Congenital Heart Defects and Pulmonary Arterial Hypertension

Genes, Environment and Heredity

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

aCGH	array comparative genomic hybridisation
ASD	atrial septal defect
AVSD	atrial ventricular septal defect
AI	aortic valve insufficiency
AV	aortic valve
AVR	aortic valve replacement
AVS	aortic valve stenosis
BAV	bicuspid aortic valve
BMI	body mass index
CDS	coding sequences
CHA	congenital heart anomalies
chr	chromosome
CHD	congenital heart defect
CI	confidence interval
CNV	chromosomal numerical variations
COA	coarctation of the aorta
DF	dysmorphic features
DILV	double inlet left ventricle
DMP	dorsal mesenchymal protrusion
DORV	double outlet right ventricle
EMT	epithelial-to mesenchymal-transition
FISH	fluorescence in situ hybridization
GDM	gestational diabetes mellitus
HLHS	hypoplastic left heart syndrome
HPAH	hereditary pulmonary arterial hypertension
IPAH	idiopathic pulmonary arterial hypertension
LVNC	left ventricular non-compaction
LVOTO	left ventricular outflow tract obstructions, including BAV, AVS, COA, HLHS
MAF	minor allele frequency
MCA	multiple congenital anomalies
MLPA	multiplex ligation-dependent probe amplification
MR	mental retardation
MVS	mitral valve stenosis
MVR	mitral valve replacement
NCA	non-cardiac congenital anomalies
NYHA	New York Heart Association

OR	odds ratio
PAH	pulmonary arterial hypertension
PDA	persistent ductus arteriosus
PCR	polymerase chain reaction
PA	pulmonary valve atresia
PH	pulmonary hypertension
PVD	pulmonary valve disease
PVR	pulmonary vascular resistance
PVS	pulmonary valve stenosis
RHC	right heart catheterization
RVOTO	right ventricular outflow tract obstructions
RVF	right ventricular failure
RVSP	right ventricular systolic pressure
SHH	sonic hedgehog
SLOS	Smith-Lemli-Opitz syndrome
SNP	single nucleotide polymorphism
SPS	small patella syndrome
SVAS	supravalvular aortic stenosis
TA	truncus arteriosus
TGA	transposition of the great arteries
TOF	Fallot's tetralogy
VSD	ventricular septal defect
WGA	whole genome array

GENETIC TERMINOLOGY

There are many textbooks and websites on genetics that explain the terms and techniques below, but I have added these explanations to help physicians non-geneticists who may read this thesis.

Allele: Because all DNA in humans is present *in duplo* (except for the X- and Y chromosomes in males) the term allele is used to distinguish between the two copies. In this context, the maternal allele is the allele inherited from the mother and the paternal allele is from the father.

Complex (or multifactorial) inheritance: Complex diseases are diseases caused by a combination of inherited and non-inherited factors. The inherited component is polygenic, meaning that variations in different genes, on different chromosomes contribute to the disease phenotype, whether or not in combination with known or unknown environmental factors. In other words: multiple risk factors accumulate and a disease will occur only after they reach a certain threshold. The essential difference from monogenic, Mendelian inheritance is that more than one gene causes the disease.

Epigenetics: Epigenetics concerns factors influencing the expression of genes in a reversible way, without changing the nucleotide sequence of the DNA in the nucleus.

Exome: The total of protein coding DNA (approximately 1.5% of the genome).

Expression: The clinical expression of a genetic predisposition is the way the disease presents. Variable expression means that carriers of a mutation have different symptoms of a disease. A second use of the term is to designate the expression of genes in certain tissues: whether or not a gene is translated to protein.

Gene: A stretch of DNA containing the code for one protein. A gene has exons and introns. The introns are spliced-out during transcription to RNA and the exons contain the code for the protein.

Genome: The total of DNA a person inherits from one parent. Human cells are diploid and therefore contain 2 genomes, one from each parent.

Genomic imprinting: Imprinting is the epigenetic reversible modification of genes or regions in the genome in the parental gametes, leading to functionally different expression of the two alleles. In other words: the expression of a part of the genome depends on the gender of the transmitting parent.

Imprinting may be involved in monogenic as well as complex inheritance. Imprinting may explain why the recurrence of congenital heart defects (CHD) is more frequent in the offspring of females with CHD than in the offspring of males with CHD.

Mendelian inheritance: Mendelian inheritance is the form of inheritance according to Mendel's laws, which state that a certain disease is caused by a mutation in one or both alleles of a gene. It is therefore designated monogenic inheritance. The pattern of inheritance is autosomal or X-linked and either dominant or recessive.

Large non-coding RNAs (LncRNAs): LncRNAs are large molecules of non-coding RNA of more than 200 nucleotides, regulating gene expression through several mechanisms. They are reported to play

a role in cardiac development and disease.

Micro-RNAs (miRNAs): miRNAs are small molecules of non-coding RNA of approximately 22 nucleotides, repressing translation to proteins by binding to messenger RNA. It has been proven that miRNAs play an important role in heart development.

Penetrance: The penetrance of a genetic predisposition is the proportion of persons expressing the disease. Reduced penetrance means that a mutation in a gene gives rise to symptoms in only some of the mutation carriers but not in others.

SOME GENETIC TECHNIQUES

Array-comparative genome hybridization (aCGH): aCGH is a molecular technique to compare the amount of DNA at many different points in the genome, allowing the detection of small deletions and duplications of genetic material at random positions. The test can be performed on DNA that has been isolated from EDTA blood or a tissue biopsy.

Fluorescence in situ hybridization (FISH): FISH may be used if one expects to find a deletion at a specific, known position on a chromosome. Metaphase nuclei are hybridized with a specific probe labelled with a fluorescent dye. Fluorescent microscopy is used to determine whether the two copies of a specific DNA sequence are present or not. The test can be performed on cells from a heparinised blood sample or from a tissue biopsy.

Karyotyping: Karyotyping or chromosome analysis describes the number and appearance of chromosomes in metaphase nuclei under a normal-light microscope. It detects numerical chromosomal anomalies (trisomy, monosomy) and large structural anomalies (translocations, large deletions, duplications and inversions). It can be performed on cells isolated from heparinized blood or tissues biopsies.

Next generation sequencing (NGS): Sequencing means determining the order of the nucleotide in a DNA molecule. NGS is a method for parallel sequencing of large numbers of DNA templates, reducing the amount of time and money needed for the tests. Several systems are available, all using fragmented DNA of a specific sample, binding to method-specific linkers. The automation enables testing of a complete exome or even genome of an individual. These methods facilitate gene finding in research settings and are now also being implemented in diagnostics. The tests can be performed in DNA that has been isolated from EDTA blood or a tissue biopsy.

Sanger sequencing: Sequencing means determining the order of the nucleotide in a DNA molecule. Sanger sequencing is the classical method ("first generation sequencing") of analysing DNA by adding small amounts of labelled dideoxynucleotides (ddNTPs) to the four normal nucleotides, creating labelled stops in the DNA string. The method has been adjusted to gain speed by using coloured labels and capillary and laser detection instead of fluorescent and radioactive labels, gels and fluorescence or radioactivity detection. The tests can be performed in DNA that has been isolated from EDTA blood or a tissue biopsy.

Single Nucleotide Polymorphism (SNP)array: SNP array is a molecular technique to compare nucleotides at many different points in the genome from a patient with reference DNA. It can be used for several purposes, for example for the detection of small deletions and duplications of genetic material at random positions. The test can be performed on DNA that has been isolated from EDTA blood or a tissue biopsy.