

Genetic bases of arrhythmogenic right ventricular cardiomyopathy

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ABSTRACT: *Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a heart muscle disease in which the pathological substrate is a fibro-fatty replacement of the right ventricular myocardium. The major clinical features are different types of arrhythmias with a left branch block pattern. ARVC shows autosomal dominant inheritance with incomplete penetrance. Recessive forms were also described, although in association with skin disorders.*

Ten genetic loci have been discovered so far and mutations were reported in five different genes. ARVD1 was associated with regulatory mutations of transforming growth factor beta-3 (TGFβ3), whereas ARVD2, characterized by effort-induced polymorphic arrhythmias, was associated with mutations in cardiac ryanodine receptor-2 (RYR2). All other mutations identified to date have been detected in genes encoding desmosomal proteins: plakoglobin (JUP) which causes Naxos disease (a recessive form of ARVC associated with palmoplantar keratosis and woolly hair); desmoplakin (DSP) which causes the autosomal dominant ARVD8 and plakophilin-2 (PKP2) involved in ARVD9. Desmosomes are important cell-to-cell adhesion junctions predominantly found in epidermis and heart; they are believed to couple cytoskeletal elements to plasma membrane in cell-to-cell or cell-to-substrate adhesions. (Heart International 2006; 2: 17-26)

KEY WORDS: *Arrhythmias, Sudden death, Molecular genetics, Desmosomes*

INTRODUCTION

Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) is a myocardial disease in which myocardium of the right ventricular free wall is partially or almost entirely replaced by fatty or fibro-fatty tissue (1-3). The process begins at epicardium and it spreads towards the endocardium. The anterior right ventricular outflow tract, the apex, and the inferior-posterior wall—the so-called “triangle of dysplasia”—are primarily involved (4). The degenerative process may be segmental or diffuse; on post mortem examination degeneration results to affect the left ventricle in about 50% of cases and to involve the septum only in 20% (3). Clinical manifestations of the disease mostly occur between the second and fourth decade of life; they include structural and functional abnormalities of the right ventricle,

electrocardiographic depolarization/repolarization changes and arrhythmias of right ventricular origin (5). Ventricular tachycardias are thought to be due to re-entry between the abnormal and normal areas of the right ventricular myocardium.

ARVC is a progressive heart muscle disease with different clinico-pathological patterns: a) “silent” cardiomyopathic abnormalities localized to the right ventricle in asymptomatic victims of sudden death; b) “overt” disease characterized by segmental or global right ventricular structural changes, often associated with histological evidence of left ventricular involvement and underlying symptomatic ventricular arrhythmias; c) “end-stage” biventricular cardiomyopathy mimicking dilated cardiomyopathy, which leads to progressive heart failure and may require heart transplantation (6).

A scoring system to establish diagnosis of ARVC has

been developed on the basis of the presence of major and minor criteria encompassing structural, histological, electrocardiographic, arrhythmic and genetic features of the disease (7). Drug treatment is warranted in patients with palpitations, syncope or documented sustained ventricular arrhythmias. Anti-arrhythmic drugs are used to suppress atrial and ventricular arrhythmias. Clinical investigation aims at identifying subjects at risk of complications in order to provide them proper treatment.

Prevalence and incidence of ARVC are still ill-established. Ten years ago, we estimated that prevalence rate of ARVC in the Veneto region of Italy might be about 1:1500 (8); however prevalence could be higher, because of many non-diagnosed or misdiagnosed cases. It was reported that in Italy, between 12.5 and 25% of sudden death events in young non-athletes and young athletes under the age of 35 years were caused by undiagnosed ARVC (9).

Two international registries, in Europe and USA, are presently on the way, aiming at determining clinical, pathological and genetic features of ARVC, at validating diagnostic criteria and at defining strategies for disease management and sudden death prevention (10-12).

Familial occurrence is common, and in ~50% of cases evidence has been found for autosomal dominant inheritance with variable penetrance (13). Recessive forms are also described, although in association with cutaneous disorders (14). Since the identification of the first ARVC locus in 1994 (8), ten loci were detected, but only five disease genes have been identified so far (15-19).

Recessive forms

The first ARVC gene was identified in a rare syndrome, characterized by arrhythmogenic right ventricular cardiomyopathy associated with palmoplantar keratoderma and peculiar woolly hairs, inherited as autosomal recessive trait.

This syndrome was called "Naxos syndrome", because of a peculiar concentration of cases in the Greek island of Naxos. Clinical features of the disease were first described by Protonotarios in 1986 (14); ten years later, the syndrome was recognized as a distinct human disease and as a variant of ARVD (OMIM 601214).

The pattern of disease stereotyped in all cases. At birth woolly hair is present. At that time or shortly after, palmar erythema occurs, and when the infants start to use their hands keratoderma develops. Later, when they start to ambulate, plantar erythema occurs, followed by keratoderma and producing the typical pattern.

Cardiac abnormality presents later. All patients present electrocardiographic and/ or echocardiographic abnormalities fulfilling criteria of ARVC. The symptomatic presentation is usually with syncope and/or sustained ventricular tachycardia during adolescence with a peak in young adulthood (20).

The presence of a visible defect in epidermal cells suggested that cardiac defect could be closely linked to a locus to which keratin genes were mapped. Indeed, DNA markers of the chromosomal region 17 did show positive linkage with the disease (21). Shortly later, mutation screening of plakoglobin gene, closely linked to keratin genes, succeeded in detecting in Naxos patients a two-nucleotide deletion (Pk2157del2TG) producing a frameshift which alters five amino acids and produces a stop codon (15). A genotype-phenotype correlation study, performed on 26 subjects, showed that the recessive trait is fully penetrant by adolescence (20). A minority of heterozygous carriers presented few phenotypic features consisting of woolly hair and minor electrocardiographic/echocardiographic changes, not fulfilling the criteria for ARVD.

Plakoglobin is a key component of desmosomes and adherens junctions. It is involved in tight adhesions of many cell types, cardiomyocytes included and is thought to serve as a linker molecule between the inner and outer portions of the desmosomal plaque by binding tightly to the cytoplasmic domain of cadherins. The adhesive function of plakoglobin is mediated through its interaction with other desmosomal and cytosolic proteins; highly conserved armadillo repeats, which form the central domain of plakoglobin, facilitate the association between plakoglobin and these molecules (22, 23). Plakoglobin has also been shown to directly signal structural changes in adherens junctions to the nucleus (24, 25). Normal functioning of cell-cell junctions is of utmost importance in myocardium and skin particularly of palms and soles, tissues that experience constant mechanical stress. Intercellular junctions not only maintain tissue integrity but also integrate mechanical and signaling pathways. Defects in linking

sites of plakoglobin might disturb the contiguous cell-cell adhesion and tissue integrity in skin and heart particularly when increased mechanical stress is applied on the tissue. This mechanism has been fostered in plakoglobin-null mice myocardium presenting decreased compliance and ventricular rupture under increased mechanical stress (26).

More recently, Alcalai et al identified a missense mutation in the C-terminal of desmoplakin in one Arabic family with recessive ARVC, woolly hair and a pemphigous-like skin disorders (27). Desmoplakin, together with plakoglobin, is a constituent of the desmosomal plaque, anchoring intermediate filaments to the plasma membrane and forming a scaffold that is essential for maintaining tissue integrity.

Interestingly, in two additional Arabic families, clinically affected with Naxos disease, mutations in genes encoding plakoglobin, desmoplakin, plakophilin 1, 2, and 4, types I and II keratin, desmoyokin and desmocollins/desmogleins have been excluded, thus suggesting additional genetic heterogeneity in this cardiocutaneous syndrome (28).

Dominant forms

The first ARVC gene identified in a dominant form was cardiac ryanodine receptor involved in ARVD2 (16). ARVD2 shows fibro-fatty substitution of the myocardial tissue, though much less pronounced than in the typical ARVCs. However, the distinctive feature of this form is the presence of polymorphic, effort-induced arrhythmias.

After restriction of the critical region on chromosome 1q42-q43 and the unsuccessful mutation screening of different genes mapped to the critical interval, causative mutations were finally detected in the gene encoding the monomer of the cardiac ryanodine receptor (RyR2) (16). hRyR2 is one of the largest human genes (105 exons); it encodes a 565Kda protein. The homo-tetrameric structure known as cardiac ryanodine receptor plays a pivotal role in intracellular calcium homeostasis and excitation-contraction coupling in cardiomyocytes.

Stimulation of voltage-sensitive L-type calcium channels (dihydropyridine receptors) on the outer myocardial cell membrane allows the entry of small

amounts of calcium ions, which in turn activate release of larger amounts of calcium from the sarcoplasmic reticulum lumen into the cytoplasm via RyR2. This is sufficient to initiate myocardial contraction. Thus, RYR2 channels serve to couple the excitation of myocardial cells to their actin/myosin contractile apparatus by a mechanism involving a calcium-induced calcium release (29, 30).

All RYR2 missense mutations detected in ARVD2 patients resulted in substitution involving amino acids highly conserved through evolution in domains of the protein which are critical for the regulation of the calcium channel (31).

Up to present, mutations in the human RYR2 gene have been associated with three inherited cardiac diseases: arrhythmogenic right ventricular cardiomyopathy type 2 (ARVD2; OMIM 600996) (16, 32), catecholaminergic polymorphic ventricular tachycardia (CPVT; OMIM 604772) (33, 34) and familial polymorphic ventricular tachycardia (FPVT; OMIM 604772) (35, 36). All these diseases are characterized by effort-induced polymorphic ventricular arrhythmias and a risk of sudden death. Moreover, putative pathogenic mutations in RYR2 have been reported in 20 of 240 patients with long-QT syndrome (37). All RYR2 mutations described to date show clustering in three specific domains: the N-terminal amino-acid residues 176-433, the centrally located residues 2246-2504, and the C-terminal residues 3778-4959.

The finding of RYR2 mutations in both ARVD2 and CPVT patients raised the question of the possible existence of a single genetic defect whose different phenotypes might be simply due to variable expression and incomplete penetrance. Interestingly, a 17 year-old boy, who died suddenly and at postmortem showed fibrofatty replacement in the right ventricular free wall consistent with ARVC, resulted as carrying a missense RYR2 mutation (A77V) (38). His mother and sister, carrying the same RYR2 mutation, showed effort-induced polymorphic ventricular tachycardia in the setting of normal echocardiogram consistent with CPVT. The observation of both ARVD2 and CPVT in the same family suggests that the two entities might correspond to different degrees of phenotypic expression of the same disease.

Both the ARVD2- and CPVT-RyR2 missense mutations (16, 33) would alter the ability of the calcium channel to remain closed and thus, intense adrenergic stim-

ulation due to emotional or physical stress would lead to calcium overload, thus triggering severe arrhythmias.

Leaky RyR channels might release SR Ca^{2+} during the resting phase of the cardiac cycle (diastole), resulting in membrane depolarizations that occur after repolarization of the previous action potential (delayed afterdepolarizations, DADs) (39). In support of this hypothesis it has recently been reported that DADs are known to trigger premature ventricular contractions which can initiate fatal cardiac arrhythmias (40).

Beta-blockers therapy proved to be effective in preventing fatal arrhythmias in several ARVD2 and CPVT patients (16,33). This may be explained by the fact that the rapid enhancement of cardiac output during exercise or sudden stress is based on the β -AR cascade (41). Therefore, blockage of β -AR signaling would result in improved cardiac contractility, as shown in human heart failure (42).

Several functional studies on RyR2 mutations have been reported so far. The common finding, both in CPVT- and ARVD2-associated mutations (43-46) is an increased Ca^{2+} release after stimulation.

Functional role of mutations R176Q, L433P, N2386I and T2504M, previously detected in ARVD2 patients and reported by Tiso et al 2001, was recently investigated (46). RyR2 mutants N2386I and R176Q/T2504M exhibited enhanced sensitivity to caffeine activation and increased Ca^{2+} release, in agreement with the current hypothesis that defective RyR2 cause Ca^{2+} leak. On the contrary, RyR2 L433P mutation showed reduced response to caffeine activation. This mutation might be interpreted as a "loss-of-function". Therefore, RyR2 mutations might be either "gain-of-function" or "loss-of-function", thus suggesting heterogeneity in functional consequences of RyR2 mutations.

The first disease gene linked to autosomal dominant ARVC showing the typical right ventricular phenotype was desmoplakin (17). DNA sequencing of all DSP exons in the index case of the investigated family revealed a missense mutation in exon 7 (C1176G; AGC→AGG). The residue Ser299Arg mutated in patients is at the center of a coiled, charged region, separating the two short helices of DSP sub-domain Z. It suppresses a putative phosphorylation site which is fully conserved in related proteins belonging to the same family, thus supporting the hypothesis that the amino acid change might cause a functional alteration. This mutation is

thought to disrupt a protein kinase C (PKC) phosphorylation site which is involved in plakoglobin binding and in clustering of desmosomal cadherin-plakoglobin complexes.

Desmoplakin, together with plakoglobin, anchors to desmosomal cadherins, forming an ordered array of non-transmembrane proteins, which then bind to keratin intermediate filaments (IF) (47). The primary structure of desmoplakin contains three functional domains: the N-terminal, which binds to the desmosome via connection with plakoglobin and plakophilin; the rod segment, which is predicted to form a dimeric coil; and the C-terminal domain, which binds IF's (48). Alternative splicing of the protein produces two isoforms, desmoplakin I and desmoplakin II. The cDNAs encoding these two highly related proteins differ in a 1.8 Kbase sequence that is missing in DSPII, most likely due to differential splicing of a longer transcript (49).

Actually, mutations in desmoplakin gene have been shown to underlie some cases of an autosomal dominant skin disorder (striate palmoplantar keratoderma) without cardiac involvement (50-52), a dominant form of ARVC without skin disease, an autosomal recessive condition characterized by dilated cardiomyopathy, woolly hair and keratoderma, named "Carvajal syndrome" (53), an autosomal recessive condition characterized by ARVC, woolly hair and keratoderma (27) and a left-sided ARVC named arrhythmogenic left ventricular cardiomyopathy (ALVC) (54) (Tab. I).

In 2004, Gerull et al directly sequenced all 14 exons of the PKP2 gene, including flanking intronic splice sequences. These authors identified 25 different heterozygous mutations (12 insertion-deletion, 6 nonsense, 4 missense and 3 splice site mutations) in 32 over 120 unrelated ARVC probands (18). PKP2 gene encodes plakophilin-2, an essential protein of the cardiac desmosome.

Plakophilin-2 gene was selected as candidate gene on the basis of the finding of a lethal defect in cardiac morphogenesis at embryonic day 10.75 in mice homozygous with respect to a deletion mutation of *Pkp2*. The resulting mutant mice exhibit lethal alterations in heart morphogenesis characterized by reduced trabeculation, disarrayed cytoskeleton, ruptures of cardiac walls, and blood leakage into the pericardiac cavity. In the absence of plakophilin-2, the cytoskeletal linker protein desmoplakin dissociates from the plaques of

TABLE I - KNOWN DESMOPLAKIN MUTATIONS AND ASSOCIATED DISEASE

| Disease | Inheritance | Nucleotide Change(s) | Amino acid Change(s) | References |
|---|-------------------------|----------------------|--|----------------------|
| Striate subtype of palmoplantar keratoderma | Dominant | C1323T | Q331X | Amstrong et al, 1999 |
| Striate palmoplantar keratoderma | Dominant | 939+1G>A | Mutant splice product | Whitlock et al, 1999 |
| Dilated cardiomyopathy, woolly hair and keratoderma | Recessive | 7901delG | Frameshift leading to a premature stop codon and truncated protein | Norgett et al, 2000 |
| Skin fragility and woolly hair syndrome | Compound heterozygosity | 861T>G and 2427T>A | N287K and C809X | Whitlock et al, 2002 |
| Skin fragility and woolly hair syndrome | Compound heterozygosity | 1990C>T and 7096C>T | Q664X and R2366G | Whitlock et al, 2002 |
| ARVC | Dominant | C1176G | S299R | Rampazzo et al, 2002 |
| ARVC, skin disorder and woolly hair | Recessive | G7402C | G2375R | Alcalai et al, 2003 |
| Arrhythmogenic left ventricular cardiomyopathy (ALVC) | Dominant | 2034insA | Frameshift leading to a premature stop codon and truncated protein | Normann et al, 2005 |

TABLE II - KNOWN ARVD GENES AND CAUSATIVE MUTATIONS IDENTIFIED SO FAR

| ARVD | Gene | Mutations | References |
|-------|-------|---|--|
| ARVD1 | TGFβ3 | 2 regulatory mutations (in 5' e 3' UTRs) among 33 probands | Beffagna et al, 2005 |
| ARVD2 | RYR2 | 6 mutations (missense) in affected subjects belonging to 6 families | Tiso et al, 2001; Bauce et al, 2002; d'Amati et al, 2005 |
| ARVD8 | DSP | 4 mutations (3 missense and 1 splice site) in affected subjects belonging to 4 large families | Rampazzo et al, 2002; Bauce et al, 2005 |
| ARVD9 | PKP2 | 25 mutations (missense, nonsense, insertion/deletion and splicing site) in 32 unrelated patients among 120 probands | Gerull et al, 2004 |
| NAXOS | JUP | 1 mutation (2bp-deletion) in affected patients belonging to a large family | McKoy et al, 2000 |

the adhering junctions that connect the cardiomyocytes and forms granular aggregates in the cytoplasm (55).

Plakophilin-2 is an armadillo-related protein, located in the outer dense plaque of desmosomes. It links desmosomal cadherins to desmoplakin and the intermediate filament system. Plakophilins are also present in the nucleus, where they may play a role in transcriptional regulation (56). Gerull et al speculated that lack of plakophilin-2 or incorporation of mutant plakophilin-2 in the cardiac desmosomes might impair cell-cell con-

tacts and, as a consequence, might disrupt association between adjacent cardiomyocytes, particularly in response to mechanical stress or stretch (thus providing a potential explanation for the high prevalence of the disorder in athletes, the frequent occurrence of ventricular tachyarrhythmias and sudden death during exercise, and the predominant affection of the right ventricle). The potential cellular mechanism for the initiation of ventricular tachyarrhythmias in ARVC could be the intrinsic variation in conduction properties as a re-

sult of the patchy areas of fibro-fatty cardiomyocyte degeneration.

Desmosomes are important cell-cell adhesion junctions which are predominantly found in the epidermis and heart and which couple cytoskeletal elements to the plasma membrane at cell-cell or cell-substrate adhesions. Whereas adherens junctions associate with microfilaments at cell-cell interfaces, desmosomes are tailored to anchor stress-bearing intermediate filaments at sites of strong intercellular adhesion. The resulting scaffold plays a key role in providing mechanical integrity to tissues such as epidermis and heart which experience mechanical stress. Desmosomes include proteins from at least three distinct gene families: cadherins, armadillo proteins and plakins. Desmosomal cadherins include desmogleins and desmocollins; members of both subfamilies are single-pass transmembrane glycoproteins, mediating Ca²⁺-dependent cell-cell adhesion. Armadillo proteins include plakoglobin and plakophilins (PKP1-3). The plakin family proteins include desmoplakin, plectin and the cell envelope proteins envoplakin and periplakin. Since the desmoplakin involved in ARVD8, plakophilin involved in ARVD9 and plakoglobin involved in Naxos syndrome encodes desmosomal proteins, different defects in proteins of desmosomal complex lead to ARVC. Thus, additional components of the desmosomal complex become good candidate genes for ARVC.

More recently, our group succeeded in identifying the gene involved in ARVD1. In one family fulfilling diagnostic criteria for ARVC, significant linkage to ARVD1 was detected, thus confirming the early locus assignment (57). The critical interval for ARVD1, still very large, contains 40 known genes; five of them (POMT2, KIAA0759, KIAA1036, C14orf4 and TAIL1) were unsuccessfully screened for pathogenic ARVC mutations (57, 58). Among genes mapped to ARVD1 critical region and expressed in myocardium, transforming growth factor-beta3 (TGFβ3) appeared a very good candidate, as it encodes a cytokine stimulating fibrosis and modulating cell adhesion (59). After previous analyses failed to detect any mutation in the coding region of this gene, mutation screening was extended to the promoter and untranslated regions (UTRs).

A nucleotide substitution (c.-36G>A) in 5'UTR of TGFβ3 gene was detected in all affected subjects belonging to a large ARVD1 family. After the investigation was extended to 30 unrelated ARVC index patients, an additional mutation (c.1723C>T) was identified in the 3' UTR of one proband. *In vitro* expression assays of constructs containing the mutations showed that mutated UTRs were twofold more active than wild-types (19).

TGF-β3 is a member of the transforming growth factor family. The TGF-β superfamily consist of a diverse range of proteins that regulate many different physiological processes, including embryonic development, homeostasis, wound healing, chemotaxis, and cell cycle control. Cytokines of the TGF-β superfamily are dimeric proteins with conserved structures and have pleiotropic functions *in vitro* and *in vivo* (60).

TGF-β1, -β2, -β3 are the prototypes of the TGF-β superfamily; they inhibit proliferation in most types of cells and induce the apoptosis of epithelial cells. Conversely, TGF-β1, -β2, -β3 stimulate mesenchymal cells to proliferate and produce extracellular matrix and induce a fibrotic response in various tissue *in vivo*. Thus, the TGF-β superfamily regulates wide-ranging and diverse roles in development, differentiation, and homeostasis (60).

Finding TGFβ3 mutations associated with ARVC is very interesting, as it is well established that TGFβs stimulate mesenchymal cells to proliferate and to produce extracellular matrix components. It may be hypothesized that the reported mutations in UTRs of TGFβ3 gene, which *in vitro* enhance expression, could promote myocardial fibrosis. Extensive myocardial fibrosis may disrupt electrical and mechanical behavior of the myocardium and extracellular matrix abnormalities may predispose to re-entrant ventricular arrhythmias (61). In fact, endomyocardial biopsy in the two probands shows extensive replacement-type fibrosis, in agreement with this hypothesis.

It has been shown that TGFβs modulate expression of genes encoding desmosomal proteins in different cell types. cDNA microarray analysis performed on RNA from cardiac fibroblasts incubated in the presence or in the absence of exogenous TGFβs revealed increased expression of different genes, in-

cluding plakoglobin (62). Yoshida et al reported that TGF β 1 exposure of cultured airway epithelial cells increases the content of desmoplakins I and II; this suggests that regulation of cell-cell junctional complexes may be an important effect due to TGF β s (63). Therefore, overexpression of TGF β 3, caused by UTRs mutations, might affect as well cell-to-cell junction stability, thus leading to a final outcome similar to that observed in ARVD8 (17) and in the ARVC-related Naxos syndrome (15). This would explain as well the preferential affection of the right ventricle.

Known ARVD genes and causative mutations identified so far are reported in Table II.

CONCLUSION

The reported involvement of different desmosomal proteins in ARVCs and the discovery that some RYR2 mutations may produce ARVD2, lead us to put forward a novel hypothesis on molecular pathogenesis of ARVCs (17). According to such hypothesis, the almost selective affection of the right ventricle in ARVCs might be in relation to higher extensibility of its wall, in comparison with the free wall of left ventricle.

Possibly, defective proteins in cardiac desmosomes might impair cell-to-cell contacts and, hence, might affect the response of ventricular myocardium to mechanical stretch. This alteration would occur preferably in areas subjected to high strain. These localizations, including the right ventricular outflow tract, the apex and sub-tricuspid areas, are known as "the triangle of dysplasia".

According to present knowledge, mechanical forces applied to adherens junctions activate stretch-sensitive calcium channels via cadherins' mechanical intracellular signaling (64). Data on stretch-activated channels in ventricular cardiomyocytes (65-67) point to the relevance of such channels in transduction of mechanical forces into a cellular electrochemical signal via increase in intracellular calcium concentration.

Volume overload in the right ventricle of a person carrying genetically defective intercellular junctions (as in case of mutant plakoglobin, desmoplakin or

plakophilin) would produce over-stretch and, hence, excessive calcium load. Stretching of cardiomyocytes is known to modulate the elementary calcium release process from ryanodine receptor release channels (68); therefore, a genetically impaired response to mechanical stress might adversely affect intracellular calcium concentration and the excitation-contraction coupling, producing arrhythmias. On the other hand, volume overload of the right ventricle in carriers of mutations in RYR2 (cardiac ryanodine receptor) would cause calcium overload, because of the defective Ca $^{++}$ homeostasis. The existence of a dominant form of ARVC (ARVD2) due to RyR2 mutations supports the hypothesis of a key pathogenic role of intracellular calcium overload in molecular pathogenesis of ARVDs.

The ongoing progresses of molecular genetics are leading to new concepts about the pathogenesis and diagnosis of arrhythmic heart diseases. At the same time, genetic studies on patients with inherited diseases are accompanied by the onset of several medical and ethical problems. An immediate clinical benefit from the discovery of human gene mutations in affected subjects is early and accurate diagnosis. However, in some cases, a pathological mutation can exist in the setting of healthy or borderline phenotype. The detection in apparently healthy subjects of a human gene mutation raises complex clinical management issues.

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