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Biallelic PRKAG2 Truncating Variants Are Associated with Severe Neonatal Cardiomyopathies

Alexandre Janin , PharmD, PhD; Evan Gouy , MD; Audrey Putoux , MD, PhD; Thomas Perouse-de-Monclos , MD; Philippe Chevalier , MD, PhD; Adèle Faucherre, PhD; Jourdano Mancilla Abaroa , MSc; Chris Jopling , PhD; Sophie Collardeau Frachon , MD, PhD; Jelena Radojevic, MD; Salima El Chehadeh, MD, PhD; Gilles Millat , PhD

Our study reports 3 infants who died from severe cardiogenic shock. Genetic analysis showed, for the first time, the presence of homozygous truncating variants in PRKAG2.

PAST MEDICAL HISTORY

Cases 1 and 2, arising from the same family, are children of second degree-related parents without previous familial medical history, who died, respectively, at 2.5 months old (case 1) and 24 days old (case 2) due to a cardiogenic shock. Despite normal development during 2 months, moderate feeding difficulties, weight loss, and weakness were observed. The child was subsequently hospitalized due to cardio-respiratory arrest and died despite cardiopulmonary resuscitation. Cardiac ultrasound indicated a dilated, thin-walled heart with weak contractility. Autopsy showed dilated ventricles, a thin left ventricular wall with associated fibrosis, and diffuse fibroelastosis. Bacteriological and virological tests were negative. postnatal day 24, the child became hypotonic with a rapid clinical deterioration and died during hospitalization. An autopsy was denied by the parents. Case 3 was the first child of first cousin parents from Tunisia. The first signs of heart failure were noted at 2 months of age with low blood pressure, asthenia, respiratory distress, and feeding difficulties which were responsible for weight stagnation, leading to the diagnosis of dilated cardiomyopathy. At 4 months of age, diagnosis of hypokinetic dilated cardiomyopathy was confirmed. A sinus tachycardia (155 bpm) with positive T waves on V1 and V2 leads with negative T waves on V3 to V6, a fragmented P wave, and a slightly prolonged PR interval (160 ms) was observed on the electrocardiogram. Transthoracic echocardiogram showed a thinwalled dilated and hypokinetic left ventricle with low ejection fraction at 25%.

MOLECULAR ANALYSIS

This report adheres to requirements of the Helsinki Declaration and informed consent for genetic analyses for diagnosis was obtained from all tested subjects. Genetic testing was performed by Next Generation Sequencing of a large panel of 95 genes involved in sudden cardiac death, identified 1 pathogenic variation for cases 1 and 2: a PRKAG2 homozygous truncating variant: p.Ile550Asnfs*58.1 Both parents and 3 of the 4 surviving children were heterozygous for the variant (Figure 1 [A]). Cardiac evaluations by transthoracic echocardiogram and ECG were normal for the parents (the father was 47 years old and the mother was 48 years old) and for the remaining children. Lastly case 3 harbored a homozygous 2504 bp deletion removing exon 11 and resulting in a frameshift and premature stop codon. Both parents were heterozygous for the variant (Figure 1 [B]). Cardiac evaluations by transthoracic echocardiogram and ECG were normal for the parents at 36 years old for the father and 31 years old for the mother.

PRKAG2 KNOCKOUT ZEBRAFISH MODEL

To investigate how PRKAG2 loss of function affects cardiac development in vivo, we targeted this gene in zebrafish using a CRISPR/Cas9 crisprant approach to generate f0 knockout larvae.2

Zebrafish possess 2 PRKAG2 orthologs, *prkag2a* and *prkag2b*, and we targeted both alleles simultaneously (targeting either alone had no effect). High-definition fluorescent movies of *prkag2a/prkag2b* crispant hearts at 5 days postfertilization were analyzed using ZeCardio software (data not shown).³ No significant difference was observed in either the atrial or ventricular heart rates between control and *prkag2a/prkag2b* crispant larvae (Figure [C]). However, we were able to detect a clear reduction in ventricular contractility resulting in a significantly diminished ejection fraction (Figure [C]). Furthermore, evidence of atrial fibrillation was also observed in a significant proportion *prkag2a/prkag2b* crispant larvae (Figure [C]). Lastly, our analysis also indicates that the ventricular wall of *prkag2a/prkag2b* crispants is significantly thicker than control larvae, a feature associated with cardiac hypertrophy (data not shown). Taken together, these data indicate that *prkag2a/prkag2b* loss of function disrupts ventricular contractility resulting in a significantly reduced ejection fraction which appears to be in line with the transthoracic echocardiogram observations of case 3. The data that support the findings of this study are available from the corresponding author upon reasonable request. Neonatal presentations of cardiomyopathies are rare and often associated with other syndromic features.⁴ Here, we describe, for the first time, 3 infants from 2 unrelated families, who died of cardiogenic shock most likely due to homozygous truncating variants in PRKAG2. The PRKAG2 knockout zebrafish model (*prkag2a/prkag2b*) confirms the pathogenic impact of homozygous PRKGA2 truncating variants. Missense PRKAG2 pathogenic variations are usually detected in patients with nonsarcomeric familial hypertrophic cardiomyopathy associated with Wolff-Parkinson-White syndrome but no pathogenic truncating variations have been reported in either the Human Gene Mutation Database or the Global Variome shared LOVD Database.⁵ As heterozygous carriers are asymptomatic, our study suggests that PRKAG2 truncating variants, in an autosomal recessive condition, are associated with severe and fatal neonatal cardiomyopathies. The identification of pathogenic variants in PRKAG2 will allow appropriate genetic counseling for prospective parents. Preimplantation or prenatal genetic screening should also be considered due to neonatal mortality and morbidity.

ARTICLE INFORMATION

Affiliations Laboratoire de Cardiogénétique Moléculaire (A.J., G.M.), NGS sequencing platform for molecular diagnosis (A.J., G.M.), Paediatric Cardiology and Congenital Heart Disease Department, Cardiovascular Louis Pradel Hospital (T.P.-d.-M.), and Institut de Pathologie, Hôpital Femme Mère Enfant (S.C.F.), Hospices Civils de Lyon. Université de Lyon & Université Lyon (A.J., E.G., P.C., S.C.F., G.M.). Genetics Department, University Hospital of Lyon, Bron (E.G., A.P.). Bessereau’team, MeLiS (Mechanisms in integrated Life Sciences), CNRS UMR 5284 INSERM U1314, Claude Bernard University Lyon (E.G.). Hôpital Cardiologique Louis Pradel, Service de Rythmologie, Lyon (P.C.). Institute of Functional Genomics, University of Montpellier, CNRS, INSERM, LabEx ICST, Montpellier (A.F., J.M.A., C.J.). Service de Cardiologie Congénitale, Strasbourg (J.R.). Service de Génétique Médicale, Institut de Génétique Médicale d’Alsace (IGMA), Centre de Référence des Déficiences Intellectuelles de Causes Rares, Hôpitaux Universitaires de Strasbourg, Centre de Recherche en Biomédecine de Strasbourg (CRBS), Strasbourg, France (S.E.C.).

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Disclosures None.

REFERENCES

1. Chanavat V, Janin A, Millat G. A fast and cost-effective molecular diagnostic tool for genetic diseases involved in sudden cardiac death. *Clin Chim Acta Int J Clin Chem.* 2016;453:80–85. doi: 10.1016/j.cca.2015.12.011
2. Kroll F, Powell GT, Ghosh M, Gestri G, Antinucci P, Hearn TJ, Tunbak H, Lim S, Dennis HW, Fernandez JM, et al. A simple and effective F0 knockout method for rapid screening of behaviour and other complex phenotypes. *eLife.* 2021;10:e59683. doi: 10.7554/eLife.59683
3. Dyballa S, Miñana R, Rubio-Brotos M, Cornet C, Pederzani T, Escaramis G, Garcia-Serna R, Mestres J, Terriente J. Comparison of zebrafish larvae and hiPSC cardiomyocytes for predicting drug induced cardiotoxicity in humans. *Toxicol Sci Off J Soc Toxicol.* 2019;171:283–295. doi: 10.1093/toxsci/kfz165
4. Choudhry S, Puri K, Denfield SW. An update on pediatric cardiomyopathy. *Curr Treat Options Cardiovasc Med.* 2019;21:36. doi: 10.1007/s11936-019-0739-y
5. Gollob MH, Green MS, Tang AS, Gollob T, Karibe A, Ali Hassan AS, Ahmad F, Lozado R, Shah G, Fananapazir L, et al. Identification of a gene responsible for familial Wolff-Parkinson-White syndrome. *N Engl J Med.* 2001;344:1823–1831. doi: 10.1056/NEJM200106143442403

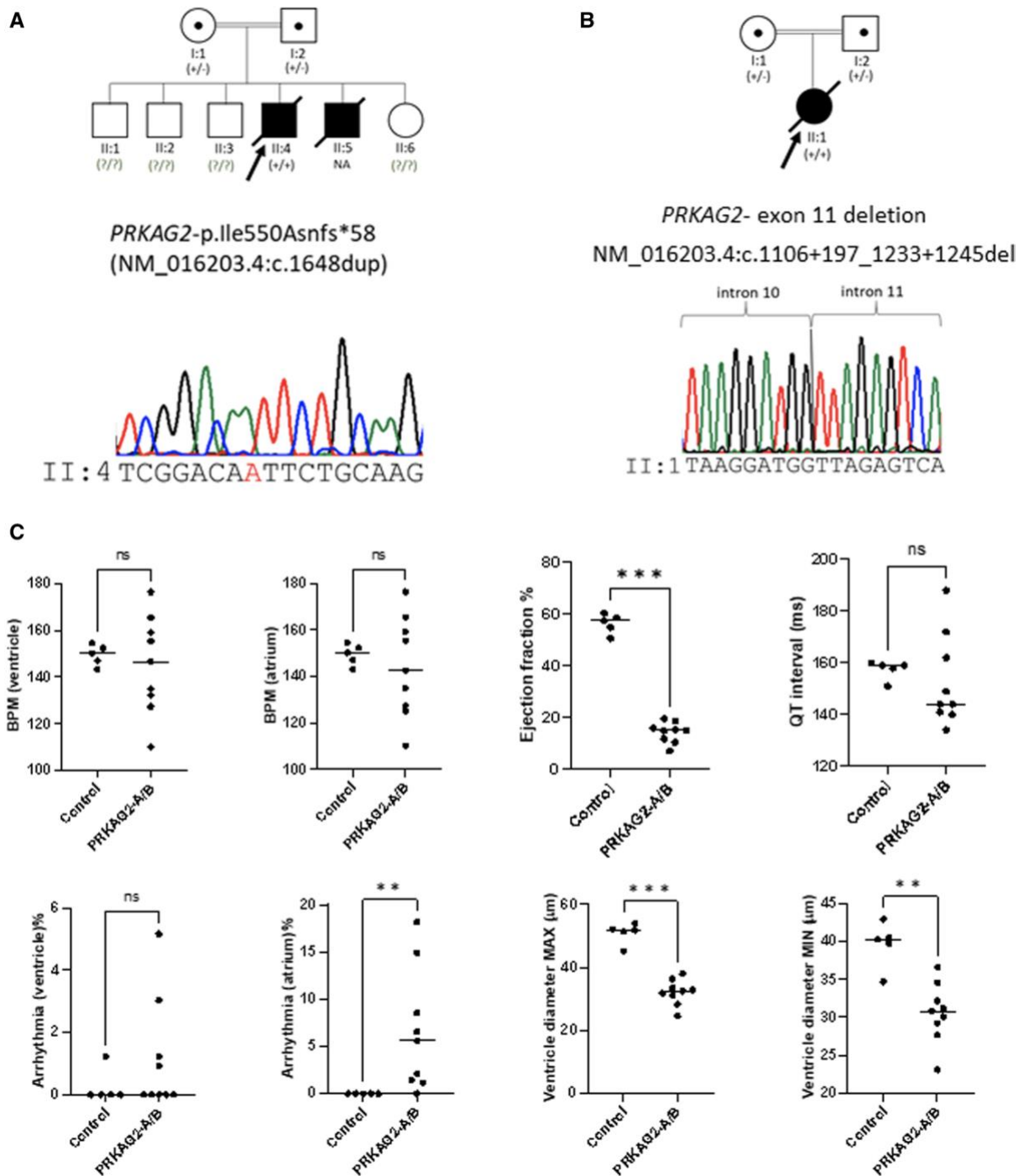


Figure 1.

A and B, Pedigrees of the families. Affected siblings are marked with black symbols, carriers of the heterozygous pathogenic variation are marked with a central dot. Electrophoregrams of probands (black arrows) are provided. **C,** Comparison of control and *PRKAG2* mutant embryos of zebrafish model. Statistical significance was assessed by Mann and Whitney nonparametric test. ns indicates nonsignificant.