information and genetic testing often lead to a definitive diagnosis and play a crucial role in determining the subsequent course of treatment. On the other hand, the complexity of the genetic background of hereditary CVD, as well as the lack of information on the pathogenicity of the variants, may also influence the decision-making process due to its ambiguity.[45] Ambiguous test results may cause confusion and uncertainty for patients and their families and may impede adaptation to the disease. To prevent these psychological impacts, it is necessary to facilitate patients' understanding of the significance and limitations of genetic testing and its relationship to clinical diagnosis at the pre-test genetic counseling session.[46]

In addition to genetic test results, uncertainty can arise in various aspects of the diagnosis, treatment, and prevention of genetic disorders.[47] Uncertainty about a disease can be an obstacle to adapting to the disease and its risks, and to adopting new lifestyle.[48] Particularly in the case of inherited CVD, a small trigger can lead to sudden death, even in apparently healthy individuals, and the psychological burden of uncertainty is high because the patient does not know when this may occur. Therefore, it is important to reduce the undue impact of uncertainty and to help patients and their families develop the ability to cope with uncertainty. Thus, it is important that cardiologists and genetic healthcare professionals have a shared understanding of the ambiguity of test results, and that they are consistent in their treatment and risk management practices to reduce the uncertainty experienced by patients and their families. The psychological burden caused by uncertainty can then be addressed by genetic counselors through psychological interventions and behavior change support.[49,50]

2.4.2 Genetic Counseling as Behavioral Change Support for Prevention of Disease Onset and Progression

Actionability of the hereditary disease is an important factor in determining the direction of genetic counseling.[51] The American Society of Clinical Genetics and Genomics listed 82 causative genes of hereditary diseases as actionable in the context of secondary findings. In the **Chapter III Table 6**,[52] about half (38 genes) of these genes are related to hereditary CVD, so genetic counseling will focuses on helping patients and their families to incorporate the diagnosis of inherited CVD (not necessarily by genetic testing) and the resulting treatment or preventive actions into the adaptation process to the disease.

2.5 Genetic Counseling for Children and Young Adults

A number of hereditary CVD require medical management from childhood and young adulthood. The purpose of genetic counseling is the same these age groups, but it is important to consider their developmental stages. Children and young adults are still forming their identities in their relationships with people around them. Having an inherited CVD requires children and young adults to take continuous medication, restrict their exercise, attend hospital frequently, and perform other self-management behaviors. The main focus of self-management behaviors is not on symptoms that can be perceived by the children themselves, but on prevention against the risk of current or future events. Therefore, it is difficult for children to recognize the necessity and significance of these behaviors, and the various burdens

associated with practicing these behaviors can make it difficult to maintain adherence. The goal of genetic counseling is to facilitate the child's understanding of his/her unique situation so that he/she is willing to continue self-management behaviors.[53] The effectiveness of genetic counseling for children, more than for adults, depends on the relationship between the child and the genetic counselor.[54] Therefore, genetic counseling for hereditary CVD requires the development of pediatric genetic counseling skills.

In addition to self-management support for the child, adjustment support for the primary caregiver (generally the parent) is also important. In families with an inherited CVD, there is often a family history of sudden death, which can lead to fear that the child is at risk. Coping with this fear may take the form of excessive restrictions on the child's life or over-intervention that interferes with the child's self-management. The goal of adaptive support for the primary caregiver is to promote an adequate understanding of the actionability of the disease and appropriate caregiver behavior. It is effective to support the primary caregiver to continue receiving treatment, to take actions in preparation for events at home or at school (e.g., installation of an AED and confirmation of location), to know what to avoid (contraindications, medications, etc.), and to take such actions that improve their sense of control. As the child develops, they will need assistance with the transition to self-management as an adult. Supporting that transition can also be considered part of the adaptive support of genetic counseling.

2.6 Genetic Counseling for At-Risk Relatives

Hereditary CVD have a high actionability and the medical benefits of early intervention are significant. In particular, when the disease-causing gene is known and a clear pathogenic variant is found in the patient, genetic testing (cascade screening) can be used to determine whether family members carry the same pathological variant, to establish risk status even if the disease has not yet developed, and to propose risk-based prevention strategies. Cascade screening and cardiovascular surveillance based on genotype are highly useful and efficient.[55,56] Adequate genetic counseling, including the usefulness of surveillance and communication support among at-risk family members, is crucial in gaining IC for cascade screening and subsequent surveillance.[57,58,59,60] Attitudes towards cascade screening vary widely among genetic healthcare providers, because of the uncertainty in the genetic risk of the diseases, and a consensus needs to be developed.[61] For example, patients with dilated cardiomyopathy tend to have lower rates of genetic counseling and cascade screening and surveillance than patients with other inherited CVD.[42,62]

Although the usefulness of cascade screening is clear, patients may feel a psychological burden of having to inform their relatives.[63] It is also important to note that family members who are uncertain before undergoing cascade screening may find it difficult to update their risk perceptions immediately and may even attempt to take unnecessary risk management actions.[64]

When hereditary CVD is suspected based on the information from the proband, such as characteristic pathology and family history, preventive interventions should be considered for blood relatives, even if genetic testing does not yield significant results, due to test ambiguity. Even in the absence of a family history, it is not uncommon for cardiovascular screening of family members to reveal lesions.[65] Regardless of the availability of genetic testing, patients should be informed about the risk of recurrence in the family and consideration should be given to providing genetic counseling for CVD in which genetic factors play a significant role.[66,67]

VI. Management in the Perinatal Period

1. Overview of Prepregnancy Genetic Counseling for Women With Cardiovascular Disease

Medical advances have significantly improved the prognosis for cardiovascular disease (CVD), and along with this, the number of fertile women with a CVD is increasing every year. In particular, the number of patients with complex CVD, who have previously rarely reached adulthood, is increasing.[68]

During pregnancy, various complications are more likely to develop due to the increase in circulating blood volume, weakening of the blood vessel walls, and thrombophilic tendency due to an increase in the amount of coagulation factors. These changes in pregnancy can cause a number of problems, including the effects of maternal CVD, fetal and neonatal effects, long-term prognosis, and responding to pregnancy-specific complications. In addition, the genetic impact on the baby and the severity of the hereditary disease of the pregnant woman herself must be taken into account. It is important to sort out these problems and to create sufficient opportunities for discussion between the medical team and the patient before pregnancy if possible. For details on the pathophysiology of CVD itself and the management of pregnancy and delivery, please refer to the Guidelines for indication and management of pregnancy and childbirth in patients with heart disease (2018 Revised Edition).[68] In this Guideline, the emphasis is on "genetic counseling for pregnancy and childbirth" for "women with hereditary cardiovascular diseases", especially from the viewpoints of "preconception care" and "prepregnancy counseling".

2. Preconception Care

"Preconception care" is based on the idea that "healthcare before pregnancy improves the health of the next generation and the health of one's own life later". In recent years, it has become known that the lifestyle and nutritional status of the mother before pregnancy can affect both the mother and fetus during pregnancy and/or the long-term health management of future children, including healthy life

expectancy. This is because various environmental factors during fetal and early postnatal development can affect future health and susceptibility to certain diseases.[69] From this perspective, understanding the health status of women and couples before pregnancy and providing them with appropriate knowledge and information will lead to improved health outcomes.

In other words, preconception care is a medical, behavioral, and social health intervention for health care for people who are in the process of growth, including puberty, regardless of gender.[70–73]

3. Purpose of Preconception Care

The purpose of preconception care is to improve the health of women and couples before pregnancy by reducing the behavioral, personal or environmental factors that worsen maternal and infant outcomes, with the ultimate goal of improving both the short- and long-term prognosis for mothers and babies.[71]

The importance of preconception care has been recognized by the Centers for Disease Control and Prevention and the World Health Organization. Common challenges to be addressed in general preconception care include family planning, weight and nutrition management through proper exercise and diet, screening for infections, appropriate vaccinations, and identifying medications that affect the fetus.

Specialized preconception care and counseling for women with underlying diseases is also important. The American College of Obstetricians and Gynecologists recommends prenatal counseling to identify comorbidities that affect pregnancy, including diabetes, hypertension, hypothyroidism, psychiatric illness, HIV, thrombosis, and past perinatal complications, together with confirmation of family and genetic history, including the presence or absence of hereditary diseases in the couple.[74] It is essential that any complications of these conditions are strictly controlled before pregnancy, and for chronic diseases, prior control is fundamental to optimize pregnancy outcomes.[75]

4. Prepregnancy Counseling for Women With Cardiovascular Disease

Prepregnancy counseling (preconception counseling) should be recommended to all women of reproductive age with known CVD. If it is only provided during pregnancy, counseling on obstetric problems caused by CVD should be provided as early as possible in thepregnancy.[76]

The aim of counselling is to reduce the risk to both the mother and fetus as much as possible. Outcomes include accurate maternal and fetal risk stratification, optimization of cardiovascular lesions, discussion of the possibility of prepregnancy interventions, review of drug safety during pregnancy, and detailed planning for pregnancy, delivery, and childbirth.[76–78] Both maternal and fetal risk assessments are needed, including cardiovascular events during pregnancy (e.g., heart failure, arrhythmias, thromboembolism, infective endocarditis, and aortic dissection), maternal risk of death during pregnancy or in the puerperium, long-term prognosis, or safe contraception when pregnancy should be avoided.

The most important medical counseling topic is the risk assessment of pregnancy. Various studies have been conducted on risk assessment methods, but in the current situation of CVD treatment in Japan, the modified World Health Organization classification is the basis.[79,80] If necessary, apply the CARPREG II risk score[77] and the ZAHARA risk score[81] to comprehensively assess the risk.[68]

Fetal risk describes the risk of congenital diseases due to drugs administered to the mother, the risk of fetal growth failure, intrauterine fetal death, and preterm birth. If necessary, drugs are changed before pregnancy.

In addition, acquired CVD such as hypertrophic cardiomyopathy and pulmonary hypertension carry high-risk pregnancy complications such as gestational hypertension nephropathy and premature birth.[82] It is also necessary to improve diabetes, obesity, hyperlipidemia, etc., which are exacerbating factors of CVD.[83]

Pregnancy is often possible with many cardiac conditions, but some CVD do not tolerate pregnancy or are contraindicated, so guidance on safe contraception methods with a low risk of blood clots is also necessary.

Some CVD carry a high risk beyond the postpartum period, and for these it is essential to fully discuss whether breastfeeding and childcare responsibilities can be reduced, whether family support is available, and the long-term prognosis.

5. General Precautions for Prepregnancy Genetic Counseling in Women With Cardiovascular Disease

For details on genetic counseling for each disease, refer to the corresponding chapters. In the case of pregnancy of female patients with CVD, it is necessary to accurately communicate the possibility of the child inheriting the disease based on genetic knowledge. In addition, genetic testing is important when the client's own disease is recommended for risk stratification based on the genetic diagnosis and treatment strategy for the particular disease; for example, connective tissue disease such as Marfan syndrome or hereditary arrhythmia such as congenital long QT syndrome (LQTS).

Prepregnancy genetic counseling for CVD should be conducted by a clinical geneticist or certified genetic counselor who has sufficient knowledge and experience in genetics, cardiology, and epidemiology, has skills in genetic counseling, and is familiar with the physiological changes associated with pregnancy and the effects of pregnancy on cardiovascular organs. Sufficient cooperation between cardiac specialists and obstetricians and gynecologists who are proficient in the treatment of CVD is essential.

6. Precautions for Prepregnancy Genetic Counseling for Each Disease Group

For details of prepregnancy counseling for CVD patients, please refer to the Guidelines for the indication and management of pregnancy and childbirth in patients with heart disease 2018 revised edition,[68] and respective sections and chapters in this guideline. Here, hereditary CVD that require particular attention in pregnancy management are described.

6.1 Congenital Heart Disease

Many congenital heart diseases are considered multifactorial and more often do not follow the Mendelian inheritance pattern. However, children born to parents with congenital heart disease are more likely to develop congenital heart disease, and it is thought that they are 3–5-fold more likely to do so than the general population. In addition, the rate of females with congenital heart disease is more than twice as high as those of males with congenital heart disease. For this reason, pregnant women with CVD are recommended to undergo fetal echocardiography screening at 18–20 weeks of pregnancy. In recent years, numerous genetic abnormalities that cause congenital heart disease have been revealed and accurate information about recurrence rates in children and possible genetic testing information can be provided.[68]

6.2 Marfan Syndrome and Related Diseases

Marfan syndrome has an autosomal dominant form of inheritance, with a 50% chance of a child being affected. The fibrillin-1 gene (*FBN1*) is responsible. Pregnancy should be considered after appropriate genetic counseling by a clinical or cardiovascular specialist who is familiar with the disease, a certified genetic counselor, or an obstetrician who is familiar with high-risk pregnancy management. The reason for this is that rapid aortic dilatation and aortic dissection may develop during pregnancy, delivery, and early puerperium. More care should be taken when the maximum diameter of the ascending aorta exceeds 4 cm at the time of pregnancy.

Angiotensin-converting enzyme inhibitors and angiotensin-receptor blockers should be discontinued due to the possibility of miscarriage or teratogenicity, and changed to β-blockers in advance. In post-mechanical valve replacement cases, the use of warfarin is not recommended due to the risk of fetal teratogenicity, intracranial hemorrhage, etc. with oral use during pregnancy. During pregnancy, unfractionated heparin is the main treatment.

A related disease of Marfan syndrome is Loeys-Dietz syndrome, caused by pathological variants of the transforming growth factor β (TGF-β) receptor (type 1 or 2) gene (*TGFBR1*, *TGFBR2*). Compared with other aortic diseases, vascular lesions occur at a younger age and tend to lead to dissection even if the aortic dilation is mild, so strict management is required for pregnancy and childbirth.

Ehlers–Danlos syndrome vascular type is led by a heterozygous pathogenic variant in *COL3A1*. The vascular form requires strict management of pregnancy and childbirth because serious complications such as arterial lesions (arterial dissection, aneurysm, rupture, carotid cavernous sinus fistula), organ rupture (intestinal, uterine), and pneumothorax can occur. Planned caesarean section is often chosen to avoid extensive tissue damage associated with vaginal delivery, but it is recommended that vascular surgeons and general surgeons be present at delivery.

6.3 Hereditary Arrhythmias

A typical hereditary arrhythmia that is relatively common in young women is congenital LQTS. In these patients, pregnancy and childbirth are not extremely risky, but especially in LQT2 patients, QT prolongation may worsen after pregnancy and childbirth, and cardiac events may occur,[84] and in high-risk patients, it is desirable to continue with β blockers after pregnancy and childbirth. Catecholamine-induced pleomorphic ventricular tachycardia is also an inherited arrhythmia that can be fatal, and it cannot be ruled out that sympathetic nervous tension associated with pregnancy and childbirth may lead to arrhythmic events, so it is desirable to continue taking β blockers during and after pregnancy in patients as with LQTS. On the other hand, in cases of cardiomyopathy with arrhythmias, such as arrhythmogenic right ventricular cardiomyopathy, pregnancy itself may be a risk to the woman. In the case of hereditary arrhythmia diseases, information on the inheritance of the disease is provided with an explanation of the risks to both mother and baby associated with pregnancy.

6.4 Venous Thromboembolism and Hereditary Thrombophilia

Pregnancy-related venous thromboembolism (VTE) remains the cause of a high proportion of maternal deaths. VTE is a disease concept that combines deep vein thrombosis (DVT) and pulmonary thromboembolism (PTE). One of the triggers for the onset of VTE is hereditary thrombophilia. Congenital deficiencies of the blood coagulation regulators antithrombin (AT), protein C, and protein S lead to the condition of coagulation predominance and are risk factors for VTE. These have an autosomal dominant inheritance. In particular, PS p.Lys196Glu is more common in Japanese people. Carriers of pathogenic variants have been reported to have an increased risk of developing DVT in early to middle pregnancy, suggesting that strict control of anticoagulation from the first trimester is necessary.[85] Hereditary thrombophilias are not currently described in the recommendations published by the American College of Medical Genetics and Genomics on secondary findings in comprehensive genetic analysis as laboratory tests, but they are "actionable" diseases that can be prevented. Therefore, it is important to provide accurate information about them.

Warfarin is not recommended for use during pregnancy due to the risk of fetal teratogenicity, intracranial hemorrhage, etc., and treatment with unfractionated heparin is the main treatment during pregnancy. Therefore, if a woman taking warfarin wishes to become pregnant, she must cease taking it before 6 weeks of pregnancy and switch to heparin. In pregnant women with AT deficiency, especially in the case of type I, which is a quantitative deficiency, supplementation with AT preparations should be considered. For detailed management of such pregnancies, refer to the clinical guidance for peripartum management of patients with hereditary thrombophilia.[86,87]

7. Types of Prenatal Tests and Diagnoses

There are 2 types of prenatal testing and diagnosis in the fetus: definitive (the diagnosis is almost confirmed) and non-definitive (more definitive tests are required for an accurate diagnosis). Chorionic villi sampling (CVS) and amniocentesis are definitive tests, whereas ultrasonography, maternal serum marker tests, combined tests and non-invasive prenatal genetic tests (NIPT) are non-definitive tests.

7.1 Scope of Implementation

Prenatal tests and diagnosis are performed when the couple wishes it in the following cases and when sufficient understanding of the significance of the test is obtained.

- If one of the couple is a carrier of a chromosomal abnormality.
- If there is a history of pregnancy or delivery of a child suffering from a chromosomal abnormality.
- In case of elderly pregnancy.
- Pregnant woman is heterozygous for serious X-linked genetic diseases that develop in the neonatal period or childhood.
- Both parents are heterozygous for serious autosomal recessive genetic diseases that develop during neonatal period or childhood.
- If one of the couple is heterozygous for a serious autosomal dominant genetic diseases that develops during neonatal period or childhood.
- In other cases of the fetus possibly suffering from serious diseases

7.2 Precautions for Genetic Tests and Diagnosis

It is important to note that genetic tests and diagnosis encompasses ethical and social issues, especially the following points.

- Explain the possibility (risk) of the fetus having the disease, the diagnostic limitations of the test, the risks to the mother and fetus, side effects, etc. before the tests and diagnosis, and provide sufficient genetic counseling.
- Tests and diagnosis should be performed by or under the guidance of an obstetrician who has undergone sufficient basic training and mastered safe and reliable testing techniques.
- Prenatal genetic tests aimed at diagnosing fetal circulatory disorders have not been approved.

7.2.1 Amniocentesis

Amniocentesis is performed after 15–16 weeks of pregnancy, and cells derived from the fetus that are suspended in amniotic fluid are collected and cultured for chromosomal and genetic diagnostic testing.

Complications such as miscarriage and fetal death occur in about 1 in 300–500 cases.[88]

7.2.2 Chorionic Villi Sampling

CVS is performed around 11–14 weeks of pregnancy, and chromosomal and genetic diagnosis are performed by collecting villi (tissue that later becomes placenta). In rare cases, confined placental mosaicism may be detected and amniocentesis may be performed after 15 weeks of pregnancy.

Complications such as miscarriage and fetal death occur in about 1 in 100 cases, but there are reports that the risk of miscarriage and fetal death of transabdominal CVS is the same as that of amniocentesis.[89]

7.2.3 Ultrasonography

There are two types of test, one is a genetic test using soft markers by ultrasound and another one is a detailed examination to diagnose fetal morphological abnormalities. Soft markers include nuchal translucency, nasal hypoplasia, etc.

In prenatal checkups, fetal arrhythmias such as fetal bradycardia and tachycardia, fetal heart construction abnormalities, myocardial densification disorders, etc. may be found by chance. In such cases, referral should be made to high-level facilities that can handle fetal CVD, or to comprehensive perinatal and neonatal medical centers or regional perinatal medical centers that can handle high-risk pregnancies.

7.2.4 Maternal Serum Marker Testing

This test estimates the probability that a fetus has trisomy 21, trisomy 18, or open neural tube malformations by measuring the concentration of hormones and proteins derived from the fetus or placenta in the maternal serum.

7.2.5 Combined Verification

By combining ultrasound soft markers and maternal serum markers at 11–13 weeks of pregnancy, it is possible to estimate the probability that the fetus suffers from trisomy 21, trisomy 18, etc.

7.2.6 Non-Invasive Prenatal Genetic Test

NIPT evaluates the possibility of fetal chromosomal abnormalities by analyzing cell-free DNA in the maternal plasma using next-generation sequencing.[90–92] There are advantages, such as being able to test in the early stages of pregnancy and being non-invasive for mother and fetus, although there are disadvantages, such as placental mosaicism may be detected by analyzing DNA derived from the placenta.

7.2.7 Preimplantation Test and Diagnosis

This is a medical practice that requires extremely high technology, and depending on the purpose of the examination, it is performed as a clinical study. Treat it more carefully from an ethical point of view.

7.2.8 Sex

Do not reveal the sex of the fetus unless the test is being performed for a serious X-linked genetic disorder.

7.2.9 Quality Control

Institutions that perform testing and diagnosis will constantly strive to improve the quality control of prenatal diagnostic technology.

VII. Congenital Heart Disease and Chromosomal Abnormalities

Congenital heart disease occurs at a frequency of 5–10 per 1,000 live births and is one of the leading causes of neonatal and infant deaths.[93] In a national survey conducted in Japan in 2021, the incidence of congenital heart disease was approximately 1.4 per 1,000 live births. Among the congenital heart diseases, 60% are due to multifactorial inheritance, while 15% are reported to be caused by gene copy number abnormalities, 13% by chromosomal abnormalities, and 12% by single gene diseases[94] and are often recognized as one of the complications of chromosomal abnormalities and congenital anomaly syndromes.[95] In recent years, the responsible regions and disease genes for chromosomal abnormalities and congenital anomaly syndromes have been successively identified, contributing to the elucidation of the pathogenesis of congenital heart disease. Chromosomal abnormalities that frequently complicate congenital heart disease are listed in **Table 12**, and congenital anomaly syndromes associated with genetic abnormalities are listed in **Table 13**. With the progress and spread of genetic diagnosis, candidate causative genes are also being identified in congenital heart diseases that are not complicated by congenital anomaly syndromes. Alterations in genes encoding transcription factors, signaling proteins, and structural proteins that are essential for cardiac development have been reported. **Table 14** lists some of them;[94,95] however, diagnosis is limited to the laboratory level at present and the cause of most of congenital heart disease is multifactorial. **Table 15** shows the current recommendation of genetic testing for congenital heart disease.[9,96]

1. Chromosomal Abnormalities

1.1 22q11.2 Deletion Syndrome

1.1.1 Disease Concept

Chromosomal microdeletion syndrome characterized by congenital heart disease, characteristic facial features, thymic hypoplasia, cleft palate/nasopharyngeal atresia, hypocalcemia, and mental retardation; includes Di George syndrome, velo-cardio-facial syndrome, and conical arterial trunk anomalous facial features syndrome. It is the second most common congenital heart disease after 21 trisomy and the most frequent cardiac outflow tract abnormality.

1.1.2 Diagnosis

Chromosomal fluorescence in situ hybridization (FISH) with a probe containing the *TUPLE1*/*HIRA* gene can prove 22q11.2 microdeletion. The test is covered by insurance and performed by a testing company.

1.1.3 Frequency of Occurrence

1 in 4,000–5,000 births. No sex or racial differences. Most frequent chromosomal microdeletion syndrome.

1.1.4 Genetic Factors

It is thought to be due to a heterozygous microdeletion in the q11.2 region of the long arm of chromosome 22, resulting in haploinsufficiency of the gene in the deleted region (1.5–3 Mb). FISH analysis shows a 3 Mb or 1.5 Mb deletion in the 22q11.2 region in >95% of patients. It is speculated that uniform deletions occur at high rates due to the presence of DNA repeats at both ends of the deleted region on the chromosome and mismatches during chromosomal recombination.[97,98] Despite the uniformity of the deletions, clinical manifestation is extremely variable and there is no correlation between genotype and phenotype. Cases of identical twins with identical deletions and genetic backgrounds but different phenotypes have been reported,[99] suggesting that differences in the in utero environment, such as differences in blood flow, may be involved.

1.1.5 Recurrence Rate

Autosomal dominant (AD: manifest) inheritance, with a 50% recurrence rate if either parent has the deletion. Most cases are sporadic, but 10–20% are familial. In familial cases, the maternal origin is more common (≈70–80%). Possible causes include low fertility in male patients, shorter life expectancy in males than in females, difficulty for male patients to carry a household, and mothers are more likely to undergo chromosome testing than fathers.[100]

1.1.6 Prenatal Diagnosis

Prenatal diagnosis is considered when (1) there is a deletion in a previous child, (2) either parent has a deletion, or (3) fetal echocardiography detects an abnormal cardiac outflow tract. Chromosomal FISH analysis of amniotic fluid test (after 15 weeks) and chorionic villus sampling (CVS: 10–12 weeks) are available. Even when testing reveals a deletion, it is difficult to predict the phenotype, severity, and prognosis of the child and a cautious approach is required.

1.1.7 Causative Genes

The deletion region contains approximately 30 genes, but *TBX1*, a T-box transcription factor, is considered the most important causal gene. Several other genes have been reported to be involved.

TBX1 is expressed in cells of the secondary heart region (differentiated into the right ventricle, cardiac outflow tract, and atrium) and primitive pharyngeal arch (differentiated into head and neck organs and aortic arch) during the embryonic period and is involved in their development by interacting with cardiac neural crest cells that migrate from the dorsal neural tube to the primitive pharyngeal arch and cardiac outflow tract. *TBX1* is considered to be the major responsible gene for the syndrome, because cardiac disease and other major symptoms of the syndrome have been observed in *Tbx1* knockout mice, and there have been cases in which mutations in *TBX1* alone have caused symptoms of the syndrome.[101] On the other hand, there are cases of *TBX1* mutations without cardiac disease, suggesting the involvement of other genes in the 22q11.2 region or even genes outside the region.[102]

1.1.8 Symptoms, Prognosis and Management

Congenital heart disease (75%), characteristic facial features such as narrow ocular cleft, flattened nasal root, failure of junction of nasal wings and nasal store, small mouth (almost all cases), cervical spine and skull abnormalities (almost all cases, including asymptomatic ones), immunodeficiency associated with hypoplastic thymus (severe 1–2%), cleft palate (9%), nasopharyngeal dysostosis (32%), hypocalcemia (asymptomatic 47%, symptomatic 12%), and mental retardation (mild 50%, moderate 15%, severe 3%) are the main physical symptoms. Other complications include short

Table 12. Major Chromosomal Abnormalities Complicating Congenital Heart Disease

Chromosomal abnormality (syndrome)	Major complicating congenital cardiovascular diseases	Complication frequency	Other major symptoms
22q11.2 deletion (22q11.2 deletion syndrome)	TOF, TrA, IAA type B, VSD, aortic arch anomaly	75%	Characteristic facial features, thymic hypoplasia, nasopharyngeal obstruction, hypocalcemia, learning disability, mental retardation, thrombocytopenia
7q11.23 deletion (Williams syndrome)	SVAS, PPS	75%	Elfin face, hoarseness, visuospatial recognition disorder, infantile hypercalcemia, dental abnormalities, hypertension
Trisomy 21 (Down syndrome)	VSD, AVSD, PDA, ASD, TOF	50%	Characteristic facial features, mental retardation, hypotonia, leukemia, hyperuricemia, atlantoaxial subluxation, hypothyroidism
Trisomy 18 (Edward's syndrome)	VSD, PDA, multiple valve dysplasia	90%	Characteristic facial features, intrauterine growth retardation, overlapping fingers, growth disturbance, mental retardation, apnea
Trisomy 13 (Patau syndrome)	VSD, PDA, ASD	90%	Characteristic facial features, hypotonia, growth disturbance, mental retardation
45 XO (Turner syndrome)	COA, BAV, AS, aortic aneurysm, HLHS	35%	Short stature, gonadal dysplasia, webbed neck, cubitus valgus, lymphedema
Deletion 1p36 (1p36 deletion syndrome)	PDA, VSD, DCM, LVNC	44%	Characteristic facial features (pointed chin), microcephaly, hypotonia, growth disturbance, mental retardation, hearing loss
Trisomy 1q32-qter	TrA, ASD, VSD	90%	Characteristic facial features (inverted triangle), growth disturbance, small head
Trisomy 2p2	AS, DORV, PDA	80%	Characteristic facial features (small, swollen eyes), small chin, skeletal abnormalities, mental retardation
Trisomy 3q2	VSD, COA, TOF+AVSD	50%	Characteristic facial features (square face), eye abnormalities, mental retardation
Trisomy 4p	ASD, SA	20%	Characteristic facial features (full moon-like), small eyes, anal atresia
Monosomy 4p (Wolf-Hirschhorn syndrome)	ASD, VSD, PDA	50%	Characteristic facial features (Greek warrier helmet appearance), giant auricles, small head, mental retardation, convulsions
Monosomy 5p (5p deletion syndrome)	VSD, PDA, ASD	20%	Characteristic facial features (round face to inverted triangle with growth), small head, high-pitched cry, mental retardation
Trisomy 5p3	ASD, VSD	50%	Small head, interorbital separation, large eyes, small mouth
Trisomy 6p2	ASD, VSD, PDA	10%	Characteristic facial features (widely protruding forehead), growth disturbance, renal malformations
Trisomy 7p2	COA, VSD, TOF	70%	Long head, hypertelorism, pointed nose, small chin
Trisomy 8	VSD	50%	Thick lips, flexures, deep eyes
Trisomy 9	VSD, PAVSD, DORV	60%	Small head, deep eyes, skeletal abnormalities
Trisomy 10p	COA, PDA	70%	Stunting, mental retardation, protruding forehead
Monosomy 11q (Jacobsen syndrome)	HLHS, COA, AS, VSD	60%	Characteristic facial features, thrombocytopenia, joint contractures, growth disturbance, mental retardation
Mosaic tetrasomy 12p (Pallister-Killian syndrome)	VSD, ASD	40%	Characteristic facial features, skin pigmentation abnormalities, diaphragmatic hernia, epilepsy, mental retardation
Trisomy 20pter-q11	VSD, TOF, PDA	40%	Mental retardation, round face, scoliosis
Trisomy 22	PS, VSD, PDA, TA	75%	Specific facial features, stunted growth, small head
Trisomy/tetrasomy 22p ter-q11 (cat-eye syndrome)	TAPVC, ASD, VSD	50%	Coloboma, anal atresia, renal anomalies, mental retardation
Trisomy 22q10-q11 and trisomy 11q23-qter (Emanuel syndrome)	VSD, ASD, TOF, PDA, TrA, TA	60%	Mental retardation, small head, stunted growth, auricular deformity, hearing loss, cleft palate, renal anomalies

AS, aortic stenosis; ASD, atrial septal defect; AVSD, atrioventricular septal defect; BAV, bicuspid aortic valve; COA, coarctation of aorta; DCM, dilated cardiomyopathy; DORV, double outlet right ventricle; HLHS, hypoplastic left heart syndrome; IAA, interruption of aortic arch; LVNC, left ventricular noncompaction; PDA, patent ductus arteriosus; PPS, peripheral pulmonary stenosis; PS, pulmonary stenosis; SA, single atrium; SVAS, supravalvular aortic stenosis; TA, tricuspid atresia; TrA, truncus arteriosus; TOF, tetralogy of Fallot; VSD, ventricular septal defect.

Table 13. Major Genetic Abnormality Syndromes Complicating Congenital Heart Disease

Congenital anomaly syndrome (disease-causing gene)	Major complicating congenital cardiovascular diseases	Complication frequency	Other major symptoms
Alagille syndrome (*JAG1*)	PS, TOF, VSD, ASD	90%	Cholestasis, skeletal abnormalities, ocular abnormalities, characteristic facial features
CFC syndrome (*BRAF* etc.)	PS, HCM, VSD, ASD	45%	Epilepsy, large head, characteristic facial features, mental retardation
Char syndrome (*TFAP2B*)	PDA	70%	Finger abnormality
CHARGE syndrome (*CHD7*)	TOF, DORV, AVSD	75%	Coloboma, atresia choanae, cranial nerve palsy, characteristic auricles growth disturbance, mental retardation, hypogonadism
Coffin-Lowry syndrome (*RPS6KA3*)	Valve abnormalities, LVNC, RCM, EFE	5–15%	Characteristic facial features, growth retardation, mental retardation, skeletal abnormalities (tapering of fingers)
Coffin-Siris syndrome (*SMARCB1* etc.)	VSD, ASD, PDA, TOF	35%	Hypoplasia or absence of the fifth finger, characteristic facial features (sparse face, thick lips), growth disturbance, mental retardation
Costello's syndrome (*HRAS*)	HCM, PS	60%	Curly and sparse hair, large head, characteristic facial features, tumors, mental retardation
Ehlers-Danlos syndrome (*COL3A1* etc.)	Valve prolapse and insufficiency, aortic dilation and aneurysm, and dissection		Fragile skin, joints and blood vessels
Ellis-van Creveld syndrome (*EVC* etc.)	AVSD, AS, SA	60%	Thoracic hypoplasia, short limbs, polydactyly, nail hypoplasia
Holt-Oram syndrome (*TBX5*)	ASD, VSD	75%	Radial hypoplasia of upper limbs
Kabuki syndrome (*KMT2D* and *KDM6A*)	AS, MS, VSD, TOF, DORV	70%	Characteristic facial features (eversion of lower eyelids), phimosis, growth disturbance, mental retardation
Loeys-Dietz syndrome (*TGFBR2* etc.)	Valve prolapse and insufficiency, aortic dilation, aneurysm and dissection		Hypertelorism, bifid uvula, arterial tortuosity (head and neck vessels)
Marfan's syndrome (*FBN1*)	Valve prolapse and insufficiency, aortic dilation, aneurysm and dissection	80%	Ectopia lentis, skeletal abnormalities, pneumothorax, lumbosacral dural ectasia
Mowatt-Wilson syndrome (*ZEB2*)	VSD, ASD, PDA	60%	Hirschsprung's disease, epilepsy, characteristic facial features, mental retardation
Noonan syndrome (*PTPN11* etc.)	ASD, PS, HCM	80%	Webbed neck, skeletal abnormalities, short stature, bleeding tendency, juvenile myelomonocytic leukemia, characteristic facial features
Rubinstein-Taybi syndrome (*CREBBP* etc.)	VSD, ASD, PDA	30%	Broad thumbs and halluces, cryptorchidism, abnormal kidneys, characteristic facial features, short stature, mental retardation
Smith-Magenis syndrome (*RAI1*)	VSD, PS, TOF	25%	Sleep disturbances, hypotonia, characteristic facial features, mental retardation, maladaptive behaviors
Sotos syndrome (*ND1*)	ASD, PDA	20%	Overgrowth, tumors, characteristic facial features, mental retardation

AS, aortic stenosis; ASD, atrial septal defect; AVSD, atrioventricular septal defect; DORV, double outlet right ventricle; EFE, endocardial fibroelastosis; HCM, hypertrophic cardiomyopathy; LVNC, left ventricular noncompaction; MS, mitral stenosis; PDA, patent ductus arteriosus; PS, pulmonary stenosis; RCM, restrictive cardiomyopathy; SA, single atrium; TOF, tetralogy of Fallot; VSD, ventricular septal defect.

stature, thrombocytopenia, seizures, strabismus, bronchomalacia, scoliosis, renal malformations, and inguinal hernia, which require comprehensive management.[101]

Congenital heart disease is characterized by anomalies of the conotruncus (cardiac outflow tract) and the aortic arch: tetralogy of Fallot (TOF) (30%), pulmonary atresia with ventricular septal defect (PAVSD) with major aortopulmonary collateral arteries (MAPCA) in 10%, interruption of aortic arc (IAA) type B (15%), ventricular septal defect (VSD) (15%) and truncus arteriosus (TrA) (10%). Anomalies of the right aortic arch and subclavian artery origin are also common complications (25%). The frequency of this syndrome in congenital heart disease is 60% for IAA type B, 55% for PAVSD with MAPCA, 35% for TA, and 15% for TOF.[101–105] The severity of cardiac disease plays a major role in the life outcome of the patient with the syndrome.

Psychiatric disorders include schizophrenia in adulthood (25%) and Parkinson's disease (6%) with young (<50 years old) onset, which makes social independence difficult.

1.2 Williams Syndrome

1.2.1 Disease Concept

Chromosomal microdeletion syndrome with characteristic fairy-like facial features in infancy, mental retardation, characteristic personality, cardiovascular lesions such as supra-aortic stenosis (SVAS) and peripheral pulmonary stenosis (PPS), and hypercalcemia in infancy.

Table 14. Genetic Abnormalities of Congenital Heart Disease (Nonsyndromic)[94,95]

Gene	Heart disease
Transcription factor	
CITED2	ASD, VSD
GATA4	ASD, VSD, AVSD, PS, TOF
GATA5	TrA, TOF
HAND1	AVSD, DORV, HLHS, ASD, VSD
HAND2	TOF, LVNC, VSD
NKX2.5	ASD, AVB, TOF, HLHS, VSD
NKX2.6	TrA
TBX1	DORV, TOF, IAA, TrA, VSD
TBX5	AVSD, TOF, BAV, COA, LVNC, ASD, VSD
TBX20	ASD, VSD, MS, DCM
MEF2C	DORV
NFATC1	TA, AVSD
ZFPM2/FOG2	AVSD, DORV, TOF, VSD
Intracellular signaling factors	
ACVR1/ALK2	AVSD, DORV, TGA, ASD
CRELD1	ASD, AVSD
GJA1	HLHS, VSD, PA
HEY2	AVSD
JAG1	TOF, PS
NODAL	TGA, DORV, TOF, VSD
NOTCH1	BAV, AS, HLHS, TOF, PS, ASD, COA, DORV
SMAD6	BAV, COA, AS
VEGFA	TOF, PDA, AS, BAV, COA, IAA, VSD
Structural protein	
ELN	SVAS
MYH6	ASD, HCM, DCM
MYH7	Ebstein disease, LVNC, HCM, DCM
MYH11	PDA, TAA

AS, aortic stenosis; ASD, atrial septal defect; AVB, atrioventricular block; AVSD, atrioventricular septal defect; BAV, bicuspid aortic valve; COA, coarctation of aorta; DCM, dilated cardiomyopathy; DORV, double outlet right ventricle; HLHS, hypoplastic left heart syndrome; IAA, interruption of aortic arch; LVNC, left ventricular noncompaction; MS, mitral stenosis; PA, pulmonary atresia; PS, pulmonary stenosis; SVAS, supravalvular aortic stenosis; TA, tricuspid atresia; TrA, truncus arteriosus; TAA, thoracic aortic aneurysm; TOF, tetralogy of Fallot; VSD, ventricular septal defect. (Source: Prepared based on Yasuhara J, et al. 2021,[94] Kodo K, et al. 2021[95])

Table 15. Recommendations and Levels of Evidence for Genetic Testing for Congenital Heart Diseases

	COR	LOE
Genetic testing for congenital heart disease concomitant with congenital anomaly syndrome	I	C
Genetic testing for isolated congenital heart disease	IIb	C

COR, Class of Recommendation; LOE, Level of Evidence.

1.2.2 Diagnosis
Chromosome testing (FISH) to prove 7q11.23 microdeletion with a probe containing the *ELN* gene. The test is performed by insurance and testing companies.

1.2.3 Frequency of Occurrence
1 in 7,500.[106]

1.2.4 Genetic Factors
It is caused by a heterozygous microdeletion of the chromosome 7 long arm 11.23 region and is thought to be a haploinsufficiency of the gene in the deletion region (1.5–1.8 Mb). As in 22q11.2 deletion syndrome, there are DNA repeats at both ends of the deletion region and the deletion is thought to occur uniformly due to mismatches during chromosome recombination.

1.2.5 Recurrence Rate
AD (manifest) inheritance, with a 50% recurrence rate if either parent has the deletion. Most cases are sporadic and familal cases are rare.[107]

1.2.6 Prenatal Diagnosis
Prenatal diagnosis is considered when (1) there is a deletion in a previous child, (2) either parent has a deletion, or (3) the child has familial SVAS. Chromosome FISH analysis of amniotic fluid test (after 15 weeks) and CVS (10–12 weeks) are available. Even when testing reveals a deletion, it is difficult to predict the phenotype, severity, and prognosis of the child and cautious approach is required.

1.2.7 Causative Genes
The deletion region of chromosome 7 q11.23 contains approximately 20 genes, including *ELN*, which encodes elastin, a major component of elastic fibers in the tunica media of arteries that are responsible for vascular elasticity; mice deficient in ELN function show luminal narrowing due to thickening of the smooth muscle layer of the tunica media throughout the body.[108] *ELN* is thought to be the disease gene for SVAS in this syndrome. *LIMK1* is highly expressed in the central nervous system and its deletion may be responsible for the mental disorders.[109]

1.2.8 Symptoms, Prognosis and Management
Growth retardation beginning with intrauterine growth retardation, mental retardation, and elfin face (thick medial eyebrows, swollen eyelids, saddle nose, thick lips; description derived from European folk tales) are present. Patients are characterized by a "cocktail-party" personality, and often have good language development and memory.

Systemic arterial stenotic lesions due to elastin abnormalities are present in 75% of cases, and SVAS and PPS are characteristic. The former is usually progressive, and the latter spontaneously resolves. It has been reported that coronary artery stenosis causes a 25–100-fold higher risk of sudden death[110] than in the general population.[111] An association between general anesthesia and sedation and cardiac arrest has also been reported,[112] so caution is required during cardiac catheterization and surgery. Other complications include hypercalcemia (mechanism unknown) (15%),[113] hypertension (40%), and urinary disorders (urinary tract stones, bladder diverticulum, vesicoureteral reflux, etc.). Renal vascular hypertension due to renal artery stenosis must be differentiated.

In adulthood, facial features change from the round face

of infancy to a long face and long neck. The average final height is approximately −2 SD. The patient is often unable to be socially independent due to intellectual disability and impaired visuospatial cognition. Anxiety disorders are also often present. In addition to congenital heart disease, hypertension, cerebrovascular disease, chronic constipation, obesity, and glucose intolerance are common. With age, neuropsychiatric symptoms and hypertension increase in frequency,[114] requiring treatment and social support.

1.3 Down Syndrome

1.3.1 Disease Concept

A congenital anomaly syndrome caused by an excess of chromosome 21 (trisomy 21).

1.3.2 Diagnosis

Chromosome testing (G banding or FISH using chromosome 21 probe). It is covered by insurance and performed by testing companies.

1.3.3 Frequency of Occurrence

It is the most frequent chromosomal abnormality among births, generally 1 in 700–1,000 births, and increases with maternal age (15–29 years: 1 in 1,500; 30–34 years: 1 in 800; 35–39 years: 1 in 270; 40–44 years: 1 in 100; ≥45 years: 1 in 50).[115]

1.3.4 Genetic Factors[116]

Due to chromosome 21 (responsible region 21q22.3) trisomy.

a. Full Trisomy [47,XY,+21 or 47,XX,+21] (92.5%)

Isolated cases are common. Maternal age is a major factor. Due to chromosome segregation at meiosis, approximately 90% of cases have an excess of chromosome 21 of maternal origin, 70% of which is due to segregation at meiosis I.

b. Mosaicism [46,XY/47,XY,+21 or 46,XX/47,XX,+21] (2.5%)

The phenotype is modified by the ratio of trisomy cells in each organ.

c. Translocation (5%)

The excess chromosome 21 is most frequently translocated to the terminal attachment type group D (chromosomes 13, 14, 15) or group G (chromosomes 21, 22) (Robertson translocation). t(14q;21q), followed by t(21q;21q), is the most common type of translocation.

1.3.5 Recurrence Rate

a. Full Trisomy

It is estimated to be approximately 1%.[115] It is also affected by the mother's older age at the time of conception of her next child.

b. Translocation Type

The recurrence rate is empirically estimated to be approximately 10–15% when the mother is a balanced Robertson translocation carrier and 1–5% when the father is a carrier.

1.3.6 Prenatal Diagnosis

If the first child is Down syndrome and the age at 2nd pregnancy is >30 years old, an amniotic fluid test is recommended. For older pregnancies (>35 years of age), prenatal diagnosis is considered.

The finding of enlarged nuchal translucency on fetal ultrasound at mid-term (10–14 weeks) is characteristic of Down syndrome and trophoblastic and amniotic fluid studies should be considered. Trisomy 18, trisomy 13, Turner syndrome (45,X), and other chromosomal abnormalities can also be seen.

The maternal serum marker test is used to estimate the possibility of Down syndrome based on the distribution of α-fetoprotein, unconjugated estriol (uE3), and human chorionic gonadotropin (hCG) concentrations in maternal serum at 15–18 weeks gestation and on maternal age.[117] A new type of prenatal diagnosis (NIPT: non-invasive prenatal genetic testing) using fetal DNA in the mother's blood is also used.

Ultrasound findings and blood test abnormalities are only presumptive, and a definitive diagnosis must be made by amniotic fluid or trophoblast chromosome analysis.

1.3.7 Symptoms, Prognosis and Management

External surface abnormalities include hypertelorism, epicanthus, macroglossia, shortened 2nd and 5th metacarpals, medial contraction of the 5th finger. Neonatal, infantile, and childhood manifestations also include decreased muscle tone and joint range of motion, psychomotor developmental delay, and short stature.[115,116]

a. Congenital Heart Disease

Congenital heart disease is present in 40%, and according to Japanese reports, the most frequent types are VSD (37%), AVSD (especially Rastelli type C) (30%), PDA (11%), ASD (10%), and TOF (7%).[118] TOF with AVSD is common in Down syndrome.[118,119] Even in cases of no apparent cardiac disease in childhood, mitral valve deviation and aortic regurgitation frequently develop in adulthood.[120]

A national survey in Japan reported that 38% of Down syndrome patients with congenital heart disease have pulmonary hypertension (PH).[118] It is also known that pulmonary vascular occlusive lesions develop early in the disease, and possible mechanisms include congenital or acquired hypoplasia of the pulmonary vascular bed, imbalance of vasoactive factors, and disruption of vascular regeneration mechanisms. On the other hand, a study of the histopathology of the pulmonary arteries in VSD and AVSD showed no morphological differences between Down syndrome and non-Down syndrome patients, and there are reports that pulmonary vascular occlusive lesions correlate with age and pulmonary artery pressure.[121] In addition, Down syndrome is often associated with pulmonary hypertensive crisis, a paroxysmal increase in pulmonary artery pressure and circulatory failure after cardiac repair surgery, which requires caution (postoperative cases of VSD: Down syndrome 1.1% vs. non-Down syndrome 0.2%).[122]

b. Other Symptoms[115,116]

i. Ocular Disorders

Cataract, strabismus, nystagmus

ii. Otolaryngological Disorders

Hearing loss (66%), exudative otitis media (60–80%)

iii. Gastrointestinal Tract Disorders

Congenital duodenal atresia (12%), anal atresia, Hirschsprung's disease

iv. Endocrine Disorders

Hypothyroidism (10–40%), hyperthyroidism, diabetes, small penis, undescended testis

v. Hematologic Disorders
Transient abnormal myeloproliferative disorders (10%, *GATA1* gene abnormalities), acute leukemia (2–3%, characterized by acute megakaryoblastic leukemia)
vi. Neurological Diseases
Epilepsy (8%)
vii. Skeletal System Disorders
Axillary joint subluxation (symptomatic 1–2%), lameness
viii. Immunodeficiency
High frequency of autoimmune diseases such as Hashimoto's disease and type 1 diabetes
ix. Respiratory Disorders
Laryngeal, tracheal, and bronchomalacia, tracheal stenosis (complete ring cartilage ring), sleep apnea
x. Mental Disorders
Depression (6%), autism (7–16%), and especially dementia due to Alzheimer's disease (50% by age 60 years); significantly more amyloid deposition in cerebral vessels in Down syndrome[123]
xi. Dental Disorders
Dental malocclusion, malocclusion

The average life expectancy for Down syndrome patients was 8 years in the 1930s, but medical advances have extended it to the 60s in recent years.[124] Infection, congenital heart disease, and leukemia significantly affect life expectancy. In addition, aging phenomena such as wrinkles, hair loss, cataracts, and hearing loss appear in early adulthood and are speculated to be caused by increased oxidative stress due to gene overexpression in the trisomy region.[125]

1.4 Trisomy 18

1.4.1 Disease Concept
A syndrome caused by duplication of all or part of chromosome 18, with various external and internal organ abnormalities and psychomotor retardation; ≥90% of cases are associated with congenital heart disease.

1.4.2 Diagnosis
Chromosome testing (G banding or FISH using chromosome 18 probe). It is covered by insurance and performed by testing companies.

1.4.3 Frequency of Occurrence
1 in 6,000–8,000. Second most frequent autosomal abnormality among births, next to Down syndrome (trisomy 21). No racial differences. Male to female ratio is 1 : 3.

1.4.4 Genetic Factors[126]
The extra chromosomal region most involved in the phenotype is 18q11-q12.

a. Full Trisomy (93.7%)
Caused by chromosome disjunctions during meiosis during gametogenesis, 95% of which are of maternal origin. Incidence increases with maternal age.

b. Mosaicism (4.6%)
Phenotype and severity vary widely depending on the proportion of mosaic cells.

c. Translocation Type (1.7%)
Partial trisomy due to unbalanced reciprocal translocation.

1.4.5 Recurrence Rate
0.5–1%; recurrence rate increases if maternal age is ≥35 years at the time of pregnancy.

1.4.6 Prenatal Diagnosis
Findings suggestive of trisomy 18 on fetal ultrasound at mid-term (10–14 weeks) include choroid plexus cysts, strawberry crania, single umbilical artery, heart disease, overlapping fingers, cerebellar hypoplasia, intrauterine growth retardation, and amniotic fluid overload. Intrauterine fetal death is common. Maternal serum triple marker test in late pregnancy with low uE3 and low hCG suggest trisomy 18; NIPT can be used to diagnose possible trisomy 18. Amniotic fluid examination is required for definitive diagnosis.

1.4.7 Symptoms, Prognosis and Management
a. Congenital Heart Disease
More than 90% of cases are complicated by congenital heart disease, most commonly pulmonary hypertrophic heart disease such as VSD, ASD, and PDA, and cardiac disease is rarely a fatal complication in the neonatal period. Approximately 10% present with complex forms of cardiac disease such as AVSD, right ventricular origin of both great vessels, aortic stenosis (AS), and hypoplastic left heart syndrome.[127] Multiple atrioventricular valves, excess semilunar valves and thickened valve leaflets are often present, but stenosis and regurgitation are often mild.

b. Pulmonary Hypertension
PH is a common complication from early infancy in heart disease with increased pulmonary blood flow, as in Down syndrome, and was found in 52% of cases in a Japanese study.[128] At autopsy, thickening of the pulmonary artery media and intimal hyperplasia were found in 8 of 25 cases (32%).[129] Histological examination of the pulmonary arteries showed hypoplasia of the small pulmonary arteries in 46.4% and loss of the tunica media of the small pulmonary arteries in 14.3%, suggesting a mechanism by which the physical stress of high pulmonary blood flow leads to fibrous thickening of the intima, leading to pulmonary vascular occlusive lesions. In addition, 75% of the patients had alveolar wall thickening and 53.5% had alveolar hypoplasia, which is considered a risk for exacerbation of PH.[130]

c. Indications for Cardiac Surgery
Because of the poor prognosis of the disease, medical therapy has been the mainstay of management. In recent years, an increasing number of facilities have begun to perform palliative and intracardiac repair with the aim of providing home care. The severity of extracardiac complications and the wishes of the family must be taken into consideration when deciding the indication of surgery. In recent years, reports of survival after intracardiac repair have increased, and a study of 65 cardiac surgery cases with trisomy 18 and 30 cases with trisomy 13 in the USA showed that the postoperative survival rate of intracardiac repair cases was significantly higher than that of palliative surgery cases (15-year survival rate 70.7% vs. 30.8%). The median postoperative survival of trisomy 18 patients is 16.2 years.[131] In a report by Nakai et al in Japan, patients with trisomy 18 and VSD with pulmonary artery banding were received intracardiac repair if the parents strongly desired it, and 14 patients (78%) survived after discharge, with a median postoperative survival of 46.3 months.[132] On

the other hand, the mortality rate for trisomy 18 patients admitted for cardiac surgery was 13%, approximately 10-fold higher than for non-trisomy 18, especially for surgery for simple cardiac diseases such as VSD.[131] A higher postoperative mortality rate has also been reported in cases requiring preoperative ventilatory management.[133] Currently, surgery is not actively performed for complex cardiac diseases such as HLHS.[134] When explaining the indications for cardiac surgery to family members, it is necessary to also inform them of the fact that surgery may improve life expectancy, but the risks associated with surgery are higher[134] than with non-trisomy 18.

d. Other Symptoms

i. Malignant Neoplasms

Wilms tumor and hepatoblastoma.[135] Chemotherapy and surgical resection are also used[135]

ii. Central Nervous System Disorders

Severe mental retardation, hypo/hypertonia, central apnea, convulsions, microcephaly, cerebellar hypoplasia, total anterior cingulate, hypoplasia/defective corpus callosum, etc.

iii. Head and Neck Disorders

Occipital protrusion, narrow cranial width, small chin, cleft lip and palate

iv. Skeletal System Disorders

2nd finger overlapping 3rd finger (overlapping finger), syndactyly, rocker-bottom feet, joint contractures, scoliosis, abnormal vertebral and rib formation

v. Respiratory Disorders

Hypoplastic laryngomalacia, tracheoesophageal fistula, tracheomalacia

vi. Gastrointestinal Tract Disorders

Esophageal atresia,[136] umbilical hernia, inguinal hernia, bowel malrotation

vii. Urogenital Disorders

Undescended testis, hydronephrosis, polycystic kidney

viii. Endocrine Disorders

Hypothyroidism, prolonged hypoglycemia

Life prognosis is poor, with 90–95% mortality within the first year of life, and even survivors have severe mental retardation.[126,137] Indications for cardiac surgery must be flexible and tailored to individual cases.[126]

1.5 Trisomy 13

1.5.1 Disease Concept

A syndrome caused by duplication of all or part of chromosome 13 and complicated by various external and internal organ abnormalities and psychomotor retardation; 90% of cases are associated with congenital heart disease. As with trisomy 18, approximately 90% of patients die before the age of 1 year due to apnea or other causes, and cardiac surgery has been discouraged because of the poor prognosis.

1.5.2 Diagnosis

Chromosome testing (G banding or FISH using chromosome 13 probe). It is covered by insurance and performed by testing companies.

1.5.3 Frequency of Occurrence

1 in 5,000–12,000. Third most frequent autosomal abnormality among births, after trisomy 21 and trisomy 18. No racial differences.

1.5.4 Genetic Factors

a. Full Trisomy (80%)

Chromosome disjunctions during meiosis in gametogenesis cause 90% of cases to be of maternal origin. Most cases occur in the first meiosis. Incidence increases with maternal age.

b. Translocation Type (5–20%)

Translocations with the terminal attachment type, group D (chromosomes 13, 14, 15) or group G (chromosomes 21, 22), are predominant with the 13;14 Robertson translocation.

c. Mosaicism (Rare)

d. Partial Trisomy

There is a 13q distal partial trisomy (13q14→qter) and a 13q proximal partial trisomy (13pter→q14), both with marked mental retardation. Life prognosis is better than with the standard type.[137]

1.5.5 Recurrence Rate

0.5–1%. Recurrence rate increases when maternal age is >35 years.

1.5.6 Prenatal Diagnosis

Findings suggestive of trisomy 13 on fetal ultrasound at midterm (10–14 weeks) include total anterior encephalocele, cleft lip and palate, polydactyly, microcephaly, navicular foot, microphthalmia, partial scalp and skull defects, and umbilical hernia. Intrauterine fetal death is common. Diagnosis is often triggered by ultrasound examination. Maternal serum triple markers are not diagnostic, but NIPT can diagnose a possible trisomy 13. Amniotic fluid examination is necessary for a definitive diagnosis.

1.5.7 Symptoms, Prognosis and Management

a. Congenital Heart Disease

Approximately 90% of cases are complicated by congenital heart disease, predominantly ASD, VSD, PDA, and TOF.[138] Compared with trisomy 18, valvular disease is less common. Cardiac disease is rarely a fatal complication in the neonatal period, but PH develops early.[128] As with trisomy 18, cardiac surgery has traditionally been reserved for this poor prognosis disease, but an increasing number of centers are performing palliative and intracardiac repair. The severity of the extracardiac complications and the family's wishes should be taken into consideration when deciding whether surgery is appropriate.

b. Indications for Cardiac Surgery

In recent years, reports of survival after intracardiac repair have increased, with a study of 30 cases of cardiac surgery for trisomy 13 in the USA showing a median survival of 14.8 years in the remote postoperative period.[131] As with trisomy 18, for surgery for simple heart disease, the mortality rate is approximately 10-fold higher than for non-trisomy,[132] and a higher postoperative mortality rate has been reported in cases requiring ventilatory management.[133] Families need to be informed of the high life risks associated with surgery as well as the prognostic benefits of surgery.[134]

c. Other Symptoms

i. Central Nervous System Disorders

Holoprosencephaly, anosmia, seizures, severe mental retardation

ii. Head and Neck Disorders
Microcephaly, scalp and skull defects, cleft lip and palate
iii. Skeletal System Disorders
Polydactyly, syndactyly, overlapping fingers
iv. Respiratory Disorders
Laryngomalacia, tracheomalacia, apnea
v. Gastrointestinal Tract Disorders
Umbilical hernia, gastroesophageal reflux, bowel malrotation, pancreatitis
vi. Urogenital Disorders
Undescended testis, polycystic kidney
vii. Endocrine Disorders
Hypothyroidism, prolonged hypoglycemia

Life expectancy is poor, with 90% dying within the first year of life, and even survivors have severe mental retardation.[137,138] As with trisomy 18, multidisciplinary treatment may improve survival rates, and there is a growing need for flexibility in individual cases.[138,139]

1.6 Turner Syndrome

1.6.1 Disease Concept

The most frequent sex chromosome abnormality, with a female phenotype. The syndrome is associated with short stature, primary amenorrhea due to gonadal dysplasia, and characteristic skeletal, soft tissue, and visceral findings.

1.6.2 Diagnosis

Chromosome testing (G banding). It is covered by insurance and performed by testing companies.

1.6.3 Frequency of Occurrence

1 in 2,000–2,500 live births of girls.

1.6.4 Genetic Factors

Due to complete deletion of one X chromosome or partial deletion including the short arm (Xp) end. Approximately 50% of cases are due to a complete deletion of the X chromosome (45,X), 25% are mosaics such as 45,X/46,XX and 45,X/47,XXX, and 20% are due to structural abnormalities of the X chromosome such as the same arm or ring chromosomes.[140] Deletions and structural abnormalities are often derived from the father's sperm. Unlike trisomy, the correlation between increasing paternal or maternal age and the development of the syndrome has not been established; one of the X chromosomes is inactivated, so genes on the X chromosome are normally expressed in one copy, but there is a pseudoautosomal region 1 (PAR1) at the short arm end of the X chromosome (Xp22.3). However, genes in PAR1 at the short end of the X chromosome (Xp22.3) escape inactivation and are only expressed in one copy when they should be expressed in two copies, resulting in clinical symptoms from haploinsufficiency.

1.6.5 Recurrence Rate

Because 45,X women are infertile due to primary gonadal dysfunction caused by gonadal dysgenesis, the recurrence rate is not significant, but there are reports of spontaneous pregnancy in approximately 5% of Turner syndrome patients.[141]

1.6.6 Prenatal Diagnosis

When fetal ultrasonography in the second trimester reveals an enlarged clear segment at the terminus, Turner syndrome is suspected in addition to Down syndrome and trisomy 18, and amniotic fluid and CVS are recommended. Turner syndrome may also be suspected in the presence of horseshoe kidney or obstructive left heart disease.

1.6.7 Causative Genes

The *SHOX* gene on PAR1 is involved in cartilage growth and its haploinsufficiency is a major cause of short stature. It has also been speculated that there are genes that escape inactivation at a different site than PAR1 on the short arm of the X chromosome, and that haploinsufficiency of the *TIMP1* gene, which encodes a matrix metalloproteinase inhibitor, is involved in BAV, aortic dilation and aneurysm formation associated with Turner syndrome.[142] A mechanism for homologous chromosome pairing defects and nonspecific mitotic defects during meiosis has also been postulated due to deletions and structural abnormalities of the X chromosome.[140]

1.6.8 Symptoms, Prognosis and Management

a. Physical Symptoms

Pterygoid neck, ectrodactyly, low hairline, shield-shaped chest, shortened 4th metacarpal, edema of dorsal feet and hands. Congenital lymphatic hypoplasia has been implicated.

b. Short Stature

The final mean height of women with Turner syndrome is approximately 20 cm lower than that of normal karyotype women[140] and there is no growth acceleration during puberty. Growth hormone replacement therapy is effective.

c. Gonadal Dysfunction

Delayed appearance of secondary sexual characteristics and amenorrhea are observed. Gonadal dysfunction is caused by damage or loss of oocytes due to homologous chromosome mismatch during meiosis.[140] Female hormone replacement therapy is effective in inducing sexual maturation and preventing osteoporosis. Secondary sexual characteristics are induced by oral estrogen preparations starting at the age of puberty, followed by Kaufmann therapy to induce menstruation.

d. Congenital Heart Disease (30–40%)

Left heart obstructive lesions such as COA (10–15%), BAV (25–30%), aortic dilation (20–30%), and AS (10%) are common. Because of the presence of signs due to lymphatic vessel hypoplasia, such as pterygium, it has been speculated that obstructed lymphatic flow is the cause of left heart lesions, but no lymphangiogenic genes on the X chromosome have been identified.[143] ASD and partial anomalous pulmonary venous connection are also often present, and adult women with Turner syndrome are at increased risk for hypertension, coronary artery disease, and aortic dissection, especially aortic dissection, which is present even in their 30s[144] and carries 100-fold higher risk than in non-Turner syndrome women.[140] Not only aortic dissection associated with aortic dilation, but dissection can occur even in young women without aortic dilation,[144] and imaging evaluation once every 5 years for those without aortic dilation and annually for those with aortic dilation is recommended.[140] Cystic medial necrosis is seen in aortic lesion tissue, as in Marfan syndrome.[144] Approximately 50% of adult women with Turner syndrome have hypertension from a young age, but the mechanism is unknown.[145]

e. Renal Disease (25–40%)
Horseshoe kidney is the most common (10%), as are duplicated renal pelvis and ureter and aplastic kidney.

1.7 1p36 Deletion Syndrome

1.7.1 Disease Concept
Deletion of the 1p36 region causes growth retardation, congenital heart disease, epilepsy, and mental retardation.

1.7.2 Diagnosis
Chromosome testing (FISH) using a probe containing a common deletion region (CDC2L1 region) at 1p36. The test is covered by insurance and is performed by a testing company.

1.7.3 Frequency of Occurrence
1 in 5,000 births. No racial differences. Slightly more common in girls. Second most frequent chromosomal microdeletion syndrome next to 22q11.2 deletion syndrome.[143]

1.7.4 Genetic Factors
95% of cases are neoplastic mutations. Deletion of chromosome 1 is 60% maternal and 40% paternal. With the widespread use of array-based comparative genomic hybridization (array CGH), a microarray chromosome test, and the increase in genetic screening tests for patients with unexplained mental retardation, 1p36 deletions are often identified. Deletion size correlates poorly with clinical symptoms.[146] Deletion of the *KCNAB2* gene, which encodes a potassium channel and is located at 1p36, is speculated to cause epilepsy.[147]

1.7.5 Recurrence Rate
Most cases are neoplastic mutations and have a low recurrence rate.

1.7.6 Symptoms, Prognosis and Management
Facial features (small head, short head, sunken eyes, flattened nasal root, pointedchin) and scoliosis of the fingers are present. Mild to severe mental retardation. Almost all cases are associated with hypotonia in the neonatal period, and approximately 50% with epilepsy. Seizures are intractable and may require multiple antiepileptic drugs. Hearing loss is present in approximately 70%, and approximately 40% are complicated by cardiac disease, of which congenital heart disease accounts for almost 70% and cardiomyopathy, such as DCM and left ventricular myocardial densification disorder, for approximately 30%. Among the congenital heart diseases, VSD, ASD, PDA, and TOF are more frequent.[143,147] In most cases, patients survive to adulthood.[146] Cardiomyopathy may be evident in adulthood and requires lifelong follow-up.[143]

1.8 4p Deletion Syndrome (Wolf-Hirschhorn Syndrome)

1.8.1 Disease Concept
A chromosomal abnormality syndrome caused by partial deletion of the short arm of chromosome 4, resulting in growth retardation, mental retardation, intractable epilepsy, and characteristic facial features.

1.8.2 Diagnosis
Chromosome testing using the G-staining method identifies deletions in 50–60% of cases, but FISH using a probe containing the region of disease responsibility (WHSCR) at 4p16 within the short arm of chromosome 4 (4p) is required for a definitive diagnosis. The test is covered by insurance.

1.8.3 Frequency of Occurrence
1 in 20,000–50,000 births. The male to female ratio is 1:2, more common in girls.[148]

1.8.4 Genetic Factors
It is caused by haploinsufficiency of a group of genes due to a deletion of the 1.5Mb region of 4p16.3 (WHSCR) on the short arm of chromosome 4.[148] In addition, the presence of the responsible region on the end side (WHSCR-2) has been reported.[149] 50–60% of cases are due to simple deletions. Other factors involved include chromosome 4 ring, mosaicism, and chromosome 4 derivatives with disproportionate translocations.

1.8.5 Recurrence Rate
For simple deletions, the recurrence rate is low. In the case of 4p-syndrome due to an unbalanced translocation, the recurrence rate is increased in the second child because the parent may be a balanced translocation carrier. The recurrence rate is approximately 10% if the mother is a balanced translocation carrier and approximately 5% if the father is a balanced translocation carrier.

1.8.6 Symptoms, Prognosis and Management[148,150]
The Greek warrior's helmet-like face with a broad nasal root extending to the forehead is characteristic and of high diagnostic value. Other features include arched, highly arched eyebrows, interocular dissection, short philtrum, and small jaw. Approximately 80% of patients have growth retardation, and short stature and poor weight gain often persist even with adequate nutrition. Epilepsy is present in >90% of cases, with a peak incidence between 6 and 12 months of age. Epilepsy is refractory and often requires multiple antiepileptic drugs. Approximately 50% have congenital heart disease, often simple heart disease such as ASD, VSD, PDA, and PS.

1.9 5p Deletion Syndrome

1.9.1 Disease Concept
A chromosomal abnormality syndrome caused by a partial deletion of the short arm of chromosome 5, resulting in a small head, growth retardation, mental retardation, hypotonia, and characteristic facial features. The name of the disease was formerly known as cri du chat syndrome, but the name is no longer used because it is considered inappropriate to include the name of an animal.

1.9.2 Diagnosis
Chromosome testing using the G-staining method may not identify the deletion; chromosome testing using a probe on the short arm of chromosome 5 (FISH method) can detect the deletion but is not performed by testing companies. Microarray testing (array CGH method) can detect copy number changes in chromosome genomes and diagnose the syndrome. The microarray test is covered by insurance and can be performed by a testing company.

1.9.3 Frequency
1 in 15,000–50,000 births.

1.9.4 Genetic Factors
Deletions range from 560 kb to 40 Mb. Simple deletions account for approximately 80–90% of cases and are most often due to chromosome breaks during spermatogenesis; 80–90% of chromosomes with deletions are of paternal origin, 10–15% are unbalanced translocations, and a small number of other cases are due to arm deletions (3–5%), mosaicism (1.4%), inversion (0.5%), or ring chromosome (0.5%).[151]

1.9.5 Recurrence Rate
For simple deletions, the recurrence rate is low. In the case of 5p-syndrome due to an unbalanced translocation, the recurrence rate is increased in the second child because the parent may be a balanced translocation carrier. The recurrence rate is approximately 10% if the mother is a balanced translocation carrier and approximately 5% if the father is a balanced translocation carrier.

1.9.6 Symptoms, Prognosis and Management[151,152]
Growth retardation, mental retardation, decreased muscle tone, and a high-pitched cat-like cry are present from neonatal and infancy, with characteristic facial features such as small head and interocular dissection. With advancing age, the decreased muscle tone becomes rather hypertonic. Scoliosis is also frequent. Approximately 30% have congenital heart disease, often of the simple type, such as ASD, VSD, and PDA.

2. Congenital Heart Disease Caused by Genetic Variants

2.1 Alagille Syndrome

2.1.1 Disease Concept
AD manifestation characterized by intrahepatic bile stasis, congenital heart disease, skeletal abnormalities, eye abnormalities, and a characteristic facial appearance caused by dysfunction of the intracellular signaling molecule Jagged 1.

2.1.2 Frequency
1 in 30,000 births.

2.1.3 Genetic Factors
Mutations in the *JAG1* gene (20p12.2), which encodes the Jagged 1 protein involved in the intracellular Notch signaling pathway, and in the *NOTCH2* gene (1p12), which encodes a receptor for Jagged 1, have been identified. Notch signaling is involved in all cell–cell interactions, and its disruption can cause a wide variety of multiorgan manifestations.[153,154] Genetic testing is covered by insurance and is contracted by the Kazusa DNA Research Institute.

2.1.4 Symptoms, Prognosis and Management
Within the first 6 months of life, symptoms of biliary stasis such as jaundice, grayish-white stools, and hepatosplenomegaly will appear. A decrease in the number of interlobular bile ducts within the liver is characteristic but may not be evident on liver histopathology in infants younger than 6 months.[153] The severity of the disease varies from asymptomatic liver dysfunction to liver failure requiring transplantation. The patient presents with facial features such as a broad, prominent forehead, depressed eyes, and a small, pointy chin. Ophthalmologic diseases such as posterior embryotoxon (≈70%) and retinitis pigmentosa are also characteristic. Approximately 90% of the patients have congenital heart disease, often with PS, and PPS.

2.2 CHARGE Syndrome

2.2.1 Disease Concept
AD manifestation in which the CHD7 protein, expressed in mesenchymal tissue derived from neural crest cells during embryonic development, disrupts the regulation of other gene expression, resulting in multiple organ defects.

2.2.2 Diagnosis
Genetic testing is covered by insurance and is contracted by the Kazusa DNA Research Institute.

2.2.3 Frequency
1 in 12,000 births.

2.2.4 Genetic Factors
Most cases are solitary with mutations in the *CHD7* (chromodomain helicase DNA-binding protein-7) gene located on chromosome 8q12.1. CHD7 affects the expression of many genes through chromatin conformational changes. CHD7 controls the expression of class 3 semaphorin (Sema 3C), interacts with Tbx1, and represses p53, and is thought to play an important role in embryonic development.[155–157] In addition, analysis of CHARGE syndrome model mice with *Chd7* mutations suggests that CHD7 regulates a number of genes involved in the development of neural crest cells and neuroaxons, including semaphorin and ephrin receptors.[158,159] The clinical manifestations of CHARGE syndrome are diverse and the correlation between genotype and phenotype is unclear.[159]

2.2.5 Symptoms, Prognosis and Management
Named after the initials of Coloboma (defects of the iris, retina, and papilla), Heart defects (congenital heart defects), Atresia choanae, Retarded Growth and development (mild to severe, sometimes with anosmia), vulvar anomalies, auricular deformities and deafness (Ear anomalies: small, cupped, and drooping ears). Other complications include cleft lip and palate, multiple cranial nerve abnormalities, feeding difficulties, nasopharyngeal dysfunction, urinary system abnormalities, umbilical hernia, tracheoesophageal fistula, rib and spinal abnormalities, deep grooves on the palms between the 2nd and 3rd fingers (field hockey stick-like palm line), pituitary and hypothalamic gonadal dysfunction, and growth hormone deficiency. Characteristic auricular and palmar line morphology is a clue to diagnosis. Management often requires tube feeding, hearing aids, and tracheostomy. Congenital heart disease complication frequency is 75–80%, with VSD and ASD being the most common, followed by AVSD, TOF, COA, and TGA.[143,160]

2.3 Holt-Oram Syndrome (Heart-Hand Syndrome)

2.3.1 Disease Concept
AD manifestation disease characterized by the radial side of upper extremity abnormalities and congenital heart disease.

2.3.2 Frequency
1 in 100,000 births.

2.3.3 Diagnosis
Genetic testing is performed only at the laboratory level.

2.3.4 Genetic Factors
Mutations in the *TBX5* gene (12q24), which encodes a T-box transcription factor, cause a tendency for mutations to accumulate in the DNA binding domain. Mutations have been reported to cause decreased DNA binding capacity, impaired transcriptional activity, impaired interaction with other transcription factors NKX2.5 and GATA4, which are essential for cardiac development, and impaired nuclear translocation.[161,162] The detection rate of *TBX5* mutations is low in patients who clinically resemble the syndrome but have abnormalities outside of the upper extremities and heart, including kidneys, feet, face, spine, and trachea.

2.3.5 Symptoms, Prognosis and Management
Upper extremity hypoplasia due to abnormalities of the medial bone of the upper extremity, such as abnormal thumb and radial defect, is characteristic. Most cases are bilateral, with no lower extremity abnormalities. Mental retardation is not seen. 75% of patients have cardiac disease, often ASD and VSD, and often associated with abnormalities of the conduction system. Sinus bradycardia and first-degree atrioventricular block (AVB) may be present from birth, progressing to complete AVB or complicated by atrial fibrillation.[163]

2.4 Kabuki Syndrome

2.4.1 Disease Concept
AD manifestation or X-linked disorder that causes characteristic facial features, congenital heart disease, and mental retardation due to dysfunction of methyltransferases and demethyltransferases involved in gene expression regulation.

2.4.2 Frequency
1 in 32,000 births.

2.4.3 Diagnosis
Genetic testing is covered by insurance and is contracted by the Kazusa DNA Research Institute.

2.4.4 Genetic Factors
It is caused by mutations in the *KMT2D*/*MLL2* gene (12q13.12), which encodes a methyltransferase involved in regulating chromatin structure and controlling gene transcription, and the *KDM6A* gene (Xp11.3), which encodes a demethyltransferase. *KMT2D* mutations account for approximately 80%.[164] Most are neoplastic mutations, with *KMT2D* mutations transmitted in an AD manifest form and *KDM6A* mutations in an X-linked form.

2.4.5 Symptoms, Prognosis and Management
It is named for the characteristic long palpebral fissures with eversion of lower eyelids, appearing a Kabuki actor's kumadori. In addition, facial features such as thin, sparse eyebrows in the outer third of the face, a collapsed nose with a short nasal bridge, and large, protruding auricles are also noted. A finger pad is also a characteristic finding. Mild to moderate mental retardation, growth retardation (short stature), and congenital anomalies involving multiple organs are common. Repeated otitis media may cause conductive hearing loss. Approximately 70% of the cases are complicated by cardiac disease, characterized by AS, MS, and other left obstructive lesions. Other common conditions include BAV, VSD, TOF, DORV, TGA, and single ventricle.[165]

2.5 Noonan Syndrome, CFC Syndrome, Costello Syndrome

2.5.1 Disease Concept
AD manifestation with characteristic facial features, congenital heart disease, skeletal abnormalities, mental retardation, and lymphatic abnormalities.

2.5.2 Frequency
1 in 1,000 to 2,500 births.

2.5.3 Diagnosis
Genetic tests are covered by insurance and are contracted by the Kazusa DNA Research Institute, a public foundation.

2.5.4 Genetic Factors
Mutations in the *PTPN11* (protein-tyrosine phosphatase nonreceptor-type 11) gene located on 12q24.1 are the most common, occurring in about half of the cases. PTPN11 is a non-receptor type protein tyrosine phosphatase, and the mutations seen in Noonan syndrome are clustered in the NSH2 (N-terminal SRC homology 2) and PTP (phosphotyrosine phosphatase) regions. It is believed that gain-of-function of PTPN11 is involved in the pathogenesis of Noonan syndrome. Noonan syndrome with multiple lentigines (NSML), previously called LEOPARD syndrome, is often associated with hypertrophic cardiomyopathy, suggesting the involvement of loss-of-function changes in the *PTPN11* gene. In addition to PTPN11, other genes involved in the RAS/MAPK signaling pathway, such as *SOS1* (11%), *RAF1* (5%), and *RIT1* (<5%), have been identified as causative genes in Noonan syndrome.[166]

Costello syndrome and cardio-facio-cutaneous (CFC) syndrome have similar clinical phenotypes, including characteristic facial features, relatively large head, congenital heart disease, hypertrophic cardiomyopathy (HCM), and psychomotor retardation. The RAS/MAPK signaling syndromes (RASopathies), together with Noonan syndrome and NSML, are now encompassed by the RAS/MAPK signaling pathway.[167] *HRAS* mutations in Costello syndrome, and *BRAF*, *MEK1/2*, and *KRAS* mutations in CFC syndrome are thought to be the causes of the diseases.

2.5.5 Symptoms, Prognosis and Management
The following complications are also seen: short stature, mild mental retardation, characteristic facial features (ptosis, hypertelorism, epicanthus, saddle nose, auricular hypotonia/deformity, small chin), webbed neck, low occipital hairline, abnormal thorax (funnel chest, pigeon chest), abnormal spine (scoliosis/folding, spina bifida), undescended testis, bleeding tendency (XI, XII, VIII coagulation factor deficiency, thrombocytopenia), juvenile myelomonocytic leukemia, lymphedema, and chylothorax.

Cardiac disease is associated with 80% of the patients, 60% of whom have congenital heart disease and 20% have HCM. PS with valve dysplasia and ASD are common, and VSD, TOF, AS, and AVSD are also seen. Noonan

syndrome patietns often present characteristic ECG findings such as left-axis deviation and left anterior thoracic induction rS pattern, which may help in the diagnosis, regardless of the presence or absence of cardiac disease.[168]

Costello syndrome is associated with HCM (60%), PS (40%), and supraventricular tachycardia (50%). CFC syndrome is associated with PS (45%), HCM (40%), ASD (25%), VSD (20%), and convulsion (50%).[166,167]

3. Nonsyndromic Congenital Heart Disease

It is difficult to separate syndromic and nonsyndromic congenital heart disease. **Table 14** shows a number of previously reported genetic variants for isolated congenital heart disease. Transcription factors, intracellular signaling molecules and structural proteins, which are essential for spatiotemporal cardiovascular development, are involved.

3.1 Transcription Factors

Homeobox transcription factor NKX2.5 was reported to be a genetic cause of ASD with AVB.[169] *NKX2.5* gene variants are also detected in VSD, TOF, and HLHS with AVB.[170–175] In the case of *NKX2.5* mutations being detected in congenital heart disease, complete AVB or cardiac sudden death may occur in the future. Zink-finger type transcription factors, GATA4, GATA5, and GATA6 are also essential for cardiac development and their mutations are reported in a variety of congenital heart diseases.[176–183] T-box type transcription factors, including TBX1 and TBX5, are genetic determinant of nonsyndromic cardiac septal defects and TOF, as well as 22q11.2DS and Holt-Oram syndrome.[184–186] *TBX20* variants are also detected in isolated congenital heart disease and cardiomyopathy.[187–189]

3.2 Intracellular Signaling Molecules

The intracellular signaling molecule, NOTCH, is required for differentiation of the cardiac chambers and for valve development. *NOTCH1* variants are reported in left-side obstruction lesions of the heart and in TOF.[190–194] *NODAL* is essential for left-right axis determination during cardiac development and its genetic variants have been detected in isolated congenital heart disease as well as in heterotaxy.[195–199]

3.3 Cardiovascular Structural Proteins

Myocardial sarcomere proteins are indispensable for maintaining myocardial structure and function. Sarcomere gene variants are found not only in cardiomyopathy but also in nonsyndromic congenital heart disease.[200–206] *ELN* variants have been found in nonsyndromic SVAS as well as Williams syndrome.[108]

VIII. Cardiomyopathy and Myocardial Disease

1. Hypertrophic Cardiomyopathy

More than 1,400 different mutations have been reported in more than 11 genes, mainly in genes encoding sarcomere component proteins, as causative genes for the development of hypertrophic cardiomyopathy (HCM). The majority of genetic mutations identified are in the cardiac β-myosin heavy chain gene (*MYH7*) and the cardiac myosin binding protein C gene (*MYBPC3*),[207–209] although involvement of genes encoding proteins other than sarcomeres has also been reported.

Approximately 60% of patients with HCM have a family history of autosomal dominant inheritance of the disease, and causative mutations are identified in genes encoding sarcomere component proteins in 40–60% of the patients

Table 16. Definitive Classifications for Hypertrophic Cardiomyopathy Gene Associations[214]

Gene	Location	Protein	Frequency	Evidence classification
MYBPC3	11p11.2	Myocardial myosin binding protein C	40–45%	Definitive
MYH7	14q11.2-q12	Cardiac myosin heavy chain	15–25%	Definitive
TNNI3	19q13.4	Cardiac troponin I	1–7%	Definitive
TNNT2	1q32.1	Myocardial troponin T	1–7%	Definitive
TPM1	15q22.2	Alpha-tropomyosin	1–2%	Definitive
ACTC1	15q.14	Myocardial alpha-actin	1–2%	Definitive
MYL2	12q24.11	Ventricular myosin regulatory light chain	1–2%	Definitive
MYL3	3p21.31	Ventricular myosin essential light chain	1–2%	Definitive
CSRP3	11p15.1	Cysteine and glycine-rich protein 3, muscle LIM protein	<1%	Moderate
JPH2	20q13.12	Junctophilin	<1%	Moderate
TNNC1	3p21.1	Cardiac troponin C	<1%	Moderate

In addition to the genes listed here, several others have been reported to be associated with hypertrophic cardiomyopathy, although the level of evidence for their etiology varies. (Source: Prepared based on Ingles et al. 2019[214])

Table 17. Recommendations and Levels of Evidence for Genetic Counseling in HCM Probands and Relatives

	COR	LOE
Genetic counseling for all patients with HCM regardless of family screening	I	B
Genetic counseling in genetic testing	I	B
Genetic counseling should be performed by professional trained in genetics with cardiovascular diseases	IIa	B

COR, Class of Recommendation; HCM, hypertrophic cardiomyopathy; LOE, Level of Evidence.

Table 18. Recommendations and Levels of Evidence for Genetic Testing in HCM Patients

	COR	LOE
Genetic testing to confirm the diagnosis in the presence of symptoms and findings of disease suggestive of secondary cardiomyopathy	I	B
Genetic testing in HCM patients, when it enables cascade genetic screening of their family members	I	B
Genetic testing for the assessment of risk of sudden cardiac death in HCM patients	IIb	B

COR, Class of Recommendation; HCM, hypertrophic cardiomyopathy; LOE, Level of Evidence.

Table 19. Recommendations and Levels of Evidence for Family Screening

	COR	LOE
Clinical evaluation of first-degree relatives	I	B
Clinical evaluation for long-term follow-up in first-degree relatives who have the same definite disease-causing mutation as the proband (at least every 1–1.5 years until age 20 and at least once every 5 years after age 20)	I	B
Cascade genetic screening in first-degree relatives of patients with a definite disease-causing mutation	I	B
Discontinuance of ongoing clinical follow-up in genotype-negative relatives of patients with a definite disease-causing mutation (but reevaluate when cardiac abnormalities are noted on medical examinations or when cardiac symptoms appear)	IIa	B
Genetic testing in relatives when the HCM proband does not have a definitive disease-causing mutation. (However, family screening including clinical evaluation and genetic testing is recommended to determine whether the variant identified in HCM proband is disease-causing or not)	III	B

COR, Class of Recommendation; HCM, hypertrophic cardiomyopathy; LOE, Level of Evidence.

with a family history.[210–212] More recently, it has been reported that whole-genome sequencing can increase the detection rate of causative mutations,[213] and attempts are now being made to increase the detection rate. On the other hand, validation of the genes reported as causative genes for HCM has shown that only 8 have strong evidence of definite disease causation (**Table 16**),[214] and thus careful interpretation of the results of genetic testing is required.

Although HCM is classically recognized as a monogenic disease, many patients with this disease have negative family histories and negative genetic testing. Recently, a genome-wide association analysis using a genetic risk score based on common variants reported that an accumulation of common variants may be involved in the pathogenesis of HCM, even in patients without pathological mutations in the sarcomere gene.[215] Thus, HCM pathology may occur even in the absence of a single causative mutation, and future developments will be of interest.

1.1 Usefulness of Genetic Testing in HCM

The following are some of the benefits of genetic testing for patients with HCM (**Tables 17**–**19**). (1) Early diagnosis: Genetic testing can facilitate cascade screening in the family if the causative mutation is identified. Confirmation of the presence or absence of the causative mutation within the family may lead to early diagnosis. If family screening reveals the presence of a causative mutation in an unaffected individual, careful follow-up should be performed. On the other hand, if the unaffected blood relatives do not have the same causative mutation, periodic follow-up should not be necessary.

(2) Diagnosis of secondary cardiomyopathies: It is useful in differentiating and diagnosing inherited secondary cardiomyopathies that present with HCM-like conditions. It is particularly meaningful to diagnose secondary cardiomyopathies for which disease-specific therapies exist. (For details, refer to **Section 4** "**Other Cardiomyopathies**" below.)

(3) Prognostic prediction: Genetic testing may lead to prognostic prediction in patients with HCM. It has been reported that patients with an identified causative mutation in the sarcomere gene have a poorer prognosis than patients without mutation.[216,217] However, the clinical presentation and clinical course often differ among patients with the same causative mutation,[218] and it is currently considered difficult to accurately predict the clinical course based solely on the causative mutation.

When genetic testing is performed, it is also important to predict the pretest probability, and the Mayo clinic has proposed a prediction score for pretest probability using 5 positive predictors (age at diagnosis, maximum wall thickness, presence of a family history of HCM, presence of a family history of sudden death, and presence of left ventricular reverse curve morphology) and 1 negative predictor (presence of hypertension). It has been shown that the detection rate of causative mutations in sarcomere genes increases in correlation with an increase in this score.[219] This score has also been validated as useful in Japanese patients.[220]

Table 20. Genes Reported to Be Associated With Dilated Cardiomyopathy

Gene	Locus	Inheritance pattern
Sarcomere		
cardiac actin (*ACTC*)	15q14	AD
cardiac β-myosin heavy chain (*MYH7*)	14q11	AD
cardiac troponin T (*TNNT2*)	1q32	AD
cardiac troponin I (*TNNI3*)	19q13	AR
cardiac troponin C (*TNNC1*)	3p21	AD
α-tropomyosin (*TPM1*)	15q22	AD
titin (*TTN*)	2q31	AD
cardiac myosin binding protein C (*MYBPC3*)	11p11	AD
cardiac α-myosin heavy chain (*MYH6*)	14q12	AD
myosin light chain kinase 3 (*MYLK3*)	16q11	AD
Z disc and related components		
αB-crystallin (*CRYAB*)	11q22	AD
four and a half LIM protein 2 (*FHL2*)	2q12	AD
muscle LIM protein (*CSRP3*)	11p15	AD
T-cap (telethonin) (*TCAP*)	17q12	AD
cypher/ZASP (*LDB3*)	10q22-q23	AD
α-actinin-2 (*ACTN2*)	1q42-q43	AD
BCL2-associated athanogene 3 (*BAG3*)	10q25-q26	AD
nexilin (*NEXN*)	1p31	AD
myopalladin (*MYPN*)	10q21	AD
CARP (*ANKRD1*)	10q23	AD
Cytoskeleton		
δ-sarcoglycan (*SGCD*)	5q33	AD
β-sarcoglycan (*SGCB*)	4q12	AD
dystrophin (*DMD*)	Xp21	X-linked
desmin (*DES*)	2q35	AD
fukutin (*FKTN*)	9q31	AD
metavinculin (*VCL*)	10q22-q23	AD
laminin-α4 (*LAMA4*)	6q21	AD
integrin-linked kinase (*ILK*)	11p15	AD
lamin A/C (*LMNA*)	1q21	AD
emerin (*EMD*)	Xq28	X-linked
Ion channel/calcium handling		
phospholamban (*PLN*)	6q22	AD
KATP channel (*ABCC9*)	12p12	AD
cardiac sodium channel (*SCN5A*)	3p21	AD
Transcription factor		
Eya4 (*EYA4*)	6q23	AD
Others		
presenilin 1 (*PSEN1*)	14q24	AD
presenilin 2 (*PSEN2*)	1q31	AD
RNA binding motif protein 20 (*RBM20*)	10q25	AD
Mitochondria		
mitochondrial DNA	Mitochondria	Maternal

AD, autosomal dominant; AR, autosomal recessive. (Adapted from Kitaoka H, et al. 2021[210])

1.2 Genetic Testing Issues

In practice, it is often difficult to determine whether the identified variant is the true cause of the disease. In the future, it will be necessary to construct a genetic medical examination system, and methods for determining the pathogenicity of variants, to guarantee the accuracy of genetic testing, and to establish a database of genetic analysis results linked to clinical information.

2. Dilated Cardiomyopathy

Genetic factors are involved in the development of dilated cardiomyopathy (DCM) and linkage analysis of DCM families has revealed the responsible chromosomal loci for lamin (*LMNA*), titin (*TTN*), β-myosin heavy chain (*MYH7*), cardiac sodium channel (*SCN5A*), transcription factor EYA4 (*EYA4*), and RNA-binding motif protein 20 (*RBM20*). Except for them, the "causal mutation" was identified only using the candidate gene approach. Therefore, there are some cases in which the proof of causal relationship between identified mutation and DCM is not sufficient. To date, "causal/pathogenic variants" have been reported for about 40 genes[210] (**Table 20**). The list of causative genes suggests that DCM is a disease category with a heterogeneous mixture of etiologies and pathogenic mechanisms. In addition, because cases of different symptoms and severity have been experienced even in a pedigree in which the same mutation as the proband is shared with the affected relatives, caution should be exercised when interpreting the results of genetic testing.

2.1 Lamin A/C Gene Mutation

Among the causative genes of DCM, the lamin gene (*LMNA*) is the most well-established. Lamin is a protein component of the inner nuclear membrane and regulates mechanosensing, signal transduction, nuclear–cytoplasmic transport, and DNA replication. DCM positive for lamin mutations is typically accompanied by atrioventricular block. Therefore, testing for *LMNA* is strongly recommended in DCM with conduction defects. In Japan, truncation mutations in the lamin gene have been shown to be a risk for conduction defects in patients younger than 50 years, atrial arrhythmias in patients younger than 60 years, and reduced left ventricular ejection fraction (<50%) in patients younger than 60 years.[221] In recent years, cardiomyopathy due to lamin mutations has gained attention as a disease category called arrhythmogenic cardiomyopathy. Lamin mutation positivity is an excellent predictor of ventricular tachyarrhythmia[222] and may be a valid basis for early treatment with an implantable cardioverter defibrillator. In addition, lamin mutation-positive DCM patients show progression to heart failure as well as lethal arrhythmia, and a poor prognosis.[223] Thus, detection of *LMNA* mutations is effective in predicting the prognosis and choosing the best therapeutic strategy for the patient.

2.2 Titin Gene Mutation

Titin is a giant elastic protein that connects the Z-disc and M-line of the sarcomere and acts as a sensor of myocardial tension as well as a regulator of passive tension.[224] *TTN* mutations are the most frequent "causative mutations" of

DCM, and truncating variants that induce the generation of C-terminal defective titin proteins are found in 25% of familial DCM and 18% of sporadic DCM cases.[224] The *TTN* truncating variant is found in 3% of healthy individuals,[225] acting as a genetic risk affecting cardiac function and cardiac remodeling in the general population.[226,227] Furthermore, the involvement of *TTN* truncating variants in the development of secondary cardiomyopathies such as peripartum cardiomyopathy, alcoholic cardiomyopathy, and cancer therapeutics-related cardiomyopathy has become evident. It is recommended to consider the possibility that the "weakly effective" variant may not be sufficient enough to cause cardiomyopathy on its own, and that other genetic factors or environmental risk factors must coexist to cause cardiomyopathy (2-hit hypothesis). Taken together, caution must be exercised when interpreting the clinical significance of the *TTN* truncating variant.

2.3 Determination of the Pathological Significance of Variants

With the use of next-generation sequencing, many variants have been identified in DCM patients. The "variant classification" step, in which the pathological significance of the variants identified by genome sequencing should be accurately determined, contributing to genetic diagnosis, is now the biggest bottleneck. There are a considerable number of variants that cannot be determined as "causative mutations" with a causal relationship to the disease and have to be classified as "variants of uncertain significance" due to the reasons such as small pedigree or inability to analyze the genome of relatives. It is realistic to consider variants with low frequency in the general population, which have been previously reported as DCM causative mutations, or variants that have been shown to co-segregate with clinical phenotype among relatives to be variants that are significant in terms of medical care.

Genetic testing for idiopathic DCM using whole-exome analysis (including previously reported causative genes) is recommended to differentiate it from secondary cardiomyopathies. We recommend Class I for the detection of lamin A/C gene mutations because of the established evidence in Japanese patients. If further studies on other gene mutations are conducted and evidence is established in Japanese patients, a Class I recommendation for them will be considered. As of October 2023, genetic testing and genetic counseling for idiopathic DCM are not reimbursed by insurance (**Tables 21**,**22**).

Table 21. Recommendations and Levels of Evidence for Genetic Testing in Dilated Cardiomyopathy

	COR	LOE
Detect lamin A/C gene mutations	I	B
In families with a confirmed causative mutation in the proband, relatives are tested for the presence or absence of that causative mutation	I	B
Consider detection of causative mutations such as titin, *RBM20*, phospholamban, filamin C, *BAG3*, desmin, desmoplakin, *SCN5A* gene mutations, and sarcomere gene mutations	IIa	B

COR, Class of Recommendation; LOE, Level of Evidence.

Table 22. Recommendations and Levels of Evidence for Genetic Counseling in Dilated Cardiomyopathy

	COR	LOE
Provide genetic counseling for patients with dilated cardiomyopathy regardless of whether cascade screening is performed	I	B
Provide genetic counseling for genetic diagnosis (explanation of the significance and possible risks of genetic diagnosis)	I	B
Consider genetic counseling by experts familiar with the genetics of cardiovascular disease	IIa	B

COR, Class of Recommendation; LOE, Level of Evidence.

3. Arrhythmogenic Right Ventricular Cardiomyopathy

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a disease caused by fibrofatty replacement of the myocardium, mainly in the right ventricle, resulting in ventricular arrhythmias arising from right ventricle and right ventricular enlargement. Recently, it has been classified as an arrhythmogenic cardiomyopathy (ACM).[228] The incidence is approximately 1 in 2,000–5,000 persons, with a slightly higher incidence in males.[229] Because strenuous exercise affects the progression of ARVC[230] in Europe and the USA, it is considered an important cause of sudden death in athletes.

3.1 Diagnosis

The average age of onset of ARVC is in the 30s to 40s, and is often diagnosed by ventricular tachycardia/ventricular fibrillation and associated syncope or cardiopulmonary arrest. The diagnosis of ARVC is based on the 2010 Task Force Criteria.[231] The criteria consist of 6 items, each with major and minor criteria, and the diagnosis is made on a 3-point scale of Definite, Borderline, or Possible, depending on the number of items meeting the criteria. The 6th criterion is family history, which includes not only the presence of an ARVC patient in the family, but also the identification of a causative genetic mutation.

3.2 Causative Genes

The main cause of ARVC is mutations in genes encoding desmosomes (**Figure 3**), which contribute to intercellular adhesion. The desmosome comprises 5 proteins: desmoglein and desmocollin expressed on the plasma membrane, plakophilin and plakoglobin present inside the cell, and desmoplakin. The genes encoding these proteins expressed in the heart are *DSG2*, *DSC2*, *PKP2*, *JUP* and *DSP*, all of which have been reported to cause ARVC.[232] Other genes associated with the sarcomere and cytoskeleton have been reported as causes of ARVC, but a recent review by ClinGen (https://clinicalgenome.org/) classified most of them as having little pathogenic potential.[233] Besides the desmosome-related genes, only *TMEM43* is classified as a definite, and *DES* and *PLN* as moderately pathogenic causative genes.

Although *PKP2* is the major causative gene in Western countries, *DSG2* mutations are more frequent in Japanese ARVC patients. Furthermore, homozygous or compound

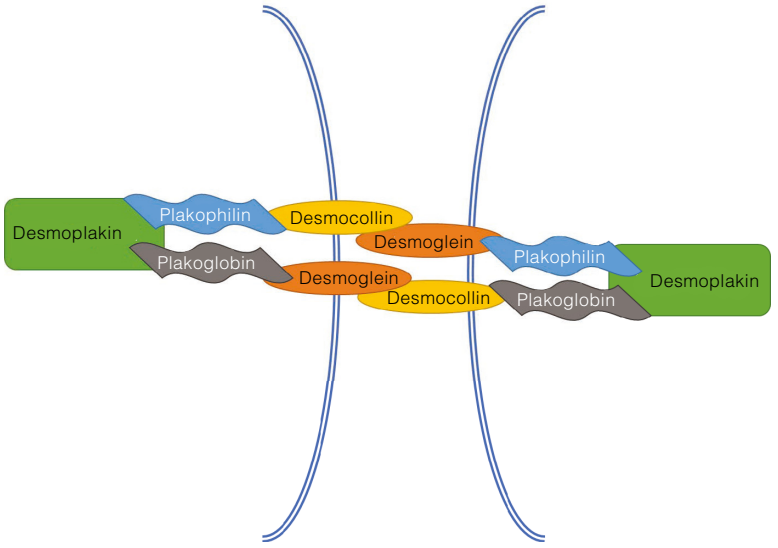

Figure 3. Desmomes contribule as adhesion molecules.

Table 23. Recommendations and Levels of Evidence for Genetic Testing for Arrhythmogenic Right Ventricular Cardiomyopathy

	COR	LOE
Comprehensive genetic testing is recommended for all patients with consistent phenotypic features of an ACM, including cases diagnosed post-mortem, whatever the familial context	I	C
Genetic testing of first-tier definitive disease-associated genes is recommended	I	C
Family history taking for 3 generation by genetic counselors and specialized clinicians	I	C
Interpretation of the results of genetic testing by a team of experts in genetics and cardiology	IIa	C

ACM, arrhythmogenic cardiomyopathy; COR, Class of Recommendation; LOE, Level of Evidence.

heterozygous *DSG2* missense mutations are more common in Japan.[234,235] *DSG2* mutations identified in Japanese ARVC patients require caution in genetic counseling when identified as a secondary finding, because heterozygous carriers are also present in the general population cohort. On the other hand, there are many cases of ARVC that are considered solitary cases but the patients are carriers of *DSG2* homozygous or compound heterozygous mutations. Therefore, genetic testing is useful even in cases of ARVC with no family history and may lead to an early diagnosis of siblings. Specific mutations in *TMEM43* and *PLN* (p.S358L[236] and p.R14del[237]) are frequently identified in some regional populations in Europe and the USA, and are very rarely identified in Japanese.

The Joint Consensus of the Arrhythmia Societies of the Four Continents published in 2022[238] states that genetic testing is useful in ACM for diagnosis, prognostication, and treatment decisions, and recommends genetic testing for diagnosed ACM and screening for definite causative genes (as of 2022: *PKP2*, *DSP*, *DSG2*, *DSC2*, *JUP*, *TMEM43*, *PLN*, *FLNC*, *DES*, *LMNA*) is recommended. Although genetic analysis for ARVC is not reimbursed in Japan as of 2024 February, it is included in the diagnostic criteria for ARVC and is recommended to be performed when ARVC is suspected (**Table 23**).

4. Other Cardiomyopathies

4.1 Secondary Cardiomyopathy

Secondary cardiomyopathies may have a prognosis that differs from that of idiopathic cardiomyopathies and may include disease-specific treatments available for their causes. Therefore, it is very important to make an accurate diagnosis.[239] Secondary cardiomyopathy is thought to be latent in 5–10% of patients presenting with HCM-like morphology,[210] and there are an even wider variety of disease types presenting with DCM-like morphology. There are certain diseases in which genetic testing plays an important role in the diagnosis of a secondary cardiomyopathy. Before genetic testing, it is also important to evaluate clinical information such as history, family history, extracardiac symptoms, and laboratory findings to increase the pretest probability of genetic testing. **Table 24** lists the hereditary secondary cardiomyopathies with HCM- or DCM-like morphology. Genetic testing should be performed for definitive diagnosis in patients with suspected hereditary secondary cardiomyopathies (**Table 25**).

4.2 Hereditary Transthyretin Amyloidosis

This disease is caused by an abnormality in the *TTR* gene, which encodes transthyretin (TTR), with an autosomal dominant inheritance.[240] More than 150 mutations have been reported and Val30Met is known to be the most frequent representative mutation.[241,242] The age of onset in Japan was considered to be ≤50 years of age, but now the age of onset and prognosis vary depending on whether or not the disease occurs in local areas (Kumamoto and Nagano prefectures are endemic areas), and whether or not

Table 24. Hereditary Secondary Cardiomyopathies and Phenotypic Manifestations		
Hereditary Secondary Cardiomyopathy With HCM-Like Manifestations	Infiltrative disease	Hereditary ATTR amyloidosis
	Metabolic disease	Fabry disease Danon disease Pompe disease PRKAG2 cardiomyopathy
	Neuromuscular disease	Friedreich's ataxia
	Mitochondrial disease	MELAS, MERRF
	Malformation syndrome	Noonan syndrome
Hereditary Secondary Cardiomyopathy With DCM-Like Manifestations	Infiltrative disease	Hereditary ATTR amyloidosis (end-stage)
	Metabolic disease	Fabry disease (end-stage) Pompe disease Hurler syndrome, Hunter syndrome
	Systemic disease	Mitochondrial disease, muscular dystrophy, laminopathy

it is due to a Val30Met mutation.[243–245] It is also reported that cardiac syndrome complications are rarely seen in the patients with the early-onset type in the endemic areas, but vary from 2% to 4% for those with Val30Met in the non-aggregating areas to 35% for those with non-Val30Met type in the non-aggregating areas. In addition to hereditary forms, wild-type forms of TTR cardiac amyloidosis exist. With recent improvements in diagnostic techniques, the diagnosis of wild-type amyloidosis is increasing, and genetic testing is essential to distinguish between hereditary and wild-type amyloidosis for a definitive diagnosis.[246] (For more information on this disease, see also the Guidelines for the treatment of cardiac amyloidosis[246]).

Table 25. Recommendation and Level of Evidence for Genetic Testing in Secondary Cardiomyopathies		
	COR	LOE
Genetic testing for definitive diagnosis of hereditary secondary cardiomyopathy	I	B

COR, Class of Recommendation; LOE, Level of Evidence.

4.3 Fabry Disease

This is an X-linked genetic disorder caused by mutations in the *GLA* gene encoding α-galactosidase, and >1,000 variants of *GLA* have been reported.[247] The disease occurs when α-galactosidase activity is reduced and Gb3 accumulates in the tissues.[248,249] Those with symptoms such as acroparesthesia, hypohidrosis and corneal opacities from childhood, as well as those showing cardiac, renal, and cerebrovascular dysfunction, have the so-called classical type of Fabry disease. However, there is a late-onset form of Fabry disease with a relatively late age of onset in middle-age and symptoms are limited to the heart.[250] In male patients, the diagnosis can be made by demonstrating a decrease in plasma or leukocyte α-galactosidase activity. Female patients, on the other hand, are heterozygous, so clinical symptoms can vary from mild to severe phenotypes, and enzyme activity can be normal.[251] Therefore, genetic testing may be useful in female patients, as some cases are difficult to diagnose based on enzyme activity alone. Currently, enzyme replacement therapy and pharmacological chaperone therapy are available as disease-modifying therapies for this disease. Genetic testing is also important in the selection of therapy, as pharmacological chaperone therapy is used only when the patient has a potentially valid mutation (amenable mutation).

4.4 Mitochondrial Disease

The cardiac lesions can be HCM-like, DCM-like, or restrictive cardiomyopathy-like.[252,253] All mitochondrial DNA is passed from mother to child. Therefore, when the disease is caused by mutations in mitochondrial DNA, it takes the form of matrilineal inheritance. More than 250 mitochondrial disease-related nuclear gene mutations have been reported so far, and all forms of inheritance have been reported, including autosomal dominant inheritance, autosomal recessive inheritance, and X-linked inheritance. It is also known that childhood is the most common age of onset of the disease when it is caused by nuclear gene mutations.[254] Based on the Ministry of Health, Labour and Welfare MHLW's diagnostic criteria,[255] some cases are difficult to diagnose based on clinical findings alone, without genetic testing. Therefore, genetic testing should be considered in cases in which mitochondrial disease is suspected based on clinical findings. Genetic testing for some mitochondrial diseases is reimbursed by insurance.

4.5 Pompe Disease

Pompe disease is an autosomal recessive inherited disease in which an abnormality in the *GAA* gene results in reduced or defective activity of acid α-glucosidase, which causes glycogen storage in lysosomes.[256] This disease is characterized by cardiomyopathy, muscle weakness and hepatomegaly. In classical Pompe disease, which is a complete enzyme deficiency, a rapidly progressive cardiomegaly develops. Untreated, the child dies before the age of 1 year due to respiratory and cardiac failure. In late-onset Pompe disease with residual enzyme activity, cardiomyopathy is less common.[257,258]

4.6 Danon Disease

Danon disease is an X-linked disorder caused by an

abnormality in the lysosome-associated membrane protein 2 (*LAMP2*) gene. It is characterized by a triad of skeletal muscle disorders, cardiomyopathy, and intellectual disability.[259] Males hemizygous for *LAMP2* abnormality have an earlier onset disease and more severe prognosis than female patients.[260,261]

4.7 Muscular Dystrophy

Duchenne and Becker muscular dystrophies have an X-linked form of inheritance and are caused by dystrophin (*DMD*) gene abnormalities. Both diseases show skeletal muscle weakness and a DCM-like cardiomyopathy.[262]

IX. Arrhythmia Diseases

1. Long QT Syndrome

1.1 Clinical Diagnosis

Long QT syndrome (LQTS) is a disorder characterized by prolonged QT interval and abnormal T waveform on ECG, resulting in polymorphic ventricular tachycardia called torsade de pointes (TdP), which can cause syncope attacks and sudden cardiac death. Congenital LQTS is clinically diagnosed by a Schwartz score ≥3.5 points[263] or a QTc ≥500 ms on repeated 12-lead ECG recordings, and can also be diagnosed in the presence of pathological variants in LQTS-related genes (**Table 26**).[264] Congenital LQTS is relatively common among the hereditary arrhythmic disorders, with a prevalence of approximately 1 in 2000 reported in Europe and the USA.[265] In Japan, approximately 1 in 1,100 infants are diagnosed with congenital LQTS during infant health examinations.[266] In addition to congenital LQTS, there is acquired (secondary) LQTS, in which QT prolongation and TdP are induced by drugs, hypokalemia, or bradycardia, even though the baseline QT interval is nearly normal or borderline. The same genetic abnormalities as congenital LQTS are found in nearly 30% of patients with acquired LQTS.[267] Therefore, the distinction between them is blurring.

1.2 Genetic Background

Congenital LQTS can be divided into 2 types of inheritance: Romano-Ward syndrome (RWS), an autosomal dominant form, and Jarvell and Lange-Nielsen syndrome (JLNS), an autosomal recessive form with congenital deafness, although most cases of congenital LQTS are RWS. The causative gene has been identified in 70–80% of patients with a clinical diagnosis of congenital LQTS.[268] In most cases (90%) the affected genes are the potassium channel genes *KCNQ1* and *KCNH2*, and the sodium channel gene *SCN5A*. In addition to these 3 genes, *CALM1-3* is also an important causative gene for congenital LQTS[43,269] (**Table 26**). In addition, *TRDN* is a possible cause in homozygous cases, *KCNQ1* and *KCNE1* are thought to cause JLNS, and *KCNE1* and *KCNE2* are thought to cause acquired LQTS.

Other phenotypic complications besides QT prolongation include Timothy syndrome (TS), in which some variants of the calcium channel gene *CACNA1C*, such as G406R and G402S, are associated with characteristic facial features, syndactyly, bradycardia, and congenital heart malformations, and *KCNJ2* is an important cause of Andersen-Tawil syndrome with periodic tetraplegia, marked U waves and skeletal malformation Other pathological variants of *CACNA1C* also cause non-TS LQTS, but the evidence is

Table 26. LQTS Causative Genes (From EHRA/HRS/APHRS/LAHRS Expert Consensus Statement on Genetic Testing)[269]

Gene	Location of chromosome	Phenotype	Protein (dysfunctional)	Frequency	ClinGen Classification
KCNQ1	11p15.5	LQTS, JLNS	IKs channel (↓)	40–55%	Definitive
KCNH2	7q35-36	LQTS	IKr channel (↓)	30–45%	Definitive
SCN5A	3p21-p24	LQTS	Nav1.5 channel (↑)	5–10%	Definitive
CALM1	14q32.11	LQTS	L-type Ca channel (↑)	<1%	Definitive
CALM2	2p21	LQTS	L-type Ca channel (↑)	<1%	Definitive
CALM3	19q13.32	LQTS	L-type Ca channel (↑)	<1%	Definitive
TRDN	6q22.31	Recessive LQTS	L-type Ca channel (↑)	<1%	Strong
KCNE1	21q22.1	LQTS, JLNS, a-LQTS	IKs channel (↓)	<1%	Strong in aLQTS, Definitive in JLNS
KCNE2	21q22.1	a-LQTS	IKs channel (↓)	<1%	Strong in aLQTS
KCNJ2	17q23	ATS	IK1 channel (↓)	<1%	Definitive in ATS
CACNA1C	12p13.3	TS, LQTS	L-type Ca channel (↑)	<1%	Definitive in TS, moderate in LQTS

Abnormal function: (↓) loss of function or (↑) gain of function. a-LQTS, acquired long QT syndrome; ATS, Andersen-Tawil syndrome; JLNS, Jervell and Lange-Nielsen syndrome; RWS, Romano-Ward syndrome; TS, Timothy syndrome. (Adapted from Wilde AAM, et al. 2022[238,269])

Table 27. Recommendations and Levels of Evidence for Genetic Testing for Long QT Syndrome

	COR	LOE
Molecular genetic testing for definitive disease-associated genes (currently *KCNQ1*, *KCNH2*, *SCN5A*, *CALM1*, *CALM2*, and *CALM3*) should be offered to all index patients with a high probability diagnosis of LQTS, based on examination of the patient's clinical history, family history, and ECG characteristics obtained at baseline, during ECG Holter recording and exercise stress test	I	C
Analysis of specific genes should be offered to patients with a specific diagnosis as follows: *KCNQ1* and *KCNE1* in patients with Jervell and Lange-Nielsen syndrome, *CACNA1C* in Timothy syndrome, *KCNJ2* in Andersen-Tawil syndrome, and *TRDN* in patients suspected to have triadin knockout syndrome	I	C
Variant-specific genetic testing is recommended for family members and appropriate relatives following the identification of the disease-causing variant	I	C
Analysis of *CACNA1C* and *KCNE1* may be performed in all index patients in whom a cardiologist has established a diagnosis of LQTS with a high probability, based on the patient's clinical history, family history, and ECG characteristics obtained at baseline, during ECG Holter recording and exercise stress test	IIa	C
Molecular genetic testing for definitive disease associated genes (currently *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNE2*) should be offered to all patients with acquired LQTS*	IIa	C
Genetic testing for all or definite genes responsible for LQTS in asymptomatic LQTS patients with Schwartz score: 1.5–3.0 or 12-lead ECG showing 480≥QTc>460 ms (minors) or 500≥QTc>480 ms (adults)	IIb	C

*TdP induced by drugs, low potassium, bradycardia, etc. and QTc >440 ms (male), >450 ms (female) on ECG without influence of suspect drugs, etc. COR, Class of Recommendation; LOE, Level of Evidence.

somewhat weak.[270]

The penetrance of congenital LQTS is not 100%, and some cases have pathological variants *without* QT prolongation on ECG. In addition, the degree of QT prolongation and the presence or absence of symptoms differs for each variant of the same gene. Ion channel dysfunction differs by genotype, with loss-of-function variants of *KCNQ1*, *KCNH2*, and *KCNJ2*, and gain-of-function variants of *SCN5A* and *CACNA1C* (**Table 26**). In *SCN5A*, the gain-of-function variant shows the phenotype of LQTS, whereas the loss-of-function variant results in Brugada syndrome, sick sinus syndrome, and progressive cardiac conduction defects. Furthermore, some variants, for example, *SCN5A*-E1784K, have both gain- and loss-of-function features, and even within the same family, the phenotypes of LQTS and Brugada syndrome, conduction defects, etc. may coexist.[271]

Pathological variants are diverse, including missense, nonsense, and frameshift mutations. Although the type of variant is not always associated with disease severity, some variants (e.g., *KCNQ1*-A341V and *SCN5A*-V411M) are known to increase the risk of arrhythmic events.

1.3 Indications for Genetic Testing

The indication for genetic testing for congenital LQTS is based on the QT interval on 12-lead ECG, with initiative testing in cases of significant QT prolongation (children and adolescents: QTc ≥480 ms, adults: QTc ≥500 ms) and testing in cases of a high possibility of QT prolongation (children and adolescents: 480 >QTc ≥460 ms, adults: 500 >QTc ≥480 ms).[264] On the other hand, the recent 4-continent (EHRA/HRS/APHRS/LAHRS) statement[269] strongly recommended genetic testing for patients with definite LQTS and a Schwartz score ≥3.5 or suspected JLNS, TS, Andersen-Tawil syndrome, or Triadin syndrome. However, congenital LQTS is not always phenotypic, and genetic testing is also recommended for patients in whom the cardiologist strongly suspects LQTS. Genetic testing is strongly recommended for genes that are certain to cause LQTS (*KCNQ1*, *KCNH2*, *SCN5A*, *CALM1*, *CALM2*, and *CALM3*), but other LQTS-related genes (*KCNE1*, *KCNE2*, *CACNA1c*, *TRDN*) may also be tested on a case-by-case basis (**Table 27**).

Patients with suspected LQTS (Schwartz score: 1.5–3.0 or 12-lead ECG QTc: 460–480 ms [children and adolescents], 480–500 ms [adults]) may also be considered for genetic testing for definite LQTS genes. In addition to LQTS, it is also important to differentiate catecholaminergic polymorphic ventricular tachycardia (CPVT) as a cause of loss of consciousness attacks, ventricular tachycardia, and ventricular fibrillation during exercise or stress in younger patients. LQTS and CPVT are known overlap, so *RYR2* may be added to the list of target genes in young patients with suspected LQTS with symptoms (syncope attacks, ventricular fibrillation, etc.).[268]

In severe cases of LQTS, seizures may occur as early as age 0. Genealogical studies have been recommended for LQTS-causing gene mutations identified in the proband,[272] and the revised guidelines also recommend prophylactic genetic testing for children in the family, regardless of age.

1.4 Genetic Testing

LQTS is covered by insurance as a genetic test (D006-4) with extremely complex processing (8,000 points). In accordance with the revision of the Medical Service Act, genetic testing for LQTS as an insured procedure can only be performed in laboratories or branch laboratories within medical institutions that meet the standards for quality and accuracy control, or in sanitary laboratories that meet the standards. In principle, genetic testing can be conducted only once per patient, but if it is performed more than once, the medical necessity of the test must be stated in the relevant section of the medical fee schedule.

1.5 Interpretation of Results and Genetic Counseling

In LQTS, the genotype and variant type can infer the recommendations for the patient's lifestyle, risk assessment,

medications, and prognosis. Therefore, it is very important to understand the clinical usefulness of the genetic test before testing. If the result cannot be explained correctly to the patient and/or family, it should not be readily performed. In any case, the test should be performed only after written informed consent has been obtained.

In genetic testing for LQTS, there are many novel variants that have not been previously reported or variants of uncertein significance (VUS). In such cases, the consistency of the genotype with the phenotype within the family is informative. When correct understanding between VUS and disease phenotype is difficult, having a genetic expert panel with extensive experience for genetic testing for LQTS would be helpful. Furthermore, if genetic testing shows no pathological variants, it does not completely rule out LQTS.

Genetic testing for siblings and blood relatives requires special attention because LQTS does not necessarily have 100% phenotypic penetrance (i.e., some carriers do not show QT prolongation on a normal resting ECG). However, even with a seemingly normal ECG, QT prolongation in carriers of pathological variants of LQTS genes may be manifested by sudden stress, drugs, etc., and may result in fatal arrhythmias, so family screening should be performed to diagnose carriers if possible. Cardiac events of LQTS are generally more common during the school years and adolescence, so testing should be initiatively recommended, especially for younger siblings (**Table 27**).

2. Other Arrhythmias

In addition to congenital LQTS, other inherited arrhythmias include Brugada syndrome (BrS), CPVT, congenital short QT syndrome (SQTS), which produces lethal ventricular arrhythmias, progressive cardiac conduction disease (PCCD) and familial bradycardia, which produces bradycardia, and atrial fibrillation (AF) have also been reported to be hereditary.

2.1 Brugada Syndrome

BrS is characterized by ST-segment elevation in the right side precordial leads on the resting ECG (Brugada-type ECG), which is more common in men of mature age. In 1998, *SCN5A*, which encodes a cardiac sodium channel, was reported as the causative gene,[273] and since then, more than 20 causative genes have been identified. However, the most recent ClinGen study has identified *SCN5A* as the only definite causative gene.[274]

SCN5A is also the causative gene for LQTS type 3 (LQT3); *SCN5A* mutations identified in BrS are loss-of-function type mutations, which cause ventricular fibrillation (VF) by reducing the cardiac sodium channel current. On the other hand, *SCN5A* mutations identified in LQT3 are gain-of-function type mutations and cause QT prolongation by increased delayed sodium current. However, some *SCN5A* mutations, such as p.E1784K, have both loss- and gain-of-function features.[275] Therefore, it is important to observe not only the identified mutations but also the clinical picture in detail.

Although the frequency of Brugada-type ECG in the general population is as high as 0.1–0.3%, the frequency of asymptomatic patients developing VF is approximately 0.3% per year, making it important to identify patients who present with Brugada-type ECG and are at high risk. Recent reports suggest that *SCN5A* mutations are useful in predicting events.[276] Furthermore, if the mutation identified is a loss-of-function type, its predictive power is improved.[277] Although there is currently no insurance coverage for BrS in Japan, international recommendations for genetic testing are summarized in **Table 28**.[238]

The identification rate of genetic mutations in BrS is low: approximately 30% in Europe and 10% in Japan.[276] However, even in families in which no significant *SCN5A* mutations have been identified, the disease may be hereditary, suggesting the possibility of multifactorial inheritance. A genome wide association study (GWAS) was conducted to elucidate the phenomenone,[278] and published in 2013, which identified single-nucleotide polymorphism (SNPs) associated with the *SCN5A-10A* region in Chr. 3 and the *HEY2* region in Chr. 6 in 312 European BrS patients, and these SNPs were also found in 208 Japanese BrS patients. In 2022, a GWAS was performed in 2,820 European BrS patients, and 10 new loci were identified in addition to the previously reported loci. This means that BrS has both a monogenetic aspect of *SCN5A* and a polygenetic aspect. The identified SNPs are weighted according to their association with BrS, and the polygenic risk score (PRS) will be used to assess the risk of developing VF in the future.

Table 28. Recommendations and Levels of Evidence for Genetic Testing for Brugada Syndrome

	COR	LOE
Genetic testing with sequencing of *SCN5A* is recommended for an index case diagnosed with BrS with a type I ECG in standard or high precordial leads occurring either spontaneously or induced by sodium-channel blockade in the presence of supporting clinical features or family history	I	C
Variant-specific genetic testing is recommended for family members and appropriate relatives following the identification of the disease-causative variant	I	C

BrS, Brugada Syndrome; COR, Class of Recommendation; LOE, Level of Evidence.

2.2 Catecholaminergic Polymorphic Ventricular Tachycardia

CPVT is characterized by bidirectional or polymorphic ventricular tachycardia triggered by exercise or emotional stress. The major causative gene is *RYR2*, which encodes a cardiac ryanodine receptor channel, and >90% of causative gene mutations identified in patients with CPVT are in *RYR2*. Mutations in *RYR2* cause excessive Ca^{2+} release from the sarcoplasmic reticulum, resulting in delayed after-depolarization and ventricular arrhythmias. The genetic form of CPVT differs according to the gene, with *TRDN* and *TECRL* being autosomal recessive (AR), and *CASQ2* being both AR and autosomal dominant (AD), although previously only AR was considered to be involved.[279] CPVT caused by *TRDN* and *TECRL* is very rare and has never been reported from Japan.

For CPVT, an evaluation of causative genes was also performed in ClinGen.[280] *RYR2* in AD, and *CASQ2*, *TRDN*, and *TECRL* in AR are considered certain, *CALM1*,

CALM2, *CALM3* and *CASQ2* in AD are considered moderate, and *KCNJ2* is disputed. Phenotypically, *RYR2* and *CASQ2* mutation carriers rarely produce the QT prolongation seen in the analogous disease LQTS, but CPVT patients caused by mutations in other genes may show QT prolongations. In particular, *KCNJ2* is the causative gene in Andersen-Tawil syndrome, in which premature ventricular contractions (PVCs) occur frequently even at rest, a phenotype that may differ from that of CPVT, in which PVCs occur frequently only during exercise.

About half of the *RYR2* mutations identified in CPVT are de novo mutations.[281] *RYR2* mutations are identified when the clinical presentation is typical of CPVT, even if is no family history. Mosaic mutations in *RYR2* can also cause the disease.[282] When there are multiple CPVT patients in the same family but no significant mutation is identified in the parents, mosaicism should be suspected and testing should be recommended.

As of February 2024, genetic testing for CPVT is not covered by insurance in Japan, but its usefulness is recognized in European and American guidelines and other guidelines, which recommend screening for causative genes, including *RYR2*, when CPVT is clinically diagnosed[238] (**Table 29**).

Table 29. Recommendations and Levels of Evidence for Genetic Testing for Catecholaminergic Polymorphic Ventricular Tachycardia

	COR	LOE
In any patient satisfying the diagnostic criteria for CPVT, molecular genetic testing is recommended for the currently established definite/strong evidence CPVT-susceptibility genes: *RYR2*, *CASQ2*, *CALM1-3*, *TRDN*, and *TECRL*	I	C
Variant-specific genetic testing is recommended for family members and appropriate relatives following the identification of the disease-causative variant	I	C
Predictive genetic testing in related children at risk of inheriting a P/LP variant is recommended from birth onward (any age)	I	C

COR, Class of Recommendation; CPVT, catecholaminergic polymorphic ventricular tachycardia; LOE, Level of Evidence; P/LP, pathogenic/likely pathogenic.

2.3 Congenital Short QT Syndrome

SQTS is a relatively new disease, first described in 2000, characterized by short QT interval and VF on ECG, which is often associated with AF. The criterion for QT shortening is defined in the consensus report in 2013 as ≤330–340 ms, or ≤360 ms if the patients carry a pathogenic variant or has family history of SQTS.[283] The SQTS follows an AD form, and the first causative genes *KCNH2*[284] and *KCNQ1*[285] were reported in 2004, and *KCNJ2*[286] in 2005. In addition, *SLC4A3*[287] was reported in 2017 as a novel causative gene; the L-type calcium channel-related genes (*CACNA1C*, *CACNA2D1*, and *CACNB2*) were also reported as causative genes, but were ruled negative in ClinGen published in 2022.[280]

Among the causative genes, *KCNH2*, *KCNQ1*, and *KCNJ2* encode potassium channels and are also causative genes for congenital LQTS. Mutations identified in LQTS are loss-of-function type, whereas those identified in SQTS are gain-of-function. *SLC4A3*, on the other hand, encodes a Cl-HCO_3 exchanger (anion exchange protein 3, AE3) that is expressed on the plasma membrane and is thought to be responsible for QT shortening due to changes in pH.[287] However, only 1 mutation (p.R370H) in 2 families has been identified so far, and future studies are needed to determine the detailed pathogenic mechanism.

2.4 Progressive Cardiac Conduction Disease

PCCD is an age-related conduction defect that can range from prolonged PR interval and widening of the QRS to various levels of atrioventricular block. The causative mutations vary depending on the presence or absence of cardiomyopathy; PCCD caused by mutations in *SCN5A* and *TRPM4* is not associated with cardiomyopathy. However, *SCN5A* mutations can cause dilated cardiomyopathy (DCM). An important causative gene in PCCD associated with cardiomyopathy is *LMNA*. Although lamin cardiomyopathy caused by *LMNA* mutations is a DCM with a poor prognosis,[210] the initial symptoms are often bradycardia and atrioventricular block. Therefore, screening for *LMNA* is recommended when lamin cardiomyopathy is suspected after detailed family history taking. There are other PCCDs associated with various cardiomyopathies; see the previous guideline for details.[264]

2.5 Atrial Fibrillation

AF is the most prevalent arrhythmia, and the heritability of AF must be considered as a monogenic disease with family history, and as a multifactorial disease with increasing incidence with age.

Familial AF is often caused by mutations in *SCN5A* or *KCNQ1* and is often associated with BrS or congenital SQTS. It can also be associated with various cardiomyopathies.[238]

On the other hand, for AF as a multifactorial disease, many disease susceptibility loci have been identified by GWAS, and this information is useful not only for predicting the risk of developing AF, but also for predicting the response to drugs[288] and the risk of recurrence after ablation therapy.[289]

A region near *PITX2* at 4q25 has been strongly correlated in AF since the early days of GWAS.[290] *PITX2* is a transcription factor that is involved in cardiac asymmetry and influences gene expression, including many ion channels.[288]

A GWAS study of 8,180 Japanese patients with AF published in 2017 found that in addition to 7 previously reported regions, 9 new loci including *KCND3*, and *HAND2*, were found to be associated with AF.[291] In 2018, an international collaboration study including Japanese, Westerners and Americans identified 97 disease susceptibility loci involved in cardiac development, electrophysiological function, myocardial contraction and structural function, contributing to the understanding of the biological mechanisms underlying the development of AF.[292] Subsequent study in 2023 further increased the number of Japanese subjects and identified 31 disease susceptibility loci, including 5 new regions. Combined with studies conducted in Europe, 150 disease susceptibility loci were identified, including 33 new regions.[293]

The PRS for AF has been used to improve the accuracy

of predicting disease onset in the absence of clinical risk factors[294–296] and to predict early detection of disease.[297,298] Some usefulness of the PRS has been reported, especially among Westerners (see General Discussion 1.1.3 for more information on the PRS). However, the frequency of individual variants differs by race, and race-specific disease susceptibility loci were identified in the GWAS used to calculate the PRS. In other words, the question is whether the PRS calculated from a Western GWAS can be applied to the Japanese population as is. Therefore, a 2023 study examined whether the PRS calculated from a race-specific GWAS or a cross-racial GWAS would perform better in the Japanese population. First, the PRS calculated from a Japanese-only GWAS was compared with the PRS calculated from a GWAS covering only Westerners, and it was shown that the PRS derived from the Japanese-only GWAS performed significantly better for Japanese. Next, a comparison was made with the PRS calculated by combining the results of the Japanese GWAS with those of the European and American GWAS. The results showed that the PRS calculated from the cross-racial GWAS was superior to the PRS calculated from the Japanese GWAS alone. Furthermore, this PRS for AF was associated not only with cardiogenic stroke, but also with cardiovascular and stroke deaths.[293]

Thus, it has been suggested that a PRS calculated from GWAS of AF may be useful as a risk assessment for unaffected individuals. However, as of 2023, the PRS has not been calculated clinically, and no preventive interventions for AF based on the PRS have been implemented.[299]

X. Aortic/Arterial Diseases and Connective Tissue Disorders

1. Syndromic Familial Aortic Diseases

1.1 Marfan Syndrome

1.1.1 Disease Concept

Marfan syndrome is an autosomal-dominant inherited disorder with predominant phenotypes in the musculoskeletal, ocular, and cardiovascular systems.[300,301] The incidence of Marfan syndrome is estimated to be at least 1 in 5,000, with no sex difference.

1.1.2 Genetic Abnormalities

Fibrillin-1 (*FBN1*) mutation causes this syndrome.[302,303] Analysis of genetically engineered mice[304] and studies of genetic mutations in related diseases suggest that chronic activation of the TGF (transforming growth factor) β-smad signaling pathway may be responsible for the pathogenesis of these diseases. Although many kinds of genetic variants have been reported in the literature, missense variants at cysteine residues involved in protein disulfide bonds are considered to have high pathological significance.[305] In the case of neonatal Marfan syndrome, which often develops early in the neonatal period and demonstrates a severe phenotype, pathogenic variants are often found in exons 23–32.[306] A recent report from Japan demonstrated that the patients with haplo-insufficient type *FBN1* mutations showed faster aortic aneurysm expansion, compared with those with dominant-negative type *FBN1* mutations.[306] However, among the latter dominant-negative group, mutations related to cysteine residues in exons 25–36 and 43–49 are reported to progress faster.[307]

In Japan genetic diagnosis of Marfan syndrome is currently reimbursed by insurance, and a panel analysis is often performed not only for *FBN1*, but also for the series of genes in the *TGF*-smad family and for genes related to familial aortic aneurysm and dissection (FTAAD), as described later. Genetic diagnosis is especially important in children and young adults, because the phenotypes of Marfan syndrome and related disorders are not always consistent, and therefore, early genetic diagnosis and intervention is highly recommended. However, it should be noted that the absence of mutations does not rule out such hereditary aortic diseases (connective tissue disorder).

1.1.3 Diagnostic Criteria

In 2010, a revised Ghent criteria (revised Ghent nosology)[308] was proposed, and more emphasis was placed on aortic phenotypes,[309,310] and a Z score of 2 or 3 higher in the aortic diameter is considered to be significantly dilated), lens deviation, family history and genetic abnormalities. In addition, the following factors are also considered important: ectopia lentis, family history, and genetic abnormality. The additional clinical manifestations, including physical and orthopaedic features, mitral valve prolapse, striae cutis, pneumothorax, and lumbosacral dural ectasia are also considered diagnostic (**Table 30**).

An outline of the criteria is presented.

Diagnose Marfan syndrome if any of the following are met.

If there is no family history

(1) Aortic enlargement and ectopia lentis
(2) Aortic enlargement and *FBN1* abnormality
(3) Aortic enlargement and systemic score ≥7
(4) Ectopia lentis and *FBN1* mutation associated with aortic root dilatation

If there is a family history

(1) Ectopia lentis and family history
(2) Systemic score ≥7 and family history
(3) Aortic dilation + family history

The systemic score is defined as significant if ≥7 points are added for the following manifestations:

- Positive wrist sign and positive thumb sign: 3 points
  1 point for one side only
- Pectus carinatum deformity: 2 points
  1 point for pectus excavatum or chest asymmetry
- Hindfoot deformity: 2 points, flat foot only: 1 point
- Pneumothorax: 2 points

Table 30. Recommendation and Level of Evidence for Genetic Testing for Marfan Syndrome (MFS)

	COR	LOE
Genetic testing to confirm the diagnosis of MFS	I	B

COR, Class of Recommendation; LOE, Level of Evidence.

- Dural dilation: 2 points
- Acetabular protrusion: 2 points
- Decreased supranodal to infranodal ratio and increased finger pole length/stature ratio (without severe scoliosis): 1 point
- Scoliosis or thoracoabdominal kyphosis: 1 point
- Impairment of elbow extension: 1 point
- Facial features: 1 point (≥3 of the following: long head, zygomatic hypoplasia, eye socket, mandibular retraction, or external downward sloping of the eyelid cleft)
- Skin striae: 1 point
- Myopia greater than −3D: 1 point
- Mitral valve prolapse: 1 point

1.1.4 Cardiovascular Abnormalities

As indicated in the diagnostic criteria, enlarged sinus of Valsalva, aortic dissection and aortic aneurysm (especially in the thoracic region) are the main phenotypes, but mitral valve prolapse is also frequent. Rarely, mitral valve annulus calcification and pulmonary artery dilation are also observed.

1.1.5 Clinical Examination and Evaluation

For diagnosis and follow up, echocardiography, computed tomography (CT) or magnetic resonance imaging (MRI) are recommended. MRI is particularly advantageous because there is no radiation exposure. If there is concern about the progression of aortic aneurysm or enlargement, the frequency of examination should be increased. Also it is essential to evaluate the systemic phenotypes: ophthalmologic examination and CT/MRI for evaluation of not only cardiovascular abnormalities but also lung abnormalities, skeletal manifestations, and the presence of dural ectasia. There are ethnic phenotypic differences between Caucasians and Japanese, with a relatively low frequency of physical phenotypes and a relatively high incidence of pneumothorax in Japanese patients.[311,312]

1.1.6 Disease Progression and Serious Cardiovascular Events

The progression of aortic aneurysm and aortic root enlargement should be noted. Enlarged vessel diameter is a risk factor for aortic dissection, but dissection may occur even in the absence of vascular enlargement. Aortic regurgitation with aortic root enlargement or mitral regurgitation with mitral valve deviation may be accompanied by progression of mitral regurgitation, which may be associated with decreased left heart function and heart failure, so caution is required.

1.1.7 Treatment and Prevention

Beta-blockers and angiotensin-receptor blockers are effective in preventing the progression of aortic enlargement[313,314] and in pediatric patients both drugs are administered from childhood. In this regard, genetic diagnosis may be useful in deciding whether or not to provide therapeutic intervention not only in adults but also in children. Aortic enlargement, dissection, and aneurysm are treated surgically.[315] In cases of aortic root enlargement or dissection including aortic valve insufficiency, Bentall surgery with prosthetic valve replacement has been conventionally performed, but autologous valve-sparing aortic root reimplantation has become popular and is often selected.

Exercise restrictions are to avoid heavy lifting, exercise requiring breath holding, and strenuous exercise, especially when there is aortic enlargement or with dissection.

With regard to pregnancy and delivery, a Valsalva sinus diameter <40 mm is considered acceptable, but ≥44 mm is a contraindication to pregnancy.[316] In female patients, it is essential to educate them about the permissibility of pregnancy, especially from puberty. For more information, see Guidelines for the Indication and Management of Pregnancy and Childbirth in Patients with Cardiac Disease (revised 2018).

1.1.8 Preimplantation Diagnosis

Preimplantation diagnosis for this syndrome is not permitted in Japan.

1.1.9 Prognosis

Recent surgical outcomes, including remote outcomes, have been excellent, and it seems possible for patients to survive beyond the age of 70 years with appropriate medical/surgical interventions and daily life management, even if cardiovascular lesions develop. Reoperation due to progression or development of vascular lesions in other parts of the body is common, and the 10-year reoperation avoidance rate is said to be approximately 60%, with a tendency toward reoperation or additional surgery for residual lesions, especially when aortic dissection is involved. Careful follow-up and appropriate therapeutic intervention are important.

1.1.10 Related Diseases and Differential Diagnoses

Other symptomatic aortic aneurysms and dissections include Marfan syndrome-related diseases with genetic abnormalities of the TGF-β receptor/ligand and smad molecules and vascular Ehlers-Danlos syndrome (EDS). Nonsyndromic familial aortic aneurysms and dissections without other systemic findings may also occur. Please refer to the appropriate section.

1.2 Loeys-Dietz Syndrome

1.2.1 Disease Concept

Loeys-Dietz syndrome,[317–319] a Marfan syndrome-associated disorder with genetic mutations in *TGFBR1*/*TGFBR2*, has prominent tortuous vessels and severe aortic lesions at a younger age, making early differentiation important. The phenotype of this syndrome differs from that of Marfan syndrome, and is characterized by characteristic facial features: cleft palate, bifid uvula, craniosynostosis and dilatation/dissection of the ascending aorta, and aortic/arterial tortuosity; genetic abnormalities of TGF-β ligands (*TGFB2*,[320] *TGFB3*,[321]) and *SMAD3*[322] are also known to cause similar clinical phenotypes (**Table 31**).

The genetic diagnosis of Loeys-Dietz syndrome, as for Marfan syndrome, is reimbursed by insurance, and a panel of genes including *TGFBR1*, *TGFBR2*, *FBN1*, and TGF-smad genes is often analyzed. Loeys-Dietz syndrome is often phenotyped in infancy, and is often associated with vascular tortuosity and vascular lesions in other organs, such as the cerebral vasculature.

In the genetic analysis of Loeys-Dietz syndrome (including

Table 31. Recommendation and Level of Evidence for Genetic Testing for Loeys-Dietz Syndrome (LDS)

	COR	LOE
Genetic testing to confirm the diagnosis of LDS	I	B

COR, Class of Recommendation; LOE, Level of Evidence.

Table 32. Classification of Ehlers-Danlos Syndrome[324]

	Clinical subtype	Abbreviation	Inheritance pattern	Causative gene	Affected protein
1	Classical	cEDS	AD	Major: *COL5A1*, *COL5A2* Rare: *COL1A1*	Type V collagen Type I collagen
2	Classical-like	clEDS	AR	*TNXB*	Tenascin-X
3	Cardiac-valvular	cvEDS	AR	*COL1A2*	Type I collagen
4	Vascular	vEDS	AD	Major: *COL3A1* Rare: *COL1A1*	Type III collagen Type I collagen
5	Hypermobile	hEDS	AD	Unknown	Unknown
6	Arthrochalasia	aEDS	AD	*COL1A1*, *COL1A2*	Type I collagen
7	Dermatosparaxis	dEDS	AR	*ADAMTS2*	ADAMTS-2
8	Kyphoscoliotic	kEDS	AR	*PLOD1* *FKBP14*	LH1 FKBP22
9	Brittle cornea syndrome	BCS	AR	*ZNF469* *PRDM5*	ZNF469 PRDM5
10	Spondylodysplastic	spEDS	AR	*B4GALT7* *B3GALT6* *SLC39A13*	β4GalT7 β3GalT6 ZIP13
11	Musculocontractural	mcEDS	AR	*CHST14* *DSE*	D4ST1 DSE (DS-epi1)
12	Myopathic	mEDS	AD, AR	*COL12A1*	Type XII collagen
13	Periodontal	pEDS	AD	*C1R* *C1S*	C1r C1s

AD, autosomal dominant inheritance; AR, autosomal recessive inheritance. (Adapted from Malfait F, et al. 2017[324])

TGF-β ligand and SMAD gene abnormalities, but not limited to TGF-β receptor gene abnormalities) in Europe and the USA, the frequency of pathological variants identified in *TGFBR1* (20–25%), *TGFBR2* (55–60%), *SMAD3* (5–10%), *TGFB2* (5–10%), and *TGFB3* (1–5%) was reported,[323] indicating the need for a panel analysis of these gene groups.

1.2.2 Diagnostic Criteria

The clinical manifestations of Loeys-Dietz syndrome are as follows.

Cardiovascular: Aortic aneurysm, small and medium-sized aneurysm, tortuosity

Congenital heart malformations (patent ductus arteriosus, atrial septal defect, ventricular septal defect, aortic bicuspid valve)

Characteristic Facial Features: Interorbital openings, cleft palate and bifid palate, small jaw and chin retraction, hypoplastic zygomatics, sloping eyelid cleft

Blue sclera, strabismus, high palate, plexus teeth, premature aging-like facial features, etc.

Skeletal System: Scoliosis, funnel and pigeon chest, elongated fingers (spider toes), congenital clubfoot

Cranial deformity due to premature fusion of skull sutures, cervical instability and cervical bony deformity

Joint hypermobility, joint contractures, flat feet, etc.

Skin: Thin skin with visible venous vessels, hernia, delayed wound healing, etc.

Other: Ectopia lentis is not observed. Pneumothorax is possible but infrequent. Spinal dural dilation is often present. Intellectual development is considered normal except in cases associated with premature cranial suture fusion or hydrocephalus.

1.2.3 Cardiovascular Abnormalities

Enlarged sinus of Valsalva, aortic dissection, and aortic aneurysm (especially in the thoracic region) are the major phenotypes, but most cases occur in children and infants, and the progression is generally rapid. It is said that even small aortic diameters can develop dissection, but on the other hand, there are cases in which large diameter vessels do not dissect even if the diameter is large in some individuals. However, aortic dissection tends to occur in children with Loeys-Dietz syndrome, so caution is required. Aneurysm formation or dissection may also occur in branch arteries such as the common iliac artery, subclavian artery, superior mesenteric artery, cerebral artery, and coronary artery, requiring systemic vascular screening. Arterial tortuosity is prominent and is particularly frequent in the arteries of the head and neck, such as the radial artery.

1.2.4 Clinical Examination and Evaluation

Chest X-rays, ECG, echocardiography, CT or MRI are performed, but it is essential to screen not only the heart and aorta, but also all blood vessels in the body, including the brain. Evaluation often begins in childhood, and MRI is highly beneficial when feasible.

1.2.5 Disease Progression

The progression of aortic aneurysm and aortic root enlargement, the presence of aortic dissection, and aneurysm and dissection of branching vessels should be noted.

1.2.6 Treatment and Lifestyle Management

As in Marfan syndrome, β-blockers and angiotensin-receptor blockers are used and thought to be effective. Surgery is indicated for aortic enlargement, dissection, and aneurysm at an earlier stage (less extensive) than in Marfan syndrome, in accordance with the 2020 revised guidelines for the treatment of aortic aneurysms and dissection.[314]

1.2.7 Preimplantation Diagnosis

It is not permitted and will not be implemented in Japan.

1.2.8 Prognosis

Prognosis depends substantially on vascular involvement, especially aortic involvement. Early therapeutic intervention can improve the prognosis of aortic complications. On the other hand, many cases go undiagnosed in childhood and are diagnosed only after aortic dissection, a serious complication, develops in adulthood.

1.2.9 Related Diseases and Differential Diagnosis

Symptomatic aortic aneurysms and dissections associated with other physical findings include the Marfan syndrome described earlier, those associated with genetic abnormalities of the TGF-β receptor/ligand and its intracellular signaling system, the SMAD group described in this section, and the vascular EDS described in detail next. There are also familial aortic aneurysms and dissections without other physical findings. Please refer to the appropriate section.

1.3 Ehlers-Danlos Syndrome

1.3.1 Disease Concept

Ehlers-Danlos syndrome (EDS) represents a group of disorders characterized by skin hyperextensibility, joint hypermobility, and tissue fragility;[324,325] the International Classification and Nomenclature published in 2017 presented 13 subtypes[324] (**Table 32**) followed by an inclusion of another subtype caused by biallelic variants in *AEBP1* encoding ACLP (classical-like EDS type 2).[325] The prevalence, including all subtypes, is estimated to be 1/5,000: 1/20,000 for classical EDS, 1/50,000 for vascular EDS, and 1/5,000 to 20,000 for hypermobile EDS.

1.3.2 Causative Genes

The causative genes for EDS include those encoding fibrillar collagen molecules (types I, III, and V), enzymes (LH1) and molecules (FKBP22, C1r, C1s) involved in folding and cross-linking at the ribosome, enzymes (ADAMTS2) involved in intracellular processing, glycosylation enzymes of collagen fibrils (β4GalT7, β3GalT6, D4ST1, DSE), or non-fibrillar collagen (type XII) and other molecules (ACLP) that constitute the extracellular matrix (**Table 32**). The genetic basis of hypermobile EDS has not been identified.

1.3.3 Diagnosis

The 2017 International Classification provided diagnostic criteria for EDS,[24] and the diagnostic criteria for vascular EDS revised for the designated intractable diseases are shown in **Table 33**. The diagnosis is based on the results of genetic testing, except for hypermobile EDS. Panel-based next-generation sequencing, which include all causative genes for EDS and those for Marfan syndrome, Loeys-Dietz syndrome, and other inherited connective tissue diseases, is useful[324,326,327] (**Table 34**).

1.3.4 Cardiovascular Abnormalities

Vascular EDS is complicated by serious vascular lesions that can threaten life and daily living. The lesions include arterial rupture, aneurysm, and dissection, and arteriovenous fistula (carotid-cavernous sinus fistula). Arterial rupture may occur following an aneurysm/dissection or arteriovenous fistula, or it may occur spontaneously. The most common site of arterial rupture is the thorax and abdomen (66%), followed by the head and neck (17%) and extremities (17%).[327,328]

Cardiovascular lesions can occur in other subtypes of EDS. In a systematic review published in 2018, 77 of 467 patients (17%) developed a total of 100 vascular lesions, including hematoma (53%, frequent in musculocontractural EDS and classical EDS), intracranial bleeding (18%, frequent in dermatosparaxis EDS), arterial dissection (16%, frequent in kyphoscoliosis EDS), and aneurysm (5%), and there were 8 vascular lesion-related deaths (2%).[329] Postural orthostatic tachycardia syndrome (POTS) is common in hypermobile EDS. Congenital heart defects are occasionally observed in musculocontractural EDS (25%, most commonly atrial septal defect).[330]

Nonvascular lesions in vascular EDS include gastrointestinal perforation (sigmoid colon is common), pneumo(hemo)thorax, muscle/tendon rupture, and talipes equinovarus.

Table 33. Diagnostic Criteria for Vascular Ehlers-Danlos Syndrome, Revised for the Designated Intractable Diseases

"Definite" cases are certified
A. Symptoms
<Major Criteria>
1. Arterial rupture at a young age
2. Intestinal perforation
3. Uterine rupture during pregnancy
4. Carotid-cavernous sinus fistula (CCSF)
5. Family history of vascular EDS
<Minor Criteria>
1. Bruising
2. Thin, translucent skin
3. Facial features
4. Spontaneous pneumo(hemo)thorax
5. Acrogeria
6. Talipes equinovarus
7. Congenital hip dislocation
8. Hypermobility of small joints
9. Tendon and muscle rupture
10. Keratoconus
11. Gingival recession and fragility
12. Early-onset varicose veins
B. Laboratory Findings
Biochemical findings: abnormal type III procollagen production in cultured dermal fibroblasts
C. Genetic Testing
Pathogenic variants in *COL3A1*
<Diagnostic Category>
Definite 1: ≥2 of (A) symptoms are recognized* and (B) is applicable
Definite 2: ≥2 of (A) symptoms are recognized* and (C) is applicable
*Any item in the major or minor criteria is acceptable

(Source: Prepared based on Malfait F, et al. 2017[324])

Table 34. Recommendation and Level of Evidence for Genetic Testing for Ehlers-Danlos Syndrome (EDS)

	COR	LOE
Genetic testing to confirm the diagnosis of EDS	I	B

COR, Class of Recommendation; LOE, Level of Evidence.

1.3.5 Prognosis

A large study in the US of vascular EDS showed that 25% of patients developed major complications by age 20 years and 80% by age 40, and that the median survival time was 48 years.[331] Patients with EDS caused by haploinsufficiency of *COL3A1*, which accounts for approximately 5–10% of cases, tended to have milder symptoms, with a later onset of disease and more favorable life expectancy.[332–334]

1.3.6 Management

a. Surveillance

In vascular EDS, surveillance of arterial lesions by contrast-enhanced CT or MRI is recommended at diagnosis and periodically or at the onset of pain.[327,328]

Aortic root dilation and valve abnormalities (e.g., mitral valve deviation) are observed in other subtypes (classical EDS, cardiac valvular EDS, hypermobile EDS, and musculocontractural EDS), and recommended to be evaluated by echocardiography at diagnosis and periodically if present.[330,335,336] Evaluation for congenital heart defects is recommended at diagnosis in musculocontractural EDS.[330]

b. Treatment and Lifestyle Management

In vascular EDS, the usefulness of celiprolol, a β-blocker with vasodilator properties, was demonstrated in studies, mainly in Europe, regarding the development of arterial lesions.[337–339] Celiprolol is started with 100 mg/day and increased to 400 mg/day if possible. Acute arterial lesions (aneurysms, dissection) are recommended to be treated conservatively as much as possible with antihypertensive therapy on admission, but endovascular therapy should be considered if the lesions are still progressive. If surgery is unavoidable, an extreme attention has to be paid to vascular and tissue vulnerability.[327,328]

Patients of all subtypes are recommended to avoid strenuous and contact exercises and activities with a risk of falling in order to reduce the risk of skin and joint trauma.[335,336] Patients with vascular EDS are recommended to avoid traumatic activities (e.g., collision sports, heavy weight lifting) that can cause increased and unstable blood pressure.[327]

c. Genetic Counseling

Genetic counseling for patients with vascular EDS includes sharing the severity of the disease, developing a responsible attitude to provide the best possible treatment (e.g., regular checkups, celiprolol administration, and emergency response), and psychosocial support (e.g., application for a designated incurable disease). Women with vascular EDS are recommended to have counseling before pregnancy by an obstetrician, a cardiologist, a cardiovascular surgeon, and a geneticist, given the increased mortality rate (5%) associated with uterine and arterial rupture (i.e., whether the pregnancy is permitted or not, and if so, what mode of delivery, including artificial preterm birth before onset of labor, should be used). Presymptomatic genetic diagnosis of at-risk relatives, especially children, may be useful for them to adjust to a preferable lifestyle, such as avoiding strenuous exercises. Further evidence on the efficacy of celiprolol prophylaxis is expected to accumulate in the future. However, because of the psychological impact, careful genetic counseling at experienced institutes is recommended.

2. Nonsyndromic Aortic Diseases (Familial Thoracic Aortic Aneurysm/Dissection [FTAAD])

2.1 Synopsis

In general, the incidence of aortic aneurysm and aortic dissection tends to be higher in the elderly, but it can also occur in young people, especially in the thoracic region, where the heritability and familial nature of the disease is relatively high, with nearly 20% of cases reported to have intrafamilial aggregation.[340,341] In the case of aortic aneurysms and dissections, hypertension, obstructive sleep apnea, aortitis, syphilis, and other infections are also causes, and if these causes can be excluded, the possibility of hereditary aortic disease is relatively high.

Thoracic aortic aneurysms and dissections can be caused by syndromes based on connective tissue abnormalities such as Marfan syndrome, Loeys-Dietz syndrome and vascular Ehlers-Danlos syndrome, and a comprehensive evaluation of the patient's physical examination and imaging studies are essential to determine if the patient has any characteristic physical findings.

On the other hand, FTAAD is a common type of aortic aneurysm and dissection that has no physical syndromic characteristics.

2.2 Causal Genes for FTAAD

Although more than a dozen causative genes have been reported to date as being associated with the development of aortic aneurysm and dissection, it should be noted that there are still a certain number of undetected causative genes and that undetected mutations in known causative gene diagnoses cannot exclude familial aortic aneurysm and dissection.

Mutations in the genes encoding vascular smooth muscle contractile proteins (*ACTA2* and *MYH11*) are known to be the major causative genes. *ACTA2* mutations[342–344] are the most frequent (12–21% of all cases), and depending on the site and type of mutation, some cases present with coronary artery disease, cerebrovascular disease, or cerebral artery malformation similar to moyamoya disease. Some cases are known to show pupil constriction disorder. In

Table 35. Causal or Candidate Genes for Familial Aortic Aneurysm and Dissection

Definitive and strong evidence	*ACTA2, MYH11, MYLK* *COL3A1, FBN1, TGFBR1, TGFBR2, SMAD3* *TGFB2, LOX, PRKG1*
Possibly useful with moderate evidence	*EFEMP2, ELN, FBN2, FLNA, TGFB3* *NOTCH1, SLC2A10, SMAD4, SKI*
Limited evidence	CBS, COL4A5, PKD1, PKD2

(Source: Prepared based on Salmasi MY, et al. 2023[354])

particular, those with mutations of Arg179 residues have a variety of physical phenotypes, including mydriasis (pupil constriction disorder), proximal dilatation and distal stenosis of the internal carotid artery, abnormal bowel rotation, and flaccid bladder, as well as a tendency to have a severe and early aortic phenotype, which requires careful attention.[345] Although infrequent (≈1%), some carriers of *MYH11* mutations[206] have been associated with patent ductus arteriosus, and similar phenotypes have been observed in genetically modified mice, suggesting a background of contractile dysfunction and structural abnormalities of adhesion molecules and contractile proteins.[346,347] Mutations in the myosin light chain phosphatase *MYLK*[348] and *PRKG1* genes, which encodes a cGMP-dependent protein kinase related to vascular smooth muscle relaxation, have also been implicated as causative genes of aortic aneurysms and dissections.[349] Other genes, such as *NOTCH1*, *LOX*, and *FOXE3*, as well as aortic bicuspid valve (involving *NOTCH1*), multiple cystic kidney disease, and Turner syndrome have been reported to be associated with aortic dissection and aneurysms.[194,350–353]

Genetic testing for FTAAD is now covered by insurance, and the major causative genes and those for Marfan syndrome, Loeys-Dietz syndrome, and vascular-type Ehlers-Danlos syndrome are also evaluated (panel analysis). However, the penetration rate is not necessarily high, so genetic analysis of multiple affected and unaffected members of the same family should be considered. Genes to be analyzed are mainly those whose pathogenicity is known, but those that have been suggested to be related and those for which there is little evidence are shown in **Table 35**.[354,355]

2.3 Cardiovascular Abnormalities

Aortic dissection and aortic aneurysm (especially in the thoracic region) are the major phenotypes. *ACTA2* mutations are typical of the phenotypes, with coronary artery disease, cerebrovascular disease, and cerebral arterial malformations resembling moyamoya disease; *MYH11* mutations are associated with patent ductus arteriosus in some carriers.

2.4 Clinical Examination and Evaluation

A chest X-ray, electrocardiography, echocardiography, CT or MRI scan should be performed. The absence of an enlarged aortic diameter does not rule out this disease. In addition to a thorough systemic examination to differentiate this disease from symptomatic aortic aneurysms and dissections, it is important to evaluate the systemic phenotype during ophthalmologic examination and CT/MRI, including pulmonary involvement (bulla), skeletal abnormalities (scoliosis, funnel chest, etc.), and the presence of dural ectasia.

2.5 Disease Progression

The progression of aortic aneurysm and aortic enlargement should be noted. Enlargement of vessel diameter is a risk factor for aortic dissection, but dissection can occur even in the absence of vessel enlargement. It has been reported that aortic aneurysms enlarge significantly faster (0.2 cm/year compared with 0.1 cm/year in Marfan syndrome), so regular follow-up is important. The site of onset and age are not always the same, even within the same family. Women who wish to become pregnant should be advised that aortic dissection may occur during pregnancy, childbirth, and the postpartum period, even if the aortic diameter is not significantly enlarged.

2.6 Treatment and Lifestyle Management

Treatment is based on physical phenotype, and β-blockers are often used to prevent the progression of aortic enlargement, but there is a lack of evidence for specific agents such as angiotensin-receptor blockers for Marfan syndrome.

Aortic enlargement, dissection, and aneurysms are treated surgically with artificial graft vessels (see the 2020 Japanese Circulation Society Revised Guidelines for Aortic Aneurysm and Dissection).[356]

Exercise restrictions are to avoid heavy lifting, exercise requiring breath holding, and strenuous exercise, especially when there is aortic enlargement or with dissection.

2.7 Preimplantation Diagnosis

Not applicable. No implementation in Japan.

2.8 Prognosis

Recent surgical outcomes, including remote outcomes, are excellent, and long-term survival is possible with appropriate surgical treatment, drug therapy, and daily life management, even in cases of cardiovascular disease, but when aortic dissection or rupture occurs suddenly without a physical phenotype, it is often difficult to save the patient's life.

2.9 Related Diseases and Differentials

Symptomatic aortic aneurysms and dissections associated with other physical findings include Marfan syndrome (*FBN1*), Loeys-Dietz syndrome, a Marfan syndrome-associated disorder (Loeys-Dietz syndrome) associated with genetic abnormalities of the TGF-β receptor/ligand and its intracellular signaling system, the smad group, and vascular Ehlers-Danlos syndrome and others. Please refer to the relevant sections.

3. Vasculitis and Peripheral Arterial Disease

3.1 Vasculitis

3.1.1 Takayasu Arteritis

Takayasu arteritis is more common in Asia and the Middle East than in North America except Mexico. The association with HLA-B*5201 (odds ratio of ≈2–3) has long been pointed out in Japanese studies,[357–359] and similar results have been obtained overseas.[360,361] In addition, 2 Japanese centers have reported that HLA-B*6701, a rare allele found in East Asia, is also independently associated with Takayasu arteritis.[362,363]

In 2013, 2 GWAS analyses of Takayasu arteritis from Japan and overseas were published simultaneously, and *IL12B* was identified as a disease susceptibility region shared between ethnicities.[359,361] The odds ratio for the *IL12B* region SNP is approximately 1.75, which is high compared with disease susceptibility genes of other autoimmune diseases, and it is also suggested to be associated with disease severity.[359,364,365] *IL12B is* also a disease susceptibility gene for psoriasis and Crohn's disease. A GWAS from Japan in 2018 newly identified *PTK2B*, *LILRA3*/*LILRB2*, *DUSP22*, *KLHL33*, *HSPA6*/*FCGR3A* and chr21q22 as disease

susceptibility regions.[366] In overseas GWAS, in addition to HLA and *IL12B*, *FCGR2A*/*FCGR3A*, *IL6*, *RPS9*/*LILRB3*, chr21q22, *PTK2B*, *VPS8*, *SVEP1*, *CFL2*, and chr13q21 have been reported.[361,367,368]

3.1.2 Giant Cell Arteritis

Together with Takayasu's arteritis, giant cell arteritis is classified as a large-vessel vasculitis in the revised Chapel Hill Consensus Conference (CHCC) Classification 2012.[369] Takayasu's arteritis is more common in Japan than in Europe and North America, whereas giant cell arteritis is less common in Japan and more common in Europe and North America. In a 1998 epidemiological survey in Japan, the prevalence among those aged over 50 years old was 1.48/100,000 persons, which is less than the 200 in the USA and 60 in Spain.[370] Giant cell arteritis has been reported to be associated with HLA-DRB1*0401 and HLA-DRB1*0404.[371,372] These alleles are more common in Caucasians and less common in Japanese, which may explain the low prevalence in Japan. In addition to HLA, *PLG* and *P4HA2* have been reported from overseas as disease susceptibility regions,[373] but there are no reports from Japan.

3.1.3 Kawasaki Disease

Kawasaki disease is classified as a medium-sized vasculitis, together with polyarteritis nodosa in the revised CHCC classification 2012. The prevalence of Kawasaki disease is high in East Asia and low in the USA and Europe, and both infectious and genetic factors are under investigation.[374] In a nationwide survey in Japan in 2016, the incidence rate among children aged less than 5 years old was 309.0 per 100,000 people, while in the USA in 2006, hospitalizations for Kawasaki disease among children in the same age group were only 20.8/100,000.[375] Similar ethnic differences were reported in a comparison between Japanese Americans and Caucasians living in Hawaii, suggesting the involvement of genetic factors.[376] There are several reports on the association with specific HLAs, but the results are inconsistent. A GWAS from Japan has shown an association with SNPs between HLA-DQA2 and HLA-DAB.[377]

Many GWAS have been reported from Japan and overseas,[377–380] and including genes identified by candidate gene search, more than 20 disease susceptibility genes have been reported so far. A SNP in the first intron of the *ITPKC* gene reported from Japan in 2008 is associated with Kawasaki disease onset and coronary artery complications, and is thought to cause lymphocyte hyperactivation due to decreased mRNA splicing efficiency.[381] Other disease susceptibility genes identified by GWAS include *FCGR2A*, *BLK*, and *CD40*. In addition, SNPs in *ITPKC* and *CASP3* have been reported to be associated with the risk of treatment resistance and coronary artery disease complications in Japanese patients.[382]

3.1.4 Polyarteritis Nodosa

Reports on genetic factors of nodular polyarteritis are scarce. In 2014, a loss-of-function mutation in the *ADA2* gene was reported to cause a condition similar to polyarteritis nodosa in childhood. The mode of inheritance is autosomal recessive.[383–386]

3.1.5 Behcet's Disease

The prevalence is high from Japan to China, Central Asia, the Middle East, and the Mediterranean coast. HLA-B*5101 is known to be the strongest genetic factor beyond racial differences, and HLA-A*26 has been reported from Japan to be independently associated with HLA-B*5101.[387] In 2010, the first GWAS were reported simultaneously from Japan and overseas,[388,389] and since then many genes, including *IL23R-IL12RB2*, *IL10*, *CCR1*, *STAT4*, *KLRC4*, and *ERAP1* have been identified as disease susceptibility genes for Behçet's disease.[390–392]

3.1.6 Buerger's Disease

Buerger's disease is more common in South Asia, East Asia, and Turkey than in Central Europe, North America, South America, and Africa. Although there are regional differences and a strong association with smoking, no obvious genetic predisposition has been reported.

3.2 Peripheral Arterial Disease

Several GWAS have been reported so far, but their number is small compared with those for coronary artery disease.[393–397] In a report from Japan in 2015, *IPO5*/*RAP2A*, *EDNRA*, and *HDAC9* were identified as disease susceptibility genes, and the association for *HDAC9* was confirmed in subsequent overseas analyses.[394] In the 2019 GWAS from overseas, 19 genes were identified, including lipid-related *LDLR*, *LPL*, *LPA*, *CELSR2*/*SORT1*, diabetes-related *TCF7L2*, thrombosis-related *F5* (Leiden mutation), smoking-related *CHRNA3*, and others such as *HLA-B*, *HDAC9*, *IL6*, *ABO*, *CDKN2B-AS1*, *MMP3*, *CREB3L1*, *PTPN11*, *RP11-359M6.3*, *COL4A1*, *SMOC1*, *LOC732538*, with the most strongly associated gene being *LPA* with an odds ratio of 1.26.[396] A 2021 GWAS report similarly identified 5 associations with *LPA*, *CDKN2B-AS1*, *SH2B3*/*PTPN11*, *HDAC9*, and CHRNA3.[397] Among 19 genes in the 2019 report, 11 genes, including *LDLR*, *LPL*, and *LPA*, were common to both coronary and cerebrovascular disease, and 4 genes, *RP11-359M6.3*, *HLA-B*, *CHRNA3*, and *F5*, were specific to peripheral artery disease. This suggests that smoking (*CHRNA3*) and thrombosis (*F5*) may have a stronger influence on the development of peripheral arterial disease. *CHRNA3* encodes a subunit of the nicotinic acetylcholine receptor and is known to be associated with nicotine dependence.[398] *F5* encodes coagulation factor 5, and a mutation in which the arginine at position 506 is replaced by glutamine (p.R506Q) is called the Leiden mutation.[399] It is a major risk factor for thrombosis in Western Caucasians, but this mutation is very rare in Asians.

3.3 Peripheral Arterial Calcification

NT5E, *ABCC6*, and *ENPP1* deficiencies are autosomal recessive disorders that cause severe calcification of the aorta and peripheral arteries. *NT5E* encodes CD73, which hydrolyses extracellular adenosine monophosphate to adenosine and phosphate. Its homozygous mutation results in calcification around the joints of the hands and feet and marked calcification of abdominal and lower extremity arteries, which is called calcification of joints and arteries (CALJA) or arterial calcification due to deficiency of CD73 (ACDC).[400] The diagnosis is often made after the age of 40 years, and cases of aortic stenosis have been reported from Japan.[401] Homozygous mutations of *ABCC6* are known as pseudoxanthoma elasticum. In typical cases, characteristic skin lesions (yellowish papules on the cervical,

axillary, and inguinal regions) and ocular lesions (peau d'orange and angioid streaks) appear in childhood and adolescence, followed by intermittent claudication and myocardial infarction in adulthood due to calcification of arteries.[402,403] Homozygous mutations in *ENPP1* cause generalized arterial calcification of infancy 1 (GACI1), and *ENPP1* is also known as one of the causative genes of autosomal recessive hypophosphatemic rickets. It causes extensive calcification of large to medium-sized blood vessels, often resulting in death within the first 6 months of life.[404,405] The most severe cases of *ABCC6* homozygous mutations described above also show similar phenotypes as GACI1,[406] and are called GACI2.

XI. Ischemic Heart Disease

Ischemic heart disease (IHD) is classified into organic and functional coronary artery disease (CAD), of which functional CAD includes epicardial coronary vasospasm and coronary microvascular dysfunction, which manifest as vasospastic angina (VSA) and microvascular angina (MVA), respectively. Each of these IHD has different risk factors and backgrounds, and the degree and mechanisms of genetic predisposition are naturally different. Therefore, the following is an overview of disease susceptibility genes and variants for each IHD.

1. Obstructive CAD

Obstructive CAD, which develops against a background of coronary risk factors such as hypertension, diabetes, and dyslipidemia, is a multifactorial disease, and develops as a result of the interaction between environmental factors and multiple genetic factors. Regarding the intrafamilial accumulation of obstructive CAD, it has been reported that the risk of obstructive CAD in first-degree relatives of the initiator is 2–4-fold,[407] and that, if the proband is a male under the age of 45 years, the risk of his peers developing CAD by age of 55 years is 6.7–11.4-fold.[408] From Japan, Yamada et al reported that the connexin 37, plasminogen activator inhibitor 1 (PAI-1), and matrix metalloproteinase 3 (MMP3) variants were predictors of myocardial infarction occurrence in a case–control study.[409]

Recently, the genome-wide approach has been frequently used in Japan, in which variants are examined across the entire genome, and many findings have been obtained regarding variants associated with IHD. Previous genome-wide association studies (GWAS) have reported more than 250 susceptibility loci involved in IHD (angina pectoris and myocardial infarction), mainly in the USA and Europe.[410–418] These loci include not only genes involved in hypertension, diabetes, dyslipidemia, and obesity, which are conventional coronary risk factors, but also genes involved in immune response and inflammation, vasoconstriction, angiogenesis, vascular remodeling, thrombus, and cell proliferation and transcriptional regulation.

From Japan, furthermore, a GWAS in approximately 25,000 CAD patients using genomic data from Biobank Japan reported 48 disease susceptibility loci, including 8 regions not identified in previous studies in Western populations, and many of these were shown to contain lipid-related genes such as the low-density lipoprotein receptor gene (*LDLR*) and the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene and other lipid-related genes.[419] In addition, a meta-analysis of 2 GWAS using genetic data from a Japanese population totaling 50,000 individuals identified 18 disease susceptibility loci associated with IHD.[420] Moreover, a cross-racial meta-analysis identified 76 disease susceptibility loci, including 3 new regions: the *CTSS* locus on 1q21[421,422] and the *RDX-FDX1* locus on 11q22,[423] which are involved in the development of atherosclerosis via the immune system, and the *WDR11-FGR2* locus on 10q26,[424] which affects the lipid system. With regard to these 76 regions, there are differences in the effects on organs and tissues involved in the occurrence of myocardial infarction between Japanese and Western populations, suggesting that there are racial differences in genetic factors in IHD. Furthermore, a cross-sectional GWAS of >210,000 Japanese subjects with 42 diseases using genome data from Biobank Japan identified 320 disease susceptibility loci associated with the development of 27 diseases in total, of which a missense variant of the ATG16L2 gene on chromosome 11 associated with CAD missense variants were not detectable in GWAS in Western populations.[425]

The identification of these disease susceptibility loci, which are not found in Westerners, is expected to help elucidating the etiology of IHD unique to the Japanese. In addition, the GWAS in IHD conducted by Tcheandjieu et al included large-scale analyses in African and Hispanic ethnic groups in addition to Westerners, revealing heterogeneity in the genetic basis of each ethnic group.[417] These results indicate that genomic analysis in diverse ethnic groups is important for elucidation of comprehensive disease mechanisms.

2. Functional CAD

2.1 Vasospastic Angina/Coronary Spastic Angina

Among the IHD, it has long been reported that the incidence of vasospatic angina (VSA)/coronary spastic angina (CSA), in which the epicardial coronary spasm, is high among Japanese and low among Westerners.[426,427] It has already been reported that smoking is an important environmental factor involved in coronary spasm.[428,429] However, it is thought that the involvement of genetic background in addition to these lifestyle factors may cause regional and racial differences in VSA/CSA.

Yoshimura and Nakayama et al. have shown that vascular endothelial dysfunction is involved in the pathogenesis of coronary spasm,[430–431] and that a variant of endothelial nitric oxide (NO) synthase (eNOS, *NOS3*), which synthesizes NO in the vascular endothelium. Specifically, a single-nucleotide polymorphism (SNP) of 894G/T (Glu298Asp) in the 7th exon of the eNOS gene and -786T/C in the 5′ untranslated region are associated with VSA/CSA.[431,432] The frequency of these 2 eNOS variants

differs by race, suggesting that they may contribute to racial differences in the pathogenesis of IHD.[433,434]

There have been several reports of variants other than eNOS associated with coronary spasm in Japanese. Both variants of phospholipase C delta-1 (*PLCD1*) increases intracellular calcium concentration by acetylcholine,[435–437] and a variant of ornithine transcarbamylase (*OTC*), the main enzyme in the urea cycle, increases ergonovine-induced coronary spasm[438] and has been reported to be involved in VSA/CSA. There is also a report using GWAS that a SNP in the untranslated region, rs10498345, was associated with coronary spasm in Japanese women.[439]

Furthermore, when 29 candidate genes were searched in Japanese patients with VSA/CSA, only the variant of cytochrome b-245 α chain (*p22 phox*) was found to be associated with this disease in males. In contrast, in females, variants of the atherosclerosis-related factors, *MMP3* and interleukin 6 (*IL6*), were reported to be associated with coronary spasm, suggesting a contributing factor to the sex difference in VSA/CSA.[440] In addition, it has been shown that inactivated polymorphism *ALDH2*2* of aldehyde dehydrogenase 2 (*ALDH2*), which is common in East Asians, is closely associated not only with coronary spasm but also with ST-elevation acute myocardial infarction,[441,442] and that smoking synergistically increases the risk.[443] It has been suggested that not only decreased NO production and increased oxidative stress due to vascular endothelial dysfunction, but also stagnation of aldehyde metabolism due to *ALDH2* variants are involved in the pathogenesis of coronary spasm in the Japanese population.

In addition, *RNF213* was reported as a gene associated with risk development in Japanese patients with coronary spasm.[444] In a case–control study of 1,088 patients with CAD, including 66 cases of VSA/CSA registered in the National Cardiovascular Center Biobank, the *RNF213* p.R4810K variant was found in 7 patients (10.6%) and was significantly associated after adjustment for age, sex, hypertension, diabetes, dyslipidemia and smoking. This variant was originally identified as the founder variant of moyamoya disease in East Asia, including Japan[445] and is found to be present in approximately 2% of Japanese but rarely found in the West, suggesting that it may explain some of the racial differences in coronary spasm. The variant has also been suggested to be associated not only with moyamoya disease, but also with peripheral arterial disease, including head and neck vascular stenosis,[446,447] and has been reported as a frequently associated gene in Japanese hereditary pulmonary arterial hypertension.[448] The RNF213 protein has been implicated in various processes such as lipid metabolism, angiogenesis, cellular and autonomic immunity. Therefore, variants of *RNF213* could be a background factor for various cerebral and cardiovascular diseases, and VSA/CSA may also be a part of a systemic RNF213-related vascular disease phenotype.

As described above, there are still many unknowns and different reports on disease susceptibility genes for VSA/CSA, so further identification of disease susceptibility genes using GWAS and other methods using genome data unique to Japanese people and further elucidation of the pathogenesis of the disease are needed.

2.2 Microvascular Angina

As opposed to obstructive CAD and coronary spasmodic heart disease, microvascular angina (MVA) has not been directly proven by whole-genome analysis, and there are few reports on candidate genes. However, several groups have reported that genetic mutations in paraoxonase 1 (*PON1*), which protects lipoproteins from oxidation, are involved in MVA in Japanese patients.[449,450] Furthermore, it has been reported that there are significantly more poor metabolizers of cytochrome P450 (*CYP2C19*) variants in the liver, which occur in approximately 15% of Japanese (vs. 3–5% of Westerners), in MVA patients, and that there are more poor metabolizers among these patients.[451] This suggests the existence of a disease susceptibility gene unique to Japanese people with MVA.

Such a genetic predisposition is very rarely brought about by a single variant and is thought to be caused by the accumulation of a large number of disease-related gene variants in addition to environmental factors. A number of IHD susceptibility loci have been identified using a comprehensive approach centered on GWAS, and recently, using the results of such GWAS, a polygenic risk score (PRS), a score of risk based on the accumulation of genetic factors of each individual, has been attempted. The PRS is expected to be applied to personalized medicine for the prediction of disease onset and treatment stratification.

3. Polygenic Risk Score in Ischemic Heart Disease

In IHD, genetic factors account for 50–60% of disease pathogenesis in overseas twin studies,[452] and a risk score based on GWAS could improve the accuracy of clinical diagnosis and be useful in determining treatment strategy. The genetic risk score (GRS) is the result of adding up the number of risk alleles of the variants identified in GWAS, weighted by effect sizes, and the PRS is an extension of the GRS to include many genetic markers that may be associated with disease development.

The potential clinical application of a PRS has recently been widely reported, especially for IHD, and Khera et al reported that using the PRS in CAD had comparable ability of risk stratification to lifestyle risk, and that lifestyle modification can reduce coronary events even in patients with a high PRS.[453] They have also shown that adding the PRS to conventional coronary risk factors improves the prediction of future coronary events.[454,455] Furthermore, Koyama et al have shown that the PRS in CAD is useful not only for predicting the risk of CAD onset but also for risk stratification of cardiovascular death.[419] It has also been reported that the PRS in IHD could stratify the risk of sudden and arrhythmic death in post-myocardial infarction patients with moderately impaired cardiac function.[456]

Although there have not been many trials examining the effect of using a PRS on treatment strategy, Kullo et al reported that discussing the PRS with IHD patients, in addition to a traditional clinical risk score, had a high lipid-lowering effect in patients with a high PRS and even stronger effects in those with a very high PRS,[457] indicating a behavior change as a result of recognizing genetic risk. In addition, patients with higher PRS in IHD showed a stronger effect of statin therapy in reducing coronary-related events[458,459] and greater prognosis-improving effects of PCSK9 inhibitors (alirocumab[460] and evolocumab[461]). With regard to health-care costs, evaluation using Markov models suggests that the addition of PRS to standard treatment regimens is effective in reducing both costs and disease-related events.[462]

From these results, it is expected that applying the PRS should contribute to improvements in the treatment of IHD, drug selection, and treatment strategy decisions in the future.

On the other hand, because the PRS is a relatively new concept, there are many issues that need to be resolved in order to apply it in daily practice, including accuracy of interpretation, application of use including cost, and ethical issues.[463–466] In particular, because most of the studies of PRS have been conducted mainly in Westerners, from the viewpoint of ethnic specificity of the genome, we must accumulate genomic information for the Japanese population and establish original clinical evidence in order to create a high-performance PRS for Japanese patients with IHD. At present, there is a lack of evidence of the PRS in IHD for it to be articulated in this guideline, but with the accumulation of evidence, it is highly expected that the usefulness of the PRS risk assessment of IHD will be established and included in the guideline in the future.

XII. Pulmonary Hypertension

1. Diagnostic Classification and Genetic Testing

Table 36 summarizes the recommendations and levels of evidence for genetic testing and genetic counseling in pulmonary hypertension (PH). However, genetic testing for PH is not covered in Japan as of March 2024. When conducting the test, appropriate genetic counseling is required, taking into consideration the fact that pulmonary arterial hypertension (PAH) often occurs in patients in their teens to 30s, and that the test may also affect the life planning of the patient.

In PH, after the diagnosis is made by hemodynamic evaluation, the differential diagnosis is subdivided according to cause. Among Group 1 PAH cases with no history of underlying disease or cardiovascular disease that may be the cause, those with confirmed pathological variants in known PAH-related genes or familial onset are subclassified as hereditary PAH.[467–469] Therefore, genetic testing for idiopathic/hereditary PAH is recommended for accurate diagnostic classification (Class I).

2. PAH-Related Genes

2.1 *BMPR2*

2.1.1 Epidemiology and Penetration

The PAH-related genes are listed in **Table 37**[448,468,469] and of them, the frequency of the BMP receptor type II (bone morphogenetic protein receptor type 2; *BMPR2*) gene is the highest, and an analysis of >1,000 PAH patients belonging to the European PAH cohort reported that approximately 15% of patients had a variant of *BMPR2*. The other genes are reported to be present in <1–2% each.[470] Reports from overseas indicate that the lifetime risk of developing PAH in *BMPR2* variant holders is approximately 20%, with a higher penetration rate in women (42%) than men (14%).[471–473] The male/female penetration rate of the *BMPR2* variant was similar in a report of Japanese patients: 12.5% in males and 43.8% in females.[474]

2.1.2 Variant Types

It has been reported that hereditary PAH due to *BMPR2* is caused not only by single nucleotide substitutions in

Table 36. Recommendations and Levels of Evidence for Genetic Counseling and Genetic Testing in Pulmonary Hypertension

	COR	LOE
Genetic counseling and genetic testing for diagnostic classification of idiopathic/hereditary pulmonary arterial hypertension (PAH)	I	B
Implementation of genetic testing for *BMPR2*, a frequent causative gene for hereditary PAH, capable of detecting copy number variations (exon deletions and duplications) as well as single nucleotide substitutions	I	B
Counseling and annual screening tests on the risk of developing PAH for those who are positive for mutations in PAH-related genes (unexposed mutation carriers) and for first-degree relatives of hereditary PAH patients[468,469]	I	C
Genetic testing combined with clinical findings, imaging, blood gas analysis, and pulmonary function tests for the diagnosis of PAH with signs of pulmonary veno-occlusive disease/pulmonary capillary hemangiomatosis (PVOD/PCH)[468,469]	I	C
Identification of both allele *EIF2AK4* variants by genetic testing for diagnosis of hereditary PVOD/PCH[468,469]	I	B
Establishment of treatment including genetic counseling at a facility specializing in pulmonary hypertension[468,469]	I	C
Genetic counseling at a specialized pulmonary hypertension facility for decision-making and psychological support for patients and families of women with PAH who wish to become pregnant or who are pregnant[468,469]	I	C

COR, Class of Recommendation; LOE, Level of Evidence.

Table 37. Genes Associated With Pulmonary Arterial Hypertension (PAH)[448,468,469]

Gene	Diagnostic classification of pulmonary hypertension and related diseases	Presumed molecular mechanisms	Genetic form	Patient class
BMPR2	Hereditary/idiopathic PAH	Haploinsufficiency	AD	Pediatric and adult
ATP13A3		Unknown	AD	Adult
AQP1		Unknown	AD	Adult
ABCC8		Haploinsufficiency	AD	Adult
KCNK3		Haploinsufficiency	AD	Adult
SMAD9		Haploinsufficiency	AD	Adult
Sox17	Hereditary/idiopathic PAH Congenital heart disease	Unknown	AD	Pediatric and adult
CAV1	Hereditary/idiopathic PAH Lipodystrophy	Gain of function; dominant inhibition	AD	Pediatric and adult
TBX4	Hereditary/idiopathic PAH Patellofemoral syndrome Substantial lung disease Bronchopulmonary dysplasia COPD	Unknown	AD	Pediatric and adult (but adults less frequently)
EIF2AK4	Pulmonary veno-occlusive disease/ pulmonary capillary hemangiomatosis	Loss of function	AR	Adult
KDR	Hereditary/idiopathic PAH	Loss of function	AD	Older-onset adult
ENG	Hereditary/idiopathic PAH Hereditary hemorrhagic peripheral vasodilatation	Unknown	AD	Pediatric and adult
ACVRL1		Haploinsufficiency	AD	Pediatric and adult
GDF2		Haploinsufficiency	AD	Pediatric and adult
RNF213 (p.R4810K)	Hereditary/idiopathic PAH Peripheral pulmonary artery stenosis Moyamoya disease (rare, progressive cerebrovascular disorder caused by blocked arteries in the basal ganglia)	Functional acquisition	AD (but AR in peripheral pulmonary artery stenosis and some moyamoya diseases)	Pediatric and adult

AD, autosomal dominant; AR, autosomal recessive. (Source: Prepared based on Suzuki H, et al. 2018,[448] Humbert M, et al. 2022,[468] Humbert M, et al. 2023[469])

BMPR2, but also by copy number variations (CNVs), such as exon deletions and duplications, in a small number of cases.[475] According to a report on Japanese patients, CNVs account for 23.1% of all cases of hereditary PAH due to *BMPR2*.[474] Therefore, it is important to consider not only single nucleotide substitutions but also CNVs in order to diagnose *BMPR2* variant-positive cases without missing any cases (Class I).

2.1.3 Treatment Responsiveness

In Japanese adult patients with PAH who required continuous intravenous or continuous subcutaneous pulmonary vasodilator therapy, it has been reported that patients with *BMPR2* variants have a better prognosis than other patients.[476] However, a meta-analysis report from overseas shows that patients with *BMPR2* variants have a poorer prognosis than other patients.[477] These conflicting results may be due to differences in the dosage and speed of dose escalation of pulmonary vasodilators between Japan and the West, the fact that CNVs were also not detected in all studies included in the meta-analysis, and the fact that *RNF213* p.R4810K variant-positive patients, who are resistant to pulmonary vasodilator therapy as described later, are more common among Japanese patients than those with the R4810K variant, and almost absent in Western patients.

2.1.4 Screening

In the Guidelines for the diagnosis and treatment of pulmonary hypertension published in Europe in October 2022, it is recommended to provide counseling on the risk of PAH and annual screening tests to those who are positive for variants of PAH-related genes (whether *BMPR2* or not) (unaffected variant holders) and to first-degree relatives (parents, children, brothers, sisters) of hereditary PAH patients (Class I)[468,469] and the same recommendation is made in this guideline.

In particular, the development of hereditary PAH by *BMPR2* is known to show genetic anticipation. Although there is no established screening method to assess for the development of PAH in unexposed variant carriers, echocardiography at specialized pulmonary hypertension centers is often performed annually abroad in asymptomatic next of kin who are variant-positive.[478,479] A study at an overseas facility that prospectively screened unaffected variant carriers and their next of kin reported a 2.3%/year incidence of PAH, but the study included not only echocardiography but also electrocardiography, NT-proBNP measurement, and other screening procedures.[471] Appropriate screening methods need to be further investigated in the future.

2.2 *RNF213*

RNF213 is reported as the second most frequently associated gene after *BMPR2* in Japanese patients with hereditary PAH.[448] In a validation of 139 Japanese PAH patients, the *RNF213* p.R4810K (p.Arg4810Lys) variant was found in 11 patients (7.9%), all of whom were resistant to treatment with pulmonary vasodilators and had a worse prognosis than *BMPR2* variant-positive PAH patients.[480] The *RNF213* p.R4810K variant is a founder mutation in East Asia, including Japan, and would not be reported from a Western PAH cohort, suggesting how important it is to accumulate findings in Japanese patients. Furthermore, the *RNF213* p.R4810K variant has been associated with moyamoya disease and peripheral pulmonary artery stenosis,[445,481] and may be a phenotype of PAH as part of systemic vascular disease (RNF213-related vascular disease).[482] In particular, peripheral pulmonary artery stenosis has been reported to be associated with the development of the *RNF213* p.R4810K variant in homozygosity.[481]

2.3 *EIF2AK4*

Group 1 PAH cases in which the primary lesion is located in the pulmonary veins rather than the pulmonary arteries are subdivided into PVOD and/or PCH as a subtype of group 1 PAH. The diagnosis of PVOD/PCH is often based on clinical and imaging findings, but appropriate diagnosis is required because pulmonary vasodilator therapy for PVOD/PCH carries the risk of pulmonary edema complications. For a proper diagnosis of PVOD/PCH, it is useful to detect variants (homozygous or compound heterozygous) of both alleles in the eukaryotic translation initiation factor 2 α kinase 4 (*EIF2AK4*) gene,[483] and genetic testing is an important part of this process. In recent years, cases of variants of both alleles in *EIF2AK4* have been reported in patients diagnosed with PAH.[470,484,485] A study using a large European consortium found both allele variants of *EIF2AK4* in 9 of 864 PAH patients (1.04%), which was not observed in the general population, and furthermore, these patients had reduced pulmonary diffusing capacity, which is considered one of the characteristics of PVOD/PCH, and the life expectancy and prognosis were significantly worse than that of PAH patients without both allele variants.[484]

In conclusion, some patients diagnosed with PAH have pulmonary vein involvement based on the *EIF2AK4* variant, and the treatment strategy should be based on PVOD/PCH when both *EIF2AK4* allele variants are detected by genetic testing. The European guidelines for the diagnosis and treatment of pulmonary hypertension published in October 2022 recommend the use of genetic testing in combination with clinical findings, imaging, blood gas analysis, and pulmonary function testing for the diagnosis of PAH with signs of PVOD/PCH (Class I), and the use of genetic testing for the diagnosis of hereditary PVOD/PCH with identification of both allelic *EIF2AK4* variants by genetic testing (Class I) is also recommended, and the same recommendations are made in this guideline.

2.4 Other PAH-Related Genes

In Japanese adult patients with PAH, *BMPR2* and *RNF213* have the highest mutation frequencies, in that order, and other genes are found at frequencies of a few percent or less; *ACVRL1* and *ENG* are genes associated with the development of hereditary hemorrhagic telangiectasia (HHT; Osler Weber Rendu syndrome), and HHT is known to cause PAH. However, these gene variants can also be found in PAH patients without symptoms or a family history of HHT.[486] *SOX17* has also been reported as a PAH-associated gene from a European PAH cohort[470] and the presence of pathological variants has been reported in Japanese patients with PAH,[487] in association with congenital heart disease such as atrial septal defect.[488]

3. Specialized Pulmonary Hypertension Facilities and Treatment Systems

The Guidelines for the diagnosis and treatment of pulmonary hypertension published in Europe in October 2022 recommended the establishment of a PH care system, including genetic counseling, at facilities specializing in PH (Class I). Although pregnancy and childbirth have traditionally been contraindicated in female patients with PAH, it is now recommended that genetic counseling be provided at specialized PH facilities for female patients with PAH who wish to become pregnant or who are pregnant, in order to support decision-making and psychological support for the patient and family (Class I). The same recommendation is made in this guideline, and it is considered necessary to continue working in Japan toward the appropriate establishment of specialized facilities and treatment systems.

XIII. Venous Thrombosis

1. Disease Concept

Venous thrombosis is caused by the formation of blood clots in the veins. Rudolf Virchow proposed 3 factors involved in intravascular thrombus formation: (1) stagnation of blood flow, (2) vascular endothelial damage, and (3) increased coagulability. Venous thrombosis occurs when there is an imbalance between coagulation and fibrinolysis in veins, resulting in a hypercoagulable state. It may be caused by a combination of environmental and genetic factors. Typical hereditary thrombophilic predispositions for venous thrombosis in Japan include deficiencies of antithrombin (AT), protein C (PC), and protein S (PS),[489] which are blood coagulation regulators, and impaired function of these factors results in the inability to suppress increased coagulation factor activity, leading to venous thrombosis. Thrombosis associated with these hereditary coagulation control factor deficiencies is called idiopathic thrombosis and can cause serious thrombosis in rare cases. In April 2017 idiopathic thrombosis was designated by the Ministry of Health, Labour and Welfare in Japan as

"Designated Intractable Disease 327: Idiopathic Thrombosis (limited to those caused by hereditary thrombophilia)". The disease was recognized as a designated intractable disease as characteristics that are suspicious for hereditary thrombophilia include: (1) young onset (<50 years of age) with no or weak exposure factors, (2) family history of venous thrombosis (especially onset in young people), (3) repeated ocurrence of venous thrombosis, and (4) venous thrombosis in rare locations (such as mesenteric veins and sagittal veins of the brain).[490] When (1)–(4) are present, an aggressive search for an inherited thrombophilic predisposition should be performed. The coagulation factor V Leiden variant (R506Q variant) and the prothrombin G20210A variant are known genetic factors for venous thrombosis in Caucasians, but not in East Asians including Japanese. On the other hand, the PS p.K196E variant, a PS deficiency disorder, is a pathological variant that has been found in approximately 1 in 55 Japanese. In addition, a prothrombin gene variant to be involved in the development of venous thrombosis was recently reported for the first time from Japan,[491] and subsequent reports from Japan have attracted much attention.[492–494]

Table 38. Recommendations and Levels of Evidence for Genetic Testing for Venous Thrombosis

	COR	LOE
Measurement of antithrombin, protein C and protein S activity in patients suspicious of hereditary thrombophilic predisposition	I	C
Proactive genetic testing if AT, PC and PS activity is reduced without other triggers	IIa	C

AT, antithrombin; COR, Class of Recommendation; LOE, Level of Evidence; PC, protein C; PS, protein S.

Table 39. Acquired Clinical Factors That Reduce Activity/Antigen Levels of Antithrombin, Protein C and Protein S

- Administration of anti-vitamin K antagonist such as warfarin
- Impaired vitamin K absorption due to decreased bile secretion (e.g., biliary obstruction)
- Impaired protein production by the liver itself (e.g., liver cirrhosis)
- Disseminated intravascular coagulation
- Consumption due to acute thrombosis
- Pregnancy
- Estrogen administration (e.g., oral contraceptive)
- After major surgery, burns
- Nephrotic syndrome
- Administration of unfractionated heparin and L-aspartic acid

2. Screening Tests for Hereditary Thrombophilic Predisposition

Especially when the patient has the characteristics of hereditary thrombophilia (see (1)–(4) above), the measurement of AT, PC, and PS activities as coagulation tests should be performed aggressively. If each of these activities is low, the possibility of idiopathic thrombosis should be suspected. Additional measurement of AT, PC, and PS antigen levels will also allow for classification of deficiency type. In AT deficiency, quantitative deficiency is reported to have an earlier age of onset than qualitative deficiency and is less likely to be venous thrombosis event-free during the lifetime of the patient. It is available at.[495] However, the activity and antigen measurements may be low due to various acquired factors. **Table 38** shows the recommendations and levels of evidence for genetic testing for venous thrombosis, and **Table 39** shows the various acquired factors that affect AT, PC, PS activity and antigen levels. The presence of these acquired factors should be evaluated to determine whether a closer examination for hereditary thrombophilic predisposition should be performed. The type of anticoagulant and the method of measurement also affect the activity value, as shown in **Table 40**.[496] The lower normal limits of AT, PC, and PS activity in neonates to children under 6 years of age have been reported to be lower than the lower normal limits in adults.[497] When measuring activity in patients (or family members) under 6 years of age, these differences should be kept in mind.

3. Genetic Testing

Genetic testing for AT deficiency, PC deficiency, and PS deficiency can be performed as an insured procedure (5,000 insurance points per test, as of March 2023), using the *SERPINC1* gene for suspected AT deficiency, the *PROC* gene for suspected PC deficiency, and the *PROS1* gene for suspected PS deficiency. Before conducting genetic testing, it is necessary to comprehensively evaluate the patient's background (age of onset, mode of onset, family history,

Table 40. Effect of Oral Anticoagulants on Antithrombin, Protein C and Protein S Measurements[496]

Coagulation test	Measurement methods	Thrombin antagonist	Xa antagonist	Vitamin K antagonist
Protein C activity	Coagulation time	False high	False high (rivaroxaban), unaffected	Lower
	Synthetic substrate method	Unaffected	Unaffected	Lower
Protein S activity	Coagulation time	False high	False high (rivaroxaban, edoxaban), unaffected	Lower
Antithrombin activity	Synthetic substrate method (thrombin method)	False high	Unaffected	Unaffected
	Synthetic substrate method (Xa method)	Unaffected	False high	Unaffected

(Adapted from Kadohira Y, et al. 2018[496] with modifications)

etc.), activity and antigen levels in the screening test, and the presence or absence of decline due to acquired factors. Designated Intractable Disease 327: Idiopathic thrombosis (limited to those caused by hereditary thrombophilic predisposition) The genetic test is one of the definite items in the diagnosis criteria of "327: Idiopathic thrombophilia (limited to those caused by inherited thrombophilia)".

4. Notes on Genetic Diagnosis

A confirmed diagnosis of hereditary thrombophilia is made when a reduction in AT, PC, or PS activity is observed and a gene variant with a clear causal relationship can be identified. However, there are cases in which gene variants cannot be identified on AT, PC, or PS genetic testing, but hereditary thrombosis should not be completely ruled out. The diagnosis must be made comprehensively in light of the patient's background. At present, there is no stratification of disease risk based on differences in gene variants.

5. Explanation to the Patient and Family

When genetic testing is performed, the individual as well as family members should be informed of the possibility of having a predisposition to thrombophilia. Another benefit of genetic testing is that the individual who has developed venous thrombosis may better understand the pathogenesis of the disease and future treatment strategies, and thus assist family members. If an unaffected family member is found to be a carrier of a pathological variant, this will lead to preventive measures for venous thrombosis depending on the risk of developing thrombosis, such as pregnancy or surgery. When genetic testing is performed, the individual and family should be fully informed, and a system of genetic counseling should be in place.

XIV. Familial Hypercholesterolemia

1. Disease Concept

Familial hypercholesterolemia (FH) is a genetic disease caused by mutations in genes involved in the low-density lipoprotein (LDL) receptor pathway. Except for autosomal recessive hypercholesterolemia (ARH), which is extremely rare (only in a few families in Japan), the disease is inherited in a dominant form.

FH heterozygotes show hyperLDL-cholesterolemia (hyperLDL-Cemia) from the time of birth, and have progressive coronary atherosclerosis due to premature atherosclerosis.[498,499] The risk of developing CAD is approximately 13-fold higher in untreated FH heterozygotes than in non-FH.[500] HyperLDL-C itself is not a cause of CAD, but because hyperLDL-Cemia itself is asymptomatic, FH should always be kept in mind when examining a patient with hyperLDL-Cemia, and early diagnosis, appropriate treatment, and family screening (cascade screening) can help prevent death in young people. A recent meta-analysis of molecular epidemiological studies suggests that the frequency of FH in the general population worldwide is approximately 1 in 300,[501,502] that is the case in Japan. Healthcare providers treating patients with FH should be aware that the disease is primarily an autosomal dominant inherited disorder and should be involved in the diagnosis and treatment of the patients' families.

FH homozygotes show marked hyperLDL-Cemia from the time of birth and often have characteristic cutaneous xanthomas. The frequency is estimated to be approximately 1 in 300,000 in the general population. Achilles tendon xanthomas, corneal rings, and systemic atherosclerosis develop markedly in childhood. Atherosclerosis progresses not only to the coronary arteries but also to the aortic valve, forming characteristic supravalvular stenosis and valve stenosis.[503] Supravalvular aortic stenosis, valve stenosis, and coronary artery stenosis are known to appear in infancy and progress to cause angina pectoris, myocardial infarction, and sudden death by age 30 years.

2. Causative Genes

FH is caused by pathogenic genetic mutations in the LDL receptor pathway such as LDL receptor (*LDLR*), apolipoprotein B-100 (*APOB*), and pathogenic gain-of-function mutations in the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene. Mutations in these genes have been identified in 60–80% of clinically diagnosed FH heterozygotes.[504,505] The presence of pathogenic mutations in these genes together with hyperLDL-Cemia, gives a definite diagnosis. Diagnostic criteria are shown in **Table 41** and **Table 42**.[506,507] Genetic testing is more reliable for the diagnosis of FH, but there are limited number of facilities to perform.

FH homozygotes are defined as having pathogenic mutations in the 2 alleles of *LDLR*, *APOB*, or *PCSK9*. ARH is an autosomal recessively inherited disease caused by pathogenic mutations in the LDL receptor adaptor protein 1 (*LDLRAP1*) gene, and its homozygotes are clinically included in the FH homozygote.

Recently, in addition to FH associated with rare pathogenic genetic mutations of the *LDLR* and its related genes as described above, the existence of so-called polygenic FH associated with the superposition of high-frequency genetic polymorphisms at loci involved in LDL metabolism has been suggested and reported.[508] However, there is no evidence, considering any known combination at this time, that the accumulation of high-frequency gene polymorphisms alone causes so-called FH, which may modify the phenotype of FH (and other hyperLDL-C blood disorders) rather than being involved in the pathogenesis of FH.

2.1 LDL Receptor

LDLR was the first gene identified as a cause of FH which accounts for 50–60% of cases of clinically diagnosed as heterozygous FH. So far, more than 2,000 pathogenic gene mutations have been reported as causes of FH worldwide

Table 41. Diagnostic Criteria for Heterozygous FH in Adults (≥15 Years of Age)[506]

1. HyperLDL-cholesterolemia (untreated LDL-C level ≥180 mg/dL)
2. Tendon xanthomas (dorsal hand, elbow, knee, etc. or Achilles tendon thickening) or cutaneous nodular xanthomas
3. Family history of FH or premature CAD (first degree relatives)

- Diagnosis is made after excluding other primary and secondary dyslipidemias
- If the patient is already on drug therapy, refer to the lipid level that triggered the therapy
- Achilles tendon thickening is diagnosed by radiography at ≥8.0 mm in men and ≥7.5 mm in women, or by ultrasound at ≥6.0 mm in men and ≥5.5 mm in women
- Cutaneous nodular xanthomas do not include xanthelasmas

If a patient meets ≥2 of the above-mentioned criteria, the condition should be diagnosed as FH. In the case of suspected heterozygous FH, making a diagnosis using genetic testing is desirable

- Xanthelasma is not included in xanthoma tuberosum
- Achilles tendon hypertrophy is maximum diameter ≥8.0 mm in men and ≥7.5 mm in women by radiography or ≥6.0 mm in men and ≥5.5 mm in women by ultrasonography
- Premature CAD is defined as CAD that develops at younger than 55 years of age in men and younger than 65 years of age in women
- FH is diagnosed when two or more items are met
- Even if two or more items are not met, if those whose LDL-C is 250 mg/dL or higher, or if 2 or 3 are met and LDL-C is 160 mg/dL or higher, they are classified as probable FH
- LDL-C level ≥250 mg/dL strongly suggests FH
- Diagnosis of FH is made in the presence of FH pathogenic gene mutations
- If HoFH is suspected, genetic testing is recommended. Genetic testing is also useful for suspected HeFH, which are more difficult to diagnose
- This diagnostic criterion also applies to HoFH

CAD, coronary artery disease; FH, familial hypercholesterolemia; HeFH, heterozygous familial hypercholesterolemia; HoFH, homozygous familial hypercholesterolemia; LDL(-C), low-density lipoprotein (cholesterol). (Adapted from Harada-Shiba M, et al. 2023[506]) Copyright © 2023 Japan Atherosclerosis Society. CC BY-NC-SA

Table 42. Diagnostic Criteria for Pediatric FH[507]

1. Hyper-LDL cholesterolemia (untreated LDL-C level ≥140 mg/dL, confirmed multiple times)
2. Family history of FH (Parents or siblings)
3. Parental LDL-C ≥180 mg/dL or family history of premature CAD (Grand parent or parent)

After ruling out other primary and secondary Hyper-LDL cholesterolemia,

- Diagnose FH with items 1 and 2
- Diagnose probable FH with items 1 and 3. If the individual's LDL-C is 180 mg/dL or higher, diagnose FH
- Even if only item 1 is used, ≥250 mg/dL is diagnosed with FH and ≥180 mg/dL is diagnosed with probable FH

- Differentiate HoFH when LDL-C is ≥250 mg/dL or xanthomas are present
- Diagnose FH if the individual has a pathogenic gene mutation for FH. If a parent, a brother, or a sister is found to have a pathogenic gene mutation for FH, that is considered to be the family history of FH (item 2)
- Premature CAD is defined as CAD occurring at less than 55 years of age in men and less than 65 years of age in women
- Probable FH cases require further scrutiny and lipid-lowering therapy

CAD, coronary artery disease; FH, familial hypercholesterolemia; HoFH, homozygous familial hypercholesterolemia; LDL-C, low-density lipoprotein cholesterol. (Adapted from Harada-Shiba M, et al. 2023[507]) Copyright © 2023 Japan Atherosclerosis Society. CC BY-NC-SA

(http://www.hgmd.cf.ac.uk/ac/gene.php?gene=LDLR). In Japan alone, more than 100 mutations have been reported.[509] The LDL receptor is located on the cell surface and binds to apolipoprotein B100 in LDL, which allows LDL to enter the cell.

2.2 Proprotein Convertase Subtilisin/Kexin Type 9

PCSK9 was originally known as a neurotrophic factor, but linkage analysis of a FH family with normal LDL receptors revealed that PCSK9 is highly involved in cholesterol metabolism.[510] PCSK9 is involved in LDL receptor degradation, and its gain-of-function mutations cause LDL receptor depletion and hyperLDL-Cemia. In Japan, the E32K mutation, which is a mild gain-of-function mutation and causes relatively mild LDL-C elevation, is observed in 1–2% of the general population and 6% of clinically diagnosed FH patients.

2.3 LDL Receptor Adapter Protein 1

LDLRAP1 is involved in endocytosis of the LDL receptor as an adapter protein. Pathogenic mutations in *LDLRAP1* from both parents cause a very rare disorder, Autosomal Recessive Hypercholesterolemia: ARH. A patient who shows severe hypercholesterolemia with giant xanthomas is suspected as having homozygous FH, but both parents do not have hyperlipidemia, ARH should be considered.[511,512] This disease should be treated as FH homozygous.

3. Diagnosis of FH

Diagnosis should be made after excluding other primary or secondary dyslipidemias. FH is diagnosed by the presence of hyperLDL-Cemia, Achilles tendon thickening and/or cutaneous xanthomas, and family history (**Tables 41**,**42**).[506,507] Achilles tendon thickening is diagnosed by visual inspection and palpation, but if there is any doubt about the diagnosis, radiography or ultrasonography is performed to measure the maximum diameter of the Achilles tendon. Cutaneous and tendon xanthomas tend to occur on the extensor surfaces of the hands, elbows, and knees. Because premature CAD often runs in the family, a full family history is extremely important for making the correct diagnosis. In addition, screening of blood relatives (cascade screening) should always be considered when FH is diagnosed.

FH homozygotes are characterized by having serum total cholesterol levels ≥600 mg/dL, skin and tendon xanthomas, atherosclerotic diseases from the childhood and their parents being heterozygous FH. Xanthomas may be the first dermatological presentation in childhood, and it is extremely important that dermatologists do not miss FH homozygotes at this time. The diagnosis of FH homozygotes does not necessarily require genetic testing.[507]

FH is diagnosed when FH pathogenic gene mutations are present; genetic testing for FH has been covered by insurance since April 2022.

4. Genetic Counseling in FH

Genetic counseling should be provided at the appropriate time. Because it is not only about providing information, but also psychological and social support to enable the patient/subject to make an informed choice, counseling should be conducted as part of team medicine, with the cooperation of a physician with extensive experience in the treatment of the disease and a skilled genetic counselor. Because the basic form of FH is an autosomal dominant inherited disease, 3 etiologic genes (*LDLR*, *APOB*, and *PCSK9*) are described here. If the child is FH heterozygous, either parent is FH heterozygote. If one parent is FH heterozygous, there is a 50% chance that the child will also be FH heterozygote, and if both parents are FH heterozygotes, there is a 75% chance that the child will be FH heterozygote (25%: FH homozygote, 50%: FH heterozygote). When both parents of a child with FH heterozygous are non-FH, it is necessary to consider the possibility of a mutation in the affected child, mild symptoms in both parents, or the absence of a true blood relationship, such as adoption.

A single randomized intervention trial of FH genetic testing enforcement and genetic counseling has provided an evidence of effectiveness in lowering LDL-C levels[513] (Recommendation Class 1b, Level of Evidence A).

5. Treatment and Lifestyle Management

Because FH heterozygotes are at extremely high risk of developing atherosclerotic cardiovascular disease, especially CAD, primary prevention is at least equivalent to the usual secondary prevention. Therefore, the control goal for patients heterozygous for FH in primary prevention should be LDL-C <100 mg/dL. As FH heterozygotes in secondary prevention are considered to be at even higher risk, LDL-C management target should be <70 mg/dL.

Statins should be started at the usual dose and then increased while observing the effect and side effects. If statins alone do not provide an adequate response, ezetimibe or PCSK9 inhibitors should be used. If the expected LDL-C-lowering effect is not achieved after concomitant therapy with a PCSK9 inhibitor in addition to the usual oral therapy, the patient is likely to be homozygous for FH and should be referred to a specialist, including for genetic testing.

FH homozygotes require aggressive LDL-C lowering therapy starting at a young age to prevent the onset and progression of CAD. Because statins, anion exchange resins, and PCSK9 inhibitors all have the primary mechanism of action of enhancing LDL receptor activity, FH homozygotes with LDLR negative type show no LDL-C lowering effect.[514–516] Although the LDL-C-lowering effect of PCSK9 inhibitors (≈30%) has been confirmed in a study of adult FH homozygous patients,[517] PCSK9 inhibitors should be discontinued if LDL-C level is not reduced at all after several times of injection. Microsomal triglyceride transfer protein (MTP) inhibitor (e.g., lomitapide) has been reported to reduce LDL-C by approximately 50% in FH homozygotes,[518] and it is now on the market.[519] However, use of lomitapide is associated with side effects of fat deposition in the liver and diarrhea, so strict control of dietary fat and alcohol intake is essential.[520] A worldwide registry study of FH homozygotes reported that the more drugs used and the lower the LDL-C level, the better their prognosis.[521] In addition, an antibody against angiopoietin-like protein 3 (ANGPTL3) called evinacumab was shown to decrease LDL-C levels in FH homozygotes and was approved in Japan in January of 2024.

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Appendix 1. Practical Guide for the Management of Inheritable Cardiovascular Diseases

Diseases	Causative genes	Penetrance	Recommendation				Remarks
			Proband(s)	At risk relatives			
			Intervention	Age for cascade screening	Surveillance	Intervention	
Hypertrophic cardiomyopathy (HCM)	*MYH7*	moderate to high (increase with age)	β-blockers, ICDs, risk avoidance (limit competitive sports and strenuous exercise), preconception and postpartum management	Before entering junior high school	Taking medical history, ECG, echocardiography*	No intervention without the appearance of cardiac hypertrophy	* At least every 1 to 1.5 years until age 20 and at least every 5 years after age 20
	MYBPC3	moderate to high (increase with age)					
Dilated cardiomyopathy (DCM)	*LMNA*	high (increase with age)	Consider medication, ICD, heart transplant, avoid risk (limit strenuous exercise), preconception and postpartum management	Before entering junior high school	Taking medical history, ECG, annual follow up of echocardiography	No intervention without the appearance of cardiac abnormalities	
	TTN	moderate	Medications, risk avoidance (limit strenuous exercise), preconception to postpartum management				
Long QT sydrome (LQTS)	*KCNQ1*	moderate to high	β-blockers, mexiletine, sympathectomy, ICD, avoid drugs that prolong QT interval, exercise restriction	Infancy to preschool	Resting ECG (1–2 times a year), exercise stress ECG, ambulatory Holter ECG (timely)	Risk management # (depend on the severity of QT prolongation)	# The Risks of LQTS QT-prolonging drugs: common to each genotype *KCNQ1*: Intense exercise (especially long distance running, swimming, etc.) *KCNH2*: Mental stress, sound stimulation, pregnancy and childbirth *SCN5A*: bradycardia *CALM1–3*: Exercise
	KCNH2	moderate to high					
	SCN5A	moderate to high					
	CALM1	high					
	CALM2	high					
	CALM3	high					
Brugada syndrome (BrS)	*SCN5A*	male: moderate to high (in adults), female: low	ICD, drug therapy, use of antipyretics for fever	After entering jounior high school	Annual follow-up of ECG	Avoid high fever	
Catecholaminergic polymorphic ventricular tachycardia (CPVT)	*RYR2*	high	β-blockers, flecainide, sympathectomy, ICD, risk avoidance (limiting exercise with rapid heart rate increases, stress avoidance)	Infancy to preschool	Exercise stress ECG, ambulatory Holter ECG (timely)	β-blockers, limiting exercise that causes rapid heart rate increases, avoidance of mental stress	
	CASQ2	high (homozygote)					
		unknown (heterozygote)					
	CALM1	high					
	CALM2	high					
	CALM3	high					

(**Appendix 1** continued the next page.)

Diseases	Causative genes	Penetrance	Recommendation				Remarks
			Proband(s)	At risk relatives			
			Intervention	Age for cascade screening	Surveillance	Intervention	
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	*PKP2*	moderate	Consider *β*-blockers, heart failure medications, ICD, restrictions on participation in competitive sports, heart transplantation	Before entering junior high school	ECG, echocardiography, cardiac MRI (every 1–3 year(s))	Avoid participatiing in competitive sports	
	DSG2	high (homozygote)					
		low (heterozygote)					
Marfan syndrome (MFS) and related diseases	*FBN1*	high	*β*-blockers, ARBs, aortic root replacement, avoid risk (athletics/competitions involving physical contact, strong isometric exercise), preconception and postpartum management	Infancy to preschool	Chest X-ray, ECG, biannual follow-up of echocardiogprahy or computed tomography	*β*-blockers, ARBs, avoid risk (athletics/competitions involving physical contact, strong isometric exercise), preconception and postpartum management	
	TGFBR1						
	TGFBR2						
	SMAD3						
Ehlers Danlos syndrome, vascular type (vEDS)	*COL3A1*	high	Celiprolol, risk avoidance (competitions involving physical contact, weightlifting, high-impact strength training), preconception to postpartum management	Preschool or infancy if there are associated symptoms such as congenital clubfoot, bruisability.)	Chest X-ray, ECG, biannual follow-up of echocardiogprahy or computed tomography	Celiprolol, risk avoidance (competitions involving physical contact, weightlifting, high-impact muscle training), preconception and postpartum management	
Familial hypercholesterolemia (FH)	*LDLR*	high (heterozygote)	Life style modification including diet and exercise, statins, ezetimibe, PCSK9 inhibitors (antibody/siRNA)	Before teens	Evaluation of lipid profile including LDL-C levels/year	Liifestyle modification including diet and exercise, statins, ezetimibe, PCSK9 inhibitors	
	PCSK9	high (heterozygote)					
Osler-Weber-Rendu disease (HHT)	*ENG*	high (increase with age)	Nasal hemorrhage, AVM (various sites)	Neonatal period (search for intracranial AVMs) intracranial AVMs(+): management and genetic testing as affected intracranial AVMs(−): infancy to preschool age	SpO_2, X-ray, ECG, ultrasound, CT (evaluation of AVM) biannually	With AVM, endovascular treatment/surgery If with hypoxemia or pulmonary hypertension, oxygen, pulmonary vasodilators	
	ACVRL1						
Pulmonary arterial hypertention (PAH)	*BMPR2*	male: low, female: moderate	Oxygen, diuretics, pulmonary vasodilators		SpO_2, X-ray, ECG, echocardiography every 6–12 months	Pulmonary vasodilators immediately after confirmation of diagnosis	

low: ~40%; moderate: 50~70%; high: 80~100%.

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Appendix 3. Disclosure of Potential Conflicts of Interest (COI): JCS/JCC/JSPCCS 2024 Guideline on Genetic Testing and Counseling in Cardiovascular Disease (2021/1/1–2023/12/31)

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Notation of corporation is omitted.

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In accordance with "The JAMS COI Management Guidance on Eligibility Criteria for Clinical Practice Guideline Formulation 2023", all members have submitted their COIs for the past 3 years.
Some members (*) reported under the category of "Amount Category 3" or "Endowed departments established through donations by a company" and therefore do not have a vote in the guideline formulation process, to ensure fairness and transparency of the guidelines.

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