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## CARDIOVASCULAR DISEASES (PART A)
## INFERTILITY AND METABOLIC SYNDROMES (PART B)

**Guest Editors: Matteo Bertelli, Stefano Paolacci**

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Foreword

# Diagnostic and therapeutic implements based on advanced Biotechnology should be available in low-income countries

*Matteo Bertelli[1, 2, 3], Stefano Paolacci[4], Tommaso Beccari[5, 6], Munis Dundar[5, 7], Gabriella Sozanski[3], Stanislav Miertus[8], Jan Miertus[9], Lucio Luzzatto[10]*

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In 2015 the United Nations identified a set of 17 sustainable development goals (SDGs) as targets to be realized by the end of 2030. One of these goals is "to promote physical and mental health and well-being, and to extend life expectancy for all and to achieve universal health coverage and access to quality health care" (1). However, at just 11 years before that deadline, according to the Global Observatory on Health R&D, wide gaps and inequalities still persist. These are noticeable when comparing developed *versus* developing countries; and also when analyzing individual health issues. Such inequalities make it difficult for developing countries to achieve the WHO objectives of efficient, cost-effective and robust means of preventing, diagnosing and treating major diseases. Improving this situation requires adoption of appropriate public health policies (2). We wish to promote the notion that biotechnology-based diagnostics and therapeutic interventions should become available in low-income countries.

About 10-30% of infant mortality in developing countries is due to genetic diseases; however, due to currently prohibitive costs (3), genetic screening programs are in practice only feasible in middle- and high-income countries. For example, immunochemical tests (*e.g.* ELISA kits) for diagnostic purposes can be of immense value in low-income countries, because they can target antigens specific for endemic pathogenic viruses or bacteria. In the case of yellow fever, an ELISA kit has been developed, whereby a test with an accuracy of >90% can be carried out in 3.5h (4). During the outbreak of Ebola in 2014-2016, trials of a recombinant vaccine conducted in Guinea resulted in robust immunity within ten days of a single injection (5), raising the potential for disease prevention. Similar viral-based vector vaccine strategies could be utilized and adapted for different antigens derived from pathogens.

Another promising approach, is that of nucleic-acid-based compounds. This may be highly relevant to low-income countries as siRNA-based treatment is under investigation, and may provide a new category of small molecules therapeutics for Ebola or other viral infections (6). This treatment could target proteins responsible for viral RNA transcription and replication, and can be adapted in order to tackle viral variants (6).

The above are mere examples of reasons why biotechnological research targeting developing countries should be appropriately funded. Genetic and immunochemical tests, as well as recombinant vaccines and biological drugs should be made fully accessible to low-income countries with the concerted support of global UN programs (UNCTAD.WHO), non-for-profit organizations, charity foundations, and projects

of international cooperation. A complementary way to achieve an improvement of the health outcomes is to promote drug production in developing countries, as suggested by the director for investment and enterprise at the UN Conference on Trade and Development in Geneva (14–17 October 2014) (7). Another important tool to reduce the biotechnology divide between developed and developing countries is the education, postgraduate training, knowledge transfer and capacity building including innovation in low-income countries. One of the few examples worldwide in this respect is the program of International Centre for Genetic Engineering and Biotechnology (8), but such programs should be by far strengthened, possibly also by support of EC, G7 etc.

An overarching consideration, is that low-income countries take political decisions with priority given to matters concerning the health of their citizens, which includes health services. With demand originating from developing countries of need, international organizations can better respond to informed requests from individual or groups of countries, rather than taking their own decisions.

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Review

# Genetics and pharmacogenetics in the diagnosis and therapy of cardiovascular diseases

*Geraldo Krasi[1], Vincenza Precone[2], Stefano Paolacci[3], Liborio Stuppia[4], Savina Nodari[5], Francesco Romeo[6], Marco Perrone[6], Vilma Bushati[1], Astrit Dautaj[1], Matteo Bertelli[2]*

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**Summary.** Cardiovascular diseases are the main cause of death worldwide. The ability to accurately define individual susceptibility to these disorders is therefore of strategic importance. Linkage analysis and genome-wide association studies have been useful for the identification of genes related to cardiovascular diseases. The identification of variants predisposing to cardiovascular diseases contributes to the risk profile and the possibility of tailored preventive or therapeutic strategies. Molecular genetics and pharmacogenetics are playing an increasingly important role in the correct clinical management of patients. For instance, genetic testing can identify variants that influence how patients metabolize medications, making it possible to prescribe personalized, safer and more efficient treatments, reducing medical costs and improving clinical outcomes. In the near future we can expect a great increment in information and genetic testing, which should be acknowledged as a true branch of diagnostics in cardiology, like hemodynamics and electrophysiology. In this review we summarize the genetics and pharmacogenetics of the main cardiovascular diseases, showing the role played by genetic information in the identification of cardiovascular risk factors and in the diagnosis and therapy of these conditions. (www.actabiomedica.it)

**Key words:** molecular genetics, cardiovascular diseases, risk factors, NGS, pharmacogenetics

## Introduction

Cardiovascular diseases (CVDs) are the principal cause of death worldwide. They include coronary heart disease, cerebrovascular disease, peripheral arterial disease, rheumatic and congenital heart diseases and venous thromboembolism (1). CVDs are complex genetically heterogeneous conditions resulting from many gene-gene and gene-environment interactions (2). Molecular genetics and pharmacogenetics play a key role in the diagnosis, prevention and treatment of CVDs. Genetic testing is normally used to identify underlying genetic etiology in patients with suspected cardiovascular disease and to determine who in the family has inherited the causal variant and is therefore at risk of developing CVD. Genetic testing should be carried out in well phenotyped individuals, ideally coupled with comprehensive family evaluation to aid in interpretation and application of the results (3). Molecular genetics technologies applied to cardiovascular studies have enabled chromosome mapping and identification of many genes involved in primary etiology, as well as significant risk factors for the development of CVDs, including environmental risk factors. Cardiovascular diseases and related risk factors may be monogenic or polygenic (4). Since many genetic variations have an association with CVDs, routine genetic testing of patients with these conditions is important.

Pharmacogenetics is the study of interpatient genetic variations associated with different responses to drugs, including toxicity. Pharmacogenetic testing reveals variations in drug metabolism genes. These genes encode metabolic enzymes that can be defined as either "poor metabolizers" or "rapid metabolizers" in relation to the efficiency of their activity. Identifying how a patient metabolizes a medication enables personalized and safer treatments, leading to improved clinical outcomes and reduced medical costs. Pharmacogenetic testing can be performed prior to prescription to guide drug selection and dosage (5,6) or after unsuccessful treatment. For instance, platelet aggregation inhibitors (PAI), oral anticoagulants (OA), anti-hypertensive and cholesterol-lowering drugs are abundantly prescribed for cardiovascular disease, but individual responses may vary significantly, since genetic variability is partly responsible for such differences in efficacy (7). Pharmacogenetics and pharmacogenomics can be expected to optimize therapy and reduce toxicity through individualized genetically guided therapy (8).

This brief review summarizes the principal cardiovascular diseases and the role molecular genetics and pharmacogenetics can have in the identification of cardiovascular risk factors, and in the diagnosis and therapy of cardiovascular diseases.

## Monogenic forms

In monogenic CVDs, a single gene determines the onset of symptoms, although genotype-phenotype correlation can be complex due to genetic phenomena (pleiotropy and variable penetrance and expressivity) and environmental factors (4).

*Cardiac conduction defects*

- Long QT syndrome (LQTS) is a genetic heart disease characterized by prolongation of the QT interval that can lead to arrhythmia, palpitations, syncope or sudden death. It typically manifests in patients under 40 years of age, and sometimes in early infancy (9). LQTS follows two distinct patterns of inheritance: autosomal dominant (Romano-Ward syndrome) with an estimated prevalence between 1:2000 and 1:5000 (10,11) and autosomal recessive (Jervell and Lange-Nielsen syndrome) with an estimated prevalence between 1:1,000,000 and 1:4,000,000 in the general population (11), although depending strongly on the study population (12).
- Short QT syndrome (SQTS) is a channelopathy characterized by an abnormally short QT interval and increased risk of atrial and ventricular arrhythmias and sudden death. Clinical presentation is heterogeneous, since some patients may be asymptomatic and others may have episodes of syncope or fall victim to sudden cardiac death. It may occur at any age from early infancy to old age. The prevalence is estimated at 1:1000 to 5:1000. SQTS is sporadic or has autosomal dominant inheritance (13,14).
- Brugada syndrome (BrS) is a genetic heart disorder involving ion channel dysfunction associated with progressive age-related conduction abnormalities, more prevalent among males. It is estimated to be responsible for up to 20% of all sudden deaths in individuals with an apparently normal heart. BrS usually manifests with syncope or sudden cardiac death at a young age, in the absence of structural heart anomalies, and typically has autosomal dominant inheritance. Prevalence is estimated at 5:10,000 worldwide (15).
- Familial atrial fibrillation (FAF) is a heterogeneous genetic heart disorder characterized by erratic activation of the atria and irregular ventricular response. The heterogeneous clinical presentations of FAF include palpitations, dyspnea, chest pain, dizziness and syncope. FAF increases the risk of stroke and sudden death. The prevalence of FAF is approximately 1% in the general population. FAF is genetically heterogeneous with autosomal dominant or recessive inheritance (16).
- Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited heart disorder characterized by electrical instability during acute activation of the adrenergic nervous system, in a structurally normal heart. The ECG is normal but arrhythmia may occur during physi-

cal activity or emotional stress, causing syncope or even cardiac arrest unless the disease is recognized and treated. Prevalence is estimated at 1:10,000. CPVT has autosomal dominant and autosomal recessive inheritance (17).

- Wolff-Parkinson-White syndrome (WPW) is a heart disease characterized by arrhythmia due to one or more abnormal electrical pathways in the heart, known as accessory pathways, that allow electrical signals to bypass the atrioventricular node or may transmit electrical impulses abnormally in the reverse direction. WPW may present with palpitations, dyspnea, dizziness or even syncope. In rare cases it can lead to cardiac arrest and sudden death. WPW affects 1 to 3 in 1000 persons worldwide. It may be sporadic or familial. The familial form typically has autosomal dominant inheritance (18).

Table 1 summarizes the different genes associated with cardiac conduction defects

*Cardiomyopathies*

- Hypertrophic cardiomyopathy (HCM) is a common myocardial disease characterized by hypertrophy of the left ventricle with histological features of cell hypertrophy, myofibril disarray and interstitial fibrosis. This condition can remain asymptomatic throughout life or manifest with variable symptoms. It is the most common cause of sudden cardiac death in young people. HCM affects an estimated 1 in 500 persons worldwide (45). It is most often caused by variations in genes essentially encoding sarcomeric, ion channel and metabolic regulatory proteins. Around 70% of all cases are found to be familial with dominant inheritance (46-48).
- Dilated cardiomyopathy (DCM) is characterized by dilation leading to systolic and diastolic dysfunction of the left and/or right ventricles, causing heart failure or arrhythmia. It is essentially an adult-onset disease, but has shown a highly variable age of onset. The prevalence of DCM has been estimated at 36.5 per 100,000. It has autosomal dominant inheritance in 85% of cases (49).
- Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a genetic disease characterized by the death of ventricular myocytes and their replacement with fibrous and fatty tissue. It predisposes to ventricular tachycardia and sudden death in young individuals and athletes. The

**Table 1.** Genes associated with cardiac conduction defects

| Cardiac conduction defects | Mutant genes | Reference |
|---|---|---|
| Long QT syndrome | *KCNQ1, SCN5A, AKAP9, ANK2, CACNA1C, CALM1, CALM2, CAV3, KCNE1, KCNE2, KCNJ2, KCNJ5, SCN4B, SNTA1, KCNH2* | (19) |
| Short QT syndrome | *KCNH2, KCNQ1, KCNJ2, CACNA1, CACNB2, CACNA2D1* | (14,20,21) |
| Brugada syndrome | *SCN5A, CACNA1C, CACNB2, GPD1L, KCND3, KCNE3, HCN4, SCN1B, SCN3B* | (22) |
| Familial atrial fibrillation | *KCNQ1, KCNE2, NPPA, KCNA5, KCNJ2, SCN5A, GJA5, ABCC9, SCN1B, SCN2B, SCN3B, SCN4B, MYL4, GATA4, GATA5, GATA6, PITX2, TBX5, NKX2-5, KCND3, KCNE1, KCNH2, LMNA, PRKAG2, RYR2, ZFHX3, SHOX2, PRRX1, KCNN3, NUP155* | (23-40) |
| Catecholaminergic polymorphic ventricular tachycardia | *RYR2, CALM1, ANK2, KCNJ2, CASQ2, TRDN* | (41-43) |
| Wolff-Parkinson-White syndrome | PRKAG2 | (44) |

symptoms (palpitations, shortness of breath, swelling of the legs and syncope) are not frequent in the early stages, but there is risk of sudden death during intense exercise. The estimated prevalence of ARVC is estimated at 1:1000. Most familial cases of ARVC have autosomal dominant inheritance, whereas autosomal recessive inheritance is rare (50).

- Left ventricular non-compaction (LVNC) is a rare condition characterized by prominent left ventricular trabeculae, a thin compacted layer and deep intertrabecular recesses continuous with the left ventricular cavity but separate from the epicardial coronary arteries. It is frequently diagnosed in children, being due to an arrest in cardiac development during embryogenesis (51). LVNC is estimated to affect 8 to 12 per million individuals per year. This genetically heterogeneous disorder has sporadic and familial forms (52). LVNC can have autosomal dominant, autosomal recessive, X-linked and mitochondrial inheritance (53,54).
- Restrictive cardiomyopathies (RCM) are the least common cardiomyopathies and are characterized by impaired diastolic function with restrictive filling and reduced diastolic volume of one or both ventricles, preserved systolic function, and invariably normal or mildly increased wall thickness. The prevalence of RCM is unknown. RCM can be idiopathic, familial (autosomal dominant, autosomal recessive or X-linked), or secondary to systemic disorders (55).

Table 2 summarizes the different genes associated with hereditary cardiomyopathies.

*Familial hyperlipidemia*

Dyslipidemias are a heterogeneous group of disorders characterized by abnormal levels of circulating lipids and lipoproteins. A minority of forms of dyslipidemia are monogenic. These forms are familial diseases with a well-defined hereditary component.

- Familial hypercholesterolemia (FH) is the most frequent condition and is characterized by severely elevated LDL-C and by xanthomas (patches of yellowish cholesterol buildup) that occur around the eyelids and in the tendons of the elbows, hands, knees and feet. FH has a prevalence of 1:200-250. An estimated 70-95% of cases are caused by a pathogenic variant in the

**Table 2.** Genes associated with hereditary cardiomyopathies

| Cardiomyopathies | Mutant genes | Reference |
|---|---|---|
| Familial hypertrophic cardiomyopathy | *MYH7, TNNT2, TPM1, MYBPC3, PRKAG2, TNNI3, MYL3, TTN, MYL2, ACTC1, CSRP3, TNNC1, MYH6, VCL, MYOZ2, PLN, NEXN, ACTN2, CAV3, JPH2, LDB3, MYPN, CALR3, FLNC, MYLK2, TCAP* | (46,47) |
| Dilatived cardiomyopathy | *LMNA, MYH7, MYH6, SCN5A, ACTN2, DSG2, LDB3, TNNT2, RBM20, TTN, BAG3, DES, DSP, CRYAB, EYA4, LAMA4, MYPN, SGCD, CSRP3, ABCC9, PLN, ACTC1, TCAP, MYBPC3, NEXN, PRDM16, PSEN1, PSEN2, TPM1, VCL, RAF1, NKX2-5, ANKRD1, TMPO, ILK, TNNC1, TNNI3, GATAD1, FKTN, SDHA, DSP, DMD, TAZ* | (56-62) |
| Arrhythmogenic right ventricular cardiomyopathy | *TGFB3, RYR2, TMEM43, DSP, PKP2, DSG2, JUP, CTNNA3, TTN, DES, LMNA, PLN, DSC2* | (63-68) |
| Left ventricular non-compaction | *MYBPC3, TPM1, PRDM16, MIB1, TNNT2, MYH7, ACTC1, LDB3, SOX6, LMNA, SCN5A, HCN4, DTNA, TAZ, PLEKHM2, PKP2* | (53,54, 69-77) |
| Restrictive cardiomyopathy | *TNNT2, TNNI3 ACTC1, MYH7, MYBPC3, MYPN, TPM1, MYL1, MYL2, FLNC* | (78-81) |

genes *APOB, LDLR* and *PCSK9* inherited in an autosomal dominant manner (82).

- Primary hypertriglyceridemia arises from genetic defects in the metabolism or synthesis of triglycerides. It usually presents in adulthood, except for lipoprotein lipase deficiency that presents in childhood. Disorders in this category include familial chylomicronemia, severe hypertriglyceridemia, infantile hypertriglyceridemia and hyperlipoproteinemia type 3. The incidence of primary hypertriglyceridemia is approximately 2 per 10,000 persons. Common genetic variants found in *LPL, APOC2* and *LMF1* are associated with triglyceride levels in patients with primary hypertriglyceridemia. Except for rare severe mutations in *APOE*, monogenic hypertriglyceridemia is autosomal recessive (83).
- Familial HDL deficiency is a rare genetic condition that causes low levels of "good" cholesterol (HDL) in the blood, associated with cardiovascular risk. The prevalence of familial HDL deficiency is unknown. Familial HDL deficiency is inherited by autosomal dominant transmission of variations in the *ABCA1* and *APOA1* genes (84).

*Arterial hypertension*

Hypertension is a long-term condition in which arterial blood pressure is persistently elevated. High blood pressure usually does not cause symptoms. About 30% of cases of arterial hypertension are caused by a variation in a single gene. Three mechanisms are recognized to explain the physiopathology of monogenic hypertension:

- increased sodium reabsorption leading to plasma volume expansion;
- excessive aldosterone synthesis;
- deficiencies of enzymes regulating adrenal steroid hormone synthesis and deactivation (85).

Arterial hypertension is an important risk factor for cardiovascular events including stroke, coronary artery disease, heart failure and atrial fibrillation. The monogenic forms are characterized by early-onset hypertension. Known genetic factors explain only 3% of blood pressure variability (85,86,87).

*Coronary artery disease*

Coronary artery disease (CAD) is the major cause of death and disability among all cardiovascular diseases. It comprises a wide variety of clinical entities that include asymptomatic subclinical atherosclerosis and its clinical complications, such as angina pectoris, myocardial infarction and sudden cardiac death. The long-recognized familial clustering of CAD suggests that genetic factors play important roles: the heritability of CAD and myocardial infarction are estimated at 50-60%. Based on their apparent patterns of inheritance, genetic diseases are classified in two broad categories: monogenic and polygenic. In monogenic forms, familial variation in one gene is responsible for all or most of the disease incidence. Monogenic coronary artery diseases (MCAD) include genes and mutations that are considered to be causal of CAD. Most are involved in lipid metabolism, while others are involved in inflammation, cell proliferation and vascular remodeling. The age of onset of clinical symptoms is variable, however MCAD is associated with early onset of symptoms with respect to multifactorial atherosclerosis (88,89).

**Oligogenic/polygenic forms**

Oligogenic/polygenic forms of CVDs are genetic disorders caused by the combined action of more than one gene.

*Hyperlipidemia*

In developed countries, most dyslipidemias are hyperlipidemias, i.e. an elevation of lipids in the blood. The etiology of dyslipidemias is primarily polygenic, being determined by interaction of many susceptibility genes with environmental factors. Polygenic dyslipidemias combine underlying genetic predispositions with disease states such as diabetes, thyroid disease or drug-related changes in lipid metabolism. High levels of cholesterol in the blood are one of the most widespread cardiovascular risk factors in the human population (90,91).

*Arterial hypertension*

Arterial hypertension is a significant public health problem and is principally considered a multifactorial disorder. Controlling blood pressure is a complex process and besides environmental factors, many genes presumably collaborate to influence it. About 22% of the world population has hypertension. Long-term high blood pressure is a major risk factor for coronary artery disease, stroke, heart failure, atrial fibrillation, peripheral vascular disease, vision loss, chronic kidney disease and dementia (92).

*Coronary artery disease*

A group of gene variants are responsible for the intricate patterns of inheritance of polygenic coronary artery diseases. Their interplay with each other often has little effect, whereas their interplay with a number of environmental factors may determine outcome. These genetic factors are independent of traditional risk factors, such as hypertension, diabetes mellitus, hypercholesterolemia, obesity, plasma homocysteine, low physical activity and smoking, but may contribute directly or through traditional risk factors to the development and manifestation of coronary artery disease (93).

*Thrombophilia*

Thrombophilia (also known as hypercoagulable state) is a coagulation disorder that predispose to clot formation (thrombus). Normal blood hemostasis is guaranteed by a balance between prothrombotic and antithrombotic processes, mediated by cell components, soluble plasma proteins and endothelium-derived factors. Genetic alterations that impair the production, activity, bioavailability and metabolism of specific factors can modify physiological balance in favor of thrombosis and predispose to thromboembolic events. Thrombophilia is caused by inherited or acquired conditions. Primary disorders or genetic causes of thrombophilia include factor V Leiden mutation, deficiency of antithrombin III, protein C and S deficiency, histidine-rich glycoprotein deficiency and prothrombin-related thrombophilia, while secondary disorders include heparin-induced thrombocytopenia, antiphospholipid antibody syndrome, neoplasia, oral contraceptive use, obesity, smoking and surgery (94).

## Genetic testing for monogenic and polygenic cardiovascular diseases

The characterization of genes associated with CVDs improves prevention, treatment and quality of care. Linkage studies and genome-wide linkage analysis are useful for identifying genes related to CVDs and pinpointing new causative genes may indicate targets for molecular diagnosis and therapeutic intervention (95). The distinction between monogenic and polygenic forms is important for cardiovascular risk assessment, counseling and treatment of patients.

Monogenic conditions are generally associated with higher cardiovascular risk. Early implementation of pharmacological treatment is therefore necessary to control risk (96). Genetic testing for monogenic forms has a fundamental role in identifying the molecular causes of cardiovascular diseases and in aiding prevention and treatment, also being crucial for early detection of potentially lethal cardiovascular events. The possibility of giving physicians a tool for predicting individual sensitivity or resistance to a specific pharmacological treatment (97) makes it possible to prescribe the best drug and the best dosage for each patient. This strategy is part of the complex perspective of personalized medicine (98,99).

Polygenic forms associated with most risk factors are of clinical interest due to their high frequency in the general population. Genetics is useful to define the susceptibility of single patients, although the contribution of each genetic variant to overall risk of onset is low. At present, the most important application of genetic testing for polygenic forms of CVDs is related to the possibility of predicting the effect of a specific therapy, mainly in the initial phases of treatment (99). Next generation sequencing (NGS), a rapid and cost-effective method for identifying mutations in genes associated with multigenic disorders, has revolutionized genetic testing in CVDs. Because CVDs are genetically heterogeneous, genetic testing can be performed with NGS and multigene panels targeted

at a specific phenotype, or including a broader array of genes associated with different diseases that may share overlapping features. Meta-analysis studies to identify predisposing genetic variants, enrolling thousands of CVDs patients, have led to the identification of certain gene variants having a modest contribution when taken individually but which are involved in the pathogenesis of CVDs in synergy with other variants and with environmental risk factors. Compared to the study of single genes, this approach makes it possible to more precisely predict the risk of developing CVDs (95,100).

Genetic testing should be offered to index patients who fulfill diagnostic criteria for CVDs; a comprehensive clinical evaluation should precede genetic testing, which should be performed in certified laboratories and combined with genetic counseling by trained healthcare professionals. Pre-test and post-test genetic counseling are important steps in the genetic testing process. Pre-test counseling provides the information necessary for proper informed consent, including description of the genetic test, its yield, benefits and limitations, and implications for family members, as well as the possibility of reclassifying the disease.

The results of genetic testing can be complex. Although a result may be classified as positive, negative or inconclusive, its clinical significance depends on the patient's personal and family history (95). The goals of family assessments of phenotype and genotype are to identify individuals with hitherto unrecognized disease and currently healthy family members at risk of developing disease, in the latter case through longitudinal follow-up. Phenotypic evaluation starts with first-degree relatives of affected individuals and is repeated periodically because penetrance for some conditions may be delayed and diagnostic features may not manifest until adulthood. If a pathogenic variant has been identified in the family, predictive genetic testing can be done to determine which relatives have inherited the variant. Relatives confirmed to carry the family variant should undergo serial phenotypic evaluation and be informed of the risk of transmission to offspring. A definitive diagnosis and familial disease increase the probability of positive genetic test results, but the absence of a family history of disease does not preclude genetic testing. Genetic forms of cardiovascular disease may occur without affected relatives, due to recessive inheritance, *de novo* mutations or reduced penetrance (101). Clinicians and patients should have accurate and realistic expectations about the yield of genetic testing and its role in management. The ethical, legal and social concerns of genetic testing must also be considered. Various guidelines on appropriate use of genetic testing have been published (102).

**Pharmacogenetics and cardiovascular diseases**

Pharmacogenetics is the search for genetic variations that affect responses to drug therapy and toxicity. Drug response is determined by physiological mechanisms (age, sex, nutritional status), pathological mechanisms (renal and liver function, comorbidities), environmental factors and above all individual genetic profile. Pharmacogenetic testing reveals variations in drug metabolism genes encoding metabolic enzymes that may be more or less efficient and which are defined as rapid or poor metabolizers, respectively (Table 3). Identifying how a patient metabolizes a medication enables personalized treatment, which besides being safer for the patient, decreases medical costs and improves clinical outcomes. The test can be performed prior to prescription, in order to guide medical selection and dosing, or can be performed after initial treatment that has proved inefficient (100,101).

Pharmacogenetics has many possible applications in the drug therapy of CVDs. Many studies have found associations between genetic variations and responses to cardiovascular drugs. Some of these relationships have been demonstrated in large patient populations, such as patients with ischemic heart disease receiving statins (102). Once the genetic variations that best determine the response to a particular drug are known and tests to rapidly identify these variations are available, individual patients may be screened for genetic variations before drug therapy is begun and the information used to choose agents with the greatest potential for efficacy and the least toxicity (102).

Pharmacogenomics, on the other hand, is a new field arising from the development of NGS technologies. It deals with the correlation between genetic profile and response to a drug for the purpose of devel-

**Table 3.** Genes associated with response to cardiovascular drugs

| Gene (OMIM ID) | Metabolic role | Drug | Main therapeutic effect | Reference |
|---|---|---|---|---|
| *PTGS1* (176805); | PTGS1: prostaglandin biosynthesis - ITGB3: fibrinogen receptor | Aspirin | Platelet aggregation inhibitor | (103,104) |
| *ITGB3* (173470) *CYP2C19* (124020); *P2RY12* (600515); *ITGB3* | CYP2C19: drug metabolism; P2RY12: regulation of platelet shape and aggregation | Clopidogrel | Platelet aggregation inhibitor | (105-107) |
| *CYP2C9* (601130) | Drug metabolism | Nonsteroidal anti-inflammatory drugs | Platelet aggregation inhibitor | (108) |
| *CYP2C9; VKORC1* (608547) | VKORC1: vitamin K pathway | Cumarin, warfarin, phenprocoumon, acenocoumarol | Anticoagulant | (109) |
| *CES1* (114835) | Hydrolysis of compounds containing amides or esters | Dabigatran | Anticoagulant | (110) |
| *SLCO1B1* (604843) | Eicosanoids, thyroid hormones, steroid transporters | Statin | Reduction of blood cholesterol (HMG-CoA reductase inhibitor) | (110) |
| *LPL* (609708) | Triglyceride hydrolysis, lipoprotein uptake | Lovastatin | Reduction of blood cholesterol (HMG-CoA reductase inhibitor) | (111) |
| *HMGCR* (142910) | Cholesterol biosynthesis | Pravastatin | Reduction of blood cholesterol (HMG-CoA reductase inhibitor) | (111) |
| *CYP7A1* (118455); *ABCB1* (171050); *CETP* (118470) | CYP7A1: cholesterol catabolism - ABCB1: drug-transport pump - CETP: uptake of cholesterol by hepatocytes | Atorvastatin | Reduction of blood cholesterol (HMG-CoA reductase inhibitor) | (112-114) |
| *LDLR* (606945); *SREBF1* (184756) | LDLR: low density lipoprotein receptor - SREBF1: sterol biosynthesis. | Fluvastatin | Reduction of blood cholesterol (HMG-CoA reductase inhibitor) | (115,116) |
| *LDLR* (606945) | Low density lipoprotein receptor | Lomitapide | Reduction of blood cholesterol (microsomal triglyceride transfer protein inhibitor) | (115) |
| *ABCB1* | Drug-transport pump | Digoxin | Inhibition of the Na+/K+ ATPase in the myocardium | (117) |
| *ADRB1* (109630) | Adrenergic receptor beta-1 | Atenolol, metoprolol, carvedilol | Adrenoceptor beta inhibitor | (118,119) |
| *ACE* (106180) | Blood pressure control | Enalapril, perindopril, imidapril, capropril | Angiotensin-converting-enzyme inhibitor | (120) |
| *CYP2C9* | Drug metabolism | Losartan | Angiotensin II receptor type 1 antagonist | (120) |

oping new drugs. Many government research groups have taken an active role in promoting pharmacogenomic research and clinical implementation. One noteworthy example is the NIH-funded Pharmacogenomics Research Network (PGRN), which focuses on understanding genetic determinants of response to various medications, including medications used to treat cardiac arrhythmias. Drug gene panels are commercially available or may be custom built for this type of approach (103).

*Genes involved in drug response*

Most variations in the genes in Table 3 are single nucleotide polymorphisms (SNPs). Genetic screening of the coding sequence of *PTGS1* in 92 healthy individuals revealed five variants that conferred decreased metabolic basal activity to PTGS1 *in vitro* (121). Heterozygous variants in the genes *CYP2C9* (OMIM disease 122700), *VKORC1* (OMIM disease 122700) and *CYP2A6* (OMIM disease 122700) cause variable drug responses transmitted by autosomal dominant inheritance. Homozygous and/or compound heterozygous mutations in the genes *CYP2C19* (OMIM disease 609535) and *ADRB1* cause variable drug responses transmitted by autosomal recessive inheritance.

## Times and costs of genetic testing

The rapid expansion of genetic testing has reduced costs and increased utilization. The costs for genetic testing include genetic counseling, biotechnologists' labor time, laboratory supplies, equipment, and data interpretation and reporting. The standard protocol for molecular diagnosis of CVDs includes DNA extraction from biological samples (peripheral blood or saliva) and analysis of genetic regions of interest through automatic sequencing or polymerase chain reaction amplification with specific primers followed by enzyme digestion of the amplification. The time required to perform the analysis varies with the number of genes screened, the length of the sequence and the number of mutations analyzed. Thus costs vary, although in recent years, genetic tests have become faster and cheaper, thanks to new developments. NGS is a rapid cost-effective tool for identifying mutations in genes associated with CVDs. It enables the optimization of times and costs in specialized genetic laboratories (92,104).

## Conclusions

Genetic testing in cardiology has become an important tool for studying and understanding the etiology, pathogenesis and development of CVDs and is beginning to change clinical practice. Advances in DNA sequencing methodology have made gene-based diagnosis increasingly feasible in routine clinical practice, while maintaining clinical accuracy. There is much evidence that molecular genetics and pharmacogenetics are playing an increasingly important role in the correct clinical management of heart patients. Knowledge of these methods should not be limited to a closed group of researchers, but should be disseminated to clinical cardiologists in contact with patients who can actually benefit from genetic diagnostics. In the near future we can expect a great increment in information and tests regarding genetic diagnosis, which will be acknowledged as a true branch of cardiology, on a par with hemodynamics and electrophysiology. Third millennium cardiologists should therefore become familiar with the diagnostic and therapeutic opportunities offered by genetic testing and be prepared for the great leap forward it will bring. The genetic test is particularly important in conditions that can lead to sudden death (e.g. long QT syndrome, Brugada syndrome, arrhythmogenic cardiomyopathies). Next generation sequencing makes it possible to analyze all the causative genes in a single experiment and can become the basis for prescribing preventive devices, such as the pacemaker.

**Conflict of interest:** Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Review

# Cardiac conduction defects

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**Summary.** Defects in cardiac electric impulse formation or conduction can lead to an irregular beat (arrhythmia) that can cause sudden death without any apparent cause or after stress. In the following sections, we describe the genetic disorders associated with primary cardiac conduction defects, primarily caused by mutations in ion channel genes. Primary indicates that these disorders are not caused by drugs and are not secondary to other disorders like cardiomyopathies (described in the next section). (www.actabiomedica.it)

**Key words:** Brugada syndrome, catecholaminergic polymorphic ventricular tachycardia, long QT syndrome, short QT syndrome, Wolff-Parkinson-White syndrome, familial atrial fibrillation

## Brugada syndrome

Brugada syndrome (BrS) is a genetic heart disorder with an ion channel dysfunction associated with progressive age-related conduction abnormalities. It is more prevalent among males. It is estimated to be responsible for up to 20% of all sudden deaths in individuals with apparently normal hearts (1). BrS has a prevalence of 5:10000 (2).

Diagnosis is based on clinical and family history and electrocardiographic examination. Penetrance and expressivity are highly variable (3). Symptoms are often absent in the first year of life, and in adults usually manifest as syncope or sudden death at rest, during sleep or with fever. Sometimes they manifest on administration of drugs such as sodium channel blockers.

BrS is usually inherited in an autosomal dominant manner, however digenic or autosomal recessive inheritance in patients with mutations in *SCN5A* and *TRPM4* has been reported (4,5). The genes associated with BrS encode subunits of cardiac sodium, potassium and calcium channels and proteins involved in the trafficking or regulation of these channels (Table 1). Only ~35% of BrS patients have been found to have a well-defined genetic cause, one third of whom carry a pathogenic mutation in *SCN5A* (6). All other genes together are responsible for about 5% of BrS cases. Pathogenic variants are usually point mutations or small insertions/deletions, however partial *SCN5A* gene deletion has been reported (7). Most of the reported patients inherit the mutation from one of their parents, while *de novo* variants account for <1% (8).

We use a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the above genes, and multiplex ligation-dependent probe amplification (MLPA) assay to detect duplications and deletions in *SCN5A*. Worldwide, 81 accredited medical genetic laboratories in the EU and 57 in the US, listed in Orphanet (9) and GTR (10) databases, respectively, offer genetic tests for Brugada syndrome. The guidelines for clinical use of genetic testing are described in Genetics Home Reference (11) and GeneReviews (12).

* Authors have equally contributed to this work

**Table 1.** Genes associated with various forms of Brugada syndrome (BrS).

| Gene | OMIM gene | Disease | OMIM disease | Inheritance | Function |
|---|---|---|---|---|---|
| *SCN5A* | 600163 | BrS1 | 601144 | AD, DR | Mediates voltage-dependent $Na^+$ permeability of excitable membranes |
| *GPD1L* | 611778 | BrS2 | 611777 | AD | Decreases cardiac $Na^+$ current |
| *CACNA1C* | 114205 | BrS3 | 611875 | AD | Pore-forming, alpha-1C subunit of voltage-gated $Ca^{2+}$ channel |
| *CACNB2* | 600003 | BrS4 | 611876 | AD | Increases cardiac peak Ca2+ current, regulates voltage-dependent activation, controls alpha-1 subunit recruitment |
| *SCN1B* | 600235 | BrS5 | 612838 | AD | Regulates assembly, expression and function of $Na^+$ channel complex |
| *KCNE3* | 604433 | BrS6 | 613119 | AD | Modulates gating kinetics, stabilizes channel complex |
| *SCN3B* | 608214 | BrS7 | 613120 | AD | Modulates channel gating kinetics |
| *HCN4* | 605206 | BrS8 | 613123 | AD | Contributes to native pacemaker currents in the heart that regulate heartbeat rhythm |
| *KCND3* | 605411 | BrS9 | 616399 | AD | Pore-forming subunit of voltage-gated rapidly-inactivating A-type $K^+$ channels |
| *ABCC9* | 601439 | BrS | / | AD | Subunit of ATP-sensitive $K_+$ channels |
| *SCN10A* | 604427 | BrS | / | AD | Mediates voltage-dependent $Na^+$ permeability of excitable membranes |
| *SLMAP* | 602701 | BrS | / | AD | Excitation-contraction coupling |
| *SCN2B* | 601327 | BrS | / | AD | Assembly, expression and modulation of $Na^+$ channel complex |
| *CACNA2D1* | 114204 | BrS | / | AD | Regulates $Ca^{2+}$ current density and activation/inactivation of $Ca^{2+}$ channel |
| *KCNJ8* | 600935 | BrS | / | AD | Inward-rectifier $K^+$ channel |
| *PKP2* | 602861 | BrS | / | AD | Maintains transcription of genes that control intracellular calcium cycling |
| *TRPM4* | 606936 | BrS | / | AR, DR | $Ca^{2+}$-activated non selective cation channel that depolarizes membranes |

AD=autosomal dominant; AR=autosomal recessive; DR= digenic recessive.

## Catecholaminergic polymorphic ventricular tachycardia

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited heart disorder characterized by electrical instability in a structurally normal heart during acute activation of the adrenergic nervous system, as in physical activity or emotional stress. Release of catecholamines causes a calcium-ion channel dysfunction in myocytes, leading to ventricular arrhythmia. Episodes of ventricular tachycardia can cause dizziness and syncope. Spontaneous recovery from the arrhythmia is possible, but unless recognized and treated, ventricular tachycardia may cause cardiac

arrest and sudden death. These symptoms typically begin in childhood. The exact prevalence of CPVT in the population is not known, but is estimated at about 1:10000 (13).

Clinical diagnosis may be difficult because echocardiograms and electrocardiograms are normal in resting state. Testing must therefore be performed under stress. Differential diagnosis should consider long-QT syndrome, arrhythmogenic right ventricular cardiomyopathy, short coupled ventricular tachycardia and Andersen-Tawil syndrome.

Preventive drugs (beta-blockers and flecainide) and other treatments (implantable cardioverter defibrillator and left cardiac sympathetic denervation) are available for susceptible individuals.

The disorder may have autosomal dominant or recessive inheritance and the associated genes are involved in calcium homeostasis in myocytes (Table 2). Most pathogenic variants are point mutations or small insertions/deletions. However, large deletions/duplications and complex genomic rearrangements have been reported in RYR2 (1). Pathogenic variants in these genes account for 55-65% of CPVT cases with a penetrance of 83% for *RYR2*-mutations carrier(13).

We use a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the genes in Table 2. Worldwide, 25 accredited medical genetic laboratories in the EU and 19 in the US, listed in Orphanet (9) and GTR (10) databases, respectively, offer genetic tests for catecholaminergic polymorphic ventricular tachycardia. The guidelines for clinical use of genetic testing are described in Genetics Home Reference (11), GeneReviews (13) and EuroGentest (14).

## Long QT syndrome

Long QT syndrome (LQT) is a genetic heart disease characterized by prolongation of the QT interval in the absence of other conditions known to lengthen it (such as QT-prolonging drugs). This may lead to arrhythmia that can cause palpitations, syncope or sudden death. Typically LQTS manifests in patients under 40 years of age, sometimes in early infancy. The mean age of onset of symptoms is 12 years and earlier onset is usually associated with severer forms (15,16).

LQT follows two distinct patterns of inheritance: autosomal dominant with an estimated prevalence of 1:2000-5000 (17,18,19) and autosomal recessive (Jervell and Lange-Nielsen syndrome) with an estimated prevalence of 1:1000000-4000000 (20).

Clinical diagnosis is based on clinical findings, ECG, medical and family history. The genetic test is useful for diagnosis confirmation, differential diagnosis, recurrence risk evaluation and prenatal diagnosis.

**Table 2.** Genes associated with various forms of catecholaminergic polymorphic ventricular tachycardia

| Gene | OMIM gene | Disease | OMIM disease | Inheritance | Function |
|---|---|---|---|---|---|
| *RYR2* | 180902 | CPVT1 | 604772 | AD | $Ca^{2+}$ channel triggers cardiac muscle contraction |
| *CASQ2* | 114251 | CPVT2 | 611938 | AR | Regulates release of luminal $Ca^{2+}$ via RYR2 |
| *TECRL* | 617242 | CPVT3 | 614021 | AR | $Ca^{2+}$ transport into myocytes |
| *CALM1* | 114180 | CPVT4 | 614916 | AD | Regulates release of $Ca^{2+}$ via RYR2 |
| *TRDN* | 603283 | CPVT5 with/without muscle weakness | 615441 | AR | Regulates release of luminal $Ca^{2+}$ release via RYR1 and RYR2 |
| *KCNJ2* | 600681 | CPVT | / | AD | Establishes action potential and excitability of neurons and muscles |

AD=Autosomal dominant; AR=Autosomal recessive

Differential diagnosis should consider QT-prolonging drugs, hypokalemia, structural heart disease, sudden infant death syndrome, vasovagal syncope, seizures, familial ventricular fibrillation, hypertrophic cardiomyopathy, dilative cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, Brugada syndrome and catecholaminergic polymorphic ventricular tachycardia (21).

Syndromic LQT may have autosomal dominant (Timothy syndrome, Andersen-Twail syndrome and Ankyrin B syndrome) (22,23,24) or autosomal recessive inheritance (Jervell and Lange-Nielsen syndromes) (20). Up to 80% of cases of LQT are due to pathogenic variants in the *KCNQ1*, *KCNH2* and *SCN5A* genes. Other associated genes account for less than 5% of all cases (21) (Table 3).

**Table 3.** Genes associated with various forms of long QT syndrome

| Gene | OMIM gene | Disease | OMIM disease | Inheritance | Function |
|---|---|---|---|---|---|
| *KCNQ1* | 607542 | LQT1 | 192500 | AD | Repolarizes cardiac action potential |
| | | JLNS1 | 220400 | AR | |
| *KCNH2* | 152427 | LQT2 | 613688 | AD | Pore-forming subunit of voltage-gated inwardly rectifying $K^+$ channel |
| *SCN5A* | 600163 | LQT3 | 603830 | AD | Mediates voltage-dependent $Na^+$ permeability of excitable membranes |
| *ANK2* | 106410 | LQT4 | 600919 | AD | Coordinates assembly of Na/Ca exchanger, Na/K ATPase and InsP3 receptor in sarcoplasmic reticulum of cardiomyocytes |
| *KCNE1* | 176261 | LQT5 | 613695 | AD | Modulates gating kinetics and enhances stability of voltage-gated $K^+$ channel complex |
| | | JLNS2 | 612347 | AR | |
| *KCNE2* | 603796 | LQT6 | 613693 | AD | Modulates gating kinetics and enhances stability of voltage-gated $K^+$ channel complex |
| *KCNJ2* | 600681 | LQT7 | 170390 | AD | Establishes neuron and muscle action potentials and excitability |
| *CACNA1C* | 114205 | LQT8 | 601005 | AD | Pore-forming, alpha-1C subunit of voltage-gated $Ca^{2+}$ channel |
| *CAV3* | 601253 | LQT9 | 611818 | AD | Regulates voltage-gated $K^+$ channels |
| *SCN4B* | 608256 | LQT10 | 611819 | AD | Interacts with voltage-gated alpha subunits to change $Na^+$ channel kinetics |
| *AKAP9* | 604001 | LQT11 | 611820 | AD | Effector in regulating $K^+$ channel |
| *SNTA1* | 601017 | LQT12 | 612955 | AD | Interacts with pore-forming alpha subunit of cardiac $Na^+$ channel |
| *KCNJ5* | 600734 | LQT13 | 613485 | AD | Allows $K^+$ flow into cells |
| *CALM1* | 114180 | LQT14 | 616247 | AD | Mediates ion channel control |
| *CALM2* | 114182 | LQT15 | 616249 | AD | Mediates ion channel control |
| *CALM3* | 114183 | LQT | / | AD | Mediates ion channel control |

AD=autosomal dominant; AR=autosomal recessive; JLNS=Jervell and Lange-Nielsen syndrome

Pathogenic variants may be sequence variations (missense, nonsense, splicing, small insertions and deletions, small indels). Large deletions/duplications have been reported in *KCNH2*, *KCNQ1* and *KCNJ2* (21,23). We use a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the above genes, and MLPA assay to detect duplications and deletions in *KCNH2*, *KCNQ1* and *KCNJ2*.

Worldwide, 52 accredited medical genetic laboratories in the EU and 4 in the US, listed in the Orphanet (9) and GTR (10) databases, respectively, offer genetic tests for long QT syndrome. The guidelines for clinical use of genetic testing are described in Genetics Home Reference (11), GeneReviews (20,21,22,23,24) and EuroGentest (14).

## Short QT syndrome

Short QT syndrome (SQT) is a rare genetic heart disease characterized by an abnormally short QT interval and increased risk of arrhythmia and sudden death. Clinical presentation is heterogeneous. Some patients may be totally asymptomatic and others may have episodes of syncope or fall victim to sudden cardiac death. SQT may occur at any time of life from early infancy to old age. The estimated prevalence is 1-5:1000 (26,27,28,29).

According to the 2013 consensus statement of major world heart associations, the recommended criteria for diagnosis of SQT are QTc <330 msec or <360 msec with one or more of the following: a pathogenic mutation, family history of SQT, family history of sudden death under 40 years of age, or survival of a ventricular tachycardia/ventricular fibrillation event without underlying heart disease (30).

Differential diagnosis should consider the secondary causes of SQT interval (hyperkalaemia, hypercalcemia, hyperthermia, acidosis, effects of catecholamines or drugs such as digitalis) (31) and other arrhythmic disorders, such as Brugada syndrome, arrhythmogenic right ventricular cardiomyopathy, catecholaminergic polymorphic ventricular tachycardia, cardiac arrest and sick sinus syndrome (Table 4).

Pathogenic variants may be sequence variations (missense, nonsense, splicing, small indels). Large deletions/duplications associated with SQT have not yet been reported in the above genes.

MAGI uses a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the above genes. 26 accredited medical genetic laboratories in the EU and 32 in the US, listed in the Orphanet and GTR databases, respectively, offer genetic tests for short QT syndrome. The guidelines for clinical use of genetic testing are described in Genetics Home Reference (11).

## Wolff-Parkinson-White syndrome

Wolff-Parkinson-White syndrome (WPWS), also known as "pre-excitation syndrome", is a genetic heart disorder characterized by arrhythmia due to an abnormal electrical pathway in the heart, a so-called accessory pathway that allows electrical signals to by-

**Table 4.** Genes associated with various forms of short QT syndrome

| Gene | OMIM gene | Disease | OMIM disease | Inheritance | Function |
|---|---|---|---|---|---|
| *KCNH2* | 152427 | SQT1 | 609620 | AD | Pore-forming subunit of voltage-gated inwardly rectifying $K^+$ channel |
| *KCNQ1* | 607542 | SQT2 | 609621 | AD | Repolarizes cardiac action potential |
| *KCNJ2* | 600681 | SQT3 | 609622 | AD | Establishes action potential and excitability of neurons and muscles |

AD=autosomal dominant; AR=autosomal recessive

pass the atrioventricular node and move faster than normal from the atria to the ventricles. It may also transmit reverse electrical impulses, resulting in arrhythmias (32).

Wolff-Parkinson-White syndrome may present clinically with palpitations, dyspnea, dizziness or even syncope. In rare cases it can lead to cardiac arrest and sudden death (33). Although age of onset ranges from 11 to 50 years, complications can occur at any age. Some patients, however, are totally asymptomatic or never experience any complication associated with this condition.

In most patients, WPWS is sporadic, though in a minority of cases it can be familial (34) or complicated underlying diseases, such as Ebstein's anomaly (35), mitochondrial disease (36), hypertrophic cardiomyopathy (37) or a lethal congenital form of glycogen storage disease (38). The estimated prevalence of WPWS is 1.5-3.1:1000 in western countries (33).

Clinical diagnosis is based on clinical history, physical examination, resting 12-lead ECG and Holter monitoring. Genetic testing is useful for confirming diagnosis and for differential diagnosis, recurrence risk calculation and prenatal diagnosis in families with a known mutation. Differential diagnosis should consider other primary channelopathies and secondary causes of arrhythmia, such as electrolyte abnormalities, hyperthyroidism and/or side effects of substances such as digoxin and alcohol (39).

Familial WPWS only accounts for a small percentage of cases, most of which occur in persons with no apparent family history of the condition. The familial form has autosomal dominant inheritance and is associated with variations in the *PRKAG2* gene (OMIM gene 602743; OMIM disease 194200). Pathogenic variants may be missense, nonsense, splicing or small insertions/deletions.

No genetic tests are listed in the Orphanet database but 10 accredited medical genetic laboratories in the US, listed in the GTR database, offer genetic testing for WPWS. The guidelines for clinical use of genetic testing are described in Genetics Home Reference (11). MAGI uses an NGS approach to detect nucleotide variations in coding exons and flanking introns of the above gene.

**Familial atrial fibrillation**

Familial atrial fibrillation (FAF) is a heterogeneous genetic heart disorder characterized by chaotic electrical activity in the atria and an irregular ventricular response. This is also known as "irregularly irregular rhythm". If untreated, it can lead to reduction in cardiac output and atrial thrombus formation, which may be responsible for episodes of stroke or sudden death. Atrial fibrillation may manifest clinically with palpitations, dyspnea, chest pain, dizziness or even syncope (40). The risk of developing atrial fibrillation increases with age and complications can occur at any age. However, some patients are totally asymptomatic or never experience any complication associated with this condition. The estimated prevalence of FAF ranges from 0.4% to 1% in the general population (40) and increases with age (41).

Clinical diagnosis is based on clinical history, physical examination, ECG and Holter monitoring. Echocardiography is performed to evaluate left chamber dimensions and systolic/diastolic performance. Genetic testing is useful for confirming diagnosis, and for differential diagnosis, recurrence risk calculation and prenatal diagnosis in families with a known mutation. Differential diagnosis should consider: reversible causes of atrial fibrillation (AF), such as alcohol intake, surgery, myocardial infarction, myocarditis and pericarditis; metabolic disorders associated with AF, such as obesity and hyperthyroidism; other heart diseases associated with AF, such as valve disease, heart failure, hypertension, hypertrophic cardiomyopathy and dilated cardiomyopathy (40, 42).

Eligibility criteria for genetic testing (43) are:

1- ECG characteristics: absence of P waves; irregular R-R intervals;
2- clinical presentation: AF as major clinical manifestation (phenotype) with early onset (before age 60 years);
3- family history: at least one affected first or second-degree family member.

Familial atrial fibrillation is highly heterogeneous and can have autosomal dominant or recessive inheritance (Table 5).

Pathogenic variants may be missense, nonsense, splicing or small small indels. Large deletions/dupli-

**Table 5.** Genes associated with various forms of atrial fibrillation, familial (ATFB)

| Gene | OMIM gene | Disease | OMIM disease | Inheritance | Function |
|---|---|---|---|---|---|
| *KCNQ1* | 607542 | ATFB3 | 607554 | AD | Repolarizes cardiac action potential |
| *KCNE2* | 603796 | ATFB4 | 611493 | AD | Modulates gating kinetics and enhances stability of voltage-gated $K^+$ channel complex |
| *NPPA* | 108780 | ATFB6 | 612201 | AD | Key role in regulation of natriuresis, diuresis, vasodilation |
| *KCNA5* | 176267 | ATFB7 | 612240 | AD | Mediates transmembrane potassium transport in excitable membranes |
| *KCNJ2* | 600681 | ATFB9 | 613980 | AD | Establishes action potential and excitability of neurons and muscles |
| *SCN5A* | 600163 | ATFB10 | 614022 | AD | Mediates voltage-dependent $Na^+$ permeability of excitable membranes |
| *GJA5* | 121013 | ATFB11 | 614049 | AD | Allows passive diffusion of small molecules, including glucose, $K^+$, $Ca^{2+}$ and cAMP |
| *ABCC9* | 601439 | ATFB12 | 614050 | AD | Subunit of ATP-sensitive $K^+$ channels |
| *SCN1B* | 600235 | ATFB13 | 615377 | AD | Regulates assembly, expression, function of $Na^+$ channel complex |
| *SCN2B* | 601327 | ATFB14 | 615378 | AD | Assembly, expression, modulation $Na^+$ channel complex |
| *SCN3B* | 608214 | ATFB16 | 613120 | AD | Modulates channel-gating kinetics |
| *SCN4B* | 608256 | ATFB17 | 611819 | AD | Interacts with voltage-gated alpha subunits to change $Na^+$ channel kinetics |
| *MYL4* | 160770 | ATFB18 | 617280 | AD | Encodes a myosin alkali light chain expressed in embryonic muscle and adult atria |
| *NUP155* | 606694 | ATFB15 | 615770 | AR | Plays a role in fusion of nuclear envelope vesicles and may also be involved in heart physiology |
| *KCND3* | 605411 | ATFB | / | AD | Pore-forming subunit of voltage-gated rapidly-inactivating A-type $K^+$ channels |
| *KCNE1* | 176261 | ATFB | / | AD | Modulates gating kinetics and enhances stability of voltage-gated $K^+$ channel complex |
| *KCNH2* | 152427 | ATFB | / | AD | Pore-forming subunit of voltage-gated inwardly rectifying $K^+$ channels |
| *LMNA* | 150330 | ATFB | / | AD | Component of nuclear lamina and required for cardiac homeostasis |
| *NKX2-5* | 600584 | ATFB | / | AD | Transcription factor involved in heart formation and development |
| *PRKAG2* | 602743 | ATFB | / | AD | Energy-sensing enzyme that monitors cell energy status and functions; inhibits de novo biosynthesis of fatty acids and cholesterol |

*(continued on next page)*

**Table 5** ***(continued)*****.** Genes associated with various forms of atrial fibrillation, familial (ATFB)

| Gene | OMIM gene | Disease | OMIM disease | Inheritance | Function |
|---|---|---|---|---|---|
| *RYR2* | 180902 | ATFB | / | AD | $Ca^{2+}$ channel that triggers cardiac muscle contraction |
| *GATA4* | 600576 | ATFB | / | AD | Regulates genes involved in myocardial differentiation and function |
| *GATA5* | 611496 | ATFB | / | AD | Required for cardiovascular development |
| *GATA6* | 601656 | ATFB | / | AD | Required for cardiovascular development |
| *PITX2* | 601542 | ATFB | / | AD | May play a role in proper localization of asymmetric organs such as heart |
| *TBX5* | 601620 | ATFB | / | AD | Regulates transcription of several genes involved in heart development |
| *ZFHX3* | 104155 | ATFB | / | AD | Regulates myogenic differentiation |
| *SHOX2* | 602504 | ATFB | / | AD | Transcriptional regulator involved in pattern formation in vertebrates |
| *PRRX1* | 167420 | ATFB | / | AD | Role in establishment of diverse mesodermal muscle types |
| *KCNN3* | 602983 | ATFB | / | AD | Forms a voltage-independent $K^+$ channel activated by intracellular $Ca^{2+}$ |

AD=autosomal dominant; AR=autosomal recessive

cations have also been reported in *KCNQ1, KCNA5, KCNJ2, SCN5A, GATA4, PTX2, TBX5* and *GJA5*. MAGI uses a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the above genes and MLPA assay to detect duplications and deletions in *KCNQ1, KCNA5, KCNJ2, SCN5A, GATA4, PTX2, TBX5* and *GJA5*.

19 accredited medical genetic laboratories in the EU and 23 in the US, listed in the Orphanet and GTR databases, respectively, offer genetic tests for familial atrial fibrillation. The guidelines for clinical use of genetic testing are described in Genetics Home Reference (11).

## Conclusions

We created a NGS panel to detect nucleotide variations in coding exons and flanking regions of all the genes associated with genetic cardiac disorders. When a suspect of cardiac conduction defect is present we perform the analysis of all the genes present in this short article.

In order to have a high diagnostic yield, we developed a NGS test that reaches an analytical sensitivity (proportion of true positives) and an analytical specificity (proportion of true negatives) of ≥99% (coverage depth ≥10x).

**Conflict of interest:** Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Review

# Sudden unexplained death due to cardiac arrest

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**Summary.** Sudden unexplained death due to cardiac arrest refers to a group of heterogeneous heart disorders characterized by sudden cessation of cardiac activity followed by hemodynamic collapse. It may be associated with structural heart disease or may occur in the absence of structural abnormalities. These inherited conditions increase the risk of sudden unexplained death in living relatives when there is a family history of sudden death. It is recommended to screen other family members of sudden unexplained death victims, as studies have revealed affected individuals in 40% of families. (www.actabiomedica.it)

**Key words:** sudden unexplained death, cardiac arrest, arrhythmia

Sudden unexplained death (SUD) due to cardiac arrest refers to a group of heterogeneous heart disorders characterized by sudden cessation of cardiac activity followed by hemodynamic collapse. In the elderly, it is a relatively common cause of death, mostly due to structural heart disease. In the non-elderly it is less frequent and can be called sudden unexplained death syndrome, which includes all sudden autopsy-negative deaths occurring after the first year of life and involving previously healthy children, adolescents and young adults (1). The world incidence of SUD due to cardiac arrest is 4-5 million cases per year (4).

Sudden unexplained death may be associated with structural heart disease, such as hypertrophic cardiomyopathy, dilated cardiomyopathy, arrhythmogenic cardiomyopathy and arrhythmogenic syndromes, or may occur in the absence of structural abnormalities, as in the case of long or short QT syndrome, Brugada syndrome, catecholaminergic polymorphic ventricular tachycardia, early repolarization syndrome, idiopathic ventricular fibrillation, primary arrhythmogenic disorders, Wolff-Parkison-White syndrome or SUD in epilepsy (2,3). Approximately 80% of cases are ascribed to coronary artery disease, 10-15% are associated with non-ischemic cardiomyopathy and 5% are caused by arrhythmic disorders (4). These inherited conditions increase the risk of SUD in living relatives when there is a family history of sudden death. It is recommended to screen other family members of SUD victims, as studies have revealed affected individuals in 40% of families (5). First and second-degree relatives of the deceased should undergo comprehensive cardiovascular evaluation, physical examination, 12-lead electrocardiogram, treadmill stress test, 24-h Holter monitoring and echocardiogram, and full personal and family medical histories should be recorded (6).

Genetic testing is useful for confirming diagnosis of SUD and for differential diagnosis, recurrence risk evalutation and prenatal diagnosis in families with a known mutation. Pathogenic variants may be sequence missense, nonsense, splicing and small indels, as well as large deletions/duplications (Supplementary Table 1). A multi-gene NGS panel is used by MAGI to detect nucleotide variations in coding exons and flank-

ing introns of the above genes. Tests comprehensive of all genes associated with disorders that may lead to sudden unexplained death are not currently listed in Orphanet, but are offered by 16 accredited medical genetic laboratories in the US, listed in the GTR database. The guidelines for clinical use of genetic testing are described by the American Heart Association (7).

## Conclusions

We created a NGS panel to detect nucleotide variations in coding exons and flanking regions of all the genes associated with cardiac disorders. When a family with a previous case of sudden unexplained death comes to our attention we perform the analysis of all the associated genes.

In order to have a high diagnostic yield, we developed a NGS test that reaches an analytical sensitivity (proportion of true positives) and an analytical specificity (proportion of true negatives) of ≥99% (coverage depth ≥10x).

**Conflict of interest:** Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Review

# Cardiomyopathies

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**Summary.** The most common cardiomyopathies often present to primary care physicians with similar symptoms, despite the fact that they involve a variety of phenotypes and etiologies (1). Many have signs and symptoms common in heart failure, such as reduced ejection fraction, peripheral edema, fatigue, orthopnea, exertion dyspnea, paroxysmal nocturnal dyspnea, presyncope, syncope and cardiac ischemia (1). In all cardiomyopathies, the cardiac muscle (myocardium) may be structurally and/or functionally impaired. They can be classified as hypertrophic, dilated, left-ventricular non compaction, restrictive and arrhythmogenic right ventricular cardiomyopathies. (www.actabiomedica.it)

**Key words:** Hypertrophic cardiomyopathy, dilated cardiomyopathy, restrictive cardiomyopathy, left ventricular noncompaction, arrhythmogenic right ventricular cardiomyopathy

## Hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy (CMH) is characterized by an increase in the number of heart muscle cells. It is frequently caused by mutations in genes encoding sarcomeric proteins, leading to myocyte disarray, a hallmark of CMH (2).

Clinical symptoms range from asymptomatic left ventricular hypertrophy to progressive heart failure or sudden cardiac death, and vary from individual to individual even within the same family. Frequent symptoms include dyspnea, chest pain, palpitations, orthostasis, presyncope and syncope. Usually CMH becomes apparent during adolescence or early adulthood, although it may also develop in different stages of life such as old age, infancy or childhood (3).

Hypertrophic cardiomyopathy is a relatively common inherited heart disease with a prevalence of 1:500 in the population (4). Clinical diagnosis is based on patient history, physical examination, echocardiography and ECG to detect hypertrophy (2). The genetic test is useful for confirming diagnosis, and for differential diagnosis, recurrence risk evaluation and prenatal diagnosis in families with a known mutation. Differential diagnosis should consider acquired left ventricular hypertrophy, Danon disease, Fabry disease, cardiac amyloidosis, glycogen storage disease type II, Noonan syndrome and Friedreich ataxia (5).

The European Society of Cardiology recommends genetic testing in the following cases (6):

1 - patients meeting diagnostic criteria for CMH, when testing enables cascade genetic screening of their relatives;
2 - in first-degree adult relatives of patients with a definite disease-causing variant;
3 - in first-degree adult relatives, clinical screening with ECG and echocardiogram should be offered when genetic testing is not performed

* Authors have equally contributed to this work

in the proband, or when genetic analysis fails to identify a definite mutation or reveals one or more genetic variants of unknown significance;

4 - children of patients with a definite disease-causing mutation should be considered for predictive genetic testing after pre-test family counseling when they are at least 10 years old;

5 - when there is a family history of childhood malignancies or early-onset disease or when children have heart symptoms or are involved in particularly demanding physical activity, clinical or genetic testing of first-degree child relatives may be considered before the age of 10 years.

Hypertrophic cardiomyopathy typically has autosomal dominant inheritance. Pathogenic variants may be missense, nonsense, splicing or small indels (Table 1). Large deletions/duplications have also been reported in the *NEXN*, *TNNI3*, *MYBPC3, CAV3* and *MYH7* genes.

The mutation detection rate for the most common mutant genes is ~56% (*MYBPC3* 20-30%; *MYH7* 20-30%; *TNNT2* 3-5%; *TNNI3* 3-5%; *TPM1* 1-3%) (7). MAGI uses a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the above genes, and MLPA to detect duplications and deletions in *NEXN*, *TNNI3*, *MYBPC3*, *CAV3* and *MYH7*. Worldwide, 151 accredited medical genetic laboratories in the EU and 19 in the US, listed in the Orphanet (8) and GTR (9) databases, respectively, offer genetic tests for hypertrophic cardiomyopathy. The clinical guidelines for genetic testing are described in Genetics Home Reference (10), GeneReviews (5) and Clinical Utility Gene Card (7).

**Dilated cardiomyopathy**

Dilated cardiomyopathy (CMD) is a heart disorder characterized by dilation of at least one ventricle and systolic dysfunction. The ventricle wall becomes thinner and its contractile force decreases. Clinical signs are usually arrhythmias, thromboembolic events, such as stroke, and above all symptoms of heart failure, such as edema, orthopnea, dyspnea and fatigue. However, the symptoms take years to cause health problems and severity varies between affected individuals.

The etiology of CMD may include either inherited or acquired causes, such as myocardial infarction, valve disease, toxins, drugs, inflammatory conditions, long-standing severe hypertension and irradiation of the chest (11). Dilated cardiomyopathy is essentially an adult-onset disease, but has shown a highly variable age of onset (12). The prevalence is 1:2700 (13). It can be classified as acquired, syndromic or non syndromic.

Diagnosis is established when left ventricular enlargement and systolic dysfunction are both ascertained. Patient history, physical examination and echocardiography are also indispensable for the diagnostic process (12). The genetic test is useful for diagnosis confirmation, differential diagnosis, recurrence risk evaluation and prenatal diagnosis in families with a known mutation. Differential diagnosis should consider acquired dilated cardiomyopathies, syndromic forms and other cardiomyopathies that may present with left ventricular involvement (14). Syndromic forms include *HFE*-associated hereditary hemochromatosis, Emery-Dreifuss muscular dystrophy, Laing distal myopathy, Carvajal syndrome, Duchenne and Becker muscular dystrophy, Barth syndrome and mitochondrial dilated cardiomyopathies (15).

Dilated cardiomyopathy is a genetically heterogeneous disease and has different modes of inheritance (Table 2). Pathogenic variants may be missense, nonsense, splicing and small indels. Large deletions/duplications have also been reported in *LMNA, MYH7, SCN5A, BAG3, DES, EYA4, SGCD, MYBPC3, NEXN, PRDM16, PSEN1, TNNI3, DND, RAF1, FKTN* and *TAZ*. The mutation detection rates for the most frequently mutant CMD-related genes are *TTN* 18-25%, *LMNA* 6%, *MYH7* 4-5%, *MYH6* 3-4%, *MYBPC3* 2-4%, *TNNT2* 3%, *BAG3* 2-3%. (16).

Ourmulti-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the above genes, MLPA to detect duplications and deletions in *LMNA, MYH7, SCN5A, BAG3, DES, EYA4, SGCD, MYBPC3, NEXN, PRDM16, PSEN1, TNNI3, DND, RAF1, FKTN* and *TAZ*.

Worldwide, 49 accredited medical genetic laboratories in the EU and 44 in the US, listed in the Orphanet (8) and GTR (9) databases, respectively, offer

**Table 1.** Genes associated with various forms of hypertrophic cardiomyopathy

| Gene | OMIM gene | Disease | OMIM disease | Inheritance | Function |
|---|---|---|---|---|---|
| *MYH7* | 160760 | CMH1 | 192600 | AD | Beta heavy chain subunit of cardiac myosin |
| *CAV3* | 601253 | CMH1 | 192600 | AD | Regulates voltage-gated $K^+$ channels and plays a role in sarcolemma repair in cardiomyocytes after mechanical stress |
| *MYLK2* | 606566 | CMH1 | 192600 | AD | Cardiac $Ca^{2+}$/calmodulin-dependent myosin light chain |
| *TNNT2* | 191045 | CMH2 | 115195 | AD | $Ca^{2+}$-dependent regulator of muscle contraction |
| *TPM1* | 191010 | CMH3 | 115196 | AD | $Ca^{2+}$-dependent regulator of striated muscle contraction |
| *MYBPC3* | 600958 | CMH4 | 115197 | AD | Cardiac isoform of myosin-binding protein C found in cross-bridge-bearing zone (C region) of A bands |
| *PRKAG2* | 602743 | CMH6 | 600858 | AD | Energy-sensing enzyme that monitors cell energy status and functions. Inhibitor of de novo biosynthesis of fatty acids and cholesterol |
| *TNNI3* | 191044 | CMH7 | 613690 | AD | Cardiac mediator of striated muscle relaxation |
| *MYL3* | 160790 | CMH8 | 608751 | AD | Ventricular isoform of myosin light chain 3 |
| *TTN* | 188840 | CMH9 | 613765 | AD | Important for assembly and functioning of striated muscles, it connects microfilaments and contributes to balance of forces between two halves of sarcomere |
| *MYL2* | 160781 | CMH10 | 608758 | AD | Regulatory light chain associated with cardiac myosin beta heavy chain, promoting cardiac myofibril assembly |
| *ACTC1* | 102540 | CMH11 | 612098 | AD | ACTC1 is localized in contractile apparatus of muscle tissues |
| *CSRP3* | 600824 | CMH12 | 612124 | AD | Positive regulator of myogenesis; transcription cofactor for myogenic bHLH transcription factors |
| *TNNC1* | 191040 | CMH13 | 613243 | AD | TNNC1 encodes Tn-C that abolishes inhibitory action of Tn on actin filaments upon $Ca^{2+}$ binding |
| *MYH6* | 160710 | CMH14 | 613251 | AD | Alpha heavy chain subunit of cardiac myosin |
| *VCL* | 193065 | CMH15 | 613255 | AD | VCL encodes an actin filament-binding protein that regulates cell-matrix adhesion, cell-cell adhesion, cell-surface E-cadherin expression, mechanosensing by E-cadherin complex, cell morphology and cell locomotion |
| *MYOZ2* | 605602 | CMH16 | 613838 | AD | MYOZ2 encodes myozenin that binds proteins involved in linking Z line proteins and localizing calcineurin signaling to sarcomeres. May play a role in myofibrillogenesis |

*(continued on next page)*

**Table 1** ***(continued)*****.** Genes associated with various forms of hypertrophic cardiomyopathy

| Gene | OMIM gene | Disease | OMIM disease | Inheritance | Function |
|---|---|---|---|---|---|
| *JPH2* | 605267 | CMH17 | 613873 | AD | JPH2 is necessary for intracellular $Ca^{2+}$ signaling in cardiac myocytes via ryanodine receptor-mediated $Ca^{2+}$ release |
| *PLN* | 172405 | CMH18 | 613874 | AD | Modulates contractility of heart muscle in response to physiological stimuli via ATP2A2 regulates $Ca^{2+}$ re-uptake during muscle relaxation and $Ca^{2+}$ homeostasis in heart muscle |
| *CALR3* | 611414 | CMH19 (?) | 613875 | AD | $Ca^{2+}$-binding chaperone localized in endoplasmic reticulum |
| *NEXN* | 613121 | CMH20 | 613876 | AD | Essential for maintenance of sarcomere integrity |
| *MYPN* | 608517 | CMH22 | 615248 | AD | Component of cardiac muscle sarcomere that links nebulette to alpha-actinin in Z lines |
| *ACTN2* | 102573 | CMH23 with or without LVNC | 612158 | AD | Localized in Z-disc of cardiac muscle where it anchors myofibrillar actin filaments |
| *LDB3* | 605906 | CMH24 | 601493 | AD | Adaptor protein in striated muscle; couples protein kinase C-mediated signaling to cytoskeleton |
| *TCAP* | 604488 | CMH25 | 607487 | AD | Muscle assembly regulating factor that mediates antiparallel assembly of titin molecules at sarcomere Z-disk |
| *FLNC* | 102565 | CMH26 | 617047 | AD | Critical for myogenesis and structural integrity of muscle fibers |

CMH=hypertrophic cardiomyopathy; LVNC=left ventricular non-compaction; AD=autosomal dominant; AR=autosomal recessive

genetic testing for CMD. The guidelines for clinical use of genetic testing are described in Genetics Home Reference (10), GeneReviews (12) and Clinical Utility Gene Card (16).

## Restrictive cardiomyopathy

Restrictive cardiomyopathy (RCM) is a rare genetic heart disease characterized by restrictive ventricle filling and diastolic dysfunction due to cardiac muscle stiffness which leads to abnormal relaxation of the ventricles, although thicknesses and systolic function are usually normal until later stages of the disease (17). It can manifest at any time from childhood to adulthood. In children, the first signs may be failure to gain weight and thrive, fatigue and fainting. As the disease advances, there may be edema, ascites, hepatomegaly and lung congestion. Some children are totally asymptomatic and sudden death is the first manifestation. Adults with RCM first develop dyspnea, fatigue and reduced ability to exercise. Arrhythmia and palpitations are also typical of adults with RCM (18). Restrictive cardiomyopathy is uncommon: in the US and Europe, it accounts for less than 5% of all cardiomyopathies. Prevalence is unknown (19).

**Table 2.** Genes associated with various forms of dilated cardiomyopathies

| Gene | OMIM gene | Disease | OMIM disease | Inheritance | Function |
|---|---|---|---|---|---|
| *LMNA* | 150330 | CMD1A | 115200 | AD | Required for cardiac homeostasis |
| *MYH7* | 160760 | CMD1S | 613426 | AD | Beta heavy chain subunit of cardiac myosin |
| *MYH6* | 160710 | CMD1EE | 613252 | AD | Alpha heavy chain subunit of cardiac myosin. |
| *SCN5A* | 600163 | CMD1E | 601154 | AD | Mediates voltage-dependent $Na^+$ permeability of excitable membranes |
| *ACTN2* | 102573 | CMD1AA with/without LVNC | 612158 | AD | Localized in the Z-disc of cardiac muscle where it anchors myofibrillar actin filaments |
| *DSG2* | 125671 | CMD1BB | 612877 | AD | $Ca^{2+}$-binding transmembrane glycoprotein component of desmosomes between myocardial cells |
| *LDB3* | 605906 | CMD1C with/ without LVNC | 601493 | AD | Adaptor protein in striated muscle; couples protein kinase C-mediated signaling to cytoskeleton |
| *TNNT2* | 191045 | CMD1D | 601494 | AD | $Ca^{2+}$-dependent regulator of muscle contraction |
| *RBM20* | 613171 | CMD1DD | 613172 | AD | RNA-binding protein that regulates mRNA splicing of genes involved in heart development, such as TTN |
| *TTN* | 188840 | CMD1G | 604145 | AD | Important for striated muscle assembly and function, connects microfilaments, contributes to balance of forces between two halves of sarcomere |
| *BAG3* | 603883 | CMD1HH | 613881 | AD | Co-chaperone for HSP70 and HSC70 chaperone proteins in heart; triggers client/substrate protein release |
| *DES* | 125660 | CMD1I | 604765 | AD | Sarcomeric microtubule-anchoring protein that maintains sarcomere structure |
| *CRYAB* | 123590 | CMD1II | 615184 | AD | Has chaperone-like activity, preventing aggregation of proteins under stress conditions |
| *EYA4* | 603550 | CMD1J | 605362 | AD | Transcriptional regulator during organogenesis |
| *LAMA4* | 600133 | CMD1JJ | 615235 | AD | Mediates attachment, migration and organization of cells into tissues during embryo development by interacting with other extracellular matrix components |
| *MYPN* | 608517 | CMD1KK | 615248 | AD | Component of heart muscle sarcomere linking nebulette to alpha-actinin in Z lines |
| *SGCD* | 601411 | CMD1L | 606685 | AD | Component of sarcoglycan complex linking F-actin cytoskeleton and extracellular matrix |
| *CSRP3* | 600824 | CMD1M | 607482 | AD | Positive regulator of myogenesis; transcription cofactor for myogenic bHLH transcription factors |
| *ABCC9* | 601439 | CMD1O | 608569 | AD | Activates and regulates cardiac and smooth muscle-type KATP channels |

*(continued on next page)*

**Table 2** ***(continued)*****.** Genes associated with various forms of dilated cardiomyopathies

| Gene | OMIM gene | Disease | OMIM disease | Inheritance | Function |
|---|---|---|---|---|---|
| *PLN* | 172405 | CMD1P | 609909 | AD | Modulates contractility of heart muscle in response to physiological stimuli via ATP2A2; regulates $Ca^{2+}$ re-uptake during muscle relaxation and $Ca^{2+}$ homeostasis in heart muscle |
| *ACTC1* | 102540 | CMD1R | 613424 | AD | Localized in contractile apparatus of muscle tissue |
| *MYBPC3* | 600958 | CMD1MM | 615396 | AD | Cardiac isoform of myosin-binding protein C found in cross-bridge-bearing zone (C region) of A bands |
| *PRDM16* | 605557 | CMD1LL | 615373 | AD | Transcriptional cofactor essential for heart development |
| *PSEN1* | 104311 | CMD1U | 613694 | AD | Expressed in heart and critical for heart development |
| *PSEN2* | 600759 | CMD1V | 613697 | AD | Expressed in heart and critical for heart development |
| *TPM1* | 191010 | CMD1Y | 611878 | AD | $Ca^{2+}$-dependent regulator of striated muscle contraction |
| *VCL* | 193065 | CMD1W | 611407 | AD | Encodes an actin filament-binding protein that regulates cell-matrix adhesion, cell-cell adhesion, cell-surface E-cadherin expression, mechanosensing by the E-cadherin complex, cell morphology and cell locomotion |
| *TNNC1* | 191040 | CMD1Z | 611879 | AD | Encodes Tn-C that abolishes inhibitory action of Tn on actin filaments upon $Ca^{2+}$ binding |
| *RAF1* | 164760 | CMD1NN | 615916 | AD | Promotes cardiomyocyte survival |
| *DSP* | 125647 | CMD with woolly hair, keratoderma, tooth agenesis | 615821, 605676 | AD, AR | Obligate component of functional desmosomes |
| *TCAP* | 604488 | CMD | / | AD | Muscle assembly regulating factor that mediates antiparallel assembly of titin molecules at sarcomeric Z-disk |
| *ANKRD1* | 609599 | CMD | / | AD | Nuclear negative transcription factor that regulates expression of cardiac genes |
| *TMPO* | 188380 | CMD | / | AD | Regulates expression patterns of major cardiac transcription factors |
| *ILK* | 602366 | CMD | / | AD | Migration and survival of myocardial and endothelial cells |
| *TNNI3* | 191044 | CMD2A, CMD1FF | 611880, 613286 | AR | Cardiac mediator of striated muscle relaxation |

*(continued on next page)*

**Table 2** ***(continued)*****.** Genes associated with various forms of dilated cardiomyopathies

| *Gene* | OMIM gene | Disease | OMIM disease | Inheritance | Function |
|---|---|---|---|---|---|
| *GATAD1* | 614518 | CMD2B | 614672 | AR | Regulates gene expression by binding to a histone modification site |
| *FKTN* | 607440 | CMD1X | 611615 | AR | Glycosylation of alpha-dystroglycan in skeletal muscle |
| *SDHA* | 600857 | CMD1GG | 613642 | AR | Major catalytic subunit of succinate-ubiquinone oxidoreductase located in mitochondrial respiratory chain |
| *DMD* | 300377 | CMD3B | 302045 | XLR | Anchors extracellular matrix to cytoskeleton via F-actin |
| *TAZ* | 300394 | CMD | / | XLR | Involved in cardiolipin metabolism |

CMD=dilated cardiomyopathy, LVNC=left ventricular non-compaction AD=autosomal dominant; AR=autosomal recessive; XLR=X-linked recessive.

Clinical diagnosis is based on medical and family history, physical examination, chest X-ray, echocardiography, ECG, Holter monitoring, stress test, cardiac MRI, cardiac catheterization, coronary angiography and myocardial biopsy (18). Genetic testing is useful for confirming diagnosis, and for differential diagnosis, recurrence risk evaluation and prenatal diagnosis in families with a known mutation. Differential diagnosis should consider constrictive pericarditis, idiopathic forms, such as Loeffler eosinophilic endomyocardial disease, secondary forms, such as infiltrative disease (amyloidosis, sarcoidosis, hemochromatosis, Fabry disease, Danon disease and Friedreich ataxia) and treatment-induced RCM (post-irradiation fibrosis and drug-induced RCM) (19).

Restrictive cardiomyopathy typically has autosomal dominant inheritance (Table 3). Pathogenic variants may be missense, nonsense, splicing and small indels. Large deletions/duplications have been reported in *TNNI3, MYBPC3* and *MYH7*. MAGI uses a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the above genes, and MLPA to detect duplications and deletions in the *TNNI3, MYBPC3* and *MYH7* genes. 6 accredited medical genetic laboratories in the US, listed in the GTR (9) database, offer genetic tests for RCM. The guidelines for clinical use of genetic testing are described in Genetics Home Reference (10).

## Left ventricular noncompaction

Left ventricular noncompaction (LVNC) is a heart disorder that affects the cardiac muscle, mostly the left ventricle, which acquires a thick spongy appearance. The disease is considered to be a consequence of an arrest in heart development during embryogenesis (20). The abnormal cardiac muscle does not function properly, leading to progressive systolic and diastolic dysfunction. LVNC may be isolated or an element of other heart diseases.

The disorder has a variety of symptoms. Some patients may be entirely asymptomatic, while others fall victim to sudden death. Other symptoms or signs may be arrhythmia, palpitations, abnormal blood clots, fatigue, dyspnea and lymphedema (21). Although the disease is genetic, age of onset is variable and diagnosis may be made from birth to late adulthood. The prevalence of LVNC is less than 0.25% (22).

Clinical diagnosis is mainly based on structural features observed by cardiac imaging. Echocardiography is used for diagnosis and follow-up. MRI can

**Table 3.** Genes associated with various forms of restrictive cardiomyopathy

| Gene | OMIM gene | Disease | OMIM disease | Inheritance | Function |
|---|---|---|---|---|---|
| *TNNI3* | 191044 | RCM1 | 115210 | AD | Cardiac mediator of striated muscle relaxation |
| *TNNT2* | 191045 | RCM3 | 612422 | AD | Ca2+-dependent regulator of muscle contraction |
| *MYPN* | 608517 | RCM4 | 615248 | AD | Component of the heart muscle sarcomere linking nebulette to alpha-actinin in Z lines |
| *FLNC* | 102565 | RCM5 | 617047 | AD | Critical for myogenesis and structural integrity of muscle fibers |
| *ACTC1* | 102540 | RCM | / | AD | Localized in contractile apparatus of muscle tissue |
| *MYH7* | 160760 | RCM | / | AD | Beta heavy chain subunit of cardiac myosin |
| *MYBPC3* | 600958 | RCM | / | AD | Cardiac isoform of myosin-binding protein C found in cross-bridge-bearing zone (C region) of A bands |
| *TPM1* | 191010 | RCM | / | AD | $Ca^{2+}$-dependent regulator of striated muscle contraction |
| *MYL1* | 160780 | RCM | / | AD | Regulatory light chain of myosin |
| *MYL2* | 160781 | RCM | / | AD | Regulatory light chain associated with cardiac myosin beta heavy chain, promoting cardiac myofibril assembly |

RCM=restrictive cardiomyopathy; AD=Autosomal dominant.

be useful in cases with poor echocardiogram findings. Genetic testing is useful for confirming diagnosis and for differential diagnosis, recurrence risk evaluation and prenatal diagnosis in families with a known mutation. Differential diagnosis should consider dilated cardiomyopathy, hypertensive heart disease, apical hypertrophic cardiomyopathy, infiltrative cardiomyopathy, eosinophilic endomyocardial disease, localized left ventricular hypertrophy, left ventricular thrombi, cardiac metastases, endocardial fibroelastosis and Barth syndrome (23).

Left ventricular noncompaction is a genetically heterogeneous disorder with sporadic and familial forms (24). Autosomal dominant inheritance seems more common than X-linked inheritance (25). Autosomal recessive inheritance and mitochondrial inheritance have also been observed (26). Current evidence suggests that in most cases, an association with genetic cardiomyopathy (CMP) and/or congenital heart disease (CHD) is more likely than a causal role. Consequently, the genetic basis coincides or overlaps with those of CMP or CHD (27). LVNC has mostly autosomal dominant inheritance, but may also have autosomal recessive inheritance (Table 4).

Pathogenic variants may be sequence variations (missense, nonsense, splicing, small insertions and deletions, small indels). Large deletions/duplications have also been reported in *MYBPC3, MYH7, PKP2* and *PRDM16*. MAGI uses a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the above genes, and MLPA to detect duplications and deletions in the same genes.

Worldwide, 40 accredited medical genetic laboratories in the EU and 4 in the US, listed in the Orphanet (8) and GTR (9) databases, respectively, offer genetic testing for LVNC. The guidelines for clinical use of genetic testing are described in Genetics Home Reference (10).

**Table 4.** Genes associated with various forms of left ventricular noncompaction

| Gene | OMIM gene | Disease | OMIM disease | Inheritance | Function |
|---|---|---|---|---|---|
| *MYH7* | 160760 | LVNC5 | 613426 | AD | Beta heavy chain subunit of cardiac myosin |
| *MYBPC3* | 600958 | LVNC10 | 615396 | AD | Cardiac isoform of myosin-binding protein C found in cross-bridge-bearing zone (C region) of A bands |
| *TPM1* | 191010 | LVNC9 | 611878 | AD | $Ca^{2+}$-dependent regulation of striated muscle contraction |
| *PRDM16* | 605557 | LVNC8 | 615373 | AD | Transcriptional cofactor essential for heart development |
| *MIB1* | 608677 | LVNC7 | 615092 | AD | Involved in heart looping process |
| *TNNT2* | 191045 | LVNC6 | 601494 | AD | $Ca^{2+}$-dependent regulator of muscle contraction |
| *ACTC1* | 102540 | LVNC4 | 613424 | AD | Localized in muscle tissue contractile system |
| *LDB3* | 605906 | LVNC3 | 601493 | AD | Adapter protein in striated muscle; couples protein kinase C-mediated signaling to cytoskeleton |
| *DTNA* | 601239 | LVNC1 | 604169 | AD | Component of dystrophin-associated protein complex; localized in sarcolemma |
| *LMNA* | 150330 | LVNC | / | AD | Required for cardiac homeostasis |
| *SCN5A* | 600163 | LVNC | / | AD | Mediates voltage-dependent $Na^{+}$ permeability of excitable membranes |
| *HCN4* | 605206 | LVNC | / | AD | Necessary for heart pacemaking |
| *PLEKHM2* | 609613 | LVNC | / | AR | Regulates conventional kinesin activity |
| *PKP2* | 602861 | LVNC | / | AR | Plays a role in junctional plaques |
| *SOX6* | 607257 | LVNC | / | AR | Transcriptional activator required for maintenance of cardiac muscle cells |
| *MT-ND1* | 516000 | LVNC | / | MT | Core subunit of mitochondrial membrane respiratory chain NADH dehydrogenase |

LVNC=left ventricular noncompaction; AD=Autosomal dominant; AR=Autosomal recessive; MT=Mitochondrial.

## Arrhythmogenic right ventricular cardiomyopathy

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a genetic heart disease characterized by replacement of right ventricular myocytes with fibrous and fatty tissue. This predisposes young persons and athletes to ventricular tachycardia and even sudden death. Symptoms are not frequent in the early stages, but there is nevertheless risk of sudden death during intense exercise. When symptoms occur, they often include palpitations and syncope. Shortness of breath, swelling of the legs or heart failure are typical of a later stage of the disease. Patients usually develop symptoms between the second and fifth decade. The mean age at diagnosis is 31 years (28).

Prevalence of ARVC is estimated at 1:1000-1250

in the general population (29), but in countries with intensive family screening this disease appears to be much more common (30). Study of a population in which males and females were equally distributed revealed that males were 3.3-fold more likely to be associated with episodes of arrhythmia (31). Expression of the disease is variable, while penetrance is incomplete and age-related (32).

To establish diagnosis, an International Task Force proposed criteria for clinical diagnosis of ARVC/dysplasia that facilitated recognition and interpretation of its often nonspecific clinical features. Structural, histological, electrocardiographic, arrhythmic and familial features of the disease were incorporated into the criteria, divided into major and minor categories according to the specificity of their association with ARVC/dysplasia. This provided a standard on which to base clinical research and genetic studies (33). Differential diagnosis should consider idiopathic right ventricular outflow-tract tachycardia, cardiac sarcoidosis and congenital heart disease leading to right ventricular volume overload (34).

Arrhythmogenic right ventricular cardiomyopathy has mostly autosomal dominant inheritance and only rarely autosomal recessive or digenic inheritance (28). Pathogenic variants in the genes listed in Table 5

**Table 5.** Genes associated with various forms of arrhythmogenic right ventricular cardiomyopathy

| Gene | OMIM gene | Disease | OMIM disease | Inheritance | Function |
|---|---|---|---|---|---|
| *TGFB3* | 190230 | ARVC1 | 107970 | AD | Involved in embryogenesis, differentiation, wound healing |
| *RYR2* | 180902 | ARVC2 | 600996 | AD | $Ca^{2+}$ channel that releases $Ca^{2+}$ from sarcoplasmic reticulum into cytoplasm and triggers cardiac muscle contraction |
| *TMEM43* | 612048 | ARVC5 | 604400 | AD | Maintains nuclear envelope structure |
| *DSP* | 125647 | ARVC8 | 607450 | AD | Forms obligate component of functional desmosomes |
| *PKP2* | 602861 | ARVC9 | 609040 | AD | Plays role in junctional plaques |
| *DSG2* | 125671 | ARVC10 | 610193 | AD | $Ca^{2+}$-binding transmembrane glycoprotein components of desmosomes between myocardial cells |
| *JUP* | 173325 | ARVC12 | 611528 | AD | Common constituent of desmosomes and intermediate junctions |
| *CTNNA3* | 607667 | ARVC13 | 615616 | AD | Involved in formation of cell-cell adhesion complexes in muscle cells |
| *TTN* | 188840 | ARVC | / | AD | Important for striated muscle assembly and functioning; connects microfilaments and contributes to balance of forces between two halves of sarcomere |
| *DES* | 125660 | ARVC | / | AD | Sarcomeric microtubule-anchoring protein that maintains sarcomere structure |
| *LMNA* | 150330 | ARVC | / | AD | Required for cardiac homeostasis |
| *DSC2* | 125645 | ARVC11 | 610476 | AD, AR | Major components of desmosomes (cell-cell junctions found in mechanically-stressed cells) |

ARVC=arrhythmogenic right ventricular cardiomyopathy; AD=Autosomal dominant; AR=Autosomal recessive.

have autosomal dominant inheritance (35). Pathogenic variants may be missense, nonsense, splicing, small indels and gross deletions or duplications. MAGI uses a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the above genes, and MLPA to detect duplications and deletions in *DSP* and *PKP2*.

Worldwide, 46 accredited medical genetic laboratories in the EU and 22 in the US, listed in the Orphanet (8) and GTR (9) databases, respectively, offer genetic testing for ARVC. The guidelines for clinical use of genetic testing are described in Genetics Home Reference (10) and Clinical Utility Gene Card (35).

## Conclusions

We created a NGS panel to detect nucleotide variations in coding exons and flanking regions of all the genes associated with cardiac disorders. When a suspect of cardiomyopathy is present, we perform the analysis of all the genes present in this short article.

In order to have a high diagnostic yield, we developed a NGS test that reaches an analytical sensitivity (proportion of true positives) and an analytical specificity (proportion of true negatives) of ≥99% (coverage depth ≥10x).

**Conflict of interest:** Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Review

# Hereditary thrombophilia

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**Summary.** Thrombophilia is a group of disorders in which blood has an increased tendency to clot. It may be caused by inherited or acquired conditions. Thrombophilia is associated with risk of deep venous thrombosis and/or venous thromboembolism. Factor V Leiden thrombophilia is the most common inherited form of thrombophilia and prothrombin-related thrombophilia is the second most common genetic form of thrombophilia, occurring in about 1.7-3% of the European and US general populations (3). Thrombophilia may have autosomal dominant, autosomal recessive or X-linked inheritance. Genetic testing is useful for confirming diagnosis and for differential diagnosis, recurrence risk evaluation and asymptomatic diagnosis in families with a known mutation. (www.actabiomedica.it)

**Key words:** thrombophilia, deep venous thrombosis, venous thromboembolism

Thrombophilia is a group of disorders in which blood has an increased tendency to clot. It may be caused by inherited or acquired conditions. Secondary disorders include heparin-induced thrombocytopenia, antiphospholipid antibody syndrome, neoplasia, oral contraceptive use, obesity, smoking and surgery. Primary disorders or genetic causes of thrombophilia include factor V Leiden mutation, deficiency of antithrombin III, protein C or S, histidine-rich glycoprotein deficiency and prothrombin-related thrombophilia.

Thrombophilia is associated with risk of deep venous thrombosis and/or venous thromboembolism. Sometimes the thrombosis occurs in uncommon sites, such as the splanchnic veins, cerebral veins and retinal vein, however the clinical expression of hereditary thrombophilia is variable. Some individuals never develop thrombosis, others may remain asymptomatic until adulthood and others have recurrent thromboembolism before 30 years of age.

Factor V Leiden thrombophilia is the most common inherited form of thrombophilia. The prevalence in the US and European general populations is 3-8% for one copy of the factor V Leiden mutation; about 1:5000 persons have two copies of the mutation (1). Moderate protein S deficiency is estimated to affect 1:500 individuals. Severe deficiency is rare and its prevalence is unknown (2). Moderate protein C deficiency affects about 1:500 individuals. Severe deficiency occurs in about 1:4000000 newborns (2). Prothrombin-related thrombophilia is the second most common genetic form of thrombophilia, occurring in about 1.7-3% of the European and US general populations (3). Hereditary antithrombin III deficiency has a prevalence of 1:500-5000 in the general population (4).

Clinical diagnosis is based on medical history, physical examination, laboratory data and imaging. Genetic testing is useful for confirming diagnosis, and for differential diagnosis, recurrence risk evaluation and asymptomatic diagnosis in families with a known

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mutation. Differential diagnosis should consider the above conditions and secondary causes of thrombosis.

Thrombophilia has autosomal dominant, autosomal recessive, or X-linked inheritance (Table 1). Pathogenic variants may be missense, nonsense, splicing or small indels. Large deletions/duplications have been reported in *F5, SERPINC1, PROS1, PROC, F9, FGA*, *FGB*.

MAGI uses a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the above genes and MLPA to detect duplications and deletions in *F5, SERPINC1, PROS1, PROC, F9, FGA* and *FGB*. Worldwide, 78 accredited medical genetic laboratories in the EU and 27 in the US, listed in the Orphanet (5) and GTR (6) databases, respectively, offer genetic tests for thrombophilia. The guide-

**Table 1.** Genes associated with various forms of thrombophilia

| Gene | OMIM gene | Disease | OMIM disease | Inheritance | Function |
|---|---|---|---|---|---|
| *F5* | 612309 | THPH2 | 188055 | AD | Activation of prothrombin to thrombin |
| *F2* | 176930 | THPH1 | 188050 | AD | Coagulation and maintenance of vascular integrity |
| *SERPINC1* | 107300 | AT3D | 613118 | AD | Inhibition of thrombin, regulation of blood coagulation cascade |
| *HRG* | 142640 | THPH11 | 613116 | AD | Adaptor protein involved in coagulation, fibrinolysis |
| *PROS1* | 176880 | THPH5, THPH6 | 612336, 614514 | AD, AR | Prevention of coagulation, stimulation of fibrinolysis |
| *SERPIND1* | 142360 | THPH10 | 612356 | AD | Thrombin, chymotrypsin inhibitor |
| *PROC* | 612283 | THPH3, THPH4 | 176860, 612304 | AD, AR | Regulation of blood coagulation by inactivating factors Va and VIIIa |
| *F13B* | 134580 | Deficiency of B subunit of factor XIII | 613235 | AR | B subunit of factor XIII, stabilizes fibrin clots |
| *F9* | 300746 | THPH8 | 300807 | XLR | Activates factor X |
| *PLAT* | 173370 | THPH9 | 612348 | AD | Involved in tissue remodeling, degradation |
| *THBD* | 188040 | THPH12 | 614486 | AD | Regulation of amount of thrombin |
| *FGB* | 134830 | Congenital dysfibrinogenemia | 616004 | AD | Beta component of fibrinogen. After vascular injury, fibrinogen is converted into thrombin to form fibrin (major component of blood clots) |
| *FGG* | 134850 | Congenital dysfibrinogenemia | 616004 | AD | Gamma component of fibrinogen. After vascular injury, fibrinogen is converted into thrombin to form fibrin (major component of blood clots) |
| *HABP2* | 603924 | THPH1 | 188050 | AD | Role in coagulation and fibrinolysis systems |
| *MTHFR* | 607093 | THPH1 | 188050 | AD | Conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate |

THPH=thrombophilia; AT3D=antithrombin III deficiency; AD=autosomal dominant; AR=autosomal recessive; XLR=X-linked recessive

lines for clinical use of genetic testing are described in Genetics Home Reference (2).

## Conclusions

We created a NGS panel to detect nucleotide variations in coding exons and flanking regions of all the genes associated with cardiac disorders. When a suspect of thrombophilia is present, we perform the analysis of all the genes present in this short article.

In order to have a high diagnostic yield, we developed a NGS test that reaches an analytical sensitivity (proportion of true positives) and an analytical specificity (proportion of true negatives) of ≥99% (coverage depth ≥10x).

**Conflict of interest:** Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Review

# Monogenic hyperlipidemias

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**Summary.** Monogenic hyperlipidemias are a group of inherited disorders characterized by elevated plasma concentrations of lipids and lipoproteins. High plasma concentrations of lipids are the most frequent risk factor for cardiovascular disease. Monogenic hyperlipidemias are a minor cause with respect to multifactorial hyperlipidemias. Diagnosis is based on clinical findings and lipid panel measurements. Genetic testing is useful for confirming diagnosis and for differential diagnosis, recurrence risk calculation and prenatal diagnosis in families with a known mutation. Monogenic hyperlipidemias can have either autosomal dominant or recessive inheritance. (www.actabiomedica.it)

**Key words:** hyperlipidemia, cholesterol, triglycerides, LDL, HDL

Monogenic hyperlipidemias are a group of inherited disorders characterized by elevated plasma concentrations of lipids, such as cholesterol and triglycerides (TG), and lipoproteins, such as chylomicron, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) (1). High plasma concentrations of lipids, especially low density lipoprotein cholesterol (LDL-C), lead to early onset atherosclerosis and are the most frequent risk factor for cardiovascular disease (2), whereas high plasma levels of HDL-C are associated with a lower risk of cardiovascular disease (2). Monogenic hyperlipidemias are a minor cause with respect to multifactorial hyperlipidemias (3).

Monogenic hyperlipidemias are classified on the basis of the primary lipid or lipoprotein anomaly, such as elevated concentrations of LDL-C, low concentrations of HDL-C, or elevated TG. Primary disorders with elevated plasma concentrations of LDL-C include familial hypercholesterolemia (FH), autosomal dominant hypercholesterolemia types 2, 3, 4 and 5, and autosomal recessive hypercholesterolemia. The most frequent condition is FH, characterized by very high LDL-C and xanthomas (patches of yellowish cholesterol build-up) around the eyelids and in the tendons of the elbows, hands, knees and feet. Heterozygous FH has a prevalence of 1:200-250, while homozygous FH (including true homozygosis and compound heterozygosis) is much rarer with a prevalence of 1:160000-250000 (4).

Since plasma levels of HDL-C are inversely related to cardiovascular risk, hereditary disorders that decrease HDL levels are of clinical importance, though rare. They include Tangier disease and homozygous deficiencies in apolipoprotein A-1 or lecithin-cholesterol acyltransferase (2).

Primary hypertriglyceridemias result from genetic defects in metabolism or synthesis of TG. Their prevalence is estimated at less than 0.2%. Except for lipoprotein lipase deficiency, which manifests in child-

hood, they usually manifest in adulthood (5). Disorders in this category include familial chylomicronemia (associated with deficiencies in LPL or APOC2), severe hypertriglyceridemia (associated with deficiencies in APOA5, LMF1 or GPIHBP1), infantile hypertriglyceridemia and hyperlipoproteinemia type 3. Clinical findings may include eruptive or palmar xanthomas and very high TG levels which are associated with increased risk of recurrent pancreatitis and premature cardiovascular disease.

**Table 1.** Genes associated with various forms of monogenic hyperlipidemia

| *Gene* | OMIM gene | Disease | OMIM disease | Inheritance | Function |
|---|---|---|---|---|---|
| *APOB* | 107730 | Hypercholesterolemia, B | 144010 | AD | Major protein constituent of chylomicrons, LDL, VLDL |
| *LDLR* | 606945 | FH | 143890 | AD | Endocytosis of LDL |
| *PCSK9* | 607786 | FH3 | 603776 | AD | Crucial regulator of plasma cholesterol homeostasis |
| *LDLRAP1* | 605747 | ARH | 603813 | AR | Endocytosis of LDLR in hepatocytes and lymphocytes |
| *APOE* | 107741 | Hyperlipoproteinemia, type III | 617347 | AD | Lipoprotein-mediated lipid transport between organs via plasma and interstitial fluids |
| *USF1* | 191523 | Combined hyperlipidemia 1 | 602491 | AD | bHLH transcription factor that binds pyrimidine-rich initiator elements, E-box motifs |
| *ABCA1* | 600046 | Primary hypoalphalipoproteinemia | 604091 | AR | Cholesteral efflux pump for lipid removal from cells |
| *APOA1* | 107680 | Primary hypoalphalipoproteinemia | 604091 | AR | Promotion of cholesterol efflux from tissues to liver |
| *LCAT* | 606967 | FED | 136120 | AR | Esterifying enzyme required for cholesterol transport |
| *LPL* | 609708 | Hyperlipoproteinemia type I | 238600 | AR | Hydrolysis of triglycerides of circulating chylomicrons, VLDL |
| *APOC2* | 608083 | Apolipoprotein C-II deficiency | 207750 | AR | Activator of lipoprotein lipase |
| *GPIHBP1* | 612757 | Hyperlipoproteinemia type ID | 615947 | AR | Lipolytic processing of chylomicrons |
| *GPD1* | 138420 | HTGTI | 614480 | AR | Synthesis of glycerol-3-phosphate, $NAD^+$ |
| *LMF1* | 611761 | Combined lipase deficiency | 246650 | AR | Maturation and transport of lipoprotein lipase |
| *APOA5* | 606368 | Familial hypertriglyceridemia | 145750 | AD | Regulator of plasma triglyceride levels |

FH=familial hypercholesterolemia; ARH=autosomal recessive hypercholesterolemia; FED=Fish-eye disease; HTGTI=transient infantile hypertriglyceridemia; AD=autosomal dominant; AR=autosomal recessive.

Diagnosis is based on clinical findings and lipid panel measurements, including total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglycerides. Genetic testing is useful for confirming diagnosis and for differential diagnosis, recurrence risk calculation and prenatal diagnosis in families with a known mutation. Differential diagnosis should consider secondary causes of hypercholesterolemia and hypertriglyceridemia, such as diabetes mellitus (types I and II), obesity, metabolic syndrome, hyperthyroidism, medications, nephrotic syndrome, acute hepatitis, alcohol abuse and pregnancy. Primary causes of hyperlipidemia also require differential diagnosis among themselves.

Monogenic hyperlipidemias can have autosomal dominant or autosomal recessive inheritance (Table 1). Pathogenic variants may be missense, nonsense, splicing or small indels. Large deletions/duplications have been reported in *APOB, LDLR, LDLRAP1, APOE, ABCA1, APOA1, LCAT, LPL, APOC2, GPIHBP1* and *APOA5*.

We use a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the above genes, and MLPA to detect duplications and deletions in *APOB, LDLR, LDLRAP1, APOE, ABCA1, APOA1, LCAT, LPL, APOC2, GPIHBP1* and *APOA5*. Worldwide, 30 accredited medical genetic laboratories in the EU and 8 in the US, listed in the Orphanet (6) and GTR (7) databases, respectively, offer genetic tests for monogenic hyperlipidemias. The guidelines for clinical use of genetic testing are described in Genetics Home Reference (8).

## Conclusions

We created a NGS panel to detect nucleotide variations in coding exons and flanking regions of all the genes associated with cardiac disorders. When a suspect of hyperlipidemia is present, we perform the analysis of all the genes present in this short article.

In order to have a high diagnostic yield, we developed a NGS test that reaches an analytical sensitivity (proportion of true positives) and an analytical specificity (proportion of true negatives) of ≥99% (coverage depth ≥10x).

**Conflict of interest:** Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Review

# Monogenic hypertension

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**Summary.** Hypertension is a significant public health problem. Thirty percent of cases are caused by a single genetic mutation. Hypertension is the predominant and usually the only manifestation in monogenic hypertension Monogenic hypertension may involve mineralcorticoid-dependent or -independent increase in $Na^+$ transport. Diagnosis is based on routine physical examination, blood pressure measurement and laboratory analysis of renin, aldosterone, cortisol and potassium. Genetic testing is useful for confirming diagnosis and for differential diagnosis. Monogenic hypertension has autosomal dominant or autosomal recessive inheritance. (www.actabiomedica.it)

**Key words:** hypertension, apparent mineralocorticoid excess, hyperaldosteronism, congenital adrenal hyperplasia, Liddle syndrome, pseudohypoaldosteronism

Hypertension is a significant public health problem and is defined as average systolic and/or diastolic blood pressure ≥95$^{th}$ percentile for gender, age and height on at least three occasions (1). It is considered a multifactorial disorder, but approximately 30% of cases are caused by a single genetic mutation. Hypertension is the predominant and usually the only manifestation in monogenic hypertension. Three mechanisms are recognised to explain the physiopathology of monogenic hypertension: increased sodium reabsorption leading to plasma volume expansion, excessive aldosterone synthesis, and deficiencies of enzymes that regulate adrenal steroid hormone synthesis and deactivation (2).

Monogenic hypertension may involve: a) increased Na+ transport induced by a mineralocorticoid effect, including apparent mineralocorticoid excess (AME) syndromes, glucocorticoid-remediable aldosteronism and congenital adrenal hyperplasia due to 11β-hydroxylase or 17α-hydroxylase deficiency; or b) increased Na+ transport independent of mineralocorticoids, including Liddle's and Gordon's syndromes.

Apparent mineralocorticoid excess is a rare disorder arising from impaired activity of 11-beta-hydroxysteroid dehydrogenase type II (HSD11B2). This enzyme is responsible for converting active cortisol to inactive cortisone at aldosterone binding sites. In AME patients, the persistence of cortisol leads to increased mineralocorticoid activity due to higher cortisol affinity for the mineralocorticoid receptor. The consequences are hypokalemia, metabolic alkalosis, low plasma renin activity and low plasma aldosterone levels (3).

Glucocorticoid-remediable aldosteronism is a rare disorder caused by unequal crossover of two adjacent genes, *CYP11B1* and *CYP11B2* (coding for 11βhydroxylase and aldosterone synthase, respectively). The chimeric gene encodes a hybrid protein that stimulates aldosterone production, independent of renin, resulting in upregulation of Na+ reabsorption and $K^+$ secretion. Although plasma renin is low, plasma concentrations of aldosterone may be normal. Patients show mild hypokalemia and metabolic alkalosis (1).

* Authors have equally contributed to this work

Congenital adrenal hyperplasia presents with hypertension and may be due to deficiency of 11β-hydroxylase or 17α-hydroxylase. Excess production of steroid intermediaries with mineralocorticoid effects lead to hypernatremia, hypokalemia and low-renin HT.

Liddle syndrome (pseudo-hyperaldosteronism) is a rare disorder due to an epithelial $Na^+$ channel gain of function. Enhanced epithelial $Na^+$ channel activity causes increased $Na^+$ reabsorption, increased intravascular volume, suppression of renin activity and reduction of aldosterone levels. Additional abnormalities include metabolic alkalosis and hypokalemia (4). Patients respond well to K-sparing diuretics such as triamterene and amiloride, but spironolactone is ineffective for blood pressure control in patients with this syndrome.

Gordon syndrome (pseudo-hypoaldosteronism type II) is caused by gain-of-function pathogenic variants in four genes that regulate Na-K-Cl cotrans-

**Table 1.** Genes associated with various forms of monogenic hypertension

| Gene | OMIM gene | Disease | OMIM disease | Inheritance | Function |
|---|---|---|---|---|---|
| *CYP11B1* | 610613 | HALD1, congenital adrenal hyperplasia | 103900, 202010 | AD, AR | Drug metabolism, synthesis of cholesterol, steroids, other lipids |
| *KLHL3* | 605775 | PHA2D | 614495 | AD, AR | Regulator of ion transport in the distal nephron |
| *CUL3* | 603136 | PHA2E | 614496 | AD | Role in late endosome maturation |
| *NR3C2* | 600983 | PHA1A | 177735 | AD | Receptor for aldosterone, corticosterone, cortisol |
| *SCNN1B* | 600760 | LIDLS1, PHA1B | 177200, 264350 | AD, AR | Essential role in electrolyte and blood pressure homeostasis |
| *SCNN1G* | 600761 | LIDLS2, PHA1B | 618114, 264350 | AD, AR | Essential role in electrolyte and blood pressure homeostasis |
| *WNK1* | 605232 | PHA2C | 614492 | AD | Regulation of electrolyte homeostasis and cell signaling, survival, and proliferation. Activator of sodium-coupled chloride cotransporters, inhibitor of potassium-coupled chloride cotransporters |
| *WNK4* | 601844 | PHA2B, PHA2A | 614491, 145260 | AD, AR | Regulation of electrolyte homeostasis and cell signaling, survival, and proliferation. Activator of sodium-coupled chloride cotransporters, inhibitor of potassium-coupled chloride cotransporters |
| *PDE3A* | 123805 | HTNB | 112410 | AD | Regulation of vascular smooth muscle contraction and relaxation |
| *HSD11B2* | 614232 | AME | 218030 | AR | Conversion of cortisol to the inactive metabolite cortisone |
| *CYP17A1* | 609300 | Congenital adrenal hyperplasia | 202110 | AR | Catalysis of reactions involved in drug metabolism; synthesis of cholesterol, steroids, other lipids |
| *SCNN1A* | 600228 | PHA1B | 264350 | AR | Electrolyte and blood pressure homeostasis |

AD=autosomal dominant; AR=autosomal recessive; HALD=familial hyperaldosteronism; PHA=pseudohypoaldosteronism; LIDLS=Liddle syndrome; HTNB=hypertension and brachydactyly syndrome; AME=apparent mineralocorticoid excess.

porter activity in the distal convoluted tubules of the kidney, with overexpression of Na-Cl cotransporters at the apical surface of the cells, causing increased $Na^+$ reabsorption (5). Affected individuals show early-onset hyperkalemia, normal $Na^+$ levels, hyperchloremia, metabolic acidosis and hypercalciuria. Plasma renin activity is suppressed and aldosterone levels are inappropriately low in relation to hyperkalemia. Thiazides are recommended to correct electrolyte abnormalities and blood pressure.

Diagnosis is based on routine physical examination, blood pressure measurement and laboratory analysis of renin, aldosterone, cortisol and potassium. Genetic testing is useful for confirming diagnosis and for differential diagnosis. Differential diagnosis should consider hypertension secondary to renal parenchymal disease, renal artery stenosis, adrenal gland neoplasia, hyperthyroidism, alcohol abuse and excessive dietary salt intake.

The estimated global age-standardized prevalence of hypertension in adults aged ≥20 years was 32.6% in the period 2009-2012. Genetic factors are thought to contribute to 30-60% of blood pressure variations, although known genetic factors explain only 3% of blood pressure variability (6).

Monogenic hypertension has autosomal dominant or autosomal recessive inheritance (Table 1). Pathogenic variants may be missense, nonsense, splicing or small indels. Large deletions/duplications have also been reported in *CYP11B1, CYP11B2, NR3C2, SCNN1B, WNK1, HSD11B2, CYP17A1* and *SCNN1A*.

We use a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the above genes, and MLPA to detect duplications and deletions in *CYP11B1, CYP11B2, NR3C2, SCNN1B, WNK1, HSD11B2, CYP17A1* and *SCNN1A*.

Worldwide, 10 accredited medical genetic laboratories in the EU and 13 in the US, listed in the Orphanet (7) and GTR (8) databases, respectively, offer genetic tests for monogenic hypertension. The guidelines for clinical use of genetic testing are described in Genetics Home Reference (9).

## Conclusions

We created a NGS panel to detect nucleotide variations in coding exons and flanking regions of all the genes associated with cardiac disorders. When a suspect of hypertension is present, we perform the analysis of all the genes present in this short article. In order to have a high diagnostic yield, we developed a NGS test that reaches an analytical sensitivity (proportion of true positives) and an analytical specificity (proportion of true negatives) of ≥99% (coverage depth ≥10x).

**Conflict of interest:** Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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| Gene | OMIM gene | Disease | OMIM disease | Inheritance | Function |
|---|---|---|---|---|---|
| *SCN5A* | 600163 | BrS1 | 601144 | AD, DR | Mediates voltage-dependent $Na^+$ permeability of excitable membranes |
| *GPD1L* | 611778 | BrS2 | 611777 | AD | Decreases cardiac $Na^+$ current |
| *CACNA1C* | 114205 | BrS3 | 611875 | AD | Pore-forming, alpha-1C subunit of voltage-gated $Ca^{2+}$ channel |
| *CACNB2* | 600003 | BrS4 | 611876 | AD | Increases cardiac peak $Ca^{2+}$ current, regulates voltage-dependent activation, controls alpha-1 subunit recruitment |
| *SCN1B* | 600235 | BrS5 | 612838 | AD | Regulates assembly, expression and function of $Na^+$ channel complex |
| *KCNE3* | 604433 | BrS6 | 613119 | AD | Modulates gating kinetics, stabilizes channel complex |
| *SCN3B* | 608214 | BrS7 | 613120 | AD | Modulates channel gating kinetics |
| *HCN4* | 605206 | BrS8 | 613123 | AD | Contributes to native pacemaker currents in the heart that regulate heartbeat rhythm |
| *KCND3* | 605411 | BrS9 | 616399 | AD | Pore-forming subunit of voltage-gated rapidly-inactivating A-type $K^+$ channels |
| *ABCC9* | 601439 | BrS | / | AD | Subunit of ATP-sensitive $K^+$ channels |
| *SCN10A* | 604427 | BrS | / | AD | Mediates voltage-dependent $Na^+$ permeability of excitable membranes |
| *SLMAP* | 602701 | BrS | / | AD | Excitation-contraction coupling |
| *SCN2B* | 601327 | BrS | / | AD | Assembly, expression and modulation of $Na^+$ channel complex |
| *CACNA2D1* | 114204 | BrS | / | AD | Regulates $Ca^{2+}$ current density and activation/inactivation of $Ca^{2+}$ channel |
| *KCNJ8* | 600935 | BrS | / | AD | Inward-rectifier $K^+$ channel |
| *PKP2* | 602861 | BrS | / | AD | Maintains transcription of genes that control intracellular calcium cycling |
| *TRPM4* | 606936 | BrS | / | AR, DR | $Ca^{2+}$-activated non selective cation channel that depolarizes membranes |
| *RYR2* | 180902 | CPVT1 | 604772 | AD | $Ca^{2+}$ channel triggers cardiac muscle contraction |
| *CASQ2* | 114251 | CPVT2 | 611938 | AR | Regulates release of luminal $Ca^{2+}$ via RYR2 |
| *TECRL* | 617242 | CPVT3 | 614021 | AR | $Ca^{2+}$ transport into myocytes |
| *CALM1* | 114180 | CPVT4 | 614916 | AD | Regulates release of $Ca^{2+}$ via RYR2 |
| *TRDN* | 603283 | CPVT5 with/without muscle weakness | 615441 | AR | Regulates release of luminal $Ca^{2+}$ release via RYR1 and RYR2 |

| | | | | | |
|---|---|---|---|---|---|
| *KCNJ2* | 600681 | CPVT | / | AD | Establishes action potential and excitability of neurons and muscles |
| *KCNQ1* | 607542 | LQT1 | 192500 | AD | Repolarizes cardiac action potential |
| | | JLNS1 | 220400 | AR | |
| *KCNH2* | 152427 | LQT2 | 613688 | AD | Pore-forming subunit of voltage-gated inwardly rectifying $K_+$ channel |
| *SCN5A* | 600163 | LQT3 | 603830 | AD | Mediates voltage-dependent $Na_+$ permeability of excitable membranes |
| *ANK2* | 106410 | LQT4 | 600919 | AD | Coordinates assembly of Na/Ca exchanger, Na/K ATPase and InsP3 receptor in sarcoplasmic reticulum of cardiomyocytes |
| *KCNE1* | 176261 | LQT5 | 613695 | AD | Modulates gating kinetics and enhances stability of voltage-gated $K_+$ channel complex |
| | | JLNS2 | 612347 | AR | |
| *KCNE2* | 603796 | LQT6 | 613693 | AD | Modulates gating kinetics and enhances stability of voltage-gated $K_+$ channel complex |
| *KCNJ2* | 600681 | LQT7 | 170390 | AD | Establishes neuron and muscle action potentials and excitability |
| *CACNA1C* | 114205 | LQT8 | 601005 | AD | Pore-forming, alpha-1C subunit of voltage-gated $Ca_{2+}$ channel |
| *CAV3* | 601253 | LQT9 | 611818 | AD | Regulates voltage-gated $K_+$ channels |
| *SCN4B* | 608256 | LQT10 | 611819 | AD | Interacts with voltage-gated alpha subunits to change $Na_+$ channel kinetics |
| *AKAP9* | 604001 | LQT11 | 611820 | AD | Effector in regulating $K_+$ channel |
| *SNTA1* | 601017 | LQT12 | 612955 | AD | Interacts with pore-forming alpha subunit of cardiac $Na_+$ channel |
| *KCNJ5* | 600734 | LQT13 | 613485 | AD | Allows $K_+$ flow into cells |
| *CALM1* | 114180 | LQT14 | 616247 | AD | Mediates ion channel control |
| *CALM2* | 114182 | LQT15 | 616249 | AD | Mediates ion channel control |
| *CALM3* | 114183 | LQT | / | AD | Mediates ion channel control |
| *KCNH2* | 152427 | SQT1 | 609620 | AD | Pore-forming subunit of voltage-gated inwardly rectifying $K_+$ channel |
| *KCNQ1* | 607542 | SQT2 | 609621 | AD | Repolarizes cardiac action potential |
| *KCNJ2* | 600681 | SQT3 | 609622 | AD | Establishes action potential and excitability of neurons and muscles |
| *KCNQ1* | 607542 | ATFB3 | 607554 | AD | Repolarizes cardiac action potential |
| *KCNE2* | 603796 | ATFB4 | 611493 | AD | Modulates gating kinetics and enhances stability of voltage-gated $K_+$ channel complex |

| | | | | | |
|---|---|---|---|---|---|
| *NPPA* | 108780 | ATFB6 | 612201 | AD | Key role in regulation of natriuresis, diuresis, vasodilation |
| *KCNA5* | 176267 | ATFB7 | 612240 | AD | Mediates transmembrane potassium transport in excitable membranes |
| *KCNJ2* | 600681 | ATFB9 | 613980 | AD | Establishes action potential and excitability of neurons and muscles |
| *SCN5A* | 600163 | ATFB10 | 614022 | AD | Mediates voltage-dependent $Na^+$ permeability of excitable membranes |
| *GJA5* | 121013 | ATFB11 | 614049 | AD | Allows passive diffusion of small molecules, including glucose, $K^+$, $Ca^{2+}$ and cAMP |
| *ABCC9* | 601439 | ATFB12 | 614050 | AD | Subunit of ATP-sensitive $K^+$ channels |
| *SCN1B* | 600235 | ATFB13 | 615377 | AD | Regulates assembly, expression, function of $Na^+$ channel complex |
| *SCN2B* | 601327 | ATFB14 | 615378 | AD | Assembly, expression, modulation $Na^+$ channel complex |
| *SCN3B* | 608214 | ATFB16 | 613120 | AD | Modulates channel-gating kinetics |
| *SCN4B* | 608256 | ATFB17 | 611819 | AD | Interacts with voltage-gated alpha subunits to change $Na^+$ channel kinetics |
| *MYL4* | 160770 | ATFB18 | 617280 | AD | Encodes a myosin alkali light chain expressed in embryonic muscle and adult atria |
| *NUP155* | 606694 | ATFB15 | 615770 | AR | Plays a role in fusion of nuclear envelope vesicles and may also be involved in heart physiology |
| *KCND3* | 605411 | ATFB | / | AD | Pore-forming subunit of voltage-gated rapidly-inactivating A-type $K^+$ channels |
| *KCNE1* | 176261 | ATFB | / | AD | Modulates gating kinetics and enhances stability of voltage-gated $K^+$ channel complex |
| *KCNH2* | 152427 | ATFB | / | AD | Pore-forming subunit of voltage-gated inwardly rectifying $K^+$ channels |
| *LMNA* | 150330 | ATFB | / | AD | Component of nuclear lamina and required for cardiac homeostasis |
| *NKX2-5* | 600584 | ATFB | / | AD | Transcription factor involved in heart formation and development |
| *PRKAG2* | 602743 | ATFB | / | AD | Energy-sensing enzyme that monitors cell energy status and functions; inhibits *de novo* biosynthesis of fatty acids and cholesterol |
| *RYR2* | 180902 | ATFB | / | AD | $Ca^{2+}$ channel that triggers cardiac muscle contraction |
| *GATA4* | 600576 | ATFB | / | AD | Regulates genes involved in myocardial differentiation and function |
| *GATA5* | 611496 | ATFB | / | AD | Required for cardiovascular development |
| *GATA6* | 601656 | ATFB | / | AD | Required for cardiovascular development |
| *PITX2* | 601542 | ATFB | / | AD | May play a role in proper localization of asymmetric organs such as heart |

| | | | | | |
|---|---|---|---|---|---|
| *TBX5* | 601620 | ATFB | / | AD | Regulates transcription of several genes involved in heart development |
| *ZFHX3* | 104155 | ATFB | / | AD | Regulates myogenic differentiation |
| *SHOX2* | 602504 | ATFB | / | AD | Transcriptional regulator involved in pattern formation in vertebrates |
| *PRRX1* | 167420 | ATFB | / | AD | Role in establishment of diverse mesodermal muscle types |
| *KCNN3* | 602983 | ATFB | / | AD | Forms a voltage-independent $K_+$ channel activated by intracellular $Ca^{2+}$ |

**Table S1.** Genes associated with various forms of Brugada syndrome (BrS), catecholaminergic polymorphic ventricular tachycardia (CPVT), short/long QT syndrome (SQT/LQT), Jervell and Lange-Nielsen syndrome (JLNS), atrial fibrillation (ATFB). AD=autosomal dominant; AR=autosomal recessive; DR= digenic recessive.

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Review

# Atrial septal defects, supravalvular aortic stenosis and syndromes predisposing to aneurysm of large vessels

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**Summary.** Atrial septal defect is a persistent interatrial communication. It is the second most common congenital heart defect and is detected in 1:1500 live births. Clinical course is variable and depends on the size of the malformation. Clinical diagnosis is based on patient history, physical and instrumental examination. Atrial septal defect is frequently sporadic, but familial cases have been reported. The disease has autosomal dominant inheritance with reduced penetrance, variable expressivity and genetic heterogeneity. Supravalvular aortic stenosis is a congenital narrowing of the lumen of the ascending aorta. It has an incidence of 1:20000 newborns and a prevalence of 1:7500. Clinical diagnosis is based on patient history, physical and instrumental examination. Supravalvular aortic stenosis is either sporadic or familial and has autosomal dominant inheritance with reduced penetrance and variable expressivity. It is associated with mutations in the ELN gene. Syndromes predisposing to aneurysm of large vessels is a group of inherited disorders that may affect different segments of the aorta. They may occur in isolation or associated with other genetic syndromes. Clinical symptoms are highly variable. Familial thoracic aortic aneurysm and dissection accounts for ~20% of all cases of aneurysms. The exact prevalence is unknown. Clinical diagnosis is based on medical history, physical and instrumental examination. Genetic testing is useful for confirming diagnosis of these syndromes and for differential diagnosis, recurrence risk evaluation and prenatal diagnosis in families with a known mutation. Most syndromes predisposing to aneurysm of large vessels have autosomal dominant inheritance with reduced penetrance and variable expressivity. (www.actabiomedica.it)

**Key words:** atrial septal defect, supravalvular aortic stenosis, aneurysm of large vessels

## Atrial septal defect

Atrial septal defect is a persistent interatrial communication (1). It is the second most common congenital heart defect and accounts for approximately 10% of all cardiac malformations. It includes ostium secundum (~75% of cases), ostium primum (15-20%) and sinus venosus (5-10%) (1). It is detected in 1:1500 live births, with a female-to-male ratio of 2-4:1. Its estimated prevalence in the general population is 1:25000 (2). Atrial septal defect is often associated with paradoxical embolism, cerebral abscess, pulmonary hypertension, conduction disturbances, cardiomyopathies, complex congenital heart defect and sudden cardiac death (3).

Clinical course is variable and depends on the size of the malformation. Most very small atrial septal defects (diameter <5 mm) do not have clinical consequences, whereas a defect of 5-10 mm may lead to symptoms in the fourth or fifth decade of life. Larg-

er defects (generally >10 mm) typically present with symptoms in the third decade of life (3).

Clinical diagnosis is based on patient history, physical examination, two-dimensional transthoracic echocardiography and transesophageal echocardiogram, cardiac computed tomography and magnetic resonance imaging (4,5). Differential diagnosis should consider Klippel-Feil syndrome and Eisenmenger syndrome, which features systolic flow murmur in the pulmonary valve region due to increased pulmonary flow (6, 7).

Atrial septal defect is almost always sporadic, but familial cases have been reported. The disease has autosomal dominant inheritance with reduced penetrance, variable expressivity and genetic heterogeneity of familial atrial septal defects (7) (Table 1).

MAGI uses a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the above genes.

Other authors have reported sensitivities of 90% and 100% in 11 ventricular septal defects and five atrial septal defects, respectively (8) and 80% sensitivity in a combined series of 10 atrial septal defects and ventricular septal defects (9).

## Supravalvular aortic stenosis

Supravalvular aortic stenosis (SVAS) is a congenital narrowing of the lumen of the ascending aorta (10). It is often associated with stenosis of other vessels, typically the pulmonary artery, and also with arrhythmia. It may occur as an isolated condition or as a feature of syndromes such as Williams-Beuren (11) or cutis laxa syndrome (12). Its severity varies: some affected patients never experience symptoms and others die in infancy. Although clinical presentation is heterogeneous and severity is variable, surgical treatment is often needed. If not treated, aortic stenosis may lead to dyspnea, chest pain and heart failure. Supravalvular aortic stenosis has an incidence of 1:20000 newborns (13) and a prevalence of 1:7500.

Clinical diagnosis is based on patient history, physical examination, echocardiography, electrocar-

**Table 1.** Genes associated with various forms of atrial septal defect

| Gene | OMIM gene | Disease | OMIM disease | Inheritance | Function |
|---|---|---|---|---|---|
| *GATA4* | 600576 | ASD2 | 607941 | AD | Regulation of genes involved in myocardial differentiation and function |
| *TBX20* | 606061 | ASD4 | 611363 | AD | Transcriptional activator and repressor required for heart development, and for functional and structural phenotypes in adult heart |
| *MYH6* | 160710 | ASD3 | 614089 | AD | Alpha heavy chain subunit of cardiac myosin |
| *ACTC1* | 102540 | ASD5 | 612794 | AD | Major constituent of contractile apparatus of muscle tissue |
| *TLL1* | 606742 | ASD6 | 613087 | AD | Essential for interventricular septum formation |
| *NKX2-5* | 600584 | ASD7, with/ without AVCD | 108900 | AD | Transcription factor necessary for heart formation and development |
| *CITED2* | 602937 | ASD8 | 614433 | AD | Regulatory gene indispensable for prenatal development |
| *GATA6* | 601656 | ASD9 | 614475 | AD | Important in gut, lung and heart development |
| *NKX2-6* | 611770 | ASD | / | AD | Role in embryonic development of heart in conjunction with NKX2-5 |

ASD=atrial septal defect; AVCD=atrioventricular conduction defects; AD=autosomal dominant.

diography and angiographic evidence of progressive narrowing of the aorta and/or pulmonary artery lumen (14). Differential diagnosis should consider Williams-Beuren syndrome, in which SVAS is identical to the isolated form but associated with behavioral disorders, typical facial features and hypercalcemia (15).

Supravalvular aortic stenosis is either sporadic or familial and has autosomal dominant inheritance with reduced penetrance and variable expressivity. It is associated with more than 60 variations in the ELN gene (OMIM gene 130160; OMIM disease 185500) (16). Pathogenic variants may be missense, nonsense, splicing or small indels. Large deletions/duplications have also been reported in the ELN gene. MAGI uses NGS to detect nucleotide variations in coding exons and flanking introns of the ELN gene and MLPA to detect duplications and deletions.

Worldwide, 6 accredited medical genetic laboratories in the EU and 10 in the US, listed in the Orphanet (17) and GTR (18) databases, respectively, offer genetic testing for SVAS. The guidelines for clinical use of genetic testing are described in Genetics Home Reference (19).

**Syndromes predisposing to aneurysm of large vessels**

Syndromes predisposing to aneurysm of large vessels are a group of inherited disorders that may affect different segments of the aorta, such as the aortic root, ascending aorta, aortic arch or descending aorta. They manifest as dilation, aneurysm or dissection of these segments. Aneurysms in the abdominal aorta, peripheral artery and cerebral artery are also reported (20).

These syndromes may occur in isolation or associated with other genetic syndromes, including Marfan syndrome, Loeys-Dietz syndrome and Ehlers-Danlos syndrome. Some patients show one or more of the following clinical signs: congenital heart abnormalities, inguinal hernia, scoliosis and livedo reticularis.

Clinical symptoms vary widely. Aortic aneurysm usually has no symptoms, but depending on its growth rate, location and size, it may express as chest, back, jaw or neck pain, upper limb edema, dyspnea and/or dysphagia. Aortic dissections usually cause sudden severe chest or back pain and may be followed by hemorrhagic shock.

Familial thoracic aortic aneurysm and dissection is a frequent disorder. It is estimated to cause about 20% of all cases of thoracic aortic aneurysm and dissection (21). The exact prevalence is not known as most aortic aneurysms do not cause symptoms unless there is dissection.

Clinical diagnosis is based on medical history, physical examination, transthoracic/transesophageal echocardiography, spiral computed tomography and invasive imaging methods such as left ventricular angiography and aortography (22). Genetic testing is useful for confirming diagnosis of these aneurysm predisposition syndromes and for differential diagnosis, recurrence risk evaluation and prenatal diagnosis in families with a known mutation. Differential diagnosis should consider inherited disorders such as Marfan syndrome, Loeys-Dietz syndrome, Ehlers-Danlos syndrome and acquired disorders of thoracic aortic aneurysm, such as severe hypertension, atherosclerosis and infectious causes.

Syndromes predisposing to aneurysm of large vessels typically have autosomal dominant inheritance with reduced penetrance and variable expressivity (23) (Table 2).

The mutation detection rate in patients with predisposition for aneurysm of large vessels is about 40% (specifically ACTA2 12-21%, FBN1 3%, FOXE3 1.4%, LOX 1.5%, MAT2A 1%, MFAP5 0.25%, MYH11 1%, MYLK 1%, PRKG1 1%, SMAD3 2%, TGFB2 1%, TGFBR1 3% and TGFBR2 5%) (24). Pathogenic variants may be missense, nonsense, splicing or small indels. Large deletions/duplications have also been reported in COL3A1, ELN, FBN1, MYH11, SLC2A10 and SMAD3.

MAGI uses NGS to detect nucleotide variations in coding exons and flanking introns in the above genes and MLPA to detect duplications and deletions in COL3A1, ELN, FBN1, MYH11, SLC2A10 and SMAD3.

Worldwide, 46 accredited medical genetic laboratories in the EU and 39 in the US, listed in the Orphanet (17) and GTR (18) databases, respectively, offer gene tests for these aneurysm predisposition syndromes. The guidelines for clinical use of genetic testing are described in Genetics Home Reference (19).

**Table 2.** Genes associated with various forms of aneurysm of large vessels

| Gene | OMIM gene | Disease | OMIM disease | Inheritance | Function |
|---|---|---|---|---|---|
| *ACTA2* | 102620 | AAT6 | 611788 | AD | Smooth muscle actin involved in vascular contractility and blood pressure homeostasis |
| *COL3A1* | 120180 | EDSVASC | 130050 | AD | Expressed in extensible connective tissues as in the vascular system |
| *ELN* | 130160 | ADCL1 | 123700 | AD | Stabilization of arterial structure |
| *FBN1* | 134797 | Marfan syndrome | 154700 | AD | Force-bearing structural support in elastic and nonelastic connective tissue as in blood vessels |
| *FLNA* | 300017 | AAT | / | XLR | Role in blood vessels and heart development |
| *FOXE3* | 601094 | AAT11 | 617349 | AD | Aortic development |
| *LOX* | 153455 | AAT10 | 617168 | AD | Crosslinking of collagen and elastin |
| *MFAP5* | 601103 | AAT9 | 616166 | AD | Regulation of growth factors maintaining large vessel integrity |
| *MYH11* | 160745 | AAT4 | 132900 | AD | Major contractile protein |
| *MYLK* | 600922 | AAT7 | 613780 | AD | Facilitation of myosin interaction with actin filaments to produce contraction |
| *PRKG1* | 176894 | AAT8 | 615436 | AD | Regulation of cardiovascular function and relaxation of smooth muscle tone |
| *SKI* | 164780 | SGS | 182212 | AD | Repressor of TGF-beta signaling, role in muscle differentiation |
| *SLC2A10* | 606145 | ATORS | 208050 | AD | Required for cardiovascular system development |
| *SMAD3* | 603109 | LDS3 | 613795 | AD | Inhibitor of wound healing |
| *SMAD6* | 602931 | AAT | / | AD | Modulation of endothelial gene expression |
| *TGFB2* | 190220 | LDS4 | 614816 | AD | Regulation of angiogenesis and heart development |
| *TGFB3* | 190230 | LDS5 | 615582 | AD | Involved in embryogenesis and cell differentiation, role in wound healing |
| *TGFBR1* | 190181 | LDS1 | 609192 | AD | Control of mesenchymal cell proliferation and differentiation, wound healing, extracellular matrix production |
| *TGFBR2* | 190182 | LDS2 | 610168 | AD | Control of mesenchymal cell proliferation and differentiation, wound healing, extracellular matrix production |
| *MAT2A* | 601468 | AAT | / | XLR | Development of aortic arches |

AAT=Familial aortic aneurysm, thoracic; EDSVASC=Ehlers-Danlos syndrome, vascular type; ADCL=autosomal dominant cutis laxa; SGS=Shprintzen-Goldberg craniosynostosis syndrome; ATORS=arterial tortuosity syndrome; LDS=Loeys-Dietz syndrome; AD=autosomal dominant; XLR=X-linked recessive.

## Conclusions

We created a NGS panel to detect nucleotide variations in coding exons and flanking regions of all the genes associated with cardiac disorders. When a suspect of cardiac or aortic structural defects is present, we perform the analysis of all the genes present in this short article.

In order to have a high diagnostic yield, we developed a NGS test that reaches an analytical sensitivity (proportion of true positives) and an analytical specificity (proportion of true negatives) of ≥99% (coverage depth ≥10x).

**Conflict of interest:** Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Review

# Combined use of medically-assisted reproductive techniques: a new bioethical issue

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**Summary.** *Background and aim:* The studies of Nobel laureate Robert Geoffrey Edwards led to the first *in vitro* fertilization and embryo transfer in 1978. Since then, reproductive medicine has made huge advances. Methods available to sterile couples now include: purchasing oocytes and sperm, uterus surrogacy, pre-implantation or pre-natal diagnosis, embryo/fetal selection. Here we highlight the fact that combinations of existing technologies could threaten the non-marketability of human life. *Methods:* We searched PubMed and websites to find articles regarding assisted reproduction techniques. *Results:* These methods, taken separately, provide support for natural fertilization, but when used together, they may lead to genuine "baby factories". In poor countries, such "factories" exist and often act illegally. *Conclusions:* We highlight the need for deeper bioethical studies and better legislation regarding the combined use of medically-assisted reproductive techniques. (www.actabiomedica.it)

**Key words:** medically assisted reproduction, *in vitro* fertilization, oocyte/sperm donation, uterus surrogacy, baby factories

## Introduction

Infertility is a widespread condition that affects the lives of millions of people. Since the pioneering research of Nobel laureate Robert Geoffrey Edwards led to the first *in vitro* fertilization (IVF) and embryo transfer in 1978 (1), reproductive medicine has made huge technological advances. In the first application of IVF, female infertility was the problem to solve. Oocytes were collected during a spontaneous cycle, but soon induction of ovulation became part of the treatment. In 1991, intracytoplasmic sperm injection was introduced, revolutionizing treatment in cases of male infertility. Development of increasingly efficient techniques for freezing sperm (2), oocytes (3) and embryos (4) has made fertilization more flexible (5). Assisted reproductive techniques are now used worldwide (6). The European Society of Human Reproduction and Embryology estimated that in 2012 five million babies were born as a result of medically assisted reproduction (MAR) (7). However, problems associated with MAR are much more extensive than is apparent and few other areas of medicine have created as many social and ethical questions or have drawn as much public interest. Society is involved at cultural, religious, social, medical and legislative levels. More than any other

field of medicine, MAR and human embryo research require the support and contribution of the entire society: legislators, physicians, human rights organizations and women's representatives (8). Medically assisted reproduction, involving IVF, has raised the expectations of many infertile couples, generated an overproduction of laws and sparked many ethical and social debates (9).

The wide range of methods now available to sterile couples includes purchasing oocytes and sperm, uterus surrogacy, pre-implantation/pre-natal diagnosis on embryos or fetuses (genetic and/or echographic analysis) and selection/abortion of imperfect embryos/fetuses. These methods offer important support to natural fertilization, but in combination may give rise to genuine "baby factories". From this perspective, the main risk is replacement of medically assisted reproductive techniques with a multi-step process consisting of programmed embryo production and sale, eliminating imperfect "products". This risk should be avoided by specific laws. Here we highlight the universal bioethical issues raised by these techniques.

*Purchasing oocytes and sperm*

The donation of oocytes and sperm is a starting point for generating an embryo. The advent of MAR brought with it the new figure of "donors" who sell their own sperm or oocytes to customers opting for artificial insemination. The term "donor" is not exactly correct, since in many countries sperm is sold rather than donated (10).

*Uterus surrogacy*

Surrogate motherhood has become a widespread social phenomenon in recent years: women from various countries, such as India, Cambodia, Thailand, Eastern Europe and the USA rent out their uterus for pregnancies that eventually end with separation of the babies from their mothers for monetary compensation (11). The most controversial practice is when the surrogate mother provides both her uterus and oocytes. In this case, the couple chooses the oocyte-and-womb donor. Surrogate mothers are also checked for genetic diseases that could be inherited by the fetuses. It is obvious why these practices are becoming more and more common in poor countries, where "labour" is abundant and cheap. These couples rent the wombs of women who have no other source of income and who live their lives in baby factories. Here they go through pregnancies on behalf of their foreign clients (12).

*Pre-implantation and prenatal genetic testing*

Pre-implantation diagnosis provides specific information about genetic diseases or the gender of embryos for implant. In some cases, however, even more specific characteristics can be identified, such as eye color (13,14). Prenatal genetic testing enables parents to know pathogenic variants in the fetus, in which case the aim of testing is to avoid the birth of babies with genetic diseases (15).

## Discussion

Since its advent, medically assisted reproduction has developed into an industry. It has revolutionized the clinical field as well as society, which now needs to consider new bioethical issues about the value of life. Although national governments are promoting policies to control these new techniques, it is hard to limit wrong practices in poorer countries, for example Nigeria (16,17), Thailand (18) and India (19,20), where genuine procreation "factories" have sprung up. Negligence, such as absence of appropriate healthcare for newborn babies, has been documented (21), as well as non-ethical practices, such as slavery, rape and violence to force women to live in these institutions (22).

Despite worldwide ethical, social and scientific debate about the medically assisted reproduction industry, to our knowledge there have been no articles on how these techniques threaten human dignity when combined in a production line (22).

Treating unborn children as goods to be traded, subject to market laws, causes a worldwide social dichotomy. A more wealthy population makes decisions about the lives of others, selecting phenotypic and genetic traits. In many cases, parents decide whether, how and when a baby should be born, and the process leading to its birth involves a purchase.

Universal regulation of medically assisted reproduction is needed to prevent violations to human dignity and should consider the inevitable consequences of leaving such clinical practices under market control. There are several examples of declarations and laws that defend human dignity against eugenic practices, such as Article 2 of the Universal Declaration on the Human Genome and Human Rights of UNESCO (11/11/1997), that states: "*Everyone has a right to respect for their dignity and for their rights regardless of their genetic characteristics*", and "*that dignity makes it imperative not to reduce individuals to their genetic characteristics and to respect their uniqueness and diversity*". The issue of the combined use of medically assisted reproduction techniques is not, however, addressed. One reason is certainly the complexity of the question, but this should not prevent thorough discussion at international level. For example, an attempt to deal with issues associated with commercial medically assisted reproduction has been made in India. The Surrogacy (Regulation) Bill was brought before Parliament in 2016 in response to human rights groups calling for action in the unregulated area of commercial surrogacy arrangements. Both houses of Parliament reviewed the Bill, which passed in December 2018 (23). The law seeks to protect women and children against exploitation and commodification (24).

## Conclusion

Medically assisted reproductive techniques are useful in many cases, such as when a couple that wishes to have children is sterile or carries pathogenic mutations that may be lethal for their children. However, these techniques may be used in illegal and unethical ways, as in the case of the increasingly common "baby factories" in poor and developing countries. Raising awareness of these issues may help reduce the legislative gap that allows "baby factories" to flourish. In 2005, UNESCO issued a "Universal Draft Declaration on Bioethics and Human Rights" as a model for national legislations. However, different religious and political views and the need to strike a reasonable balance between scientific research, the rights of parents and newborns, and respect for life make it difficult to find a common formulation (25).

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Review

# Non-syndromic monogenic male infertility

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**Summary.** Infertility is a widespread clinical problem affecting 8-12% of couples worldwide. Of these, about 30% are diagnosed with idiopathic infertility since no causative factor is found. Overall 40-50% of cases are due to male reproductive defects. Numerical or structural chromosome abnormalities have long been associated with male infertility. Monogenic mutations have only recently been addressed in the pathogenesis of this condition. Mutations of specific genes involved in meiosis, mitosis or spermiohistogenesis result in spermatogenic failure, leading to the following anomalies: insufficient (oligozoospermia) or no (azoospermia) sperm production, limited progressive and/or total sperm motility (asthenozoospermia), altered sperm morphology (teratozoospermia), or combinations thereof. Androgen insensitivity, causing hormonal and sexual impairment in males with normal karyotype, also affects male fertility. The genetic causes of non-syndromic monogenic of male infertility are summarized in this article and a gene panel is proposed. (www.actabiomedica.it)

**Key words:** male infertility, oligozoospermia, azoospermia, asthenozoospermia, teratozoospermia, spermatogenic failure, androgen insensitivity syndrome

## Introduction

Infertility is defined as failure to achieve a successful pregnancy after 12 months or more of regular unprotected intercourse (1). Its prevalence is not negligible, since about 48.5 million couples worldwide do not reach pregnancy after 5 years (2). Overall, about 50% of cases are due to a male factor infertility (3). Genetic causes have been estimated to exist in about 15% of infertile patients, especially in those with azoospermia or severe oligozoospermia (3).

Genetic causes of male infertility can be classified as pre-testicular (affecting hypothalamic-pituitary function), testicular (causing dysfunction at testicular level) and post-testicular (leading to obstruction or interfering with ejaculation of sperm). Other causes include androgen resistance and disorders of sexual development. Genetic causes of male infertility are outlined in Table 1.

Despite a proper diagnostic work-up, the etiology of male infertility remains elusive in up to 75% of cases (4). In recent years, much effort has been made to investigate new candidate genes responsible for male infertility caused by single-gene mutations (5,6). Several genes involved in meiotic and mitotic divisions and in spermiohistogenesis have been examined as potential targets. They may play a role in the pathogenesis of defects of sperm number (oligozoospermia or azoospermia), motility (asthenozoospermia) or morphology (teratozoospermia) (7).

In this review we describe genes belonging to the panels developed by us for the diagnosis of monogenic spermatogenic failure and androgen insensitivity syndrome.

**Table 1.** Main causes of genetic forms of male infertility.

| | |
|---|---|
| Pre-testicular causes | • Normosmic hypogonadotropic hypogonadism<br>• Anosmic hypogonadotropic hypogonadism (Kallmann syndrome)<br>• Prader-Willy syndrome<br>• Laurence-Moon-Biedl syndrome<br>• Others |
| Testicular forms | • Klinefelter syndrome<br>• Numerical chromosomal abnormalities<br>• Y chromosome microdeletions<br>• Chromosomal translocations<br>• Down syndrome<br>• Myotonic dystrophy (Steinert syndrome) |
| Post-testicular causes | • Kartagener syndrome<br>• Congenital bilateral deferent duct agenesis<br>• Young syndrome |
| Others | • Androgen resistance<br>• Disorders of sexual development |

**Genes involved in sperm number defects**

Sperm number defects include azoospermia and oligozoospermia. Azoospermia is the absence of spermatozoa in semen. It affects 1% of the male population and accounts for 20% of all cases of male infertility (8). In about the 40% of patients, spermatogenesis occurs in a regular way but sperm emission is impaired by seminal duct obstruction (obstructive azoospermia) (9); in the other cases, azoospermia is due to spermatogenic failure (non-obstructive azoospermia) (10). Genetic causes of azoospermia include chromosome anomalies (numerical or structural aberrations of autosomal or sexual chromosomes) that affect 5% of all infertile males and 16% of males with azoospermia or oligozoospermia (11). In 5-15% of cases, azoospermia or oligozoospermia is associated with Y chromosome microdeletions; 6-8% of cases with obstructive azoospermia are associated with mutations in the cystic fibrosis transmembrane receptor gene (*CFTR*) that causes congenital bilateral absence of the *vas deferens* (11). Point mutations that cause azoospermia were recently found in the following genes: *NR5A1, SYCP3, ZMYND15, TAF4B, TEX11 NANOS1, PLK4, MEIOB, SYCE1, USP9Y, SOHLH1, TEX15, HSF2* and *KLHL10* (12-19) (Table 2).

Frameshift mutations in *ZMYND15* cause the SPGF14 phenotype. The protein encoded by this gene is involved in temporally normal haploid gene expression during spermatogenesis (13).

A homozygous mutation in *SYCE1* is associated with the SPGF15 phenotype. This gene encodes a member of the synaptonemal complex, a structure that physically links homologous chromosomes during meiosis I (14).

Mutations in *TEX11* have been associated with meiotic arrest and azoospermia with a frequency of 1-15% in the azoospermic males. *TEX11*-encoded protein regulates the coupling of homologous chromosomes in double-strand DNA repair through formation of the synaptonemal complex and the chiasma during the crossover process (20). A similar role is performed by SYCP3 that has also been found mutated in sterile men (16).

*SOHLH1* is mutated in some cases of azoospermia and encodes a testicular transcription factor essential for spermatogenesis (21).

A mutation in *NR5A1*, encoding steroidogenic factor 1, has been reported in a Pakistani patient with meiotic arrest and normal levels of follicle-stimulating and luteinizing hormones, and testosterone (22).

Finally, a patient with spermatogenesis blocked at the spermatocyte stage had a dominant negative mutation in *HSF2*, encoding heat shock transcription factor 2 (23).

**Table 2.** Genes associated with spermatogenic failure

| Gene | Inheritance | OMIM gene | OMIM phenotype | OMIM or HGMD phenotype ID | Spermatogenic defect |
|---|---|---|---|---|---|
| *NR5A1* | AR | 184757 | SPGF8 | 613957 | AZS/OZS |
| *SYCP3* | AD | 604759 | SPGF4 | 270960 | AZS/OZS |
| *ZMYND15* | AR | 614312 | SPGF14 | 615842 | AZS/OZS |
| *TAF4B* | AR | 601689 | SPGF13 | 615841 | AZS/OZS |
| *TEX11* | XLR | 300311 | SPGFX2 | 309120 | AZS |
| *NANOS1* | AD | 608226 | SPGF12 | 615413 | AZS/OZS/OZS+ASTHZ+TZS |
| *PLK4* | AD | 605031 | / | 1556988045 | AZS |
| *MEIOB* | AR | 617670 | SPGF22 | 617706 | AZS |
| *SYCE1* | AR | 611486 | SPGF15 | 616950 | AZS |
| *USP9Y* | YL | 400005 | SPGFY2 | 400042 | AZS |
| *SOHLH1* | AD | 610224 | SPGF32 | 618115 | AZS |
| *TEX15* | AR | 605795 | SPGF25 | 617960 | AZS/OZS |
| *HSF2* | AD | 140581 | / | 702994563 | AZS |
| *KLHL10* | AD | 608778 | SPGF11 | 615081 | OZS; TZS; AZS |
| *AURKC* | AR | 603495 | SPGF5 | 243060 | TZS (macrozoospermia) |
| *DPY19L2* | AR | 613893 | SPGF9 | 613958 | TZS (globozoospermia) |
| *SPATA16* | AR | 609856 | SPGF6 | 102530 | TZS (globozoospermia) |
| *PICK1* | AR | 605926 | / | 247048065 | TZS (globozoospermia) |
| *BRDT* | AR | 602144 | SPGF21 | 617644 | ASS |
| *SUN5* | AR | 613942 | SPGF16 | 617187 | ASS |
| *SLC26A8* | AD | 608480 | SPGF3 | 606766 | AZS |
| *CATSPER1* | AR | 606389 | SPGF7 | 612997 | AZS |
| *SEPT12* | AD | 611562 | SPGF10 | 614822 | AZS; OZS+ASTHZ+TZS |
| *CFAP43* | AR | 617558 | SPGF19 | 617592 | MMAF |
| *CFAP44* | AR | 617559 | SPGF20 | 617593 | MMAF |
| *DNAH1* | AR | 603332 | SPGF18 | 617576 | MMAF |
| *PLCZ1* | AR | 608075 | SPGF17 | 617214 | OAF |

SPGF = spermatogenic failure; OZS = oligozoospermia; AZS = azoospermia; ASTHZ = asthenozoospermia; TZS = teratozoospermia; OZS+ASTHZ+TZS = oligoasthenoteratozoospermia; ASS = acephalic spermatozoa syndrome; MMAF = multiple morphological abnormalities of the flagellum; OAF = oocyte activation failure; AR = autosomal recessive; AD = autosomal dominant; XLR = X-linked recessive; YL = Y-linked; HGMD = Human Gene Mutation Database (https://portal.biobase-international.com/hgmd/pro/)

## Genes involved in defects of sperm morphology

Teratozoospermia is a heterogeneous group of disorders. Sperm morphological evaluation considers the main functional regions (head, body and tail), which may have anomalies in shape and size. Phenotype may involve a single type of malformation in a single patient or different types of malformation in the same patient (24). Recent studies in consanguineous families and small phenotypically homogeneous cohorts has made it possible to identify autosomal recessive cases of teratozoospermia.

Macrozoospermia is a rare condition observed in <1% of infertile men. It is characterized by a high percentage of sperm with large irregularly-shaped heads, multiple flagella and abnormal acrosome. Macrozoospermia is generally associated with severe oligoasthenozoospermia and a high rate of sperm chromosomal anomalies. Homozygous mutations in *AURKC* are the major cause of macrozoospermia. *AURKC* is highly expressed in male germline cells and is involved in chromosomal segregation and cytokinesis (25).

Globozoospermia is a rare condition characterized by round sperm lacking acrosomes. Genes reported mutated in patients with this condition are *SPATA16* and *DPY19L2*. *SPATA16* encodes a protein specific to Golgi apparatus, highly expressed in the testes. A genetic study in a single consanguineous family with male infertility due to globozoospermia revealed a homozygous variant in the three affected siblings (26). Subsequent studies identified mutations in *DPY19L2* in 66.7% of 54 probands with globozoospermia (25). The protein encoded by *DPY19L2* is necessary for elongation of the sperm head and acrosome formation during spermatogenesis (27).

To date, more than 20 cases of patients with acephalic sperm have been reported. Biallelic mutations in *SUN5* can be found in 47% of cases (28), and a homozygous mutation in *BRDT* has been reported in a consanguineous family (27).

Genes currently associated with morphological sperm defects are: *AURKC, ZPBP, DPY19L2, SPATA16, PICK1, BRDT* and *SUN5* (Table 2).

## Genes involved in sperm motility defects

Asthenozoospermia is a condition leading to reduced sperm motility due to defects in the flagellum. The axoneme, outer dense fibers, mitochondria or fibrous sheath of the flagellum may be affected (30). Ultrastructural defects in 9+2 axoneme structure may involve the outer or inner dynein arms, central microtubules and radial spokes.

Biallelic mutations in *DNAH1*, that encodes heavy chain 1 of axonemal dynein expressed in the testis, cause a heterogeneous group of anomalies defined as multiple morphological anomalies of the flagellum (MMAF) (31,32). Mutations in *DNAH1* are the major cause of MMAF and account for 28-44% of cases (33). In four out of 30 Chinese subjects with MMAF, Tang et al. identified mutations in either *CFAP43* or *CFAP44*. The proteins encoded, CFAP43 and CFAP44, are cilium- and flagellum-associated, almost exclusively expressed in the testis.

The genes associated with sperm motility defects are: *SLC26A8, CATSPER1, SEPT12, CFAP43, CFAP44, DNAH1* and *PLCZ1* (Table 2).

We uses a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the aforementioned genes (Table 2). Pathogenic variants may be missense, nonsense, splicing or small indels. Large deletions/duplications have been reported in *USP9Y*, *DPY19L2*, *SPATA16*, *SUN5* and *CFAP43*. We use MLPA to detect duplications and deletions in the latter genes.

## Androgen insensitivity syndrome

Androgens and their receptor are essential for the development and maintenance of the male phenotype and spermatogenesis. The gene *AR* encoding the androgen receptor is X-linked and the encoded protein is a nuclear receptor that recognizes the canonical androgen response elements on the genome (34).

Mutations in *AR* (OMIM gene ID: 313700) cause a spectrum of conditions defined as androgen insensitivity syndromes (OMIM phenotype ID: 300068), which are disorders of sexual development characterized by external female genitalia, ambiguous genitalia or virilization defects, 46,XY karyotype and

little or no response to androgens. Androgen insensitivity syndromes (AIS) may be complete or partial. Patients with the complete form have a female phenotype with little or no pubic/axillary hair or secondary sexual features and a 46,XY karyotype (35).

Mutations can be found throughout the gene, but are more frequent in five exons that encode ligand-binding domains. The androgen insensitivity syndrome phenotype is due to loss-of-function mutations in *AR*, making target cells insensitive to testosterone or dihydrotestosterone. In 95% of cases, a mutation in the *AR* gene can be detected. In 30% of cases the mutations are *de novo*. The disorder is inherited with a X-linked recessive inheritance (36).

Clinical diagnosis is based on symptoms and biochemical features of 46,XY females. The typical hormonal profile of adults includes increased basal concentrations of luteinizing hormone and testosterone. Serum levels of anti-Mullerian hormone may be normal or elevated. Subjects with partial loss-of-function mutations in *AR* (partial AIS) can have infertility as first or only symptom. Partial androgen insensitivity syndrome may be suspected in infertile males with high plasma levels of testosterone and LH, although a precise threshold has not yet been established (36).

Interestingly, mutations in *AR* can be identified in 2-3% of cases of azoospermia and oligozoospermia (38). There may be a genotype-phenotype correlation in AIS patients, as reported in the Androgen Receptor Gene Mutations Database (39), whereas a correlation in partial AIS patients is less clear and the same mutations can express as different phenotypes (37). The *AR* gene may therefore be included among candidate genes in patients with apparently idiopathic azoospermia or oligozoospermia.

## Conclusions

We created a NGS panel to detect nucleotide variations in coding exons and flanking regions of all the genes associated with infertility. When a suspect of male infertility is present we perform the analysis of all the genes present in this short article.

In order to have a high diagnostic yield, we developed a NGS test that reaches an analytical sensitivity (proportion of true positives) and an analytical specificity (proportion of true negatives) of ≥99% (coverage depth ≥10x).

**Conflict of interest:** Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Review

# Non-syndromic monogenic female infertility

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**Summary.** Infertility is a significant clinical problem. It affects 8-12% of couples worldwide, about 30% of whom are diagnosed with idiopathic infertility (infertility lacking any obvious cause). In 2010, the World Health Organization calculated that 1.9% of child-seeking women aged 20-44 years were unable to have a first live birth (primary infertility), and 10.5% of child-seeking women with a prior live birth were unable to have an additional live birth (secondary infertility). About 50% of all infertility cases are due to female reproductive defects. Several chromosome aberrations, diagnosed by karyotype analysis, have long been known to be associated with female infertility and monogenic mutations have also recently been found. Female infertility primarily involves oogenesis. The following phenotypes are associated with monogenic female infertility: premature ovarian failure, ovarian dysgenesis, oocyte maturation defects, early embryo arrest, polycystic ovary syndrome and recurrent pregnancy loss. Here we summarize the genetic causes of non-syndromic monogenic female infertility and the genes analyzed by our genetic test. (www.actabiomedica.it)

**Key words:** female infertility, premature ovarian failure, ovarian dysgenesis, oocyte maturation defects, preimplantation embryonic lethality, recurrent pregnancy loss, ovarian hyperstimulation syndrome

## Premature ovarian failure and ovarian dysgenesis

Premature ovarian failure (POF) is a frequent and heterogeneous disorder (1-2% of women under age 40 years, 1:10000 women under age 20 years and 1:1000 under 30 years) due to anomalies in follicular development. It is characterized by early functional blockade of the ovary (with respect to menopause which normally occurs after age 45 years) and menstrual cycles can be completely absent (primary amenorrhea) or end before 40 years of age (secondary amenorrhea). The most severe forms are caused by ovarian dysgenesis (50% of cases of primary amenorrhea), whereas postpubertal forms are characterized by disappearance of the menstrual cycle (secondary amenorrhea) (1).

Biochemically, premature ovarian failure is characterized by reduced levels of gonad hormones (estrogens) and increased levels of gonadotropins (LH and FSH) (2). Ovarian dysgenesis is characterized by absence of gonad development, gonadotropin resistance and normal development of the external and internal genitalia (3). Chromosome anomalies (deletions, translocations) and pre-mutation status of the *FMR1* gene are frequent causes of POF (estimated prevalence 10-13%) (4). Several studies have identified genes important for ovarian development and onset of POF (Table 1). Mutations in the *BMP15* gene have been identified in 1.5-15% of Caucasian, Indian and Chinese women with POF (5). One third of patients with POF have mutations in *PGRMC1* while changes

**Table 1.** Genes associated with primary ovarian failure and ovarian dysgenesis

| Gene | Inheritance | OMIM gene ID | OMIM phenotype | OMIM or HGMD phenotype ID | Clinical Features |
|---|---|---|---|---|---|
| *HFM1* | AR | 615684 | POF9 | 615724 | Amenorrhea |
| *FIGLA* | AD | 608697 | POF6 | 612310 | Small/absent ovaries, follicles absent, atrophic endometrium |
| *FOXL2* | AD | 605597 | POF3 | 608996 | Hypoplastic uterus and ovaries, follicles absent, secondary amenorrhea |
| *MSH5* | AR | 603382 | POF13 | 617442 | Oligomenorrhea, atrophic ovaries, follicles absent |
| *STAG3* | AR | 608489 | POF8 | 615723 | Primary amenorrhea, ovarian dysgenesis |
| *NOBOX* | AD | 610934 | POF5 | 611548 | Secondary amenorrhea, follicles absent |
| *NR5A1* | AD | 184757 | POF7 | 612964 | Irregular or anovulatory menstrual cycles, secondary amenorrhea, dysgenetic gonads, no germ cells |
| *ERCC6* | AD | 609413 | POF11 | 616946 | Secondary amenorrhea |
| *SYCE1* | AR | 611486 | POF12 | 616947 | Primary amenorrhea, small prepubertal uterus and ovaries, no ovarian follicles |
| *MCM8* | AR | 608187 | POF10 | 612885 | Absent thelarche, primary amenorrhea, no ovaries, hypergonadotropic ovarian failure |
| *BMP15* | XLD | 300247 | POF4, OD2 | 300510 | Delayed puberty, primary/secondary amenorrhea, small ovaries, follicles absent, hypoplastic uterus, hirsutism, absent pubic/axillary hair |
| *FLJ22792* | XLR | 300603 | POF2B | 300604 | Weak teeth, delayed puberty, primary amenorrhea, osteoporosis |
| *DIAPH2* | XLD | 300108 | POF2A | 300511 | Secondary amenorrhea |
| *FSHR* | AR | 136435 | OD1 | 233300 | Osteoporosis, primary amenorrhea |
| *MCM9* | AR | 610098 | OD4 | 616185 | Short stature, low weight, underdeveloped breasts, no ovaries, retarded bone age and development of pubic/axillary hair, primary amenorrhea |
| *SOHLH1* | AR | 610224 | OD5 | 617690 | Short stature, absent thelarche, primary amenorrhea, hypoplastic/no ovaries, small uterus, retarded bone age |
| *PSMC3IP* | AR | 608665 | OD3 | 614324 | Underdeveloped breasts and absent pubic hair, hypoplastic uterus, primary amenorrhea |
| *AMH* | AD | 600957 | POF | 782468699 | Primary/secondary amenorrhea |
| *AMHR2* | AD | 600956 | POF | 1454100025 | Primary ovarian insufficiency |
| *DAZL* | AR | 601486 | POF | 782468699 | Low ovarian reserves |
| *GDF9* | AR | 601918 | POF14 | 618014 | Primary amenorrhea, no breast development, delayed pubic hair development |
| *LHCGR* | AR | 152790 | POF | 1754122511 | Primary amenorrhea |

*(continued on next page)*

**Table 1** ***(continued).*** Genes associated with primary ovarian failure and ovarian dysgenesis

| Gene | Inheritance | OMIM gene ID | OMIM phenotyp | OMIM or HGMD phenotype ID | Clinical Features |
|---|---|---|---|---|---|
| *INHA* | AD, AR | 147380 | POF | 782468699 | Primary amenorrhea |
| *PGRMC1* | AD | 300435 | POF | 782468699 | Hypergonadotropic hypogonadism, amenorrhea |
| *POU5F1* | AD | 164177 | POF | 782468699 | Small ovaries without follicles |
| *TGFBR3* | AD | 600742 | POF | 782468699 | Premature ovarian failure |
| *WT1* | AD | 607102 | POF | 782468699 | Secondary amenorrhea |
| *SGO2* | AR | 612425 | POF | 141105721 | Ovarian insufficiency |
| *SPIDR* | AR | 615384 | POF | 141105721 | Hypoplastic/no ovaries |
| *EIF4ENIF1* | AD | 607445 | POF | 141105721 | Secondary amenorrhea |
| *NUP107* | AR | 607617 | OD6 | 618078 | No ovaries, small uterus, no spontaneous puberty |
| *NANOS3* | AD | 608229 | POF | 729748889 | Primary amenorrhea |

OD=ovarian dysgenesis; POF = primary ovarian failure; HGMD = Human Gene Mutation Database (https://portal.biobase-international.com/hgmd/pro/)

in levels of the encoded protein are known to cause POF through impaired activation of microsomal cytochrome P450 and excessive apoptosis of ovarian cells (6). In 1-2% of cases, mutations in *GDF9*, *FIGLA*, *NR5A1* and *NANOS3* have been identified (6). Whole exome sequencing (WES) in large families has detected mutations in genes important for homologous recombination and meiosis (*STAG3*, *SYCE1*, *HFM1*), DNA repair (*MCM8*, *MCM9*, *ERCC6*, *NUP107*), mRNA transcription (*SOHLH1*) and mRNA translation (*eIF4ENIF1*) (7).

MAGI uses a multi-gene next generation sequencing (NGS) panel to detect nucleotide variations in coding exons and flanking introns of the above genes.

## Oocyte maturation defects and pre-implantation embryonic lethality

Oocyte maturation is defined as re-initiation and completion of the first meiotic division, subsequent progression to the second phase of meiosis, and other molecular events essential for fertilization and early embryo development (8). The meiotic cell cycle begins in the neonatal ovary and stops at prophase I of meiosis until puberty, when an increase in luteinizing hormone concentrations re-initiates meiosis and ovulation. Thus the oocyte progresses from metaphase I to metaphase II. Metaphase I is completed by extrusion of a polar body. Mature oocytes are again arrested at metaphase II, the only stage at which they can be successfully fertilized (9).

Microscope observation of mature oocytes shows a single polar body, a homogeneous cytoplasm, a *zona pellucida* (ZP) and a perivitelline space. The *zona pellucida* is an extracellular matrix surrounding the oocytes of mammals and is fundamental for oogenesis, fertilization and pre-implantation embryo development. It consists of four glycoproteins (ZP1-ZP4) and ensures species-specific fertilization and induction of the sperm acrosomal reaction during fertilization. It also contains sperm receptors, contributes to blocking polyspermy and protects early embryos until implantation. Glycoprotein ZP1 connects ZP2 with ZP3. ZP2 is a structural component of the *zona pellucida* and has a role in sperm binding and penetration after the acrosomal reaction. ZP3 is a receptor that binds sperm at the beginning of fertilization and induces the acrosomal reaction (10). Oocyte maturation can be arrested in various phases of the cell cycle. Until recently, the genetic events underlying oocyte maturation ar-

rest were unknown (9). Only in the last few years have pathogenic genetic variations that cause oocyte maturation defects been found. In particular, heterozygous mutations in the tubulin beta 8 gene (*TUBB8*) cause defects in the assembly of the meiotic spindle and in oocyte maturation (11). Pathogenic variations in *TUBB8* can be found in ~30% of cases with oocyte maturation arrest; mutations in *PATL2* and genes that encode ZP proteins are less frequent (12).

Early arrest of embryo development is one of the main causes of female infertility, although diagnosis can be difficult and the genetic causes are largely unknown. Gene-disease is difficult to identify, but studies on animal models suggest that there may be hundreds. A recent study identified a homozygous mutation in *TLE6* in a case of pre-implantation embryonic lethality with reduced female fertility and embryo development arrest at the meiosis II phase of the oocyte (13). Another study found that mutations in *PADI6* cause early embryonic arrest due to lack of activation of the zygotic genome (14). PADI6 may be involved in formation of the subcortical maternal complex, essential for the embryo to go through the two-cell stage in mice as well as humans.

The current list of genes associated with oocyte maturation defects and pre-implantation embryonic lethality includes *ZP1*, *TUBB8*, *ZP3*, *PATL2*, *ZP2*, *TLE6* and *PADI6* (Table 2).

MAGI uses a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the above genes.

## Sporadic and recurrent pregnancy loss

Recurrent pregnancy loss is defined as two or more consecutive miscarriages before the 20$^{th}$ week of gestation (15) and affects 1-5% of women of fertile age. Several other conditions have been associated with recurrent pregnancy loss: chromosome anomalies in parents or embryo, prothrombotic states, structural anomalies of the uterus, endocrine dysfunction, infections and immunological factors. Although there has been progress in the clinical and biochemical diagnosis of the human infertility, it is estimated that 35-60% of cases are still considered idiopathic, suggesting that genetic, epigenetic and environmental factors contribute to the recurrent pregnancy loss phenotype (16).

Fetal aneuploidies are the most frequent cause of sporadic miscarriage and can be detected in 50-70% of miscarriages in the first trimester and 5-10% of all pregnancies. The most frequent chromosome aberrations are trisomy, triploidy and X monosomy. Chromosome anomalies can also be found in the parental karyotype in 4-6% of couples with at least two miscarriages, and are more frequent in women. The most

**Table 2.** Genes associated with oocyte maturation defect and preimplantation embryonic lethality

| Gene | Inheritance | OMIM gene ID | OMIM phenotype | OMIM phenotype ID | Clinical Features |
|---|---|---|---|---|---|
| *ZP3* | AD | 182889 | OOMD3 | 617712 | Oocyte degeneration, absence of zona pellucida |
| *TUBB8* | AD, AR | 616768 | OOMD2 | 616780 | Oocyte arrest at metaphase I or II; abnormal spindle |
| *ZP1* | AR | 195000 | OOMD1 | 615774 | Absence of zona pellucida |
| *PATL2* | AR | 614661 | OOMD4 | 617743 | Oocyte maturation arrest in germinal vesicle stage, metaphase I or polar body 1 stage; abnormal polar body 1; early embryonic arrest |
| *ZP2* | AR | 182888 | OOMD6 | 618353 | Abnormal of zona pellucida |
| *TLE6* | AR | 612399 | PREMBL1 | 616814 | Failure of zygote formation |
| *PADI6* | AR | 610363 | PREMBL2 | 617234 | Recurrent early embryonic arrest |

OOMD=oocyte maturation defect; PREMBL=preimplantation embryonic lethality.

common anomaly found in couples is unbalanced translocation. Carriers are phenotypically healthy, but about 50-60% of their gametes are unbalanced due to anomalous meiotic segregation (17).

Single genes or few genes as the main cause of recurrent pregnancy loss have been less considered. However, in couples with recurrent pregnancy loss, identification of mutations in the *SYCP3* gene, which encodes a fundamental component of the synaptonemal complex involved in meiotic segregation, has demonstrated a correlation between meiosis, aneuploidy and recurrent miscarriages. This suggests that correct segregation of chromosomes is influenced by events that take place in the fertilization phase, during meiosis I (18,19).

Recurrent miscarriage can also be linked to thrombophilia. In fact, mutations in the Leiden factor V gene (*F5*), coagulation factor II gene (*F2*) and annexin A5 gene (*ANXA5* encoding an anticoagulant protein active in placental villi), have been associated with increased risk of recurrent pregnancy loss. Finally, mutations in *NLRP7* and *KHDC3L* have been associated with hydatidiform mole, a disease of the trophoblast. Hydatidiform mole is due to a fertilization defect and is characterized by trophoblast proliferation that prevents normal embryo development. Mutations in the two genes have been reported in 1% of cases of hydatidiform mole.

The current list of genes known to be associated with recurrent pregnancy loss is reported in Table 3.

MAGI uses a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the above genes.

**Ovarian hyperstimulation syndrome**

Ovarian hyperstimulation syndrome (OHSS, OMIM phenotype: 608115) is a potentially life-threatening condition. It is a systemic disorder caused by excessive secretion of vasoactive hormones by hyperstimulated ovaries. The physiopathology is characterized by an increase in capillary permeability with leakage into the vasal compartment and intravascular dehydration. Severe complications include thrombophilia, renal and hepatic dysfunction and acute respiratory distress (20).

The syndrome is defined as having early onset when it manifests in luteal phase in response to human chorionic gonadotropin (hCG). It is defined as having late onset when it manifests at the beginning of pregnancy and endogenous hCG further stimulates the ovary. It is often induced by ovarian stimulation used for *in vitro* fertilization, although 0.5-5% of cases are spontaneous. Clinical manifestations may range from benign abdominal distension to massive, potentially lethal ovarian enlargement (21). Pathological features of the syndrome, both spontaneous and iatrogenic, include multiple serous and hemorrhagic follicular cysts surrounded by luteal cells (*iperreactio luteinalis*). The syndrome can arise from high serous levels of hCG caused by multiple or molar pregnancies. It can also be associated with pituitary or neuroendocrine adenomas stimulating follicular hormone (FSH), with hypothyroidism, or with activating mutations of the FSH receptor (FSHR) (22).

Five activating mutations in the *FSHR* gene have been described in pregnant women with OHSS. These

**Table 3.** Genes associated with recurrent pregnancy loss.

| Gene | Inheritance | OMIM gene ID | OMIM phenotype | OMIM phenotype ID | Clinical Features |
|---|---|---|---|---|---|
| SYCP3 | AD | 604759 | RPRGL4 | 270960 | Fetal loss after 6-10 weeks of gestation |
| F2 | AD | 176930 | RPRGL2 | 614390 | Recurrent miscarriage |
| ANXA5 | AD | 131230 | RPRGL3 | 614391 | |
| NLRP7 | AR | 609661 | HYDM1 | 231090 | Gestational trophoblastic disease |
| KHDC3L | AR | 611687 | HYDM2 | 614293 | |

RPRGL=recurrent pregnancy loss; PREMBL=preimplantation embryonic lethality.

mutations increase sensitivity to hCG and/or thyroid stimulating hormone (TSH). By contrast, loss-of-function mutations in *FSHR* can severely upset folliculogenesis, causing ovarian insufficiency. Recent studies reported cases with non-gestational OHSS with new mutations in *FSHR* (23-26).

To date, the only gene known to be associated with OHSS is *FSHR* (OMIM gene ID: 136435) and the phenotype has autosomal dominant inheritance.

MAGI uses a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of *FSHR*.

## Conclusions

Infertility is a significant and increasing clinical problem. Several chromosome aberrations have long been known to be associated with female infertility. Only recently have monogenic mutations been found in association with male and female infertility. Genetic tests based on parallel sequencing of several genes are becoming increasingly important in diagnostic practice.

We created a NGS panel to detect nucleotide variations in coding exons and flanking regions of all the genes associated with infertility. When a suspect of female infertility is present we perform the analysis of all the genes present in this short article. In order to have a high diagnostic yield, we developed a NGS test that reaches an analytical sensitivity (proportion of true positives) and an analytical specificity (proportion of true negatives) of ≥99% (coverage depth ≥10x).

Knowledge of the exact molecular cause helps clinicians choose the most appropriate treatments and follow-up.

**Conflict of interest:** Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Review

# Syndromic infertility

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**Summary.** Infertility due to genetic mutations that cause other defects, besides infertility, is defined as syndromic. Here we describe three of these disorders for which we perform genetic tests. 1) Hypopituitarism is an endocrine syndrome characterized by reduced or absent secretion of one or more anterior pituitary hormones with consequent dysfunction of the corresponding peripheral glands. Deficiencies in all the hormones is defined as pan-hypopituitarism, lack of two or more hormones is called partial hypopituitarism, whereas absence of a single hormone is defined as selective hypopituitarism. Pan-hypopituitarism is the rarest condition, whereas the other two are more frequent. Several forms exist: congenital, acquired, organic and functional. 2) The correct functioning of the hypothalamic-pituitary-gonadal axis is fundamental for sexual differentiation and development during fetal life and puberty and for normal gonad function. Alteration of the hypothalamic-pituitary system can determine a condition called hypogonadotropic hypogonadism, characterized by normal/low serum levels of the hormones FSH and LH. 3) Primary ciliary dyskinesia is frequently associated with infertility in males because it impairs sperm motility (asthenozoospermia). Primary ciliary dyskinesia is a group of genetically and phenotypically heterogeneous disorders that show morpho-structural alterations of the cilia. Adult women with primary ciliary dyskinesia can be subfertile and have an increased probability of extra-uterine pregnancies. This is due to delayed transport of the oocyte through the uterine tubes. (www.actabiomedica.it)

**Key words:** hypopituitarism, primary ciliary dyskinesia, hypogonadotropic hypogonadism

## Genetics of hypopituitarism

Hypopituitarism is an endocrine syndrome characterized by reduced or absent secretion of one or more anterior pituitary hormones with consequent dysfunction of the corresponding peripheral glands. Deficiencies in all the hormones is defined as pan-hypopituitarism, the lack of two or more hormones is called partial hypopituitarism, whereas the absence of a single hormone is defined as selective hypopituitarism. Pan-hypopituitarism is the rarest of the three. Several forms exist: congenital, acquired, organic and functional (1).

Combined pituitary hormone deficiency (CPHD) is characterized by impaired production of several pituitary hormones, such as growth hormone, thyroid-stimulating hormone, prolactin, adrenocorticotropic hormone and gonadotropic hormone, and is caused by mutations in transcription factors involved in pituitary ontogenesis. Congenital hypopituitarism has a low incidence with respect to secondary hypopituitarism due to pituitary adenomas, trans-sphenoidal surgery, or radiotherapy. The incidence of congenital hypopituitarism in the population is 1:3000-4000 (2). Genetic mutations associated with congenital hypopituitarism

mainly affect eight genes encoding transcription factors: *PROP1* (thyroid-stimulating, follicle-stimulating, growth, luteinizing and adrenocorticotropic hormones and prolactin are low or absent), *POU1F1* (growth and thyroid-stimulating hormones and prolactin are low or absent), *HESX1* (thyrotropin, follicle-stimulating, growth, luteinizing and adrenocorticotropic hormones are low or absent), *LHX3* (thyroid-stimulating, follicle-stimulating, growth, luteinizing and adrenocorticotropic hormones and prolactin are low or absent), and *LHX4* (thyroid-stimulating, growth, luteinizing, follicle-stimulating and adrenocorticotropic hormones are low or absent) (2).

The clinical phenotype depends on the affected hormone, the severity of pituitary impairment and age of onset. In childhood, congenital idiopathic forms are the most frequent, and are associated with developmental retardation, delay of puberty and absence of adrenarche. In adulthood, acquired forms are more frequent (3).

Loss-of-function mutations in *PROP1* are the most common cause of sporadic and familial cases of CPHD. This gene is mutated in 11% of cases. The mutation rate, however, varies considerably in relation to geographical area. The prevalence of the mutation is less than 1% in western European, American, Australian and Japanese populations, and higher in Russian and eastern European populations. Patients with mutations in *PROP1* show growth hormone (GH), prolactin (PL), and thyroid-stimulating hormone (TSH) deficiency and variable defects in the secretion of luteinizing (LH), follicle-stimulating (FSH) and adenocorticotropic (ACTH) hormones (4).

Mutations in *POU1F1* are the second most frequent cause of pituitary hormone deficiency. The phenotype associated with *POU1F1* mutations can be inherited by dominant or recessive transmission. The major mutation is the heterozygous p.Arg271Trp, found in ~30% of patients with *POU1F1* mutations. In sporadic cases, mutations in this gene are only found in 1.6% of cases. POU1F1 is a member of the POU family of transcription factors and is expressed in the anterior lobe of the pituitary gland. The phenotype associated with *POU1F1* mutations has severely low levels of GH and PRL, variable levels of TSH, short stature, facial dysmorphism, and dysphagia during infancy (5).

Another gene with occasional mutations is *HESX1*. Mutations in this gene occur in 0.45% of sporadic cases. Single heterozygous mutations causes a less severe disorder with incomplete penetrance, whereas homozygous mutations cause a severe and completely penetrant disorder (6).

Biallelic mutations in *LHX3* cause deficiencies in GH, PRL, TSH, LH, FSH and ACTH. Mutations in *LHX3* are found in 0.3% of sporadic cases and 11.1% of familial cases (7).

The pathological phenotype associated with heterozygous mutations in *LHX4* is inherited as an autosomal dominant trait with variable penetrance. Patients with CPHD have variable reductions in serum levels of GH, TSH, ACTH and gonadotropin. Cranial magnetic resonance imaging shows pituitary gland hypoplasia in most cases. However, there is a wide phenotypic variability within and between families.

Finally, mutations in the *GLI2* gene have been reported in patients with combined pituitary hormone deficiency and ectopic posterior pituitary lobe. For instance, several individuals with truncating mutations in *GLI2* show pituitary anomalies, polydactyly and subtly dysmorphic facial features. The inheritance pattern is dominant with incomplete penetrance and variable phenotype. There are mutations in *GLI2* in 1.5% of CPHD cases.

The genes associated with combined pituitary hormone deficiency are: *PROP1*, *SOX3*, *POU1F1*, *HESX1*, *LHX4*, *LHX3*, *OTX2* and *GLI2* (Table 1). Pathogenic variants may be missense, nonsense, splicing or small indels. MAGI uses a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the genes listed in the table. Our NGS test has an analytical sensitivity (proportion of true positives) and analytical specificity (proportion of true negatives) of ≥99% (coverage depth ≥10x).

**Primary ciliary dyskinesia**

Primary ciliary dyskinesia (PCD) is a genetically and phenotypically heterogeneous group of inherited disorders due to morphological and structural alterations of the cilia. It is characterized by chronic bronchorrhea with bronchiectasis and chronic sinusitis and

**Table 1.** Genes associated with combined pituitary hormone deficiency

| Gene | Inheritance | OMIM gene | OMIM phenotype | OMIM phenotype ID | Gene function |
|---|---|---|---|---|---|
| *PROP1* | AR | 601538 | CPHD2 | 262600 | Paired-like homeodomain transcription factor required for pituitary development |
| *POU1F1* | AD, AR | 173110 | CPHD1 | 613038 | Regulation of expression of genes involved in pituitary development and hormone expression |
| *HESX1* | AD, AR | 601802 | CPHD5 | 182230 | Transcriptional repressor expressed in developing forebrain and pituitary gland |
| *LHX3* | AR | 600577 | CPHD3 | 221750 | LIM-containing domain transcription factor required for pituitary development and motor neuron specification |
| *LHX4* | AD | 602146 | CPHD4 | 262700 | LIM-containing domain transcription factor required for pituitary development |
| *SOX3* | XLR | 313430 | PHPX | 312000 | Transcription factor required for pituitary function and development of CNS midline structures |
| *OTX2* | AD | 600037 | CPHD6 | 613986 | Homeodomain-containing transcription factor required for brain, craniofacial and sensory organ development |
| *GLI2* | AD | 165230 | CJS | 615849 | Zinc finger transcription factor required for embryogenesis |

CJS = Culler-Jones syndrome; CNS = central nervous system; CPHD = combined pituitary hormone deficiency; PHPX = panhypopituitarism, X-linked; AR = autosomal recessive; AD = autosomal dominant; XLR = X-linked recessive.

is the second most common congenital disease of the respiratory system after cystic fibrosis. The prevalence is estimated at around 1:20000 (8).

Ultrastructural defects of the 9+2 axoneme of cilia and flagella may be: partial or complete loss of internal dynein arms, central microtubule anomalies, and radial spoke defects. These defects cause recurrent sinusitis, bronchiectasis due to immotile cilia in the upper and lower airways, and infertility due to altered cilia in the oviduct as well as altered sperm flagella (9).

Fifty percent of patients show *situs inversus*. The association of *situs inversus,* sinusitis and bronchiectasis is the classical triad known as Kartagener syndrome. It is noteworthy that this syndrome is a subgroup of primary ciliary dyskinesia (10). In fact, we know that *situs inversus* is caused by motility failure of nodal cilia that allow lateralization of organs during early embryogenesis (11).

In some subjects, primary ciliary dyskinesia is associated with other disorders like polycystic kidney, retinitis pigmentosa, Barder-Biedl syndrome and Usher syndrome, the pathogenesis of which is linked to structural defects of the primary cilia (12). Respiratory disorders can appear at birth (neonatal respiratory distress), during infancy and rarely in adulthood, and may include chronic infections of the upper and lower respiratory tract. Bronchiectasis is not present at birth but may be a secondary effect of a chronic lung disease (8).

Severity and progression of the disease are variable among patients and depend on what ciliary substructures are altered. About 50% of male patients with PCD are infertile due to lack of sperm motility (9,13). Adult women with PCD may be subfertile and at risk of extra-uterine pregnancies due to delayed oocyte transport through the uterine tubes (10). The most fre-

quent ultrastructural defects of PCD in spermatozoa are (14,15):

- reduction and/or absence of the outer dynein arm: ~38.5% of all PCD cases;
- reduction and/or absence of both dynein arms (outer and inner): ~10.5% of all PCD cases;
- microtubule (axoneme) disorganization due to absence of the inner dynein arm and defects in the central apparatus: ~14% of all PCD cases;
- absence or interruption of central apparatus (i.e. the pair of central microtubules and/or radial spokes): ~7% of all PCD cases;
- reduction and/or absence of the inner dynein arm (rare);
- oligocilia with or without normal ultrastructure (rare).

Most cases of primary ciliary dyskinesia or Kartagener syndrome have autosomal recessive inheritance, although some cases with X-linked recessive inheritance have been reported. Currently, 39 genes are known to be involved in PCD (Table 2). The most frequent mutations are in: *DNAH5*, *DNAH11*, *CCDC39*, *DNAI1*, *CCDC40*, *CCDC103*, *SPAG1*, *ZMYND10*, *ARMC4*, *CCDC151*, *DNAI2*, *RSPH1*, *CCDC114*, *RSPH4A*, *DNAAF1*, *DNAAF2* and *LRRC6*. Table 2 shows the frequencies of biallelic pathogenic variants in affected unrelated subjects. Pathogenic variants may be missense, nonsense, splicing and small indels. MAGI uses a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the genes listed in Table 2.

**Hypogonadotropic hypogonadism**

Correct functioning of the hypothalamo-pituitary-gonadal axis is fundamental for differentiation and sexual development during the fetal period and puberty (16). Hypogonadotropic hypogonadism (HH) is caused by alterations in this axis. Such alterations cause low serum levels of sex hormones associated with normal or low levels of FSH and LH. The prevalence of HH is 1/8000 newborns (17).

Clinically, patients with HH show little or no sexual development, primary amenorrhea (women) and oligoazoospermia (men). Other possible features may be: cleft palate, tooth agenesis, visual impairment, intellectual disability (and other neurological abnormalities), and renal agenesis (18).

Hypogonadotropic hypogonadism may be considered isolated when only the gonads are impaired. There are two forms of the isolated HH: Kallmann syndrome (HH associated with anosmia) is caused by defects in embryonic migration of neurons secreting gonadotropin releasing hormone (GnRH); normosmic HH, in which HH is the only symptom and is due to altered signaling, regulation and secretion of GnRH (19).

The HH may have autosomal dominant, autosomal recessive or X-linked inheritance.

The first gene variation discovered in cases of HH was in *ANOS1* (or *KAL1*). *ANOS1* encodes an adhesion molecule (anosmin), probably involved in migration of olfactory and GnRH-secreting neurons toward the hypothalamus during embryo development. Hypogonadotropic hypogonadism associated with *ANOS1* mutations has X-linked recessive inheritance, so only males are affected. Besides HH and anosmia, patients with mutations in *ANOS1* show renal agenesis and neurological disorders such as intellectual disability, sensorineural deafness and synkinesis (20).

*GNRHR* was the first gene found to have variations in cases of normosmic HH, a disorder with autosomal recessive inheritance. The gene encodes the GnRH receptor, a protein expressed in the pituitary gland. The associated phenotype is highly variable, ranging from very severe (total absence of puberty) to partial or delayed pubertal development (21).

Since involvement of *ANOS1* and *GNRHR* in hypogonadotropic hypogonadism was discovered, 28 other associated-genes have emerged (Table 3). More than 2% of cases have mutations in *ANOS1*, *CHD7*, *FGFR1*, *GNRHR*, *IL17RD*, *PROKR2*, *SOX10* or *TACR3*. The other genes have only been found in a few families (18).

Pathogenic variants may be missense, nonsense, splicing or small indels. MAGI uses a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the above genes (Table 3).

**Table 2.** Genes associated with primary ciliary dyskinesia

| Gene | Inheritance | OMIM gene | OMIM phenotype | OMIM or HGMD phenotype ID | Frequency of biallelic variants in affected unrelated subjects (22) |
|---|---|---|---|---|---|
| *DNAI1* | AR | 604366 | CILD1 | 244400 | 2%-10% |
| *DNAAF3* | AR | 614566 | CILD2 | 606763 | <1% |
| *DNAH5* | AR | 603335 | CILD3 | 608644 | 15%-29% |
| *HYDIN* | AR | 610812 | CILD5 | 608647 | <1% |
| *NME8* | AR | 607421 | CILD6 | 610852 | <1% |
| *DNAH11* | AR | 603339 | CILD7 | 611884 | 6%-9% |
| *DNAI2* | AR | 605483 | CILD9 | 612444 | 2% |
| *DNAAF2* | AR | 612517 | CILD10 | 612518 | <1%-2% |
| *RSPH4A* | AR | 612647 | CILD11 | 612649 | 1%-2% |
| *RSPH9* | AR | 612648 | CILD12 | 612650 | <1% |
| *DNAAF1* | AR | 613190 | CILD13 | 613193 | 1%-2% |
| *CCDC39* | AR | 613798 | CILD14 | 613807 | 4%-9% |
| *CCDC40* | AR | 613799 | CILD15 | 613808 | 3%-4% |
| *DNAL1* | AR | 610062 | CILD16 | 614017 | <1% |
| *CCDC103* | AR | 614677 | CILD17 | 614679 | <4% |
| *DNAAF5* | AR | 614864 | CILD18 | 614874 | <1% |
| *LRRC6* | AR | 614930 | CILD19 | 614935 | 1% |
| *CCDC114* | AR | 615038 | CILD20 | 615067 | <2% |
| *DRC1* | AR | 615288 | CILD21 | 615294 | <1% |
| *ZMYND10* | AR | 607070 | CILD22 | 615444 | 2%-4% |
| *ARMC4* | AR | 615408 | CILD23 | 615451 | <3% |
| *RSPH1* | AR | 609314 | CILD24 | 615481 | 2% |
| *C21ORF59* | AR | 615494 | CILD26 | 615500 | <1% |
| *CCDC65* | AR | 611088 | CILD27 | 615504 | <1% |
| *SPAG1* | AR | 603395 | CILD28 | 615505 | <4% |
| *CCNO* | AR | 607752 | CILD29 | 615872 | <1% |
| *CCDC151* | AR | 615956 | CILD30 | 616037 | <3% |
| *CENPF* | AR | 600236 | STROMS | 243605 | <1% |
| *RSPH3* | AR | 615876 | CILD32 | 616481 | <1% |
| *GAS8* | AR | 605178 | CILD33 | 616726 | / |
| *DNAJB13* | AR | 610263 | CILD34 | 617091 | / |
| *TTC25* | AR | 617095 | CILD35 | 617092 | / |
| *PIH1D3* | XLR | 300933 | CILD36 | 300991 | 9.5% |
| *DNAH1* | AR | 603332 | CILD37 | 617577 | <1% |
| *STK36* | AR | 607652 | CILD | 1147369503 | / |

CILD = ciliary dyskinesia, primary; STROMS = Stromme syndrome; AR = autosomal recessive; XLR = X-linked recessive; HGMD = Human Gene Mutation Database (https://portal.biobase-international.com/hgmd/pro/)

**Table 3.** Genes associated with hypogonadotropic hypogonadism

| Gene | Inheritance | OMIM gene | OMIM phenotype | OMIM or HGMD phenotype ID | Gene function |
|---|---|---|---|---|---|
| *KISS1* | AR | 603286 | HH13 | 614842 | Stimulation of GnRH-induced gonadotropin secretion, activation of GnRH neurons |
| *HS6ST1* | AD | 604846 | HH15 | 614880 | Neuron development, neuron branching |
| *IL17RD* | AD, AR | 606807 | HH18 | 615267 | Fate-specification of GnRH-secreting neurons |
| *PROK2* | AD | 607002 | HH4 | 610628 | Chemoattractant for neuronal precursor cells in olfactory bulb |
| *GNRHR* | AR | 138850 | HH7 | 146110 | Receptor for GnRH. Stimulation of LH and FSH secretion |
| *TACR3* | AR | 162332 | HH11 | 614840 | Receptor for neurokinin B. Expressed in hippocampus, hypothalamus, substantia nigra |
| *SPRY4* | AD | 607984 | HH17 | 615266 | Regulation of neurite outgrowth in hippocampal neurons |
| *SEMA3A* | AD | 603961 | HH16 | 614897 | Inhibition of axonal outgrowth, stimulation of apical dendrite growth |
| *FEZF1* | AR | 613301 | HH22 | 616030 | Embryonic migration of GnRH-releasing neurons into brain |
| *FGF17* | AD | 603725 | HH20 | 615270 | Induction and patterning of embryonic brain |
| *GNRH1* | AR | 152760 | HH12 | 614841 | Stimulation of LH and FSH secretion |
| *FGFR1* | AD | 136350 | HH2 | 147950 | Mesoderm patterning, correct axial organization during embryo development, skeletogenesis, development of GnRH neuronal system |
| *CHD7* | AD | 608892 | HH5 | 612370 | Formation of neural crest |
| *NSMF* | AD | 608137 | HH9 | 614838 | Guidance of olfactory axon projections, migration of LHRH neurons |
| *FGF8* | AD | 600483 | HH6 | 612702 | Regulation of embryo development, cell proliferation, differentiation, migration. Brain, eye, ear, limb, GnRH neuronal system, hippocampal neuron development |
| *WDR11* | AD | 606417 | HH14 | 614858 | Regulation of GnRH production |
| *FSHB* | AR | 136530 | HH24 | 229070 | Beta subunit of FSH. Induction of egg and sperm production |
| *TAC3* | AR | 162330 | HH10 | 614839 | Central regulator of gonad function |
| *DUSP6* | AD | 602748 | HH19 | 615269 | Expression regulated by GnRH |
| *KISS1R* | AR | 604161 | HH8 | 614837 | Neuroendocrine control of gonadotropin axis |
| *LHB* | AR | 152780 | HH23 | 228300 | Promotion of spermatogenesis and ovulation by stimulating gonads to synthesize steroids |

*(continued on the next page)*

**Table 3** ***(continued)*****.** Genes associated with hypogonadotropic hypogonadism

| Gene | Inheritance | OMIM gene | OMIM phenotype | OMIM or HGMD phenotype ID | Gene function |
|---|---|---|---|---|---|
| *PROKR2* | AD | 607123 | HH3 | 244200 | Induction of tangential and radial migration of olfactory bulb interneurons |
| *FLRT3* | AD | 604808 | HH21 | 615271 | Spatial organization of brain neurons. |
| *ANOS1* | XLR | 300836 | HH1 (KS) | 308700 | Neural cell adhesion, axonal migration, patterning of mitral and tufted cell collaterals to olfactory cortex |
| *SOX10* | AD | 602229 | WS2E | 611584 | Development of neural crest, peripheral nervous system, glia |
| *AXL* | AD | 109135 | HH | 1734393901 | GnRH neuron survival and migration |
| *CCDC141* | AR | 616031 | KS | 817012261 | Neural radial migration |
| *SEMA3E* | AD | 608166 | KS | 817012261 | Ensuring synapse formation specificity |
| *SRA1* | AR | 603819 | HH | 1734393901 | Mediation of transcriptional co-activation of steroid receptors |

GnRH = gonadotropin-releasing hormone; HH = hypogonadotropic hypogonadism; KS = Kallmann syndrome; WS = Waardenburg syndrome; AD = autosomal dominant; AR = autosomal recessive; XLR = X-linked recessive; HGMD = Human Gene Mutation Database (https://portal.biobase-international.com/hgmd/pro/)

## Conclusions

We created a NGS panel to detect nucleotide variations in coding exons and flanking regions of all the genes associated with infertility. When a suspect of syndromic infertility is present we perform the analysis of all the genes present in this short article. In order to have a high diagnostic yield, we developed a NGS test that reaches an analytical sensitivity (proportion of true positives) and an analytical specificity (proportion of true negatives) of ≥99% (coverage depth ≥10x).

**Conflict of interest:** Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Review

# Hypothyroidism and hyperthyroidism

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**Summary.** Congenital hypothyroidism is a condition in which the thyroid gland does not produce enough thyroid hormones. It occurs in 1:2000-4000 newborns. Common clinical features include decreased activity and increased sleep, feeding difficulty, constipation, prolonged jaundice, myxedematous facies, large fontanels (especially posterior), macroglossia, distended abdomen with umbilical hernia, and hypotonia. Slow linear growth and developmental delay are usually apparent by 4-6 months of age. Without treatment, congenital hypothyroidism leads to severe intellectual deficit and short stature. Congenital hyperthyroidism occurs when the thyroid gland produces too much of the hormone thyroxine, which can accelerate body metabolism, causing unintentional weight loss and a rapid or irregular heartbeat. Hyperthyroidism is very rare and its prevalence is unknown. Common clinical features include unintentional weight loss, tachycardia, arrhythmia, palpitations, anxiety, tremor and sweating. Here we summarize the genes involved in congenital hypo- and hyperthyroidism and the tests we use for genetic analysis. (www.actabiomedica.it)

**Key words:** congenital hypothyroidism, non-autoimmune hyperthyroidism, thyroxine

## Congenital hypothyroidism

Congenital hypothyroidism (CH) is the most common congenital endocrine disorder. It has a prevalence of 1:2000-4000 and is more frequent in females than in males (ratio of 2:1) (1). At birth, clinical features are mild or absent, becoming apparent a few months later. The disorder is characterized by reduced physical activity, increased sleeping periods, feeding difficulties, constipation, jaundice, myxedematous face, wide fontanels, macroglossia, abdominal distention with umbilical hernia, and hypotonia. Developmental and growth delay become evident 4-6 months after birth Without therapy, the disorder leads to intellectual disability and very short stature (1).

Congenital hypothyroidism can be caused by thyroid dysgenesis (85% of cases) or defects in thyroid hormone biosynthesis (10-15% of cases) (2). Secondary congenital hypothyroidism is caused by chronic low levels of thyroid stimulating hormone (TSH) and may be due to congenital hypopituitarism. Peripheral congenital hypothyroidism is caused by defects in the transport, metabolism and action of thyroid hormones, or peripheral resistance to thyroid hormones (3). Congenital hypothyroidism can also be syndromic.

Currently, neonatal screening mainly detects elevated levels of TSH that increase in response to the reduction in thyroid hormone. This screening identifies 90% of cases of CH. Most patients have normal development after treatment with thyroxine. Besides assay of TSH, triiodothyronine (T3) and thyroxine (T4), other diagnostic tests include thyroid scanning with radioactive iodine, thyroid echography, and assay of serum thyroglobulin. These exams can help determine the etiology of the disease and differentiate permanent and transient cases (1,4). Differential

**Table 1.** Genes associated with congenital hypothyroidism

| Gene | OMIM gene | Disease | OMIM disease | Inheritance | Gene function |
|---|---|---|---|---|---|
| *THRA* | 190120 | CHNG6 | 614450 | AD | Nuclear hormone receptor mediator of T3 biological activity |
| *NKX2-1* | 600635 | CAHTP | 610978 | AD | Transcription factor for expression of thyroid-specific genes |
| *NKX2-5* | 600584 | CHNG5 | 225250 | AD | Transcription factor for thyroid organogenesis |
| *PAX8* | 67415 | CHNG2 | 218700 | AD | Transcription factor for expression of thyroid-specific genes, maintenance of thyroid cell differentiation |
| *POU1F1* | 173110 | CPHD1 | 613038 | AD, AR | Transcription factor involved in specification of lactotrope, somatotrope and thyrotrope phenotypes in developing anterior pituitary gland |
| *GNAS* | 139320 | PHP1A<br>PHP1C | 103580<br>612462 | AD | Activation of adenylate cyclase that regulates thyroid activity |
| *SECISBP2* | 607693 | Abnormal thyroid hormone metabolism | 609698 | AR | Co-translational insertion of selenocysteine into selenoproteins like type II iodothyronine deiodinase |
| *THRB* | 190160 | GRTH<br>PRTH | 188570<br>274300<br>145650 | AD<br>AR<br>AD | Nuclear hormone receptor for triiodothyronine. Mediation of thyroid hormone activity |
| *TRHR* | 188545 | Generalized thyrotropin-releasing hormone resistance | 188545 | AR | TRH receptor promoting TSH and prolactin release |
| *KAT6B* | 605880 | GTPTS<br>SBBYSS | 606170<br>603736 | AD | Histone acetyltransferase transcriptional activator and repressor, also important for thyroid organogenesis |
| *LHX4* | 602146 | CPHD4 | 262700 | AD | Early stages of pituitary development |
| *TSHB* | 188540 | CHNG4 | 275100 | AR | Control of thyroid structure and metabolism |
| *TSHR* | 603372 | CHNG1 | 275200 | AR | Major controller of thyroid cell metabolism. Receptor for thyrotropin and thyrostimulin |
| *TPO* | 606765 | TDH2A | 274500 | AR | Central role in thyroid gland function. Generation of thyroxine, T3 |
| *SLC5A5* | 601843 | TDH1 | 274400 | AR | Uptake of iodine by thyroid |
| *DUOXA2* | 612772 | TDH5 | 274900 | AR | Thyroid hormone synthesis |
| *DUOX2* | 606759 | TDH6 | 607200 | AR | Thyroid hormone synthesis |
| *IYD* | 612025 | TDH4 | 274800 | AR | Hydrolysis of thyroglobulin to release iodide |

*(continued on next page)*

**Table 1** ***(continued)*****.** Genes associated with congenital hypothyroidism

| Gene | OMIM gene | Disease | OMIM disease | Inheritance | Gene function |
|---|---|---|---|---|---|
| *PROP1* | 601538 | CPHD2 | 262600 | AR | Involved in ontogenesis of pituitary gonadotropes, somatotropes, lactotropes and caudomedial thyrotropes |
| *SLC26A4* | 605646 | PDS | 274600 | AR | Sodium-independent transporter of iodide |
| *TG* | 188450 | TDH3 | 274700 | AR | Substrate for synthesis of T4 and T3, storage of inactive forms of thyroid hormone and iodine |
| *FOXI1* | 601093 | PDS | / | AR | Transcription factor for *SLC26A4* |
| *FOXE1* | 602617 | Athyroidal/ thyroidal hypothyroidism with spiky hair, cleft palate | 241850 | AR | Thyroid morphogenesis |
| *UBR1* | 605981 | JBS | 243800 | AR | Degradation of substrate proteins |
| *SLC16A2* | 300095 | AHDS | 300523 | XLR | Cell import of T4, T3, T2 |

AHDS = Allan-Herndon-Dudley syndrome; CAHTP = choreoathetosis and congenital hypothyroidism with/without pulmonary dysfunction; CHNG = congenital nongoitrous hypothyroidism; CPHD = combined pituitary hormone deficiency; GRTH = generalized thyroid hormone resistance; GTPTS = genitopatellar syndrome; JBS = Johanson-Blizzard syndrome; PDS = Pendred syndrome; PRTH = selective pituitary thyroid hormone resistance; SBBYSS = Ohdo syndrome, SBBYS variant; TDH = thyroid dyshormonogenesis; AD = autosomal dominant; AR = autosomal recessive; XLR = X-linked recessive.

diagnosis should consider chronic fatigue syndrome, depression, dementia and heart failure (5).

Newborns diagnosed with CH should be treated with levothyroxine to ensure normal neurocognitive development. Serum levels of TSH, T4 and T3 should be measured frequently. When babies are treated soon after birth, their prognosis is excellent and their IQ normal (1). Congenital hypothyroidism is usually sporadic, but in 10% of cases it is inherited (6).

Generalized thyroid hormone resistance is a rare genetic disorder caused by a reduced peripheral response to thyroid hormones. The prevalence is 1:40000. In 85% of cases it is caused by mutations in *THRB* (7).

Twenty-five genes are currently known to be associated with congenital hypothyroidism or generalized thyroid hormone resistance (Table 1). Pathogenic variants may be missense, nonsense, splicing or small indels. We use a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the above genes.

**Nonautoimmune hyperthyroidism**

Nonautoimmune hyperthyroidism or hereditary hyperthyroidism is a rare form of hyperthyroidism, characterized by excessive thyroid activity. Major symptoms are hyperactivity, anxiety, weight loss, exophthalmos and tachycardia (8). Age of onset is highly variable. Clinical diagnosis is based on observation and measurement of plasma concentrations of thyroid hormone. Differential diagnosis is based on absence of exophthalmos and presence of myxedema, anti-TSH antibodies and lymphocyte infiltration of the thyroid (9). Hyperthyroidism can be treated with drugs that inhibit thyroid activity or with ablation therapy (surgery or radioiodine) (10).

The nonautoimmune hyperthyroidism has autosomal dominant inheritance and is caused by mutations in *TSHR* (OMIM gene: 603373; OMIM disease: 609152, 603373) (9). Prevalence is unknown: very few families (130 patients from 22 families) and

some sporadic cases, especially in Caucasian subjects, have been described (9,11).

Pathogenic variants may be missense, nonsense, splicing or small indels. MAGI uses an NGS panel to detect nucleotide variations in coding exons and flanking introns of *TSHR*.

## Conclusions

We created a NGS panel to detect nucleotide variations in coding exons and flanking regions of all the genes associated with hypo- and hyperthyroidism. When one of those suspects is present we perform the analysis of all the genes present in this short article.

In order to have a high diagnostic yield, we developed a NGS test that reaches an analytical sensitivity (proportion of true positives) and an analytical specificity (proportion of true negatives) of ≥99% (coverage depth ≥10x).

**Conflict of interest:** Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Review

# Mendelian non-syndromic obesity

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**Summary.** Obesity is highly heritable and arises from the interplay of many genes and environmental factors. It can be defined as the result of prolonged imbalance between calorie intake and energy utilization. About 5% of cases of non-syndromic obesity are monogenic (Mendelian obesity). The amount of adipose tissue in the body is mainly regulated by leptin, a hormone produced by adipocytes, and Mendelian obesity is mainly caused by mutations that disrupt the leptin/melanocortin pathway. In this article, we summarize the genes involved in genetic obesity and the test we use for genetic analysis. (www.actabiomedica.it)

**Key words:** Mendelian obesity, leptin/melanocortin pathway, adipogenesis

Obesity is a chronic disease defined by the World Health Organization as a "condition characterized by excessive body weight due to adipose tissue accumulation, which has a negative influence on health status". Obesity is the most common nutritional disorder in the western world, and its prevalence is constantly increasing in developing countries. In 2014, about 39% of the world adult population was overweight and about 13% obese (1). Obesity is associated with several metabolic co-morbidities and higher mortality risk due to onset of type 2 diabetes, hypertension, cardiopathies and some cancers (1).

In 2000, the WHO set classification criteria for obesity on the basis of body mass index (BMI). The normal range of BMI is 18.5-24.9, whereas for different types of obesity it is 25-29.9 (type I), 30-39.9 (type II) and >40 (type III) (2).

Most of the genes known to be associated with monogenic obesity belong to the leptin/melanocortin pathway and are expressed in the hypothalamus. They encode proteins involved in the food intake/energy expenditure balance. The adipocyte differentiation pathway, which involves several growth and transcription factors (3,4), is also related to obesity.

Leptin is secreted by adipose tissue and binds to the leptin receptor in the hypothalamus, where it controls food intake through the melanocortin pathway. In the hypothalamus, leptin activates neurons expressing proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript protein (CARTPT), and inhibits neurons expressing neuropeptide Y (NPY) and Agouti related neuropeptide (AGRP). POMC is cleaved into melanocortins α-, β- and γ-MSH which bind to their receptors: MC3R and MC4R (3).

Adipogenesis is the differentiation of adipocytes from mesenchymal stem cells. Differentiation into the adipocyte lineage is induced by chronic excessive energy intake and elevated glucose uptake. Many factors involved in this process have been identified, but

**Table 1.** Genes associated with various forms of Mendelian non-syndromic obesity

| Gene | OMIM gene | Disease | OMIM disease | Inheritance | Function |
|---|---|---|---|---|---|
| *UCP3* | 602044 | Severe obesity, T2D | 601665 | AR, AD | Uncoupling of oxidative phosphorylation, energy dissipation, modulation of tissue respiratory control, thermogenesis, energy balance |
| *CARTPT* | 602606 | Obesity | 601665 | AR, AD | Inhibition of feeding |
| *CEP19* | 615586 | Morbid obesity and spermatogenic failure | 615703 | AR | Required for ciliation |
| *DYRK1B* | 604556 | Abdominal obesity-metabolic syndrome 3 | 615812 | AD | Enhancement of adipogenesis |
| *KSR2* | 610737 | Severe early-onset obesity | / | AD, AR | Regulation of cell energy homeostasis through AMPK activation |
| *LEP* | 164160 | Morbid obesity | 614962 | AR | Major role in regulation of energy homeostasis |
| *LEPR* | 601007 | Morbid obesity | 614963 | AR | |
| *MC4R* | 155541 | Obesity | 618406 | AD, AR | |
| *NR0B2* | 604630 | Early-onset mild obesity | 601665 | AD, AR | Transcriptional regulator of HNF4A, involved in onset of MODY |
| *PCSK1* | 162150 | Obesity | 600955 | AR | Processing of POMC, insulin |
| *POMC* | 176830 | Obesity, adrenal insufficiency, red hair | 609734 | AR | Energy homeostasis |
| *PPARG* | 601487 | Severe obesity | 601665 | AD, AR | Regulator of adipocyte differentiation |
| *PPP1R3A* | 600917 | NIDDM, obesity | 125853 | AD | Regulation of glycogen metabolism |
| *SH2B1* | 608937 | Hyperphagia, early onset obesity, insulin resistance and short stature | / | AD | Adaptor protein involved in insulin, BDNF and leptin signaling pathways |
| *SIM1* | 603128 | Severe obesity, neurobehavioral disorder | / | AD | Transcription factors essential for formation of the hypothalamic paraventricular nucleus |
| *TUB* | 601197 | Retinal dystrophy and obesity | 616188 | AR | Involved in hypothalamic regulation of body weight |
| *NTRK2* | 600456 | Obesity, hyperphagia, developmental delay | 613886 | AD | Mediation of neuronal plasticity in hypothalamus |
| *ADCY3* | 600291 | Obesity, T2D | / | AR | Control of adipose tissue development, function and insulin secretion in beta cells |
| *BDNF* | 113505 | Hyperphagia, severe obesity, cognitive impairment, hyperactivity | / | AD | Regulation of food intake, body weight |

AD=autosomal dominant; AR=autosomal recessive; T2D=type II diabetes; MODY=diabetes of the young; NIDDM=non-insulin dependent diabetes mellitus.

PPARγ is the only factor which is necessary and sufficient for adipocyte differentiation, establishing it as the master regulator of adipogenesis (3).

The most common form of Mendelian non syndromic obesity is associated with mutations in *MC4R*, with a prevalence in the general population of 1-5:10000. Patients with mutations in this gene can also present with hyperinsulinemia and increased linear growth (5).

Other relatively frequent forms of obesity are associated with mutations in the leptin and the leptin receptor gene: *LEP* and *LEPR* (prevalence of both forms <1:1000000). Patients with mutations in these genes show hyperphagia and severe early-onset obesity, and may also have immune function alterations and hypogonadotropic hypogonadism (5). Obesity due to mutations in *POMC* has a prevalence <1:1000000. Besides obesity, patients with *POMC* mutations show hypocortisolism, hair and skin hypopigmentation, neonatal hypoglycemia, seizures, cholestasis and voracious appetite (5). Obesity linked to *PCSK1* mutations has a prevalence in the general population of <1:1000000. Mutation carriers display severe early-onset obesity, hyperphagia, hypoglycemia, hypogonadotropic hypogonadism, hypocortisolism, elevated plasma levels of proinsulin and low plasma concentrations of insulin (5).

Diagnosis is based on clinical findings and lipid panel measurements. Genetic testing is useful for confirming diagnosis and for differential diagnosis, recurrence risk calculation and prenatal diagnosis in families with a known mutation.

Mendelian non syndromic obesity can have autosomal dominant or autosomal recessive inheritance (Table 1). Pathogenic variants may be missense, nonsense, splicing or small indels. MAGI uses a multigene NGS panel to detect nucleotide variations in coding exons and flanking introns of the genes listed in Table 1.

Worldwide, 34 accredited medical genetic laboratories in the EU and 37 in the US, listed in the Orphanet (5) and GTR (6) databases, respectively, offer genetic tests for Mendelian non syndromic obesity. The guidelines for clinical use of genetic testing are available in Genetics Home Reference (7).

## Conclusions

We created a NGS panel to detect nucleotide variations in coding exons and flanking regions of all the genes associated with Mendelian obesity. When this suspects is present we perform the analysis of all the genes present in this short article.

In order to have a high diagnostic yield, we developed a NGS test that reaches an analytical sensitivity (proportion of true positives) and an analytical specificity (proportion of true negatives) of ≥99% (coverage depth ≥10x).

**Conflict of interest:** Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Review

# Genetic syndromes with localized subcutaneous fat tissue accumulation

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**Summary.** Syndromes with localized accumulation of subcutaneous fatty tissue belong to a group of genetically and phenotypically heterogeneous disorders. These diseases may show some common signs, such as nodular fat, symmetrical fat masses, obesity, fatigue, lymphedema and symmetrical lipomas (painful or otherwise). Other symptoms may be specific for the different clinical entities, enabling correct differential diagnosis. Disorders belonging to this spectrum are lipedema, generalized diffuse or nodular forms of Dercum disease, localized nodular Dercum disease and multiple symmetric lipomatosis. Here we summarize the genes involved in syndromes with localized accumulation of subcutaneous fat and the test we use for genetic analysis. (www.actabiomedica.it)

**Key words:** lipedema, Dercum disease, lipomatosis

Lipedema is an underdiagnosed chronic debilitating disease characterized by bruising, pain and excess subcutaneous fat affecting the lower and/or upper limbs of women during or after periods of hormonal change, especially puberty (1). The first guidelines on lipedema were proposed in Germany in 2015, and again in 2017 using the international classification of function, disability and health (2-4). Lipedema can easily be confused with obesity, but is distinguished by primarily affecting the lower limbs and upper extremities; the fat deposits do not reduce with a low-calorie diet and body mass index is normal (5). Lipedema can also be confused with lymphedema, but is always bilateral, whereas lymphedema can be unilateral or bilateral; pain and bruising are absent in lymphedema while lipedema patients are negative for Stemmer sign (6). The prevalence of lipedema has been reported to be 1-9/100,000 (7). Lipedema can be considered a component of a spectrum of diseases characterized by dysregulated proliferation of adipose tissue and pain: generalized diffuse form of Dercum disease (painful pearl-sized nodular subcutaneous adipose tissue throughout the body); generalized nodular form of Dercum disease (large painful nodules on the arms, trunk, and thighs); lipedema (localized form of painful fat with pearl-sized nodular fat and larger masses on the limbs); localized nodular form of Dercum disease (localized around joints); Madelung disease or multiple symmetric lipomatosis (nodular fat and lipomas on the upper part of the body (8,9). Genetic testing that includes all genes known to be involved in syndromes with localized accumulation of subcutaneous fat is useful for confirming diagnosis, and for differential diagnosis, recurrence risk evaluation and prenatal diagnosis in families with a known mutation (10).

Syndromes with localized subcutaneous fat accumulation can have autosomal dominant or autosomal recessive inheritance (Table 1). Pathogenic variants may be missense, nonsense, splicing or small indels. MAGI uses a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the genes listed in Table 1.

**Table 1.** Genes associated with various forms of localized accumulation of subcutaneous fat

| .Gene | OMIM gene | Disease | OMIM disease | Inheritance | Function |
|---|---|---|---|---|---|
| *POU1F1* | 173110 | Combined pituitary hormone deficiency 1 (1 family with lipedema) | 613038 | AD | Regulation of expression of growth hormone, prolactin, thyroid-stimulating hormone |
| *NSD1* | 606681 | Sotos syndrome 1 (1 case with lipedema) | 117550 | AD | Androgen receptor transactivation |
| *ALDH18A1* | 138250 | Cutis laxa, type III (abnormal fat pad, buttocks, upper thighs (some patients) | 616603, 219150 | AD, AR | De novo biosynthesis of proline, ornithine, arginine |
| *PALB2* | 610355 | Multiple subcutaneous familial lipomatosis (1 case) | / | AD | DNA repair |
| *TBL1XR1* | 608628 | Pierpont syndrome | 602342 | AD | Essential transcription activation mediated by nuclear receptors |
| *MFN2* | 608507 | Madelung disease | 151800 | AR | Mitochondrial membrane protein necessary for mitochondrial fusion and maintenance of mitochondrial network |
| *LMNA* | 150330 | FPLD2 | 151660 | AD | Nuclear assembly, chromatin organization, nuclear membrane and telomere dynamics |
| *PPARG* | 601487 | FPLD3 | 604367 | AD | Master regulator of adipocyte differentiation |
| *PLIN1* | 170290 | FPLD4 | 613877 | AD | Coating of lipid storage droplets in adipocytes |
| *CIDEC* | 612120 | FPLD5 | 615238 | AR | Regulation of lipid droplet enlargement by restricting lipolysis, favoring storage |
| *LIPE* | 151750 | FPLD6 | 615980 | AR | Hydrolysis of stored triglycerides to free fatty acids |
| *AKT2* | 164731 | FPLD | / | AD | Key-mediator of insulin receptor |
| *ADRA2A* | 104210 | Atypical FPLD | / | AD | Fundamental for regulation of neurotransmitter release from sympathetic nerves and adrenergic neurons in CNS |

FPLD=familial partial lipodystrophy; AD=autosomal dominant; AR=autosomal recessive; CNS = central nervous system.

## Conclusions

We created a NGS panel to detect nucleotide variations in coding exons and flanking regions of all the genes associated with localized accumulation of subcutaneous fat. When this suspects is present we perform the analysis of all the genes present in this short article.

In order to have a high diagnostic yield, we developed a NGS test that reaches an analytical sensitivity (proportion of true positives) and an analytical specificity (proportion of true negatives) of ≥99% (coverage depth ≥10x).

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Review

# Molecular foundations of chiropractic therapy

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**Summary.** *Background and aim:* Alternative medicine is a broad term used to encompass different therapies, including chiropractic. Chiropractic was called "a science of healing without drugs" by its founder, David Daniel Palmer. It is based on the idea that the body has a powerful self-healing ability and that there is a relationship between body structure and function that affects health. In particular, chiropractic assumes that the nervous system controls the human body through nerves branching from the vertebral column and spinal cord. Researchers do not fully understand how chiropractic therapies affect pain, but chiropractic is widely used today to treat chronic pain, such as back pain. Different studies with animal models have demonstrated that chiropractic therapies mediate neuroplasticity, specifically through modulation of neurotrophins. No studies have yet been published on interaction between neurotrophin gene polymorphisms and chiropractic treatment. *Methods:* We searched PubMed with the following keywords: chiropractic, neuroplasticity, neurotrophin gene polymorphism for a panorama of on the molecular mechanisms of chiropractic therapy. *Results:* From the material collected, we identified a set of genes and some functional polymorphisms that could be correlated with better response to chiropractic therapy. *Conclusions:* Further association studies will be necessary to confirm hypotheses of a correlation between single nucleotide polymorphisms in specific genes and better response to chiropractic therapy. (www.actabiomedica.it)

**Key words:** chiropractic, chiropractic therapy, neuroplasticity, neurotrophins, polymorphism

## Chiropractic – a technique based on manipulation of the spine and peripheral nerve endings

Chiropractic is a form of complementary and alternative medicine that focuses on the relationship between major body structures, such as skeleton, muscles and nerves, and patient health. Basic concepts of chiropractic are: i) the body has powerful self-healing ability and ii) body function and structure, especially the spinal cord, are closely related and this relationship may affect health. These two concepts lead to the idea that chiropractic therapy can normalize the relationship between body structure and function and can help the body to heal. The founder of chiropractic, David Daniel Palmer, called it "a science of healing without drugs" (1). Chiropractic is based on the assumption that the central nervous system controls the human body through peripheral nerves branching from the vertebral column and the spinal cord (2). In the chiropractic view, spinal cord dysfunctions can interfere with the body's innate capacity to heal. Chiropractic emphasizes spinal manipulation. The branches of chiropractic that seem most interesting from a biological point of view are those concerning spinal manipulation and acupressure/acupuncture of peripheral sensory nerve endings (3).

Recent insights have identified inflammation as a local protective response to microbial invasion or injury. It must be well regulated, because deficiencies or excesses of inflammatory response can cause morbidity and mortality. The discovery that cholinergic neurons can inhibit inflammation has changed

the way we understand the link between the nervous system and immune responses. We now know that a basic neural pathway monitors and adjusts the inflammatory response and that an inflammatory input activates a fast subconscious anti-inflammatory response. In his 2002 review, Tracey discussed evidence indicating that stimulation of the "vagus nerve" can prevent inflammation (4). Recent studies of the mechanisms that regulate inflammation have identified a neural mechanism, called the "cholinergic anti-inflammatory pathway", that inhibits macrophage activation through parasympathetic outflow. The pathway's name refers to the role of acetylcholine as the principle parasympathetic neurotransmitter, because macrophages exposed to acetylcholine are effectively deactivated (3).

**Biological foundations of chiropractic therapy**

Massage is a general term for many different techniques involving the application of bodily contact and physical pressure with therapeutic intent. The effects of massage therapy depend on the amount of pressure and the speed of the stroke. For example, slow strokes can evoke systemic relaxation whereas deep strokes increased blood flow to the area. Attempts to define and classify the extensive range of types of massage have sometimes created confusion, but as far as chiropractic is concerned, spinal manipulative therapy (SMT) is the best practice.

One of the effects of spinal manipulation during chiropractic therapy is to stretch spinal muscles. Muscle stretch is a powerful stimulus for up-regulation of a splice product (mechano-growth factor MGF) of the insulin-like growth factor (IGF-1) gene by the stretched muscle. MGF promotes muscle growth and repair (myotrophism) and the growth and repair of neurons (neurotrophism) (5).

Non-noxious mechanical skin stimulation releases nerve growth factor (NGF) in rats. Produced and secreted by brain cortex neurons, NGF is a neurotrophic factor that promotes neuron survival and function (6).

Mechanical vibration massage in rats promotes secretion of NGF by the sub maxillary gland. This secretion accelerates repair of brachial plexus injuries and slows down atrophy of skeletal muscle. Spinal manipulation is a specific hands-on approach commonly used in chiropractic. Thoracic spinal manipulation can lead to different responses involving the sympathetic nervous system, the endocrine system and the hypothalamic-pituitary axis (7).

Spinal manipulative therapy has been demonstrated to be an effective treatment for acute and chronic back pain (8, 9) although the neuronal mechanisms responsible for the pain-reducing effects are not yet understood (10). The idea of a link between SMT and spinal cord neuroplasticity is gaining interest among researchers, and different studies have demonstrated a connection (11, 12). Interestingly, Guzzetta and colleagues demonstrated that increased body massage and multisensory stimulation affects brain development in humans and rat pups. In particular, visual system maturation seems to be increased by "massage therapy" through modulation of levels of endogenous factors such as IGF-1 in infants. In rat pups, massage also proved effective in increasing IGF-1 levels in the cortex. This suggests that IGF-1 could also be a mediator of the effects of massage therapy on visual development in infants (13). The effects of massage in accelerating visual development in rat pups are not due to the simple act of removing pups from the nest, because they are absent in pups separated from the mother for the same amount of time but not massaged. This supports the idea that massage could be a promoter of brain development and the hypothesis that the level of multisensory stimulation provided by licking/grooming is an important regulator of brain development (14-18).

Chiropractic uses acupressure/acupuncture as well as spinal manipulative therapy to decrease pain and for a wide range of other complaints. Acupuncture is a technique of traditional Chinese medicine (19). Acupuncturists insert hair-thin needles in specific points of the body to balance body energy, stimulate healing and promote relaxation. Researchers do not understand how acupuncture can decrease pain, although the technique is widely used in treatment of neurological disorders such as ischemic stroke, cognitive impairment (20), Parkinson's disease (PD) (21) and prophylaxis of migraine (22).

Interestingly, insertion of a needle in the human body produced a slow increase in the skin pain thresh-

old, peaking in 30 min, followed by exponential decay after removal of the needle (23). This suggests that chemical mediators are involved, a hypothesis validated by observation of transfer of the analgesic effect on cross-infusion of cerebroventricular fluid from a donor rabbit undergoing acupuncture stimulation to a naive recipient rabbit.

There are currently two theories about how massage and acupuncture could work: i) by stimulating release of endorphins, natural pain-relieving chemicals of the body (23) and/or ii) by influencing the nervous system and the release of chemicals that regulate blood flow and pressure, reduce inflammation and calm the brain (24). It was recently shown that peripheral sensory stimulation by electro-acupuncture could improve the availability and utilization of brain nerve growth factor (NGF). The clinical efficacy of acupuncture on pain and inflammation are based on the stimulation of several classes of sensory afferent fibers. The resulting activation of physiological processes seems to be similar to those resulting from physical exercise or deep massage. Acupuncture stimulation may induce variations in neural activity throughout the nervous system, affecting the synthesis and release of different neuromodulators. A relationship between acupuncture and NGF was recently investigated with a view to synergic clinical use. Administration of NGF with acupuncture and/or SMT in neurological, endocrine and immune diseases could be an interesting therapeutic approach (25).

## Neuroplasticity and neuro modulator reflex as effector of chiropractic therapy

Various authors have demonstrated that acupuncture and massage mediate neuroplasticity in animal models (26). Neuroplasticity is defined as the ability of the nervous system to reorganize its structure and function in response to intrinsic and/or environmental demands (27). Neuroplasticity may be involved in physiological or pathological conditions: in physiological conditions, it is mainly related to brain development, learning and memory. The most famous form of physiological neuroplasticity is the process known as "adult hippocampal neurogenesis", whereby the adult hippocampus generates functional neurons throughout life (28). In pathological conditions, neuroplasticity seems to be involved in brain injury healing processes.

Neuroplasticity has been identified in the spinal cord and central nervous system, together with its prolonged forms, known as long-term potentiation (LTP) and long-term depression (LTD). In particular, observations of LTP in neurons of the spinal cord and similarities between LTP cell mechanisms and those associated with central sensitization suggest that LTP may play a significant role in establishing major pain conditions. It has also been identified as an amplifying mechanism within the pain system, suggesting its involvement both in acute and chronic pain states (10). If LTP is an important mechanism in pain system regulation, any mechanism that interferes with LTP is of clinical interest. Long-term depression (LTD), found to depend on glutamate release, activation of its NMDARs, and intracellular $Ca^{2+}$, is suggested to have a relationship with LTP. LTD was initially discovered in the hippocampus and was later identified in other body regions, such as spinal neurons, cerebellum, cortex, basal ganglia and amygdala. The identification of spinal LTD and the consequent demonstration of LTP reversal suggests that LTD is a cell mechanism able to mediate the effects of certain therapies (10).

So how can massage and acupuncture operate in the treatment of diseases and pain? Different studies attribute the underlying mechanisms of mediation of neuroplasticity by acupuncture and SMT to modulation of neurotrophins (NTs) (26). It is well known that NTs are responsible for the growth of neurons during development and for the maintenance of adult neurons. More recently, however, there has been increasing evidence that NTs are also involved in neuron survival and differentiation and even in axonal regeneration in neurological disorders (29, 30).

## Genetic determinants of chiropractic response

The expression of NTs, for example by the brain-derived neurotrophic factor *(BDNF)* gene, is induced after cerebral ischemia. Expression peaks within minutes or hours of the event, then quickly returning to normal or even below normal levels. BDNF belongs to

a family of neurotrophins that include neurotrophin-3 (NT3), neurotrophin-4 (NT4) and nerve growth factor (NGF) (31). Here we list a series of known polymorphisms, most having a demonstrated functional effect, which could be considered in genetic analysis of predisposition to a positive response to chiropractic care (Table 1). The selected polymorphisms belong to genes involved in neurotrophism, myotrophism and pain sensitivity. Polymorphisms with a weak functional effect, as evaluated by *in vitro* or association studies, were disregarded.

NGF was the first target-derived neurotrophic factor identified. It is fundamental for the development and maintenance of peripheral nervous system neurons and for the functional integrity of central nervous system cholinergic neurons (32). Moreover, NGF can affect balanced interplay between the nervous, endocrine and immune systems. It is well known that NGF concentrations increase during stress and play an important role in the hypothalamic-pituitary-adrenal axis (33).

Growing evidence suggests that different polymorphisms in the *NGF* gene can be considered risk factors for conditions such as vascular hypertension (34) and processes such as atherogenesis (35) and inflammation (36).

**Table 1.** Polymorphisms identified from the literature as potential modifiers of neurotrophin-mediated neuroplastic activity

| Gene | Protein | Class | RefSeq | Nucleotide change | Amino acid change | MAF (GnomAD) | Ref |
|---|---|---|---|---|---|---|---|
| *NGF* | Beta-nerve growth factor | NTs | rs6330 | c.104C>T | Ala35Val | A=0.37015 (91127/246188) | 39 |
| *BDNF* | Brain-derived neurotrophic factor | NTs | rs6265 | c.196G>A | Val66Met | T=0.19437 (47821/246030) | 40 |
| *NGFR* | Beta-nerve growth factor receptor | NTs | rs2072446 | c.614C>T | Ser205Leu | T=0.05165 (12700/245884) | 39 |
| *ADRB2* | Beta-2 adrenergic receptor | NTs | rs1042713 | c.46A>G | Gly16Arg | A=0.42081 (103492/245936) | 45 |
| *ADRB2* | Beta-2 adrenergic receptor | NTs | rs1042714 | c.79C>G | Gln27Glu | G=0.31729 (78116/246198) | 45 |
| *CNTF* | Ciliary neurotrophic factor | NTs | rs1800169 | c.115-6G>A | | A=0.14950 (35961/240538) | 46 |
| *MSTN* | Growth/ differentiation factor 8 | recovery | rs1805086 | c.458A>G | Lys153Arg | C=0.02732 (6704/245366) | 53 |
| *ACTN3* | Alpha-actinin-3 | recovery | rs1815739 | c.1729C>T | Arg577* | T=0.46060 (113241/245854) | 55 |
| *NTRK1* | High affinity nerve growth factor receptor | pain/ NTs | rs6334 | c.1656G>A | Gln552His | A=0.22025 (54107/245664) | 57 |
| *SCN9A* | Sodium channel protein type 9 subunit alpha | pain | rs6746030 | c.3448C>T | Arg1150Trp | A=0.12050 (18374/152480) | 60 |
| *COMT* | Catechol O-methyltransferase | pain | rs4680 | c.322G>A | Val158Met | A=0.46252 (112239/242666) | 66 |

Legend: NTs, neurotrophins; RefSeq, polymorhism accession number as recorded in the database of single nucleotide polymorphisms; MAF, minor alle frequency; Ref, reference.

A nucleotide change in *NGF*, c.104C>T, causes an amino acid change in position 35, p.Ala35Val. This amino acid change is referred to as rs6330 and the more common of the two alleles (C) encodes alanine (Ala). Several studies have indicated that this single nucleotide polymorphism (SNP) can lead to dysfunction in intracellular processing and secretion of NGF. The minor T allele seems to be associated with increased susceptibility to anxiety by association with low vagal activity, while the major C allele seems to be associated with attention deficit hyperactivity disorder (ADHD) (37, 38). More interestingly, rs6330 CC homozygotes show significantly higher NGF median plasma levels of NGF than carriers of the minor T allele (39). These findings suggest an association between certain *NGF* polymorphisms, rs6330 in particular, and response to chiropractic SMT and acupuncture.

The BDNF gene encodes a protein found in the brain and spinal cord. This protein promotes neuron survival by playing a role in the growth, differentiation, maturation and maintenance of these cells. BDNF protein is active in synapses. Synapses change and adapt in response to external factors. The BDNF protein helps regulate synaptic plasticity, which is important for learning and memory.

Several polymorphisms have been identified in *BDNF*. The non-synonymous polymorphism, known as SNP rs6265 or p.Val66Met or c.G>A196, is common and causes a valine (Val) to methionine (Met) substitution at position 66 of the pro-BDNF protein. The replacement of Val by Met impairs neuronal activity-dependent secretion of BDNF (40). Approximately 30-50% of the population is heterozygous or homozygous for this rs6265 polymorphism. The SNP occurs in the 5′ pro-domain of *BDNF* and does not affect mature BDNF protein function or BDNF constitutive release, but rather activity-dependent BDNF release, thus influencing intracellular trafficking of pro-BDNF (41).

Recent studies suggest a role of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and their receptor, nerve growth factor receptor (NGFR), in neuropsychiatric disorders, especially Alzheimer's disease (42). The rs2072446 polymorphism in *NGFR* has been associated with risk of Alzheimer's disease, but also as a functional SNP that could be involved in gene expression and protein secretion (39, 43).

The ADRB2 gene encodes beta2-adrenergic receptor and maps to the 5q32 chromosomal region. The association between polymorphisms in ADRB2 and risk of other diseases has also been studied (44). Moreover, rs1042713 (p.Arg16Gly) and rs1042714 (p.Gln27Glu), non-synonymous polymorphisms in *ADRB2*, have been associated with cognitive function and brain white matter integrity (45).

Ciliary neurotrophic factor (CNTF) is a pleiotropic cytokine of the interleukin-6 family whose myotrophic and neurotrophic effects have been extensively studied (46). Rs1800169 is the most widely studied *CNTF* polymorphism: this SNP leads to a G to A transition close to the boundary between the first intron and second exon. As a result of this change, four nucleotides are inserted in CNTF mRNA, leading to a frameshift mutation and a premature stop codon. By virtue of its functional effect, the rs1800169 polymorphism in *CNTF* could also be considered in relation to response to chiropractic treatment.

Polymorphisms in muscle-related genes can modify muscle healing after injury, so they could also be related to response to chiropractic treatment. Myostatin, encoded by *MSTN*, is a muscle inhibitor peptide that regulates myoblast differentiation with direct consequences on muscle mass and strength. *MSTN* knock-out mice show muscle hypertrophy and greater strength than wild-type mice (47). Null *MSTN* variants are known to express the same phenotype in humans (48) and other mammals (49, 50). Lack of the protein has also been associated with better muscle regeneration after injury (51, 52).

Among common polymorphisms, rs1805086 is receiving growing attention from researchers, and a number of association studies agree that it has functional significance (53, 54).

The ACTN3 gene, encoding the structural skeletal muscle protein α-actinin-3, locates on chromosome 11 and is one of the most interesting genes associated with athletic performance. Previous studies report that the ACTN3 Arg577* variant (rs1815739) seems to be associated with athletic performance in different races but the function of this SNP is unknown (55, 56). Since it is a null mutation and considering its evocative

role in muscle quality, the rs1815739 polymorphism is worth studying in relation to chiropractic response.

The NTRK1 gene, encoding the high-affinity NGF receptor TrkA, seems to be involved in gene expression and protein secretion. Szczepankiewicz and colleagues demonstrated that NGF serum levels may be influenced by the rs6334 polymorphism in NTRK1 and that the interaction between this variant and NGF indicates that this pathway may influence NGF protein levels (57). The polymorphism has also been associated with pain perception during acupunture (58). This involvement led us to consider the rs6334 polymorphism in NTRK1 as a variation that could be involved in chiropractic response.

Pain is a disturbing non-motor symptom in Parkinson disease (PD) and susceptibility to pain varies widely among these patients. *SCN9A* encodes the NaV1.7 sodium channel and is preferentially expressed in pain-signaling dorsal root ganglion (DRG) neurons (nociceptors) that play a critical role in amplifying small depolarizations, thus increasing the pain signaling gain (59).

Greenbaum and colleagues demonstrated that the non-synonymous rs6746030 polymorphism in *SCN9A* was associated with PD-related pain susceptibility and with PD central and musculoskeletal pain. Since the non-synonymous rs6746030 polymorphism has also proved experimentally to influence the excitability of nociceptive DRG neurons, thus influencing pain sensitivity and susceptibility to chronic pain (60). We focus on this SNP in relation to chiropractic response (61).

The COMT gene encodes catechol-O-methyltransferase, the enzyme mainly responsible for catecholamine metabolism and known as a major modulator of synaptic dopamine concentrations in the brain. COMT is involved in dopamine degradation at biochemical level and in complex cognitive functions such as cognitive control and working memory (61), and interestingly also pain modulation (62). Indeed, COMT is a key regulator in the pain perception pathway and polymorphisms in the gene have been studied in relation to variability in pain perception between individuals (63, 64).

Among the different polymorphisms identified in COMT, the rs4680 SNP seems to be involved in the increased synaptic dopamine concentrations in Met-allele carriers (65). The experimentally detected functional effect of rs4680 revealed a 40% decrease in COMT activity associated with the Met108 variant as a consequence of reduced COMT protein levels (66). The polymorphism has also been shown to influence the human experience of pain (67). All these findings make it a good candidate for an association study regarding chiropractic response in cases of chronic pain.

## Conclusions

As documented by the growing number of scientific papers concerning chiropractic therapy in PubMed over the last 20 years, complementary and alternative medicine and chiropractic in particular are ranking close to traditional medicine, especially but not only in the treatment of chronic pain. Different studies show utilization rates for chiropractic services of 6-12% (68-70). Interestingly, several surveys have found higher use of chiropractic services among middle-aged women (71, 72). As expected, higher chiropractic utilization is reported for patients with chronic pain and back pain (16.1% and 31.0%, respectively) (73, 74). Chiropractors provide a substantial portion of care for patients with various pathologies, including lumbar pain and neck pain (75). Today chiropractic care is available in over 100 countries, most of which have established national chiropractic associations. To the best of our knowledge, no studies regarding interaction between neurotrophin gene polymorphisms and chiropractic have been published; such studies could be interesting for understanding whether certain polymorphisms predispose for response to chiropractic, especially to acupuncture and SMT. Four genes in the family of neurotrophic factors, *BDNF, NGF, NTF3* and *NTF4*, together with other genes, could be considered for a study into the existence of a genetic association between polymorphisms in these genes and the amount of neurotrophic factors released by chiropractic techniques. Concerning the last two genes, we did not find convincing studies on functional polymorphisms in the literature and they were therefore not considered by this review.

Today it is well known that neuroplasticity plays an important role in neurological rehabilitation. SNPs with

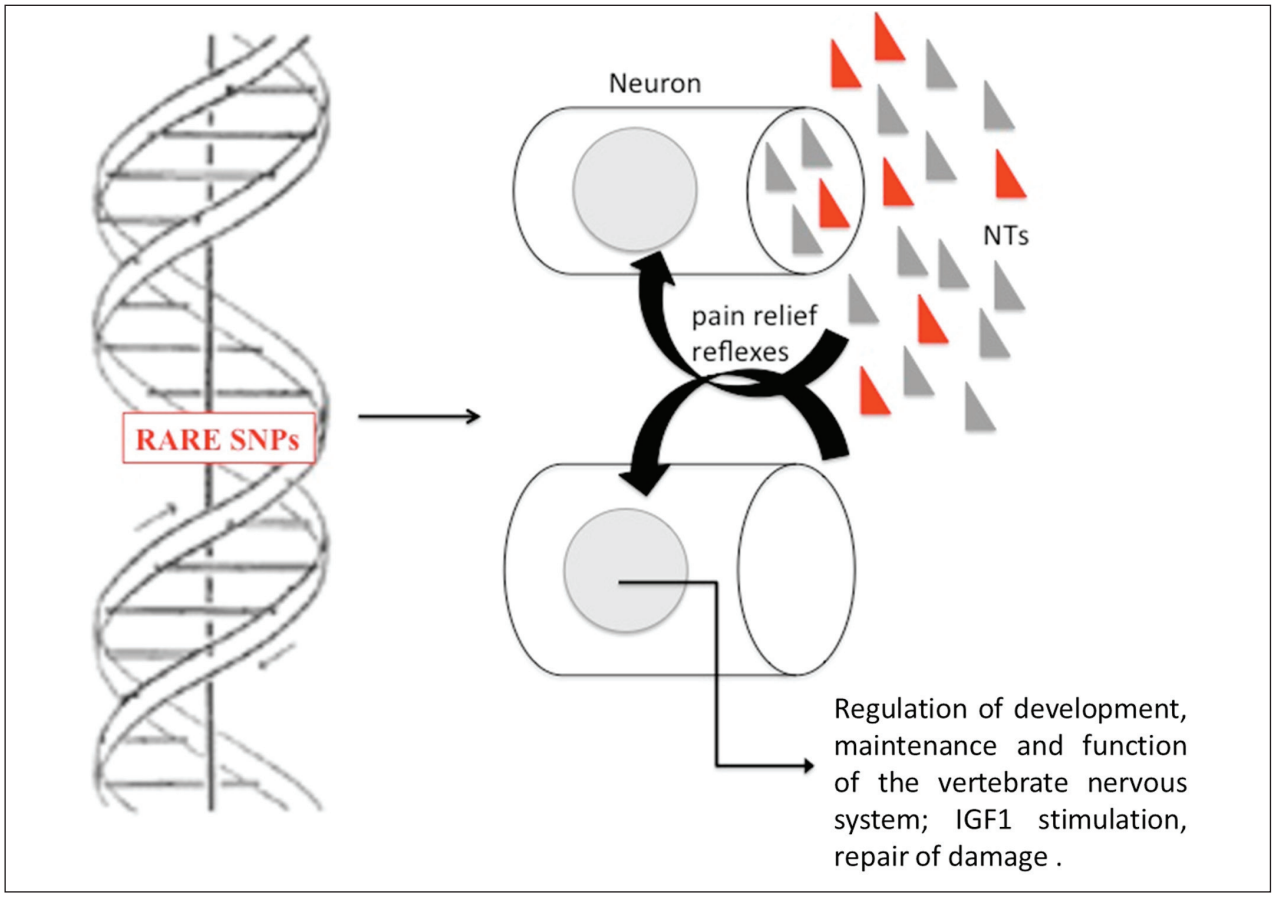


**Figure 1.** Presence of specific SNPs in genes related to brain function could increase production of neurotrophins (NTs) by neurons and consequently their neuroplasticity, leading to a better response to chiropractic therapy through regulation of development, maintenance and function of the vertebrate nervous system. These specific SNPs could also increase IGF-1 blood levels after stimulation by massage

a demonstrated effect on neuroplasticity may therefore be relevant for neurological rehabilitation (Figure 1). Different authors suggest that individuals with genetic variants associated with neuroplasticity may respond better to therapies involving neuroplastic processes than individuals without such variants (70-72).

**Conflict of interest:** Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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