Unraveling Missing Genes and Missing Inheritance in Arrhythmogenic Cardiomyopathy

See Article by Pilichou et al

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Arrhythmogenic cardiomyopathy (AC) is an enigmatic inherited cardiomyopathy characterized by fibro-fatty replacement of cardiac myocytes and ventricular arrhythmias.[1–3] When affecting predominantly the right ventricle, this disease is referred as arrhythmogenic right ventricular cardiomyopathy. The prevalence of AC in the general population has been estimated in a range from 1 in 2000 to 1 in 5000 individuals. Clinically, it is characterized by palpitations, syncope, and sudden cardiac death because of ventricular arrhythmias. This cardiomyopathy is a major cause of sudden cardiac death in the young people and in athletes.[1–3] To date, there is no effective pharmacological therapy for this deadly cardiac disease, which may require implantable defibrillators and heart transplantation at the advanced stage.[4]

AC is a hereditary autosomal dominant disease with reduced penetrance and variable clinical expression. Thus far, 5 causal genes have been identified, all coding for desmosomal proteins.[5] These known causal desmosomal genes are plakoglobin (*JUP*), desmoplakin (*DSP*), plakophilin-2 (*PKP2*), desmoglein-2 (*DSG2*), and desmocollin-2 (*DSC2*). Desmosomal proteins are structural proteins important for cell–cell attachment and communication. *PKP2* is the most common causal gene for AC, accounting for up to 43% of the cases.[6–8] Most of *PKP2* mutations are loss-of-function mutations resulting in reduced expression of PKP2 (haploinsufficiency[6,9]). Atypical forms of AC are caused by mutations in nondesmosomal genes, such as transforming growth factor-β-3 (*TGFβ3*), cardiac ryanodine receptor (*RYR2*), transmembrane protein 43 (*TMEM43*), and lamin A/C (*LMNA*). Current diagnostic strategies rely on next-generation DNA sequencing that has poor sensitivity for genomic deletions and identifies causative variants in only ≈50% of the AC cases, leaving half of cases with unknown genetic cause. To address the possibility of genomic mutations, Pilichou et al[10] turned to multiplex ligation-dependent probe amplification to quantify genomic deletions in 160 patients with AC who had negative next-generation DNA sequencing testing for the expected AC genes.

The human genome consists of >3 billion base pairs of DNA packaged into 2 sets of 23 chromosomes of which one set of chromosomes is inherited from each parent. These chromosomes contain DNA sequences encoding for ≈30 000 genes. In general, each gene is presumed to be present in 2 copies in a genome. However, recent studies have revealed different copy number variants (CNVs) of large DNA segments ranging in size from kilobases to megabases as the source of genetic diversity in the general population. Since then, several research groups have identified CNVs of large segments of chromosomes that encompass genes and that are associated with diseases.[11–14] CNVs can cause diseases through different mechanisms: variation of gene dosage through insertions or deletions (1), unmasking of a recessive allele alters the gene expression through inversions, deletions, or trans-

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locations (2), gene expression modifications through interaction with regulatory elements (3), or combination of ≥2 CNVs can produce a complex disease, whereas individually the changes produce no effect (4) which can explain missing inheritance which sometimes occurs.[15] The proband can inherit the disease-associated CNVs from an unaffected parent, which highlights the incomplete penetrance of the disease resulting from change in gene dosage. Noteworthy, in this issue, Pilichou et al[10] also showed that several family members carrying a complete *PKP2* deletion CNV remained asymptomatic during a mean 6±5 years of follow-up period, further supporting the gene dosage theory.

In their study, Pilichou et al[10] identified 9 heterozygous CNVs in 11 of the 160 AC probands of Italian descents (7% of cases) by using multiplex ligation-dependent probe amplification.[10] These patients were negative for pathogenic mutations in 5 known AC desmosomal genes screened by conventional genetic testing approaches. By using the multiplex ligation-dependent probe amplification technology, the authors screened these patients for the presence of CNVs in *PKP2*, *DSP*, *JUP*, *DSC2*, *DSG2*, *TGFβ3*, and *RYR2* and reported for the first time the identification of heterozygous partial deletion/duplications of *DSG2* and *DSC2* genes in clinically affected patients with AC. Although the authors identified for the first time a de novo duplication of *DSC2* exons 7 to 9 in a patient with definite arrhythmogenic right ventricular cardiomyopathy, which did not segregate with the disease, it is unknown whether this intragenic CNV caused a gain of function or a loss-of-function because of disruption of the reading frame. In addition, the authors also identified partial and complete deletions of *PKP2* gene in 9 of 160 probands in agreement with the fact that the majority of next-generation DNA sequencing sequence mutations cluster in *PKP2*. Among the 9 probands, 5 probands were carriers of a complete CNV deletion of the *PKP2* gene and possibly shared a founder variant as suggested by haplotype analysis. Together, these findings implicate the involvement of rare AC genomic rearrangements in a subset of patients with AC, with a mechanism of loss-of-function, as found in the majority of mutations identified by next-generation DNA sequencing.

Interestingly, the genetic analysis of Pilichou et al[10] revealed the possible involvement of digenic heterozygosity in 2 probands (H-III;1 and K-III,1). One proband was a digenic heterozygous carrier of a point mutation c.2491C>T (p.Leu831Phe) in *DSG2* and a CNV that resulted in deletion of exons 6 to 11 in *PKP2*. The c.2491C>T (p.Leu831Phe) point mutation in *DSG2* is a reported pathogenic variant that affects a nonconserved amino acid. Another proband from family K was a heterozygous carrier of c.536A>G (p.Asp179Gly) in *DSC2*, a recessive point mutation, and a deletion of 482 kb on chromosome 18q that encompassed both *DSG2* and *DSC2*. Both pathogenic point mutations were reported to derive from the unaffected father's allele. These findings raise the notion that, in some AC cases, the additive effects from 2 pathogenic variants should be considered in determining the association with the phenotype and severity of the disease. Single nucleotide polymorphisms and CNVs may act in concert on different cellular molecular mechanisms contributing to the development of the phenotype. Hence, integrative analysis by combining both single nucleotide polymorphisms and CNVs data would uncover the underlying phenotype traits of a disease.

Cascade genetic screening of the family members revealed low disease penetrance of 32% in relatives carrying heterozygous CNVs of *DSG2*, *DSC2*, and *PKP2*, implicating that other genetic and nongenetic factors could be involved in the pathogenesis of the disease. The identification of complete deletion of *PKP2* genes from affected probands in this study and by other research groups supports the hypothesis that *PKP2* haploinsufficiency is not the sole cause of the pathogenesis of AC. The authors found that several family members carrying the complete *PKP2* deletion CNV were phenotypically negative. Although, in this study, the authors have isolated mRNA from patient blood cells and performed reverse transcription on selected fragment to confirm the deletion of 44 bp in *PKP2* exon 4, allelic asymmetries are tissue specific and the presence of polymorphism in *cis*-acting elements DNA sequence and its transcription regulatory factors could contribute to the differences in transcription levels in different cell types.[16]

In summary, the identification of rare CNVs by Pilichou et al[10] highlights that CNVs causing large genomics rearrangements may contribute to the heterogeneous clinical phenotypes in the development and progression of AC. The current study expands the notion that routine clinical genetic testing should be expanded to include CNVs identification using multiplex ligation-dependent probe amplification approach in mutation screening for patients with AC because it provides a high-throughput and cost-effective tool, suitable for clinical laboratories. Although Pilichou et al[10] provided suggestive evidence of CNVs in the pathogenesis of AC, further study on the identified CNVs functionality is required to establish causality. It is clear that there is a need to bridge the gap between CNVs identified in patients with AC and their effects on gene expression levels, either directly on the affected gene or indirectly through structural alternations effects on nearby genes; thus disease models (either human cell lines or mouse models) are necessary to characterize the molecular pathways affected by the identified CNVs to provide a correct diagnosis and potentially identify therapeutic targets to treat patients with AC. Although it is beyond

the scope of the current study, Pilichou et al[10] and other research groups have raised the question of what are the genetic factors contributing to the structural rearrangement of chromosome nearby desmosomal gene loci, which still remains unknown.

AFFILIATIONS

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DISCLOSURES

None.

FOOTNOTES

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REFERENCES

1. Basso C, Corrado D, Marcus FI, Nava A, Thiene G. Arrhythmogenic right ventricular cardiomyopathy. *Lancet*. 2009;373:1289–1300. doi: 10.1016/S0140-6736(09)60256-7.
2. Marcus FI, McKenna WJ, Sherrill D, Basso C, Bauce B, Bluemke DA, Calkins H, Corrado D, Cox MG, Daubert JP, Fontaine G, Gear K, Hauer R, Nava A, Picard MH, Protonotarios N, Saffitz JE, Sanborn DM, Steinberg JS, Tandri H, Thiene G, Towbin JA, Tsatsopoulou A, Wichter T, Zareba W. Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia: proposed modification of the Task Force Criteria. *Eur Heart J*. 2010;31:806–814. doi: 10.1093/eurheartj/ehq025.
3. Thiene G. The research venture in arrhythmogenic right ventricular cardiomyopathy: a paradigm of translational medicine. *Eur Heart J*. 2015;36:837–846. doi: 10.1093/eurheartj/ehu493.
4. Marcus FI, Abidov A. Arrhythmogenic right ventricular cardiomyopathy 2012: diagnostic challenges and treatment. *J Cardiovasc Electrophysiol*. 2012;23:1149–1153. doi: 10.1111/j.1540-8167.2012.02412.x.
5. Li Mura IE, Bauce B, Nava A, Fanciulli M, Vazza G, Mazzotti E, Rigato I, De Bortoli M, Beffagna G, Lorenzon A, Calore M, Dazzo E, Nobile C, Mostacciuolo ML, Corrado D, Basso C, Daliento L, Thiene G, Rampazzo A. Identification of a PKP2 gene deletion in a family with arrhythmogenic right ventricular cardiomyopathy. *Eur J Hum Genet*. 2013;21:1226–1231. doi: 10.1038/ejhg.2013.39.
6. Gerull B, Heuser A, Wichter T, Paul M, Basson CT, McDermott DA, Lerman BB, Markowitz SM, Ellinor PT, MacRae CA, Peters S, Grossmann KS, Drenckhahn J, Michely B, Sasse-Klaassen S, Birchmeier W, Dietz R, Breithardt G, Schulze-Bahr E, Thierfelder L. Mutations in the desmosomal protein plakophilin-2 are common in arrhythmogenic right ventricular cardiomyopathy. *Nat Genet*. 2004;36:1162–1164. doi: 10.1038/ng1461.
7. van Tintelen JP, Entius MM, Bhuiyan ZA, Jongbloed R, Wiesfeld AC, Wilde AA, van der Smagt J, Boven LG, Mannens MM, van Langen IM, Hofstra RM, Otterspoor LC, Doevendans PA, Rodriguez LM, van Gelder IC, Hauer RN. Plakophilin-2 mutations are the major determinant of familial arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Circulation*. 2006;113:1650–1658. doi: 10.1161/CIRCULATIONAHA.105.609719.
8. den Haan AD, Tan BY, Zikusoka MN, Lladó LI, Jain R, Daly A, Tichnell C, James C, Amat-Alarcon N, Abraham T, Russell SD, Bluemke DA, Calkins H, Dalal D, Judge DP. Comprehensive desmosome mutation analysis in North Americans with arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Circ Cardiovasc Genet*. 2009;2:428–435. doi: 10.1161/CIRCGENETICS.109.858217.
9. Kirchner F, Schuetz A, Boldt LH, Martens K, Dittmar G, Haverkamp W, Thierfelder L, Heinemann U, Gerull B. Molecular insights into arrhythmogenic right ventricular cardiomyopathy caused by plakophilin-2 missense mutations. *Circ Cardiovasc Genet*. 2012;5:400–411. doi: 10.1161/CIRCGENETICS.111.961854.
10. Pilichou K, Lazzarini E, Rigato I, Celeghin R, De Bortoli M, Marra MP, Cason M, Jongbloed J, Calore M, Rizzo S, Regazzo D, Poloni G, Iliceto S, Daliento L, Delise P, Corrado D, Van Tintelen JP, Thiene G, Rampazzo A, Basso C, Bauce B, Lorenzon A, Occhi G. Large genomic rearrangements of desmosomal genes in Italian arrhythmogenic cardiomyopathy patients. 2017;10:e005324. doi: 10.1161/CIRCEP.117.005324.
11. Cox MG, van der Zwaag PA, van der Werf C, van der Smagt JJ, Noorman M, Bhuiyan ZA, Wiesfeld AC, Volders PG, van Langen IM, Atsma DE, Dooijes D, van den Wijngaard A, Houweling AC, Jongbloed JD, Jordaens L, Cramer MJ, Doevendans PA, de Bakker JM, Wilde AA, van Tintelen JP, Hauer RN. Arrhythmogenic right ventricular dysplasia/cardiomyopathy: pathogenic desmosome mutations in index-patients predict outcome of family screening: Dutch arrhythmogenic right ventricular dysplasia/cardiomyopathy genotype-phenotype follow-up study. *Circulation*. 2011;123:2690–2700. doi: 10.1161/CIRCULATIONAHA.110.988287.
12. Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R, Lincoln S, Crawley A, Hanson M, Maraganore D, Adler C, Cookson MR, Muenter M, Baptista M, Miller D, Blancato J, Hardy J, Gwinn-Hardy K. Alpha-synuclein locus triplication causes Parkinson's disease. *Science*. 2003;302:841. doi: 10.1126/science.1090278.
13. Zhang F, Lupski JR. Non-coding genetic variants in human disease. *Hum Mol Genet*. 2015;24:R102–R110. doi: 10.1093/hmg/ddv259.
14. Edwards JJ, Gelb BD. Genetics of congenital heart disease. *Curr Opin Cardiol*. 2016;31:235–241. doi: 10.1097/HCO.0000000000000274.
15. Feuk L, Carson AR, Scherer SW. Structural variation in the human genome. *Nat Rev Genet*. 2006;7:85–97. doi: 10.1038/nrg1767.
16. Henrichsen CN, Vinckenbosch N, Zöllner S, Chaignat E, Pradervand S, Schütz F, Ruedi M, Kaessmann H, Reymond A. Segmental copy number variation shapes tissue transcriptomes. *Nat Genet*. 2009;41:424–429. doi: 10.1038/ng.345.

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