

Exome sequencing identified a novel pathogenic RET variant with high variable expressivity and incomplete penetrance in an extended pedigree with Hirschsprung disease

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Abstract

Background: Hirschsprung disease (HSCR) is a developmental disorder characterized by the absence of ganglion cells in the gastrointestinal tract, which consequences in intestinal obstruction. HSCR has more than 80% heritability, including two major forms as sporadically which is the most common form with a complex pattern of inheritance and other forms appear with a familial/syndromic basis along with Mendelian inheritance and incomplete penetrance. The rare and common sequence variants in genes related to the enteric nervous system (ENS) was identified to play a critical role in the progress of HSCR. **Results:** This study aimed to investigate the genetic basis of an Iranian extended family with different manifestations such as inherited HSCR, chronic constipation, congenital malformations (single kidney and closed anus) to find the causal mutations. To uncover the contributing genetic variant/s, exome sequencing was performed on proband (IV-V). Finally, with the standard filtering protocol, we identified a novel heterozygous nonsense mutation, p.Y314X at exon 5 of the RET gene. This variant is located in the extracellular domain of RET tyrosine kinase and subsequently leads to no detectable RET full-length protein and so may lead to protein loss of function. This devastating variant is manifested in the proband, with long-segment form and other phenotypes such as single kidney, absent of peritoneum and pigmentation of the face. However, this variant has been segregated in the mother (III-IV, with single kidney), the grandmother (II-II, with diarrhea) and the aunt (III-V, with chronic constipation and premature ovarian failure); these members of the family didn't show HSCR. Furthermore, in other members of the family (II-IV and III-XII) with chronic constipation and no HSCR, the variant was detected. Nonetheless, this phenomenon can be interpreted by incomplete or reduced penetrance in this extended family with variable expression.

Conclusions: The present study found a novel null variant at RET gene that is associated with a wide range of phenotypes and incomplete penetrance. This can be synergistic effects of rare and/or common variants among known and/or unknown disease susceptibility genes which lead to variable severity of phenotype. Such information is important for proper genetic counseling in familial HSCR. **Keywords:** Hirschsprung disease, whole-exome sequencing, RET novel mutation, extended pedigree, incomplete penetrance.

Background

Hirschsprung's disease (HSCR) which also known as intestinal aganglionosis is a rare congenital genetic anomaly, characterized by the complete failure of enteric neural crest-derived intestinal ganglion cells to fully migrate, proliferate, or differentiate through the length of the fetal intestines during development. The disease is classified according to the length of the aganglionic segment; short-segment (S-HSCR; 80% of the cases), long-segment (L-HSCR; 15% of the cases), total colonic aganglionosis (TCA; 5% of the cases). The incidence of HSCR is approximately 1 per 5000 live birth. However, it varies significantly among different ethnic groups. The great incidence is reported in the Asian population, 2.8 per 10 000 live birth. HSCR can manifest sporadically in the most common form with highly heritable (> 80%), the complex pattern of inheritance, phenotypic variability by gender male: female ratio (M: F = 4:1), and low sex-dependent penetrance (1–3). But other forms appear with a familial/syndromic basis (30% of the cases) along with autosomal recessive and/or dominant inheritance and incomplete penetrance (4–6).

Genetic studies of HSCR have identified extensive genetic heterogeneity; So far, more than 17 HSCR susceptibility genes (RET, EDNRB, EDN3, ECE1, GDNF, NRTN, SOX10, PHOX2B, ZEB2, TCF4, KBP, L1CAM, SEMA3C, SEMA3D, PSPN, NTF3, NTRK3, ZFH1B, KIAA1279, PROK1, PROKR1, PROKR2, GFRA1, NRG1, NRG3, and DNMT3B) have been known to be associated with the disease (3). These genes are associated with protein components of signaling pathways complicated in the development of the enteric nervous system (ENS). The RET proto-oncogene is the major gene with > 80% of all known mutations that are responsible for nearly 50% of familial and up to 15% of sporadic cases, however other genes may contribute to approximately 10% of the cases (2, 7, 8).

RET encodes a receptor tyrosine kinase that regulates the proliferation, differentiation, and migration of the enteric neural crest cells to enteric neurons System. More than 100 RET mutations have been described in HSCR patients: large deletions, insertions/deletions, missense/nonsense, and splicing mutations. These mutations are scattered throughout the entire coding sequence (CDS) that can be divided into two groups. The first one is nonsense and frameshift mutations result in an early truncation of the protein and the other group is the missense mutations and small in-frame deletions result in a non-functional or minus-functional protein. In general, haploinsufficiency/loss of function is assumed to be a mutational mechanism in HSCR cases (2, 9). However, RET gain-of-function mutations are directly associated with patients who suffered from hereditary thyroid cancers (10). Additional to rare mutations in the RET gene, a common variant of rs2435357, is located in a gut-specific RET enhancer element in intron 1 to disrupt binding of SOX10, act synergistically on Hirschsprung disease risk (11).

Although HSCR is usually detected early after birth, failure to treat this disease is often fatal, therefore, the Determination of functional consequences these mutations and understanding genotype-phenotype correlation can help in better management and can use in prenatal diagnosis (PND) for HSCR in familial type. The study aims to uncover the novel pathogenic genes or variants using whole-exome sequencing (WES) as a high-throughput and cost-effective method to detect genetic defects. The filtering steps include minor allele frequency (MAF), co-

segregation in family, the mode of inheritance, pathogenicity scores by the prediction tools. So, we identified a novel nonsense variant (p.Y314X) in RET as the disease-causing candidate variant for heritable HSCR.

Materials And Methods

Subjects and Ethics Statement

DNA samples from twelve affected and unaffected family members (I-I, I-III, II-II, II-IV, III-IV, III-V, III-VI, III-IX, III-XII, IV-V, IV-VII, and IV-X) are collected for the present study. Whole exome sequencing was performed only on proband IV-V and identified variants confirmed by Sanger sequencing in other mentioned family members. The study approved by the Research Ethics Committee of Iran University of Medical Sciences. ALL subjects of the study signed the informed consent.

Sequencing Alignment And Variant Calling

DNA extraction was performed from a peripheral blood sample of the mentioned patients and healthy individuals of the family using the Salting out procedure (12). Whole Exome Sequencing (WES) was performed on patient IV-V using Agilent SureSelect V6 Target Enrichment Kit. Paired-end sequencing on reads of 150 bp using IlluminaHiSeq 4000 platform was done. The reads were aligned to the human reference genome (GRCh37/hg19 assembly) using BWA (Li and Durbin, 2010) version 0.7.12 and then duplicate reads and low-quality reads were removed (QBase < 20). Single nucleotide variants (SNVs) and short insertions or deletions (Indels) were called using the GATK (<https://www.broadinstitute.org/gatk/>) software package.

Rare Damaging Variant Filtering

We annotated VCF data using Wannovar tool (<http://wannovar.wglab.org>). Our workflow of variant filtering is shown in Fig. 1. In the beginning, the annotated file prioritized by an in house pipeline with the mode of zygosity (homozygous and heterozygous variants). Then, filtering common variants with minor allele frequency > 0.05 in dbSNP137, 1000 Genomes database, NHLBI Exome Sequencing Project, ExAC database, and gnomAD database was done. The potential impact of rare coding sequence variants on the function or structure of the protein and conservation directed by bioinformatics tools including SIFT (13, 14), PolyPhen2 (15), Mutation Taster (16), Combined Annotation Dependent Depletion (CADD) (17), DANN score (18) and GERP score (19). Among the prioritized variants; nonsense, splice site, frameshift, in-frameshift, and missense in the known HSCR genes (PubMed and OMIM were searched for ranking the genes related to HSCR) were selected and checked in HGMD and ClinVar. The identified variants were classified and interpreted according to the ACMG-AMP 2015 Standards and Guidelines (20) that facilitated by the Varsome tool (21).

Validation Of The Identified Mutations

The identified variant was validated by Sanger sequencing in the proband IV-V and segregation analysis was performed in the parents and other affected and unaffected members of the family. For this purpose, primers of the specific exons were designed using Primer3 (<http://bioinfo.ut.ee/primer3-0.4.0/>) and PCR products directed to sequencing with ABI 3500 Genetic Analyser (Applied Biosystems, Foster City, CA, USA). Chromas v2.33 software was used for data analysis of sequencing results (Technelysium, Tewantin, Qld, Australia).

Results

Clinical Features of Patients

The proband IV-V was a 10-year old girl who suffered from Hirschsprung disease and congenital right kidney aplasia. Her birth measurements (weight: 3000 g and length: 48 cm) and the neonatal history were normal at birth. But after 6 months, she had the symptoms of abdominal distension, vomiting, diarrhea, fever and chronic constipation that leads to hospitalization. Histological examination of the proband showed aganglionic myenteric plexus, rectal, sigmoid, splenic flexure large intestinal and appendix involvement that was diagnosed as HSCR. Then she underwent colectomy and gave antibiotic drugs. More investigation in family history revealed that the proband's mother (III-IV) has a single kidney. The subjects II-III, II-V, II-VII, III-VIII and III-X died after birth with congenital malformations such as closed anus and intestine obstruction. Furthermore, other family members (I-II, II-IV, II-VI III-V, III-XII) suffered from severe chronic constipation throughout their lives. Figure 2 presented the family pedigree and other abnormalities along with HSCR in family members.

Rare Variants In Coding Or Splicing Regions

Whole exome sequencing was performed for finding the genetic variants related to HSCR on the IV-V proband. A 7.2 Giga base sequence was generated and Minimum coverage of target regions (exons and the first and last 20 bps of introns) were represented 97.7, 96.2, and 92.2% for the 1X, 5X, and 10X reads, respectively. As shown in the following Fig. 1, totally we detected 113,077 variants in the VCF file of proband which include 103,620 substitutions and 9,200 indels (insertion/deletion) variations in the exome analysis. We performed the filtering of variants according to frequently found among populations, zygosity (pattern inheritance of HSCR could be autosomal dominant and recessive) and pathogenic impacts of variants using bioinformatics tools such as CADD-PHRED score and mutation taster and so on. Firstly, filtering based on zygosity, we identified 53,063 homozygous and 59,839 heterozygous variants. Then to detect the rare variants, we gained MAFs from population databases and then obtained a total of 1849 rare variants with inside mentioned mode of inheritance (MAF < 0.05).

Classification/annotation Of Novel Variations

Multiple lines of in silico-analysis were performed to evaluate the pathogenicity of dominant and recessive variants. The variants with CADD-PHRED score > 10, mutation taster (disease-causing), pathogenic scores in DANN Score (the value range is 0 to 1), and conservation prediction tools such as GERP-score (ranging from - 12.3 to 6.17) were selected. Finally, a novel heterozygous stop gained variant p.Y314X or c.C942A in exon 5 of the RET gene was identified in the proband IV-V (NM_020975, chr10-43601898).

Bioinformatics prediction analysis by VARSOME (21) and the manual examination of the ACMG-AMP 2015 guidelines (20) considered this variant as pathogenic: (1) single base exchange causing a premature termination codon (a null variant leading to nonsense-mediated mRNA decay (NMD or truncated protein)), which by itself is very strong evidence in favor of pathogenicity (PVS1); (2) this variant absent from controls in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium, and GenomAD databases (PM2) and (3) Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.) (PP3). The results for comprehensive in silico-analysis was shown in Table 1. We validated this variant by Sanger sequencing on proband and followed by co-segregation analysis in all available family members.

Table 1
In silico computational analysis and frequency in population databases for the identified variant (p.Y314X or c.C942A) in the IV-V proband.

Variant Definition	Variant name Protein Change Variant Location Chromosome Position (GRCh37) Zygosity	c.942C > A p.Y314X Exon 5 Chr10 : 43601898 Heterozygote
In-silico Predictive Tools	CADD (Phred Score) DANN GERP Mutation Taster	36 (Deleterious) 0.99 5.3 Disease-Causing
Population Databases	1000 GP ExAC GenomAD ESP	- - - -

Other Rare Variants In Hirschsprung Disease-associated Genes

Several rare variants (MAF < 0.05) were identified in the patient (IV-V) which are associated with other HSCR associated genes (literature reviews and original articles at PubMed, OMIM and phenolyzer were searched for ranking the genes related to HSCR). Table 2 shows comprehensive in silico-analysis, allele frequency in GenomAD and ACMG prediction analysis by VARSOME.

Table 2
Other filtered rare variants in identified HSCR associated genes.

Gene names	Chromosome	Position	Variant name	DANN	GERP	MAF in GenomAD	CADD	Mutation Taster	SIFT	Varsome (ACMG score)
NOTCH1	9	139401233	c.3836G > A, p.R1279H	0.99	4	0.0152	23	D	Tolerated	B
MYH9	22	36697694	c.2517G > A, p.Q839=	0.87	2.4	0.000223	16	-	-	B
POLE	12	133202740	c.6494G > A, p.R2165H	0.99	3.9	0.00468	26	D	Damaging	B
IFIH1	2	163144694	c.1046A > G, p.K349R	0.99	5.9	0.000892	22	D	Tolerated	B
FLNA	X	153586860	c.4551G > C, p.Gly1517=	0.69	2	0.0000924	11	-	-	B
MITF	3	69986984	c.366C > T, p.His122=	0.62	4.6	0.00178	11	-	-	B
SF3B4	1	149899116	c.105G > A, p.Gln35=	0.93	4.9	0.0000257	22	-	-	B
ARAF	X	47428231	c.1046A > G, p.K349R	0.99	5.9	0.000892	16	D	Tolerated	LB
CHD6	20	40074369	c.3813G > A, p.Glu1271=	0.55	3.9	0.000796	10	-	-	LB
FANCI	15	89804072	c.286G > A, p.Glu96Lys	0.99	5.4	0.00111	34	D	Damaging	LB
TSHR	14	81610235	c.1833G > A, p.P611=	0.85	2.5	GnomAD exomes = 0.0000199	15	-	-	LB
LAMA1	18	7007194	c.4204C > T, p.P1402S	0.99	5.8	0.0000956	26	D	Damaging	LB
PRICKLE2	3	64145697	c.315C > T, p.S105=	0.76	3.9	0.0000257	12	-	-	LB
RAI1	17	17700989	c.4727G > A, p.R1576H	0.99	4.7	0.000012	28	D	Damaging	VUS
GPR98	5	89977185	c.5578G > A, p.A1860T	0.99	5.8	0.000351	29	D	Damaging	VUS
SACS	13	23912136	c.5879G > T, p.C1960F	0.99	5.7	Variant not found	24	D	Damaging	VUS
MACF1	1	39763362	c.2441C > G, p.S814C	0.98	6	GnomAD exomes = 0.000131	24	D/P	Damaging	VUS

These heterozygous variants were detected after filtering values including allele frequency (minor allele frequency < 0.05 in GenomAD databases) and CADD score > 10. The potential impact of these variants on the function or structure of the protein and conservation concentrating by bioinformatics tools including SIFT (Tolerated/Damaging), Mutation Taster (Polymorphism/Disease causing), GERP (ranging from - 12.3 to 6.17, with 6.17 being the most conserved) and DANN (the value range is 0 to 1, with 1 given to the variants predicted to be the most damaging). Bioinformatics prediction analysis was performed by VARSOME based on the manual examination of the ACMG-AMP 2015 guideline; B: benign, LB: Likely benign and VUS: variant of uncertain significance.

Gene names	Chromosome	Position	Variant name	DANN	GERP	MAF in GenomAD	CADD	Mutation Taster	SIFT	Varsome (ACMG score)
MDN1	6	90359793	c.16156T > C, p.C5386G	0.94	5.8	Variant not found in GnomAD	30	D	Damaging	VUS
NOTCH2	9	120458867	c.6478T > A, p.Y2160N	0.97	5.7	Variant not found in GnomAD	22	D	Damaging	VUS
RERE	1	8421866 8415606	c.1973C > T, p.T658M c.4540G > A, p.A960T	0.99 0.99	5.4 5.9	0.0000386 Variant not found	25 32	D D	Damaging Damaging	VUS VUS

These heterozygous variants were detected after filtering values including allele frequency (minor allele frequency < 0.05 in GenomAD databases) and CADD score > 10. The potential impact of these variants on the function or structure of the protein and conservation concentrating by bioinformatics tools including SIFT (Tolerated/Damaging), Mutation Taster (Polymorphism/Disease causing), GERP (ranging from -12.3 to 6.17, with 6.17 being the most conserved) and DANN (the value range is 0 to 1, with 1 given to the variants predicted to be the most damaging). Bioinformatics prediction analysis was performed by VARSOME based on the manual examination of the ACMG-AMP 2015 guideline; B: benign, LB: Likely benign and VUS: variant of uncertain significance.

Discussion

HSCR is a complex and heterogeneous disorder with a wide spectrum of mutations responsible for HSCR. RET is considered to be the main participant gene for HSCR (2, 22, 23). The identified mutations of RET mainly include loss-of-function (LOF) mutations and de novo mutations, accounting for >80% of known pathogenic mutations in HSCR (24). A wide range of identified mutations scattered all over the full coding sequence (CDS) in RET (2). These RET CDS mutations have been account for 20% of the sporadic cases and in up to 50% in familial cases (3).

As yet, over 200 CDS rare variants in HSCR have been described (). These identified rare variants can be divided into two groups: null variants such as nonsense mutation, frame-shift, Indels or canonical ± 1 or 2 splice site variants that produce a truncated protein and variant of uncertain significance (less functional or nonfunctional protein) such as most missense mutations and small in-frame deletions. In general, these variants mainly cause loss-of-function of the RET protein (haploinsufficiency assumed to be mutational mechanism) in HSCR (9, 25–28). RET gene in human contains 21 exons that encodes a tyrosine kinase receptor with a cadherin-like extracellular domain, a cysteine-rich region, and an intercellular tyrosine kinase domain. Based on a functional assessment and location of variants, the RET mutations are classified as follows. First; mutations in coding sequences that code for the extracellular domain of RET that mutation will affect the transport of RET to the plasma membrane during translation of the protein. Second; mutations in the cysteine-rich domain of RET make a covalent dimerization of RET upon ligand activation and diminish its localization at the plasma membrane. Third; mutations targeting the kinase domain of RET that abolished RET tyrosine kinase activity. Fourth; mutations at the C-terminal end that change signaling over alteration of binding proteins, Fifth: mutations in regulatory sequences (promoter and intron 1) that reduce RET transcription. These mutations affect protein function and accordingly lead to HSCR (29).

In HSCR, two types of variants (rare and common) in RET have been observed which have a major role in the manifestation of the disease. The common variants in RET have been mainly identified in the commonest form of HSCR (S-HSCR and sporadic forms), and the rare variants in CDS of RET are often found in more severe forms of HSCR (L/TCA-HSCR and familial) (3). Unlike common variants, rare destructive variants normally have a profound effect on the risk of developing the disease and have higher penetrance. Rare damaging variants are negatively selected, and they have to be newly (de novo) generated and accumulated to exert its destructive effect on a population level (30). Since, enormous amount of genomic data has been usually generated by NGS technology (especially WES service) planning an experiment with 2 or more subjects may help to determine significant disease-causing variants, and this would probably ease the difficulty of interpreting such rare variants (31). Regarding HSCR, more than 17 HSCR susceptibility genes and three signaling pathways have been identified concerning the etiology of the disease. A comprehensive understanding of the genetics of an inherited rare complex disease is a major challenge requiring further efforts. In the present study, we performed WES on a proband (IV-V) of an extended family with variable expressivity. We found a novel nonsense mutation (c.942C > A, p.Y314X) as a null variant at exon 5 of the RET gene that is associated with a wide range of phenotypes and incomplete penetrance.

This mutation, p.Y314X, may lead to a truncated protein without protein kinase critical domain as mentioned above. In general, nonsense variants are associated with severe pathogenic impact in the genes with loss of function mechanism. This devastating variant is located in a gene with an autosomal dominant mode of inheritance, which may lead to protein loss of function. The patient IV-V with the identified causative variant had other phenotypes such as single kidney, absent of peritoneum and pigmentation of the face in addition to HSCR, in which these manifestations explain variable expression in the disease. However, this variant has been segregated in her mother (III-IV), grandmother (II-II) and aunt (III-V) of the patient (IV-V), these members of the family don't have shown HSCR. The single kidney and diarrhea were manifestations in III-IV and II-II, respectively. Also, chronic constipation and premature ovarian failure were observed in III-V. Furthermore, in the other members of the family (II-IV and III-XII) with chronic constipation and without HSCR, the novel variant p.Y314X was detected. This phenomenon described by incomplete or reduced penetrance. In a meta-analysis study of RET gene in Hirschsprung disease by Puri P and Tomuschat C, it is suggested that carrying one pathogenic RET mutation maybe share a little role in genotype-phenotype correlation on its own and that these variabilities can be more dependent upon genotypes at different loci and/or environmental factors (32, 33). As mentioned, HSCR as a complex disease is influenced by many mutations, each of which might have only a small effect (34, 35). Also, RET mutations include rare high penetrant variants (at coding sequence) and common low-penetrant variants (at introns and the promoter regions) that seem to act in a synergistic way predisposing to HSCR phenotype (8, 36–38). Another explanation could be that the majority of HSCR cases without RET rare and common variants or the presence of additive phenotypes in the family history of this study might be explained by yet unidentified mutational events in the known HSCR genes or unknown genes, acting alone or in combination. In addition to modifying genes, environmental and epigenetic factors can be described as variable expression and incomplete penetrance in multifactorial diseases such as HSCR (39). Ultimately, other members in the pedigree of family (II-III, II-V, II-VII, III-VIII and III-X) died at the birth with manifestation/congenital malformations such as closed anus.

Also, we identified some rare benign and VUS variants (heterozygous mode) in other HSCR related genes (Table 2). As mentioned above, several variants (common and rare variants) with additive effects contribute to Hirschsprung disease as a complex phenotype that could be observed with other phenotypes. So, the impact or pathogenicity of these variants should be followed by a burden test in control and patients, and functional studies in zebrafish models.

Also, approximately 18% of cases with HSCR co-occurs with other congenital malformations (40). These are often Gastrointestinal anomalies (intestinal malrotation, imperforate anus), genitourinary anomalies (cryptorchidism, inguinal hernia, hypospadias, renal malformation), cardiac anomalies (such as atrial septal defect, ventricular septal defect, patent ductus arteriosus, tetralogy of Fallot), and central nervous system anomalies (intellectual disability and microcephaly) (41). In this study, in addition to HSCR, the history of the family was shown other manifestations/malformations such as single kidney, closed anus, premature ovarian failure, chronic constipation, and so on. However, RET mutations (rare or/and common variants) plays a major role in both isolated and syndromic HSCR. Various authors have described HSCR occurrence does not seem to depend on the RET genotype alone but 1 or more other genes can be contributed in HSCR expression (42, 43). Other studies revealed that the common gene networks such as RET/GDNF signaling pathway participates in the development of the enteric nervous system and also kidneys formation. Subsequently, RET mutations can be recognized in a variety of congenital abnormalities with isolated HSCR, isolated congenital anomalies of kidney or urinary tract (CAKUT) and HSCR together (44). For example, in one case report with total colonic aganglionosis as well as right renal agenesis and oligomeganephronia, gene study in this patient revealed a heterozygous p.S811F mutation in exon 14 of RET. Also, in our study, the proband IV-V and her mother (III-IV) indicated a single kidney and HCSR together with identified p.Y314X mutation in exon 5.

Conclusions

In this study, Data from the first whole-exome sequencing in Iranian familial HSCR suggest the novel deleterious RET variant reaffirming the vital role of RET in this affected of members in the family. In this regard, to increase the number of genes identified in Hirschsprung disease with variable expression and additive effects of rare and common variants, our results show that even in extended families with variable expressivity of phenotypes and existence of incomplete penetrance, it is appropriate to be taken into account (high genetic complexity) when counseling and performing genetic tests for disorders with a presumed multifactorial etiology. Like this family with multiple affected family members and known deleterious mutation, genetic counseling should be considered as part of the disease management. Investigation on other genetic variants in noncoding regions of the genome using whole-genome sequencing, assessments of their functional significance and as well research on environmental or other factors would be related not only for identifying penetrance and variability of the Hirschsprung disease presentations but also for providing accurate genetic counseling.

Abbreviations

HSCR: Hirschsprung disease

ENS: enteric nervous system

MAF: minor allele frequency

S-HSCR: short-segment

L-HSCR: long-segment

TCA: total colonic aganglionosis

CDS: entire coding sequence

PND: prenatal diagnosis

WES: whole-exome sequencing

Indels: short insertions or deletions

SNVs: Single nucleotide variants

CADD: Depletion Combined Annotation Dependent

LOF: loss-of-function

Declarations

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Availability of data and materials

The datasets are available from the corresponding author on a reasonable request.

Authors' contributions

EM performed analysis and interpretation of the data and drafting the manuscript.

RS was involved in drafting the manuscript and data collection.

SH was involved in the analysis and interpretation of the data and in-silico analysis of datasets.

AB contributed to patients' assessments and data collection.

AM designed and supervised the study and in drafting and finalizing the manuscript.

All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The study protocol was approved by the ethical committee of the Iran University of Medical Science. The study consent forms have been attained from the participants and all participants signed informed consent forms.

Consent for publication

The consent to publish has been obtained from the participant to report individual patient data.

Competing interests

The authors declare that they have no competing interests.

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Figures

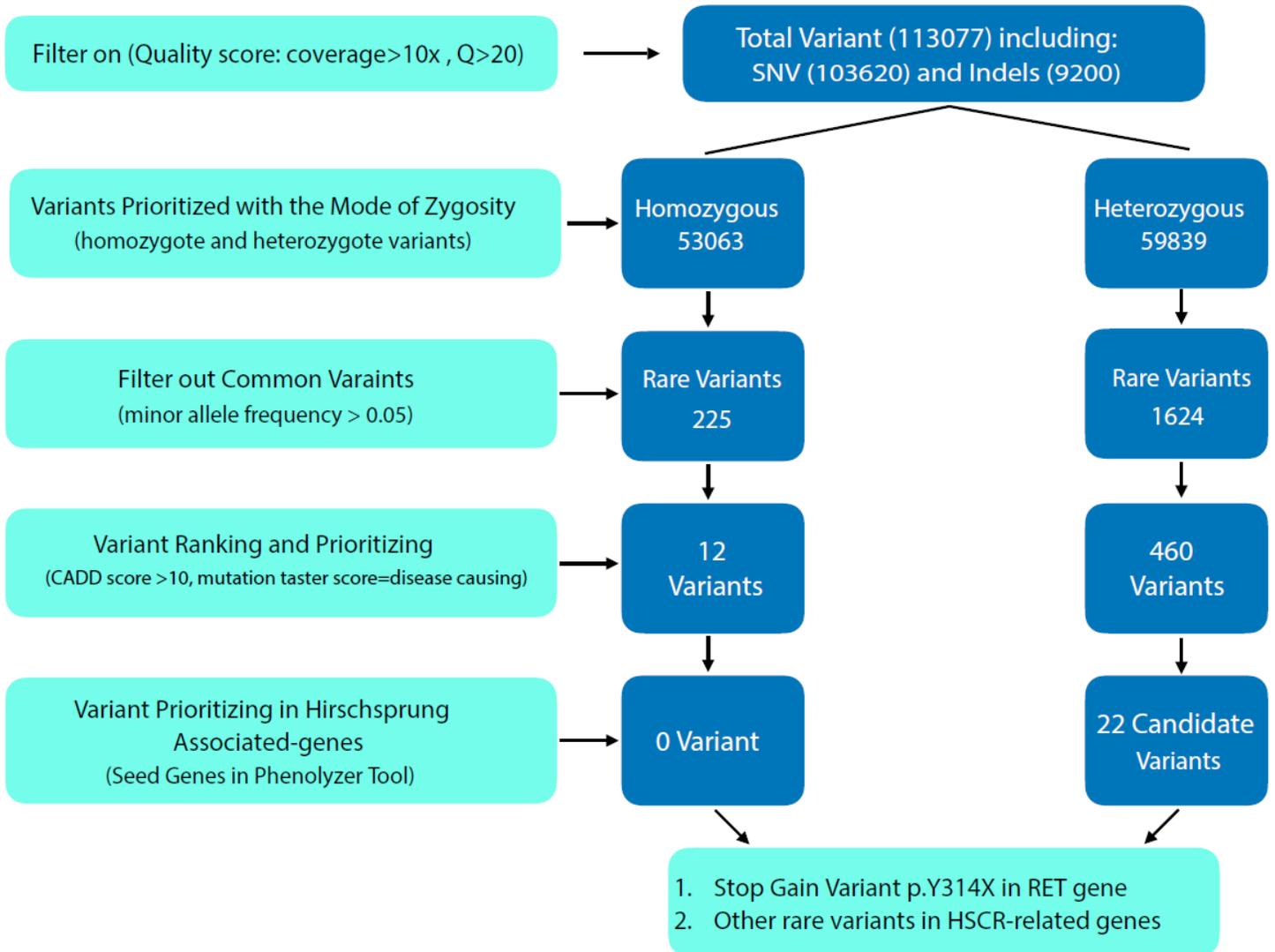


Figure 1

workflow of variant filtering

■ HSCR
 ■ Single Kidney
 ■ Constipation
 ■ Diarrhea
 ● Pigmentation of the face
 ■ Premature ovarian failure
 ■ Closed anal end
 ■ Absent of peritoneum

NO The unidentified variant in the patient.

YES The identified variant in the patient.

HR HSCR confirmation by histopathological study.

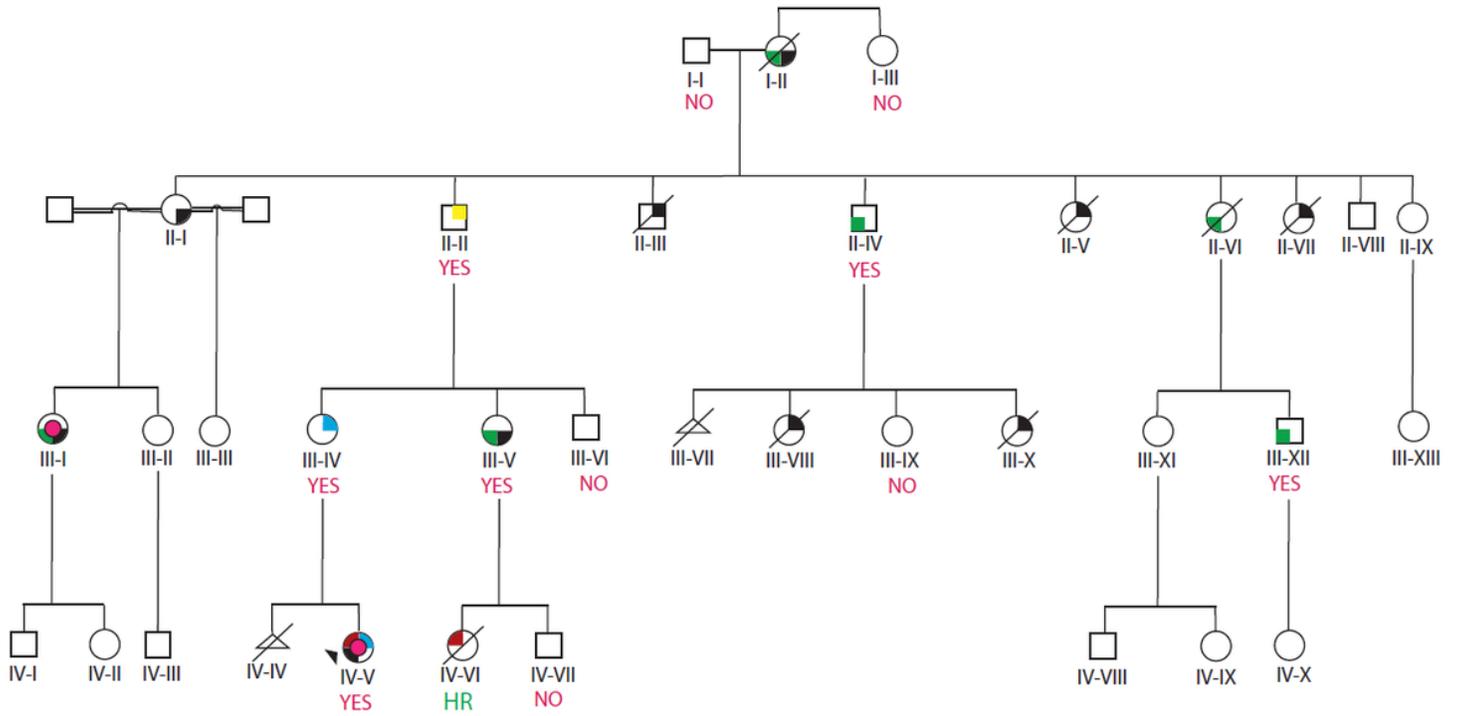


Figure 2

family pedigree and other abnormalities along with HSCR in family members