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Whole Exome Sequencing Reveals a Large Genetic Heterogeneity and Revisits the Causes of Hypertrophic Cardiomyopathy

Experience of a Multicentric Study of 200 French Patients

Hypertrophic cardiomyopathy (HCM), the most common hereditary heart disease, is autosomal dominant with incomplete penetrance and variability. Heterozygous mutations in 5 sarcomeric genes (*MYBPC3*, *MYH7*, *TNNT2*, *TNNI3*, and *MYL2*), estimated to account for 50% to 70% of HCM cases, are routinely screened for diagnosis, genetic counseling, predictive testing, and targeted follow-up of mutation carriers.¹ Secondary HCM cases caused by Fabry disease and transthyretin hereditary amyloidosis, which require specific therapies, remain underdiagnosed.²

The HYPERGEN study evaluated the diagnostic value of whole exome sequencing (WES) in HCM compared with targeted sequencing of the 5 sarcomeric genes. Two hundred unrelated patients with newly diagnosed isolated HCM were enrolled in 5 French centers (Marseille, Bordeaux, Paris, Dijon, and Rennes). An institutional review committee approved the study. All subjects gave informed consent for genetic studies. HCM echocardiographic diagnosis was considered definite in presence of left ventricular hypertrophy (maximal wall thickness ≥ 15 mm in sporadic or >13 mm in familial cases) without dilatation or any other cardiac or systemic disease. The patient cohort included 132 men and 68 women (mean age, 55 years; range, 19–91; 86.5% above 40; 66% sporadic cases).

WES was performed on the NGS Illumina HiSeq2500 platform using Agilent SureSelect V6 technology (Genewiz, United States). Bioinformatic analyses were performed in our laboratory using prediction software and comparison to mutation databases.³ We retained exonic and intronic variants whose frequencies were lower than 1% and predicted as pathogenic or likely pathogenic.⁴ The first step of WES data analysis consisted of searching for mutations in a virtual panel of 167 genes involved in cardiomyopathies and other various hereditary diseases. WES identified 418 variants located in 101 genes among the 167 genes (Figure [A]). Besides *TTN* (titin), which carried the largest number of variants ($n=88$), the most frequently mutated genes were *MYBPC3* ($n=57$, 13.6%), *MYH7* ($n=16$, 3.8%), *FLNC* ($n=14$, 3.3%), *RYR2* ($n=10$, 2.4%), *SCN5A* ($n=10$, 2.4%), *ANK2* ($n=8$, 1.9%), and *CTNNA3* ($n=7$, 1.7%); *MYH6*, *TNNT2*, *DSP*, *LAMA4*, and *NEBL* ($n=6$ each gene, 1.4% each); and 88 other genes (total $n=178$, 42.6%). Only 1 variant was found in *MYL2* (0.2%) and none in *TNNI3*. Overall, 65 variants (15.5% of all, 19.7% with *TTN* excluded) were located in 19 arrhythmia genes. The most prevalent genes were the sarcomeric genes with 96 variants in 10 genes (23%) among which the 5 most frequent sarcomeric genes accounted for 80 variants (19.1%).

Four variants in the *GLA* gene (Fabry disease) were identified in 4 patients, among which were the same benign polymorphism p.Asp313Tyr in 3 patients and a novel heterozygous c.931dupC (p.Leu311Profs*4) pathogenic mutation in 1 woman presenting with isolated HCM and no extracardiac features. Accordingly, the plasmatic Lyso-GL3 biomarker was abnormal (10.6, normal ≤ 1.8 ng/mL) and

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Key Words: amyloidosis ■ dilatation
■ Fabry disease ■ genetic counseling
■ whole exome sequencing

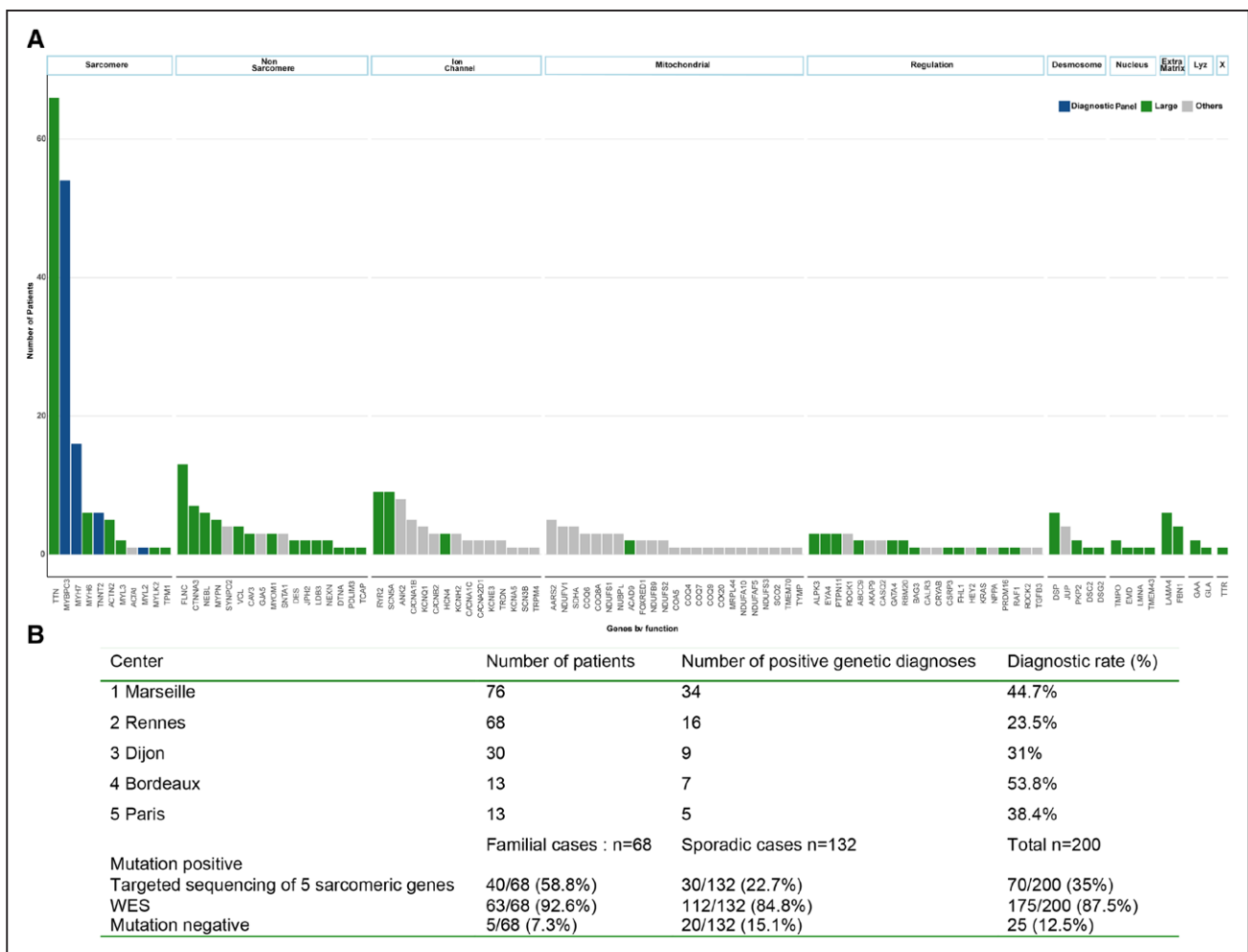


Figure. Mutations found by WES and diagnostic rate of targeted sequencing of 5 sarcomeric genes.

A, Distribution of variants (n=418). Top, The relative frequencies of genes carrying variants is presented, according to the gene function. In blue, the 4 sarcomeric genes among the 5 genes expected to be the most frequently associated with hypertrophic cardiomyopathy (HCM), because of the absence of mutations in *TNNI3* in this cohort. Green corresponds to a larger panel of 60 genes involved in various hereditary cardiac diseases. In gray, other genes involved in hereditary cardiac or systemic diseases. **B**, Diagnostic rate for the targeted sequencing of the 5 sarcomeric genes in the 5 French centers of the HYPERGEN study (**top**) and mutation detection rate in sporadic and familial cases of HCM (**bottom**). Lys indicates lysosomal; X, other; and WES, whole exome sequencing.

confirmed Fabry disease. The well-known p.Val50Met mutation in the *TTR* gene was identified in 1 patient showing isolated HCM, confirming transthyretin cardiac amyloidosis.

Interestingly, 4 variants (1%) in the *FBN1* gene were found in 4 patients, among which 1 was previously reported as a disease-causing mutation in Marfan syndrome, and all the patients had other variants in other genes. We also identified 4 variants in 2 novel genes (*ROCK1* and *ROCK2*) belonging to the Rho kinases family and not previously associated with any cardiac phenotype. All occurred in combination with variants in other genes, including *MYBPC3* and *FBN1*. Forty-six patients (23%) carried a single variant, whereas 129 (64.5%) carried >1 variant (from 2 to 7 variants).

Altogether, after targeted sequencing of the 5 sarcomeric genes, 70 patients (35%) had a diagnosis of a pathogenic mutation, whereas, by WES, 175 patients (87.5%) carried at least 1 pathogenic or likely patho-

genic mutation within the 167 genes (Figure [B]). Among the 130 patients with no mutation identified by the standard approach, WES detected at least 1 mutation in 105/130 patients (80.7%). Among the 70 patients with 1 mutation, WES detected additional mutations in 53/70 patients (75.7%).

Considering a larger targeted panel of 60 genes involved in various cardiomyopathies and arrhythmias, currently proposed to be screened as a second step of the diagnostic strategy after sequencing of the 5 sarcomeric genes, the diagnostic rate reached 79% of patients (158/200) with identification of at least 1 mutation. In 25 patients (12.5%), neither targeted sequencing nor analysis of the WES virtual panel detected any mutation. Beyond the 167 genes in the WES virtual panel, further analysis of WES data is needed to identify potential new genes.

In conclusion, the HYPERGEN study validated the yield of WES in HCM. WES detected additional muta-

tions in patients with 1 mutation identified by targeted sequencing, suggesting larger genetic heterogeneity than previously thought, and perhaps oligogenism.⁵ WES confirmed the prominent involvement of *MYBPC3* and *MYH7*¹ but also showed unexpected frequency of mutations in *TTN* and *FLNC* as well as in *SCN5A*, *RYR2*, and other ion channel genes, suggesting overlap between HCM and arrhythmia genes. Familial segregation studies will refine the interpretation of variants' pathogenicity and will be the next step of the HYPER-GEN project.

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ARTICLE INFORMATION

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Disclosures

None.

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