

# Approaching the facts between genetic mutation and clinical practice of hypertrophic cardiomyopathy

## A case report with *RAF1* 770C>T mutant

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### Abstract

**Introduction:** Hypertrophic cardiomyopathy (HCM) is one of the most common cardiomyopathies, which induces sudden cardiac death. Several mutants have been identified among HCM cases.

**Methods and results:** A 10-month female infant who experienced cough, fever, aggressive exertional dyspnea, and recurrent cyanosis was admitted to our hospital. The patient was first diagnosed with type I respiratory failure, dysfunction of heart, severe pneumonia, and also some cardiac disorders were suspected. The echocardiography, cardiac computed tomography scan, cardiac magnetic resonance imaging scan, and also electrocardiogram were performed to confirm a diagnosis of HCM. Moreover, the whole-genome sequencing and chromatin analysis have been suggested. Based on the sequencing analysis, a new heterozygous mutant of *RAF1* at c. 770C>T had been identified in absence of the same mutant in both her parents. Besides, the existence of normal karyotypes was confirmed among 3 samples.

**Conclusion:** So we first reported a single mutant of *RAF1* 770C>T with idiopathic HCM in a very early age. This patient would have suffered significant cardiac ventricular hypertrophy with more severe clinical manifestation in an extremely younger age compared with other identified mutations. However, we could only take limited advantages of deoxyribonucleic acid sequencing in HCM diagnosis and therapy. Reporting additional observations of well designed cohorts with a long-term follow-up would be very helpful to accelerate the transition of genetic molecular research on HCM.

**Abbreviations:** EDV = end diastolic volume, ESV = end systolic volume, HCM = hypertrophic cardiomyopathy.

**Keywords:** hypertrophic cardiomyopathy, mutation, *RAF1*

### 1. Introduction

Hypertrophic cardiomyopathy (HCM) is one of the most common cardiomyopathies, which would induce sudden cardiac death among children and young individuals. Typically, HCM is

defined as a kind of primary disorder of the myocardium characterized by hypertrophy, usually affecting the left ventricle, in the absence of other loading conditions such as hypertension and aortic valve stenosis.<sup>[1]</sup> In addition, it is also referred to as a “tumor of the heart” under modern definition which might demonstrate diverse clinical cardiac disorders. Such patients always appear at hospitals with a wide range of clinical spectra. For most of them, heart failure or arrhythmias would usually be the common complains, while some individuals might experience a benign and asymptomatic physiopathological course. Until now, the diagnosis of HCM has mainly been based on the echocardiographic manifestation of unexplained cardiac hypertrophy, which can be subtle or massive. However, with the rapid development of genetic and molecular technologies, we have the opportunities to pursue further understanding of the deoxyribonucleic acid (DNA) and molecular basis of cardiac diseases. During the past decades, a series of mutant genes have been identified, which focused on the functional impact of sarcomere,<sup>[2]</sup> while new mutations are obtained at the same time. Today, molecular genetics have already redefined etiology and diagnostic criteria for numerous diseases. Moreover, these new insights provide targeted and individual treatment for particular diseases. On the contrary, there are limited insights that the genetic diagnoses of HCM could provide, and a lack of precision genetic management and therapy still exists, although functional experiments have approved several core roles of mutations. Here, we would like to report a case of early-onset HCM case with a recently identified mutation of *RAF1* without any known

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syndromes. Besides, a predicament between the basic genetic researches and clinical practice is a serious concern.

## 2. Case report

This study was approved by the Ethics Committee of West China Second University Hospital of Sichuan University. A 10-month female infant was admitted to our hospital after experiencing cough and fever with a highest temperature of about 40°C for 5 days on January 21, 2016. Moreover, she suffered an aggressive exertional dyspnea and underwent recurrent cyanosis since her birth, with an adverse growth and development in the previous 2 days. The physical examination at admission observed a heartbeat rate of >160 per minute, with >70 respirations/min, blood pressure 88/44 mm Hg, edema of lower lips, 3 depression signs, rough breath sounds, enlargement of liver (4 cm below costal margin) but without splenomegaly, and a systolic murmur at the apical zone of heart. The artery blood gas analysis revealed that partial pressure of oxygen was under 40 mm Hg. Besides, levels of cardiac troponin I and brain natriuretic peptide were extremely high as 0.890 µg/L and 273.0 ng/L, respectively. So the patient was first diagnosed with type I respiratory failure, dysfunction of heart, severe pneumonia, and some kinds of disorder of the heart were suspected as well.

The echocardiography, cardiac computed tomography (CT) scan, cardiac magnetic resonance imaging (MRI) scan, and also electrocardiogram were performed following the mechanical ventilation. The echocardiography detected the hypertrophic myocardium both in left and right ventricles, especially for the right ventricular wall that indicated a poor prognosis<sup>[3]</sup> (Fig. 1A). In addition, the stenosis of left outflow tract was identified with the accelerated blood flow. However, it revealed a persisted left ventricular systolic function, but with a failure of a normal diastolic function. A similar manifestation had been confirmed both in CT and MRI scan and the data of the left ventricular ejection fraction as 73.8%, end systolic volume (ESV) as 3.0 mL, and end diastolic volume (EDV) as 11.4 mL were obtained, while the right ventricular ESV was 2.8 mL, and EDV was 8.6 mL (Fig. 1B). Moreover, the electrocardiogram showed a high voltage of left ventricular indicating hypertrophic myocardium. Given that, a diagnosis of HCM had been done.

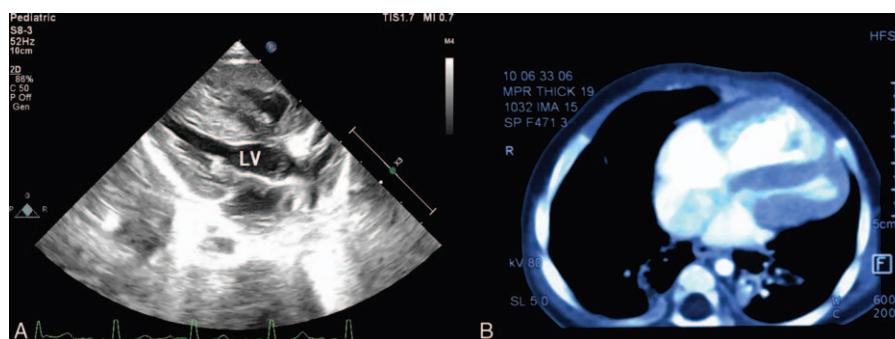
As mentioned above, the strong antibiotics treatment had been applied with Meropenem (Dainippon Sumitomo, Osaka, Japan) (0.8 g/d) and Cefoperazone (Pfizer, La Jolla, CA, USA) (0.6 g/d). The mechanical ventilation has also been applied after intubation as she suffered respiratory failure, and Budesonide (AstraZeneca,

London, UK) (1 g/d) was also given to improve the oxygen transfusion. Typically, only diuretics of Spirolactone (Minsheng, Hangzhou, China) and Hydrochlorothiazide (Fangqiang, Jiangsu, China) were provided for treating heart failure with a dosage of 8 mg/d. However, as obstruction of outflow tract was suspected, no positive inotropic medication was applied. After 3 days of treatment, the patient was still depending on the mechanical ventilation and suffering heart failure, but in a lighter condition. On the contrary, the parents refused to take any medical treatment due to financial issues as well as negative prognosis. The infant was taken back home after nonmedical purpose extubation. In addition, there were no opportunities for our doctors to administer any medication or treatment such as  $\beta$ -blocker or angiotensin-converting enzyme inhibitor to improve her prognosis.

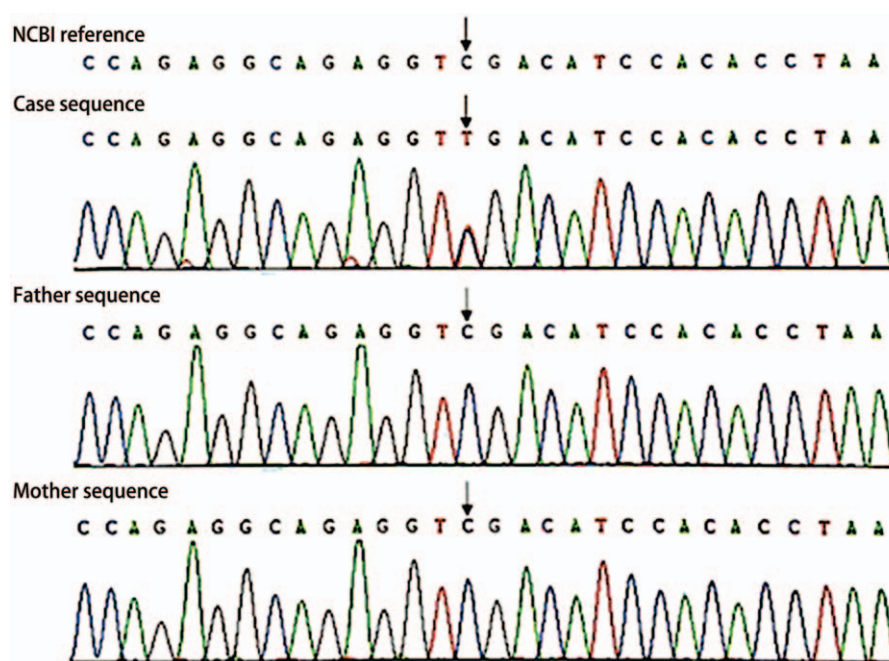
Besides, as the HCM was mostly accompanied with a kind of genetic variations or secondary to some syndromes, the whole-genome sequencing and chromatin analysis have been suggested. We obtained the blood sample from her parents and herself with informed consent from parents. DNA extraction was performed according to the standard protocols. Polymerase chain reaction amplicons were Sanger sequenced using BigDye3.1 chemistry (Applied Biosystems Foster City, CA, USA) and capillary electrophoresed on an ABI3130xl bioanalyser (Applied Biosystems Foster City, CA, USA) using standard methodologies. Resultant sequencing chromatograms were compared to the Genbank reference sequences. Parental DNA samples were screened to investigate allele transmission.

Based on the sequencing analysis, a new heterozygous mutant of *RAF1* at c. 770C>T had been identified (Fig. 2). Besides, the existence of normal karyotypes was confirmed among 3 samples. As *RAF1* mutant was usually associated with Noonan syndrome and LEOPARD syndrome,<sup>[4]</sup> we reviewed the clinical manifestations and related examinations and excluded the possibilities of these 2 syndromes. To rule out Noonan syndrome, the chromatin analysis was appropriate without any special face. And for LEOPARD syndrome, it had been excluded by the absence of various sized lentigines, pulmonary stenosis, skeletal malformation, disorders of heart conduction, and deafness. Here, we first reported an idiopathic HCM case with a single mutant of *RAF1* 770C>T in a very early age.

Following the diagnosis and management procedure,<sup>[5]</sup> the same mutation is absent between her parents, and it is not necessary to perform any follow-up, examination, or warrant risk of next offspring for such parents. However, it is also embarrassing that we could launch little things for this infant



**Figure 1.** The images of echocardiography and MRI for this patient: (A) presents the echocardiographic image indicating the severe hypertrophic ventricular walls, (B) presents the MRI images for cardiac hypertrophy.



**Figure 2.** The results of genetic sequencing that indicate a single mutant of *RAF1* 770C>T in absence of parents' finding.

based on the genetic result. The management, treatment, and prognosis are mainly approached according to her clinical and echocardiographic manifestation, not refereeing to the genetic background. So, we noticed that there is a predicament. Although the genetic testing is important in HCM, and great milestones have been achieved with basic researches, the usefulness of mutation results is really limited, failing to conduct the precision management and therapy for affected cases. However, we also performed a follow-up after the discharge, and the infant demised within 10 days due to uncontrolled heart failure.

### 3. Discussion

HCM is a common type of cardiomyopathy, and the clinical diagnosis is the most essential criterion for such disease. However, starting from the early 1990s, genetic linkage studies of HCM have been published. Since then, major advances have been made to get a better understanding of genetic and molecular basis of HCM. HCM is partly caused by an autosomal dominant mutation in genes that encode sarcomere proteins or sarcomere associated proteins. Among them, 8 genes are known to definitively cause HCM, which are associated with the motor molecules of myocardium including the  $\beta$ -myosin heavy chain (MYH7, 30–35%), myosin-binding protein C (MYBPC3, 20–30%), troponin T (TNNT2, 10–15%), troponin I (TNNI3, <5%),  $\alpha$ -tropomyosin (TPM1, <5%), actin (ACTC, <1%), regulatory light chain (MYL2, <1%), and essential light chain (MYL3, <1%).<sup>[1]</sup> Abovementioned mutations could affect the cardiac muscle force generations, velocities, ATPase supplementations, contractile kinetics, duty ratio, and the concentration of calcium.<sup>[2]</sup> Moreover, functional experiments on cells and transgenic animal models have also been carried out to demonstrate a similar physiological procedure, which provide pioneer results on genetic and molecular mechanism of cardiomyopathy.<sup>[6–8]</sup> Given that, great contributions have been made to reveal the facts of HCM at genetic and molecular levels.

However, in this case, we have not identified a single mutation of idiopathic HCM reported. Besides, a new mutant on *RAF1* 770C>T has been found. According to the guideline for diagnosis and treatment of HCM,<sup>[5]</sup> *RAF1* is not involved in the sarcomere-related genes. So, the *RAF1* 770C>T mutation is recognized as a potential HCM DNA variant that was previously unknown as a cause of HCM but has molecular characteristics in myocardial development, which are similar to recognized HCM mutations. Moreover, *RAF1* mutations that affect the *RAF1*'s kinase activity were found to cause Noonan and LEOPARD syndromes with a high prevalence of HCM.<sup>[4]</sup> Those HCM-associated mutations exhibited high kinase activities and increased extracellular regulated kinase activation in phosphorylation, particularly as it pertains to cardiac hypertrophy. Besides, it has also been confirmed to be involved in dilated cardiomyopathy.<sup>[9]</sup> Several intracellular signaling pathways regulate the procedure of cardiac hypertrophy including the RAS/mitogen-activated protein kinase cascades and calcineurin/nuclear factor of activated T cells (NFAT). Evidence showed that *RAF1* encoded a downstream RAS effector and induced hypertrophy signaling via NFAT.<sup>[4,10]</sup> As mentioned above, we seriously considered that the non-sarcomere-related mutation of *RAF1* 770C>T could be a genetic cause of this early-onset HCM case, which also supplied ideas on the differences of the prognosis and pathological cause between this early-onset cases and ordinary patients with sarcomere gene related mutations who shall experience clinical manifestation around a later age. The *RAF1* mutation might induce significant ventricular hypertrophy in a very young age, and especially for the right ventricular and outflow tract obstruction. So that there was a possibility to suffer heart failure during their infant period, which should be the main difference from other mutations-related cardiomyopathy.

Nowadays, with the incredible development of DNA-sequencing technology, we get more and more understandings of diseases, not only limited in cardiovascular diseases. Taking the advantages of genetic sequencing, several types of targeted drugs

have been developed and made into practice in a clinical procedure. However, to cardiovascular diseases, hundreds of publications have been reported with hundreds of mutations, along with a series of transgenic cell and animal investigations revealing that how such mutations exactly affected myocardial function.<sup>[11,12]</sup> But there is still no high-quality and convinced clinical research to provide suggestions on how to use the genetic background into clinical practice, especially for the affected cases of precision management and therapy. The latest guideline also pointed out that information from genetic sequencing about risk of sudden cardiac death or prognosis is still limited. So it brought us a situation that great milestones had been achieved with basic researches, but the usefulness of mutation results was really limited and failed to conduct the precision management and therapy.

After a numerous mutations have been published, researchers and doctors seem still to have a long way to approach the facts of cardiomyopathy. A decade ago, researchers strongly used to believe understanding how mutations perturb biophysical events of muscle contraction and cell-signaling pathways will inevitably allow advances in future management, treatment, and interventions. However, we are still seeking a way on how to approach the facts between the genetic mutations and clinical practice, so that investigations on the following objectives should be recalled to help guideline updating by genetic revolution: how to define the risk stages and prognosis of disease based on genetic results, large sample size observation study should be performed to find out whether particular genotypes are correlated with the survival ratio, whether separated therapeutic strategies should be carried out according to individual genomic sequencing not only based on their clinical and echocardiographic manifestations, and the treatment of HCM, as well as all kinds of cardiomyopathy calls for targeted medication, which should be the ultimate purpose of genetic research. There are still many efforts should be done to make the innovation of genetic investigation to benefit the patients with HCM.

#### 4. Conclusion

In general, this is the very first report of idiopathic HCM with newly identified mutant of *RAF1* c.770C>T in absence of Noonan and Leopard syndromes that were previously reported to be associated with *RAF1* variants. The mutation of *RAF1* would induce significant cardiac ventricular hypertrophy with

severe clinical manifestation in a young age. However, this case also brought us to review the current role of genetic screening in the diagnosis and therapy of HCM, although genetic-related researches have made great advances. It indicated that there were limited contributions which the information based on genetic background could make in defining risk stages, predict prognosis and individuals treatment, as well as little consults that physician could provide. Reporting additional observations of well designed cohorts with a long-term follow-up would be of great help to accelerate the transition of genetic molecular research on HCM.

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