

# GDNF and GUCY2C mutation associated with Hirschsprung's disease in a trio pedigree: a case report

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## Case report

**Keywords:** Hirschsprung's disease, Exome sequencing, GDNF, GUCY2C, Ganglion cells

**Posted Date:** April 6th, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-21172/v1>

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## Abstract

Background: Hirschsprung's disease (HSCR) caused by absence or dysfunction of ganglion cells in the submucosal plexus and intermuscular plexus of the intestinal wall. Studies have shown that homozygous mutations of glial cell-line derived neurotrophic factor ( GDNF ) can lead to the occurrence of HSCR. but heterozygous mutation must combine with other related abnormal genes could to cause the HSCR. The other Guanylyl cyclase C ( GUCY2C ) gene is rarely reported as a direct cause of HSCR. Our findings Provides a new perspective for application of mutation screening in the genetic counseling.

Case presentation: We report clinical and genetic findings of a trio pedigree with Hirschsprung's disease. Whole exome sequencing and Sanger sequencing were performed for propositus and all members of trio pedigree, respectively. We also collected 74 healthy control samples and used Sanger sequencing to identify that found the mutation. We first described the rare trio pedigree of HSCR caused by two special genes of GDNF (p.Arg93Gln) and GUCY2C (p.Tyr1072Cys). Studies have found that mutations in GDNF alone do not cause HSCR symptom, so is propositus' mother in this study. Common mutation of GDNF gene mutation inherited from mother of proband, but the rare mutation of GUCY2C from the father. None of the parents showed HSCR phenotype. We provide evidence for the causative role of the two variants.

Conclusions: Our study found that GDNF and GUCY2C together lead to the occurrence of HSCR. This finding expands the genotype spectrum and increases knowledge on the genetic molecular mechanisms of the HSCR. Our findings will provide help for the screening of pathogenic genes of HSCR.

## Background

Hirschsprung's disease (HSCR, OMIM 142623), also known as intestinal ganglion cell disease, is a rare congenital developmental disorder of intestinal malformation. It has been observed other organ abnormalities except for obstruction of the bowels in an infant, such as gastrointestinal malformations, cleft palate, renal dysfunction, cardiac malformations, craniofacial anomalies, and polydactyly, occurred in 18% of patients with HSCR in previous studies[1]. The average incidence of HSCR in live births is about 1/5000. There are significant racial differences, with the highest morbidity in Asia, especially in China. The incidence of HSCR in men is 2–4 times that of women. Pathologically, HSCR is characterized by congenital intestinal blockage caused by the absence or dysfunction of ganglion cells in the submucosal plexus and intermuscular plexus of the intestinal wall. The etiology of HSCR mainly focuses on the abnormal proliferation, differentiation and migration of intestinal nerve trunk [2, 3]. Intestinal neural crest cell (NCC) migrate and implant into the whole intestine at 5 to 12 weeks of gestation. HSCR is the most common digestive tract malformation in children, and it is a typical developmental disorder of enteric nervous system (ENS)[3]. Most cases of HSCR are sporadic, but there exists the inheritance mode of autosomal dominant, recessive inheritance, with incomplete dominance and diversity phenotypes[4, 5]. The main clinical manifestation is complete or incomplete intestinal obstruction. Severe cases are manifested as the failure of passing the first stool within 48 h after birth. Abdominal distention, vomiting, or neonatal enterocolitis, which seriously affects the quality of life of children and even endangers their

lives. In recent years, with the development of high-throughput sequencing and the increasing incidence of HSCR, domestic and foreign scholars attach great importance to it. Therefore, the research on the pathogenesis of HSCR has become a hot topic.

Genetic studies have shown that HSCR-related susceptibility genes are mainly derived from two major signaling pathways and one related transcription factors during ENS development[6]. One is the RET/GDNF/GFRA1 signaling pathway. The RET gene encodes a transmembrane receptor and member of the tyrosine protein kinase family of proteins. Binding of ligands such as GDNF (glial cell-line derived neurotrophic factor) and other related molecules, neurturin, artemin and persephin, these molecules are linked to glycosylphosphatidylinositol-anchored coreceptors (GFRalpha1/2/2/4), activation of downstream signaling pathways that play a role in cell differentiation, growth, migration and survival. The encoded receptor is important in development of the nervous system, and the development of organs and tissues derived from the neural crest. This proto-oncogene can undergo oncogenic activation through both cytogenetic rearrangement and activating point mutations[7, 8]. RET knockout mice exhibited intestinal neuron deletion, superior cervical ganglion deletion, renal hypoplasia or dysgenesis[9, 10]. The other is Endothelin 3-Endothelin Receptor B(EDNRB) signaling Pathway and transcriptional factor SOX10.

Guanylyl cyclase C (GUCY2C) is a transmembrane receptor protein whose expressed mainly in intestinal epithelial cells[11]. Activated GUCY2C can convert guanosine-5'-triphosphate (GTP) into cyclic Guanosine monophosphate (cGMP) and increases the amount of cGMP in cells. Increased cGMP will result in activation of a series of downstream signals, such as including cGMP-dependent protein kinases (PKGs), cyclic nucleotide-gated (CNG) channels and phosphodiesterases(PDEs). These functions are essential for the balance of electrolytes and fluids and tight junctional barrier function of the intestinal epithelium[12]. Abnormal function of GUCY2C gene may lead to autosomal dominant hereditary diarrhea and are predisposed to inflammatory bowel disease[13]. GUCY2C gene is rarely reported as a direct cause of HSCR.

In this study, we collected a trio pedigree with HSCR. The patient showed typical HSCR symptoms. Through gene sequencing, we identified the pathogenic genes of the family. Scientific guidance was made for future fertility and prenatal screening of the family, so as to provide a richer basis for the pathogenic gene spectrum of HSCR.

## Case Presentation

## Participants and clinical diagnosis

A 15-year-old girl came to our hospital because of defecation difficulties. According to their parents' complaints and relevant diagnostic data, the little girl was hospitalized in XiangYang City Central Hospital for many times due to defecation difficulties and stricture of anus since she was born. At that time, HSCR was diagnosed by experienced doctors in XiangYang City Central Hospital. We identify HSCR as a congenital disease. It has been described a correlation between the occurrence of HSCR and anorectal

malformations[14, 15]. The anal expansion operation was performed in the newborn, But the difficulty of defecating has not been eliminated. The patient's father was 41 years old and his mother was 39 years old. They were all healthy and had no HSCR related symptoms. But the mother dictates that she must pay great attention to diet, otherwise constipation will occur easily. They all live in FanCheng District, Xiangyang City, Hubei Province.

This study was approved by the Ethics Committee of Xiangyang No.1 People's Hospital, Hubei province. After obtaining informed consent from participants, we collected the trio pedigree with HSCR and age matched 74 healthy control peripheral blood samples from each member. 5 ml venous blood samples were collected and stored in an Ethylene Diamine Tetraacetic Acid (EDTA) anticoagulant tube in a refrigerator at -20°C.

## DNA extraction and Genomic analysis

Genomic DNAs (gDNAs) of the family (one affected and two unaffected members) and 74 healthy control samples were extracted from peripheral blood using TIANGEN Blood DNA extraction kit (article number: DP304-03).

Whole exome sequencing (WES) was performed on propositus(♂-1) in our trio pedigree (Fig. 1) with a mean target coverage of 100x. BGI Exome V4 kit was used to exome capture and sequencing. Firstly, we used cutadapt (v1.15) to trim adaptor sequences at the tail of sequencing reads, and then aligned sequencing reads to human reference genome (UCSC hg19) with BWA (v0.7.15). Duplicated reads were marked by Picard (v2.4.1). Qualimap[16] (v2.2.1) was used to calculate base quality metrics, genome mapping rate, and the coverage of targeted regions. Base quality score recalibration, indel realignment and variants (SNVs & InDels) calling were performed following the best practice protocol of the Genome Analysis Toolkit (GATK, v3.8). Variant filtering was done by a finely tuned in house script. Pass-filter variants were annotated using Pubvar variant annotation engine ([www.pubvar.com](http://www.pubvar.com)) and VEP[17].

In genetic analysis, we separately identified variants that fit the dominant and recessive inheritance models. Variants met anyone of the following criteria were excluded from genetic analysis: maximum population frequency was large than 0.01, genotype confidence was low, or predicted as benign by all five algorithms (SIFT[18], PolyPhen 2[19], MetaSVM[20], MCAP[21] and MutationTaster[22]). The pathogenic evidences of candidate disease-causing variants were scored by InterVar[23] (1.0.8) according to American College of Medical Genetics and Genomics(ACMG) guidelines[24].

Two highly suspicious HSCR pathogenic mutations were found in the propositus(♂-1). One is the common pathogenic mutations c.278G > A (NM\_000514, rs771970145, p.Arg93Gln) of GDNF gene. The other is the rare mutation c.3215A > G (NM\_004963, rs35179392, p.Tyr1072Cys) of GUCY2C gene. Both genes sites have very low frequency of mutation in the 1000 Genomes, gnomAD exomes and East Asian gnomAD exomes individuals( $\leq 0.004$ ). Primer-BLAST (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>) was used to design the primers contain the rs771970145 and rs35179392 (Table 1). Next, PCR was used to amplify and sanger sequence was performed on three people(♀-1, ♂-2, ♂-1) of the pedigree to affirm

GDNF and GUCY2C mutation. We found that the rare mutation of GUCY2C gene was carried by the father of the patient, but the common mutation of GDNF gene was carried by mother (Fig. 2, Table 2).

We identified DGNF and GUCY2C were heterozygous mutations, respectively. According to the evidence classification of ACMG pathogenicity, GDNF belongs to PS1 + PS4 + PM1 + PM2 + PP3, GUCY2C belongs to PM2 + PP2 + PP3. These two mutations were classified as variants of uncertain significance, but computer projections predicted them to be damaging or probably damaging by SIFT, Polyphen2, MetaSVM, MCAP and Mutation Taster(Table 3).

In order to confirm the mutation of mutant gene in healthy population, 74 healthy control samples were collected from physical examination center of XiangYang City First People's Hospital to detect mutant gene. We successfully amplified GDNF rs771970145 gene of 74 samples and GUCY2C rs35179392 of 64 samples by PCR and sanger sequence were used. No rare allele carrier was found at either locus of each sample (Fig. 2, The full sanger sequence results in the Additional file).

## Discussion And Conclusion

Most HSCR is caused by abnormal RET gene. About the RET/GDNF/GFRA1 Signalling Pathway, The RET proto-oncogene was identified as the causative gene for HSCR. In addition, there are human papillary thyroid carcinoma and multiple endocrine neoplasia (MEN) types 2A and 2B[25–28]. One of the ligands for RET is GDNF, which connect to RET via GDNF family receptor α 1(GFRα1). When the ligand and receptor have associated, RET was recruited to cholesterol-rich areas on the cell membrane where it undergoes autophosphorylation, and activation of downstream effector pathways[29]. It was found that mice with homozygous mutation of GDNF gene showed deficiency of kidney and ENS, had similar phenotypes with RET knockout mice. [30, 31]. These studies show that RET/GDNF/GFRA1 signaling Pathway played a crucial role in the development of ENS and renal agenesis or dysplasia.

In our case, the GDNF mutation was inherited from the patient's phenotypically normal mother. But she is prone to constipation. One possibility is that is a chance occurrence, the GDNF mutation does not contribute to the patient's phenotype. We consider this to be unlikely for several reasons: First, this change was not found in 74 control samples from the same race and area, but also not found in other 203 cases[32]. From these, we argue against the existence of missense polymorphisms. Second, Arg93Gln substitutes, different charge properties of two amino acids. Thirdly, this change is found in the mature peptide between the presumptive proteolytic processing side and the seven canonical TGF-B cysteine residues. The last is that heterozygous mutation of GDNF gene lead to the haploinsufficiency. In the study of a large population of mice heterozygous for GDNF null mutation, there are obvious absence of intestinal ganglion cells. This *Gdnf<sup>+/−</sup>* mutant cohort recapitulates complex features characteristic of HSCR, including dominant inheritance, incomplete penetrance, and variable severity of symptoms, HSCR-like phenotypes. As many as one fifth *Gdnf<sup>+/−</sup>* mutant mice die shortly after birth[33]. Moreover, this residue is conserved in human, mouse and rat. The mutation of the same amino acid arginine site has been reported many times before in HSCR patients[32, 34, 35]. Genetic variation in the GDNF promoter

affects its expression and modifies the severity of HSCR in rats carrying Ednrb mutations[36]. In addition, Michael A. et al. found in 33 stillborn fetuses that had bilateral or unilateral renal agenesis existed the mutations of gene RET, GDNF and GFRA1[37]. It may be that in many cases GDNF homozygous mutations cause early fetal demise, GDNF heterozygous mutations Increased risk of non cystic fibrosis meconium ileus (CF-MI). From these studies, we boldly infer that a heterozygous mutation will not sufficiently lead to HSCR and related phenotypes, but will certainly associate with it and increase the possibility of HSCR occurrence. The other gene GUCY2C mutation was inherited from the patient's phenotypically normal father. We focused on the role of GUCY2C in intestinal secretion. When GUCY2C gene is over activated, resulting in elevated levels of cGMP, that elicits a signaling cascade, activated Type I cGMP dependent protein kinase(PKGII) and the cystic fibrosis transmembrane conductance regulator(CFTR), Eventually leading to secretory diarrhea[38, 39]. On the other hand, It has also been reported that the compound heterozygous mutation of GUCY2C is related to non CF-MI[40]. There are research findings when the activity of GUCY2C is reduced or lost, the expression of cGMP will not be up-regulated, and the downstream protein kinase PKGII will not be activated, ultimately causing intestinal secretion dysfunction and non CF-MI[41]. Based on previous studies and our findings, we boldly speculate that GUCY2C activity in a balanced state plays a very important role in intestinal secretion. Its over activation will lead to diarrhea, on the contrary, it will lead to non CF-MI. Different domain mutations have different effects on the function of proteins, which lead to different disease phenotypes. Whether GUCY2C plays a role in the development of intestinal neurons remains to be further studied. Heterozygous for GDNF causes absence or dysfunction of intestinal neuron cell and Heterozygous for GUCY2C causes abnormal intestinal mucosal secretion. Our case suggests that under certain conditions, monogenic diseases may be caused by heterozygous mutations of two genes.

In conclusion, our study found that GDNF and GUCY2C together lead to the occurrence of HSCR. This finding expands the genotype spectrum and increases knowledge on the genetic molecular mechanisms of the HSCR. We provide a new perspective for the pathogenesis of HSCR. We should pay attention to this mutation in pre-pregnancy screening and prenatal diagnosis.

## Abbreviations

HSCR: Hirschsprung's disease; *GDNF*: Glial Cell Derived Neurotrophic Factor; *GUCY2C*: Guanylate Cyclase 2C; NCC: Intestinal neural crest cell; ENS: Enteric nervous system; *RET*: receptor-tyrosine kinase; WES: Whole exome sequencing; ACMG: American College of Medical Genetics and Genomics; GTP: guanosine-5'-triphosphate; cGMP: cyclic Guanosine monophosphate; CF-MI: cystic fibrosis meconium ileus.

## Declarations

### Ethics approval and consent to participate

The Ethical approval for the study was taken from Ethics Committee of Xiangyang No.1 People's Hospital. Written informed consents were obtained from the parents or guardians of the propositus and

74 healthy control cases.

### **Consent for publication**

Written informed consent for publication of individual/genetic data was obtained from the participants or parents/legal guardians of any participant under the age of 18.

### **Availability of data and materials**

All relevant data are within the paper. Further information is available from the authors on request.

### **Competing interest**

The authors declare no competing interest.

### **Funding**

This study was funded by the Natural Science Foundation of Hubei Province. Award Number: 2019AHB068, 2017CFB674, 2018CFB701. Open Project of Hubei Key Laboratory of Wudang Local Chinese Medicine Research. Award Number: WDCM2018009. The Innovative Team Project from the Institute of Medicine and Nursing at Hubei University of Medicine. Award Number: 2017YHKT02. The recipient is Ming Sang for All of the funding. MS is also the corresponding author.

### **Authors' contributions**

MS, SC and KD Conceived and designed the experiments. SC, XS and PF Performed the sample recruitment and Whole exome and sanger sequencing. QZ and QX Conducted the data analysis. MS Provided the reagents/ materials/ analysis tools to carry out the experiments. SC Wrote the paper. All authors read and approved the final manuscript.

### **Acknowledgements**

We thank the patient's family for participating in this study, Kd of Reproductive Medicine Center, Renmin Hospital, Hubei University of Medicine. We thank all the staff of Hubei Institute of Parkinson's Disease at Xiangyang No.1 People's Hospital. They were very helpful.

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## Tables

**Table 1.** The primers contain the rs771970145 and rs35179392

Gene	rs	Primer	Tm	Size
GDNF	rs771970145	F: CACCACCACTAGTCTCCCTCT R: TCTGGGCAAACATTCCCTGGG	60°C	786bp
GUCY2C	rs35179392	F: GCCTCCAATTCCAGCAGCAT R: TTTCTTTCCAGGTAGAAGCTCCC	60°C	668bp

**Table 2.** mutation information of the family members

Family members	Mutant gene	Exon	Reference sequence	Rs	cDNA	Protein
Father	GUCY2C	27	NM_004963.3	rs35179392	c.3215A>G	p.Tyr1072Cys
Mother	GDNF	3	NM_000514.3	rs771970145	c.278G>A	p.Arg93Gln
propositus	GDNF	3	NM_000514.3	rs771970145	c.278G>A	p.Arg93Gln
	GUCY2C	27	NM_004963.3	rs35179392	c.3215A>G	p.Tyr1072Cys

Table 3. computer projections predicted result

Algorithms	GDNF (c.278G>A)	GUCY2C (3215A>G)
SIFT	Tolerated	Damaging
Polyphen2	Possibly damaging	Possibly damaging
MetaSVM	Damaging	Damaging
MCAP	Damaging	Damaging
Mutation Taster	Disease causing	Disease causing

## Figures

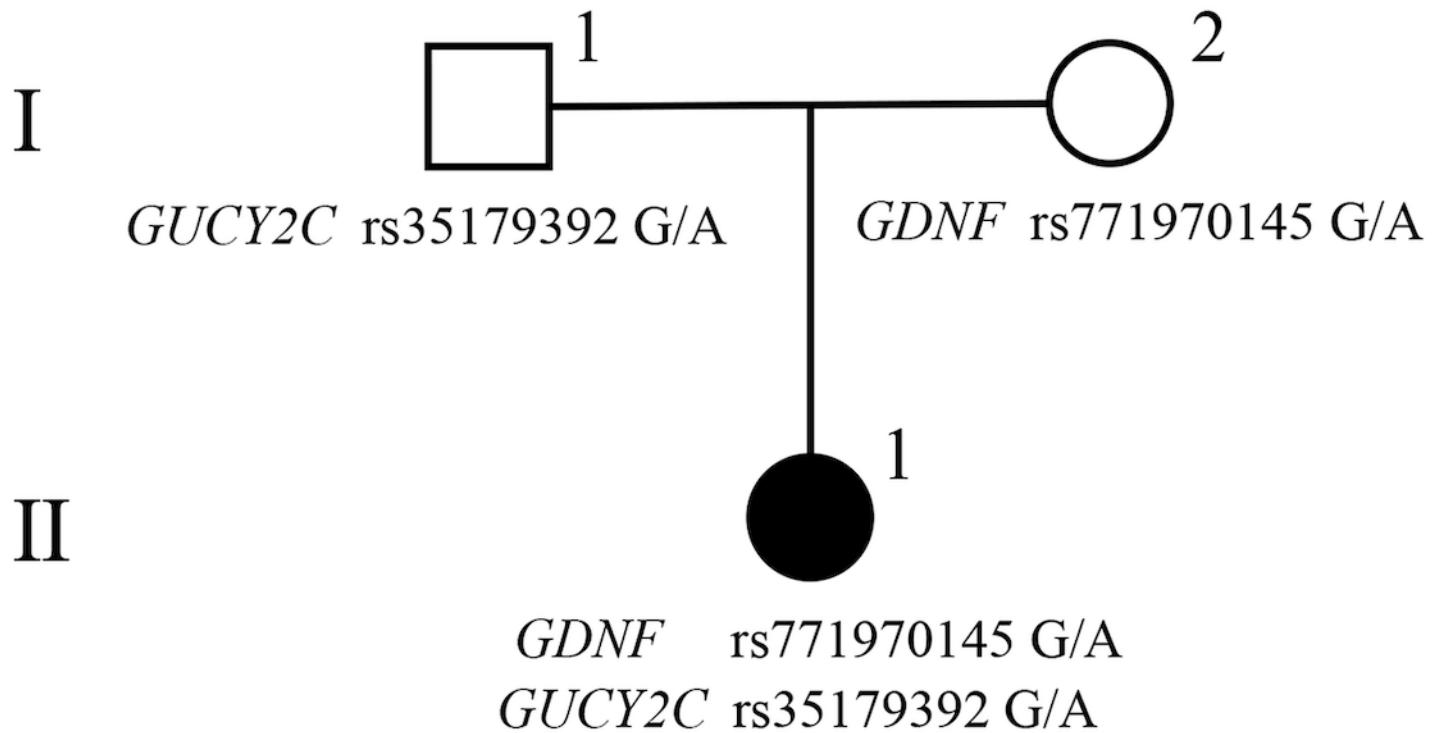
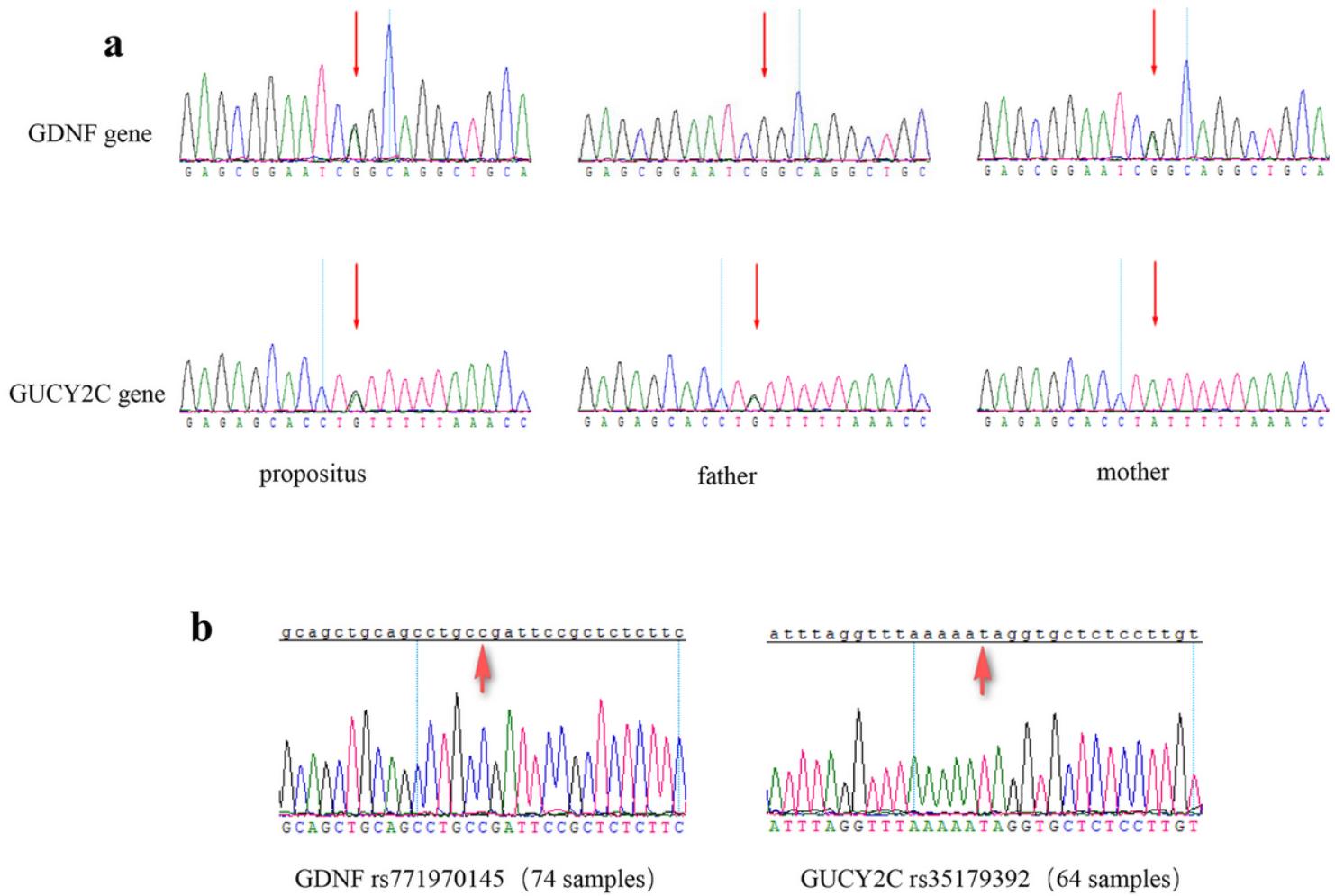


Figure 1

The trio families pedigrees, the mother(✉-2) carry the mutation of GDNF gene, the father(✉-1) carry the mutation of GUCY2C gene, the propositus(✉-1) has both mutation from the mother and father.



**Figure 2**

The Sanger sequence of two pathogenic mutations, PCR was used to amplify and sanger sequence was performed. a: GDNF and GUCY2C was sequenced in three people(♀-1,♂-2,♂-1) of the pedigree to affirm mutation. The mother carry the GDNF mutation and the father carry the GUCY2C mutation. b: 74 healthy control samples were collected to sequence GDNF and GUCY2C mutation site. No rare allele carrier was found.

## Supplementary Files

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