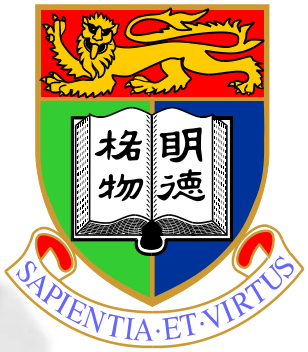
Project 01121516

Uncovering the genetic lesions underlying the most severe form of Hirschsprung (HSCR) disease by whole genome sequencing (WGS): a pilot study in 8 family trios



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What is Hirschsprung disease (HSCR)?:

- Congenital disorder ▸ abnormal development of the gut
- Lack of ganglion cells in the distal gut ▸ no peristalsis
- Surgery only available treatment ▸ lethal if untreated
- Heterogeneous phenotype
- Boys 4 times more affected than males

Aganglionic segment

Doctors remove 13kg of faeces from constipated man in China

22-year-old diagnosed with Hirschsprung's disease said he'd been suffering from constipation, stomach pain all his life

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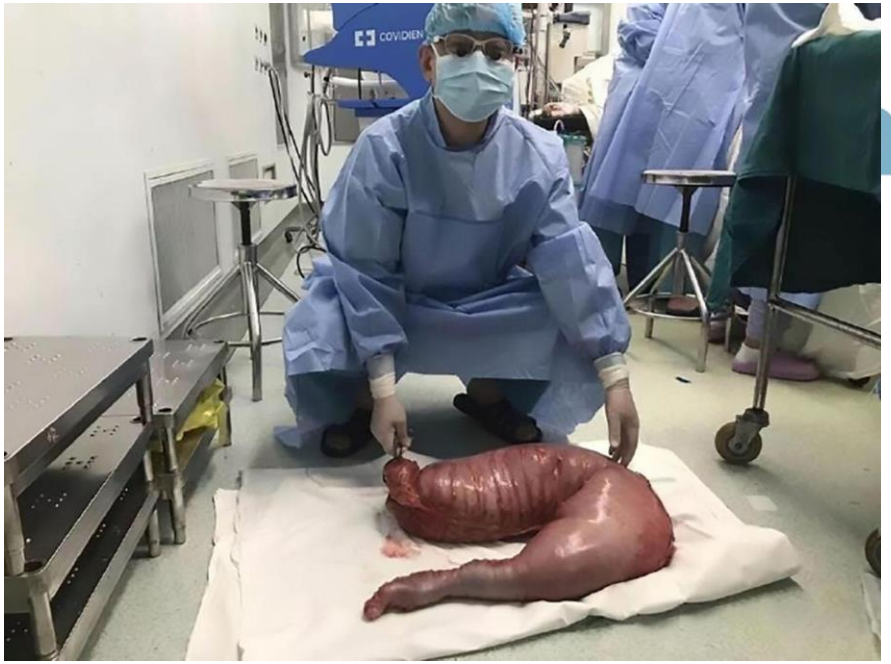
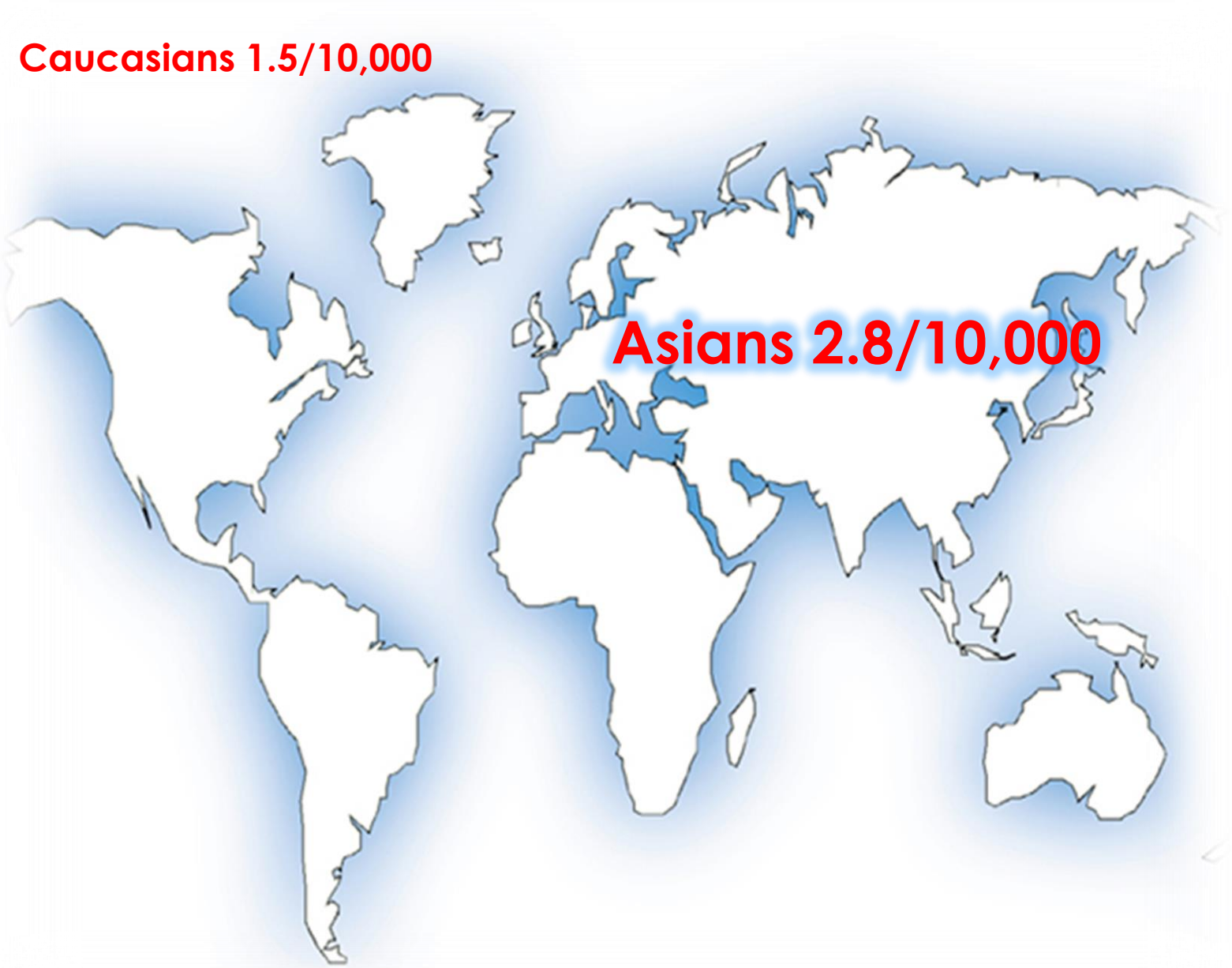
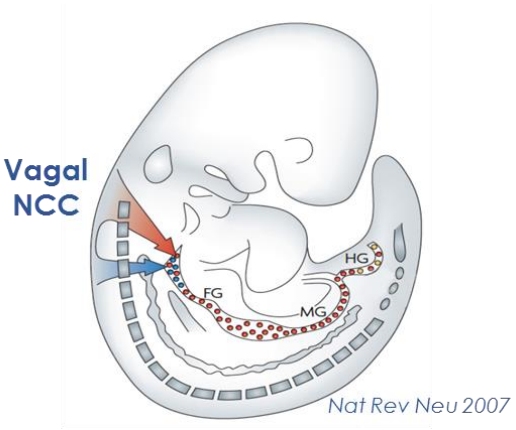



Caucasians 1.5/10,000
Asians 2.8/10,000

How/why does it happen?

Neural crest cells (NCC) give rise to enteric neurons (EN)

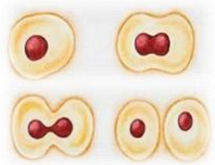


Perturbation

▼

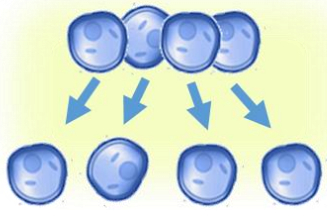
HSCR

Proliferation



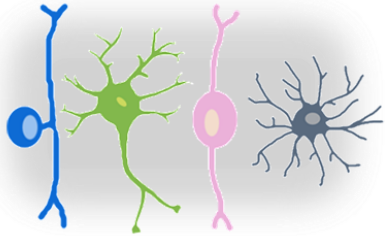
Correct population size of stem cells

Migration



Complete colonization

Differentiation

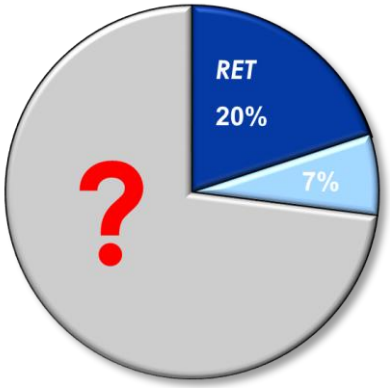


Full diversity of neurons & glia

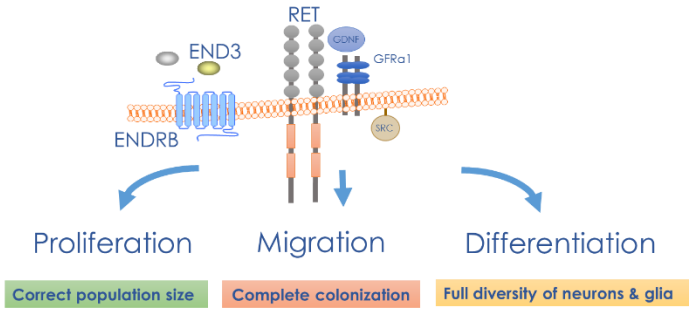
DNA variants impairing genes involved in EN development

What do we know about the genetic risk factors?

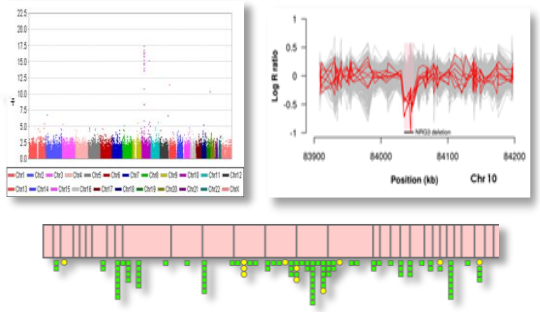
RET main gene



Other genes of interacting pathways involved in ENS



Common and rare variants involved



Oligogenic

Genetically heterogeneous

Heterogeneous phenotype

- Long segment (20%)
- Short segment (80)

Variable presentation

- Familial (20%)
- Sporadic (80)

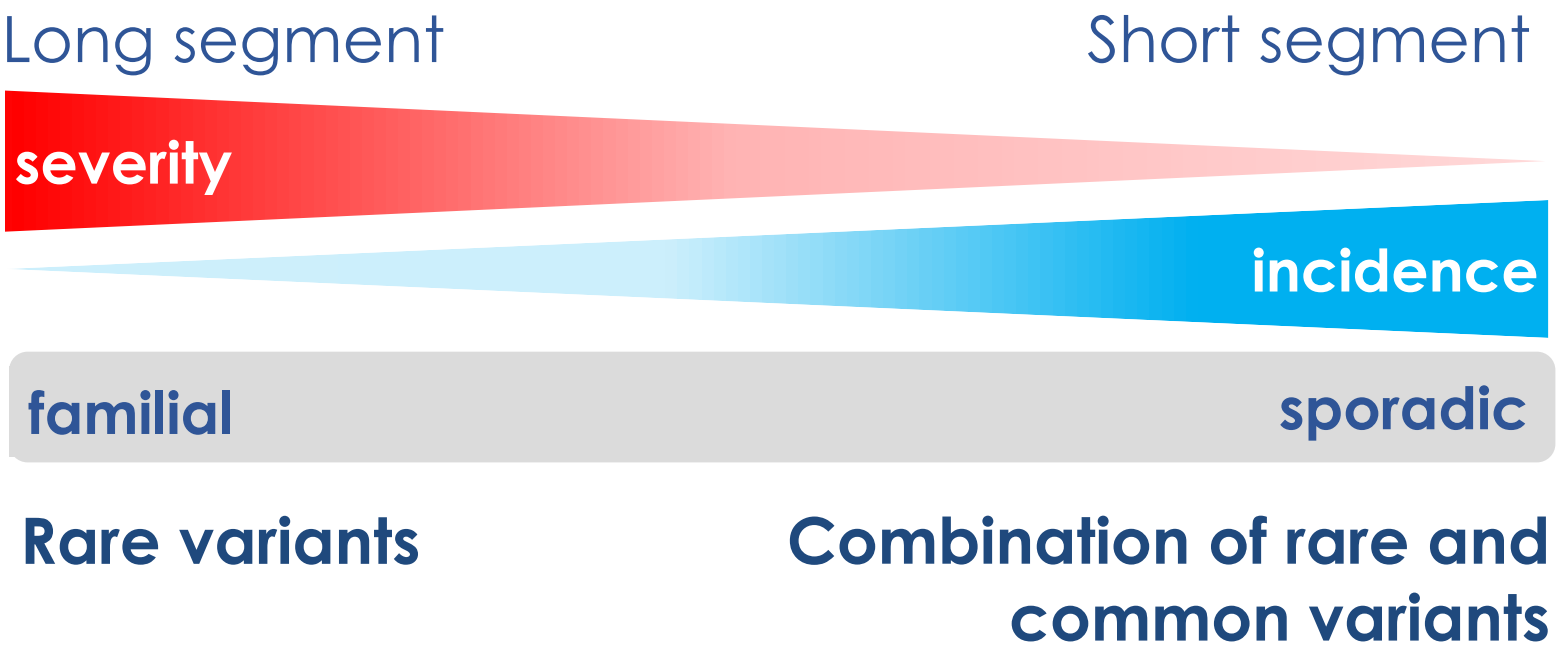
Various disease mechanisms

Affecting either:

- Proliferation
- Migration
- Differentiation

What else do we know about the genetic risk factors?

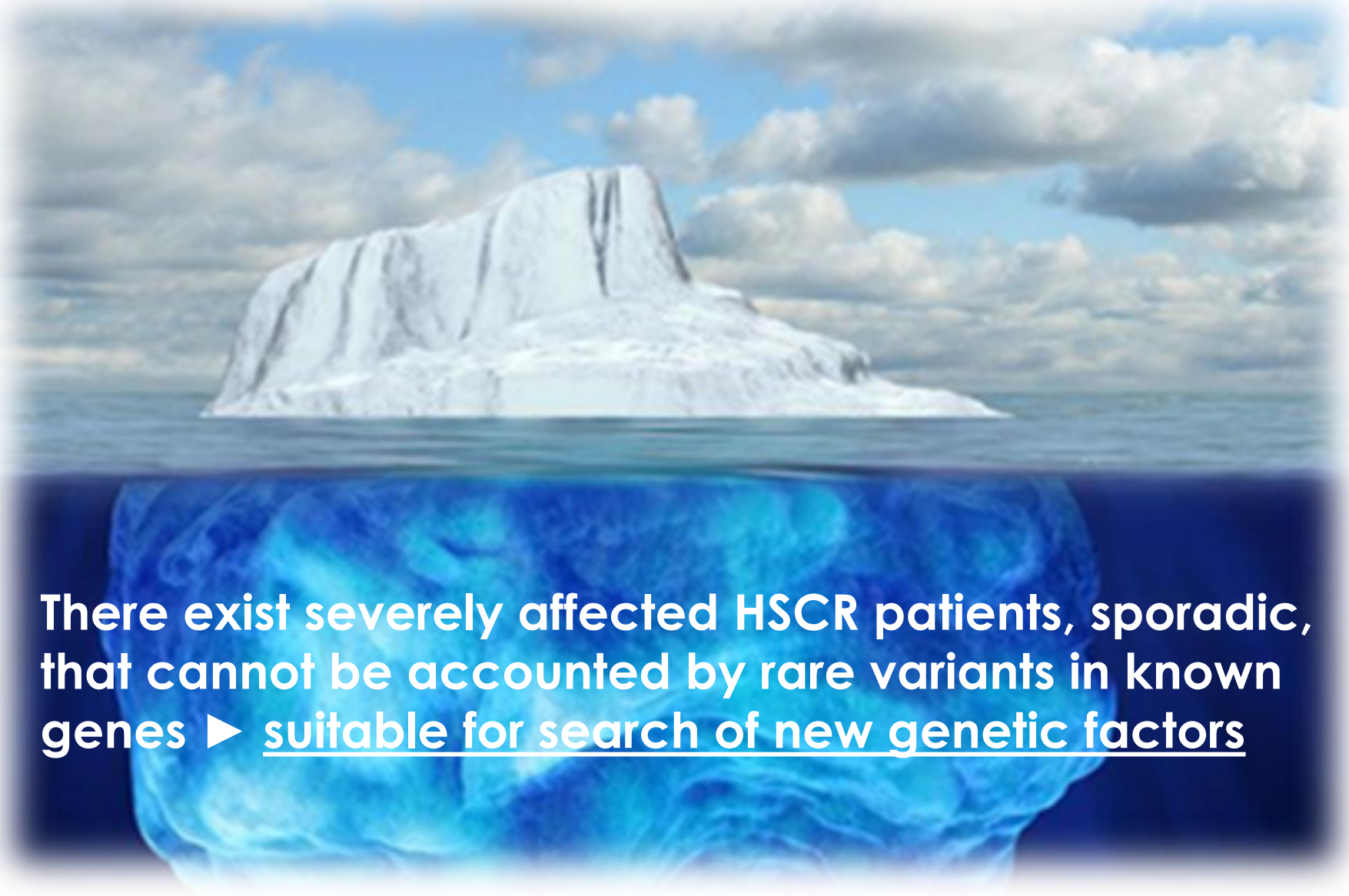
From our and others' observations:



Variants:

- Any type (small/large variants)
- Anywhere in the genome ►relevance of regulatory processes during development

Not all patients are accounted for by the known genes



- HSCR patients affected with the **most severe phenotype**
- Born to unaffected patients ► **sporadic**
- **Devoid** of damaging variants in the coding sequences of known HSCR genes
- Whole genome sequencing
- Trio-based approach ► **detection of *de novo* variants**

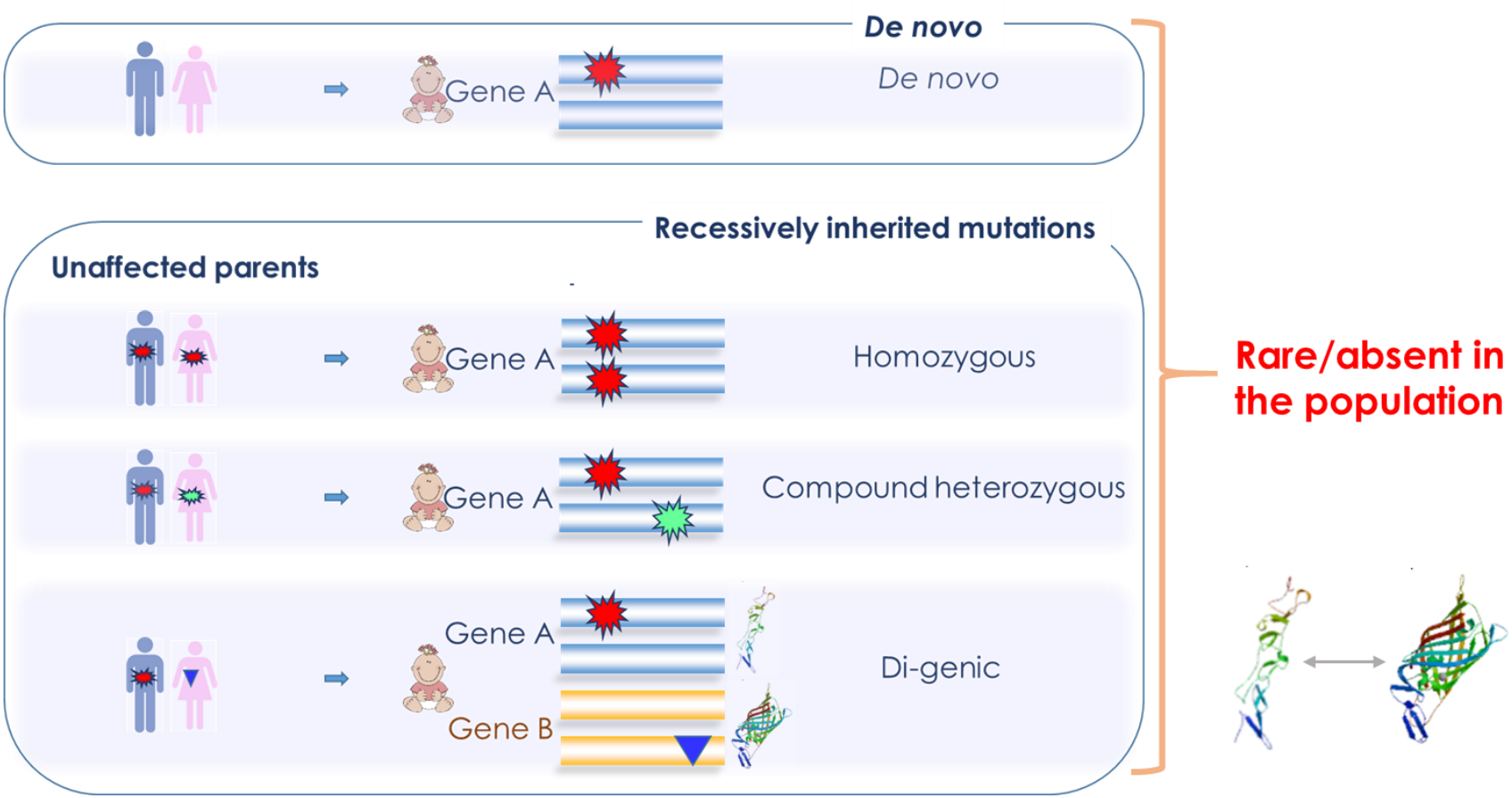
Why whole genome sequencing?

To cover in both coding and regulatory regions:

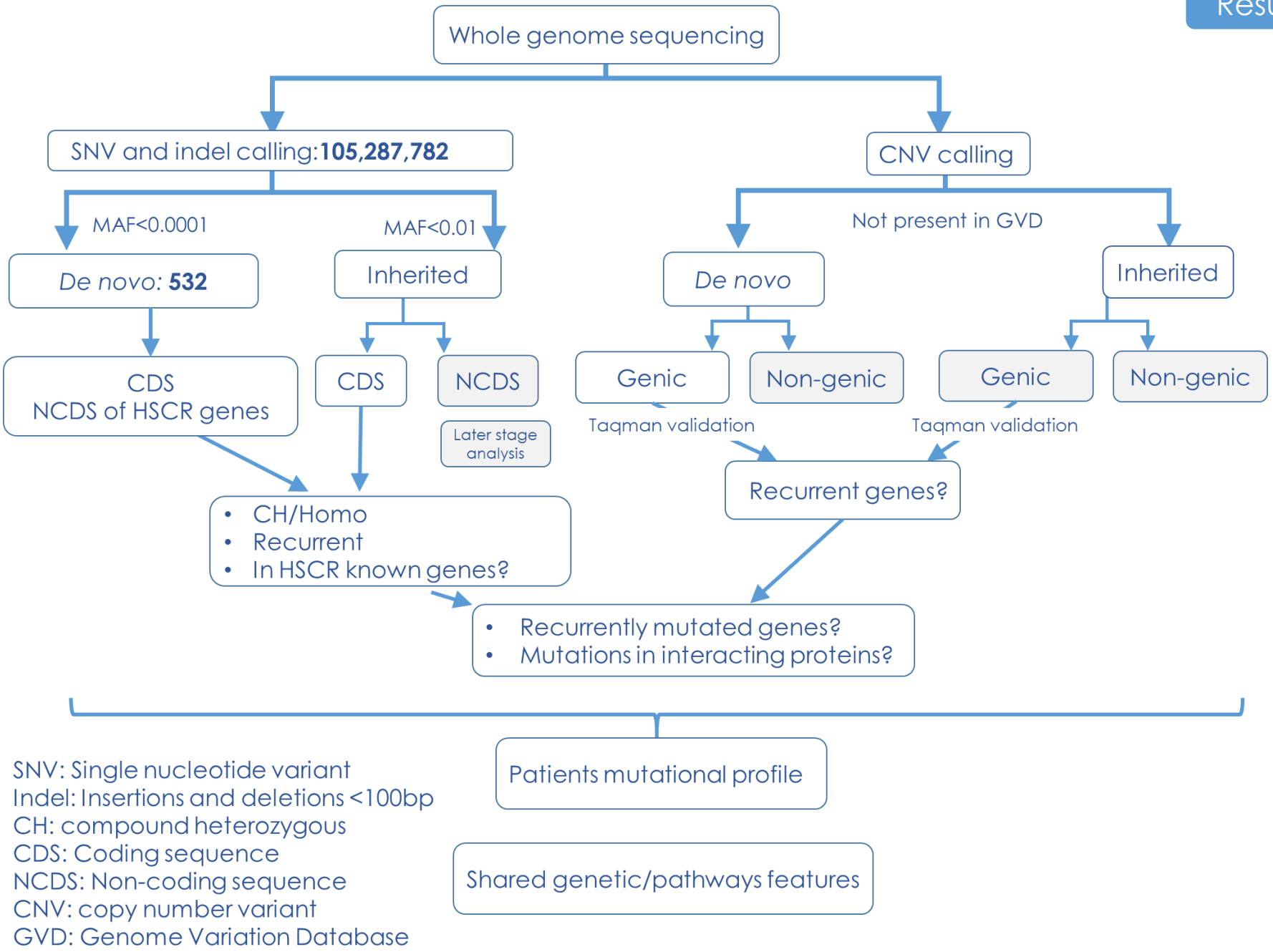
- Single nucleotide variants (SNV)
- Small deletions and insertions (Indels)
- Rare structural variations/Copy number variants (CNV)

Variants \ Platform	Common variants array	Whole exome sequencing (WES)	Whole genome sequencing (WGS)
Common variants	✔	✔ (only coding)	✔
Rare variants		✔ (only coding)	✔
CNV	✔		✔

Long segment HSCR = Rare + Sporadic disorder

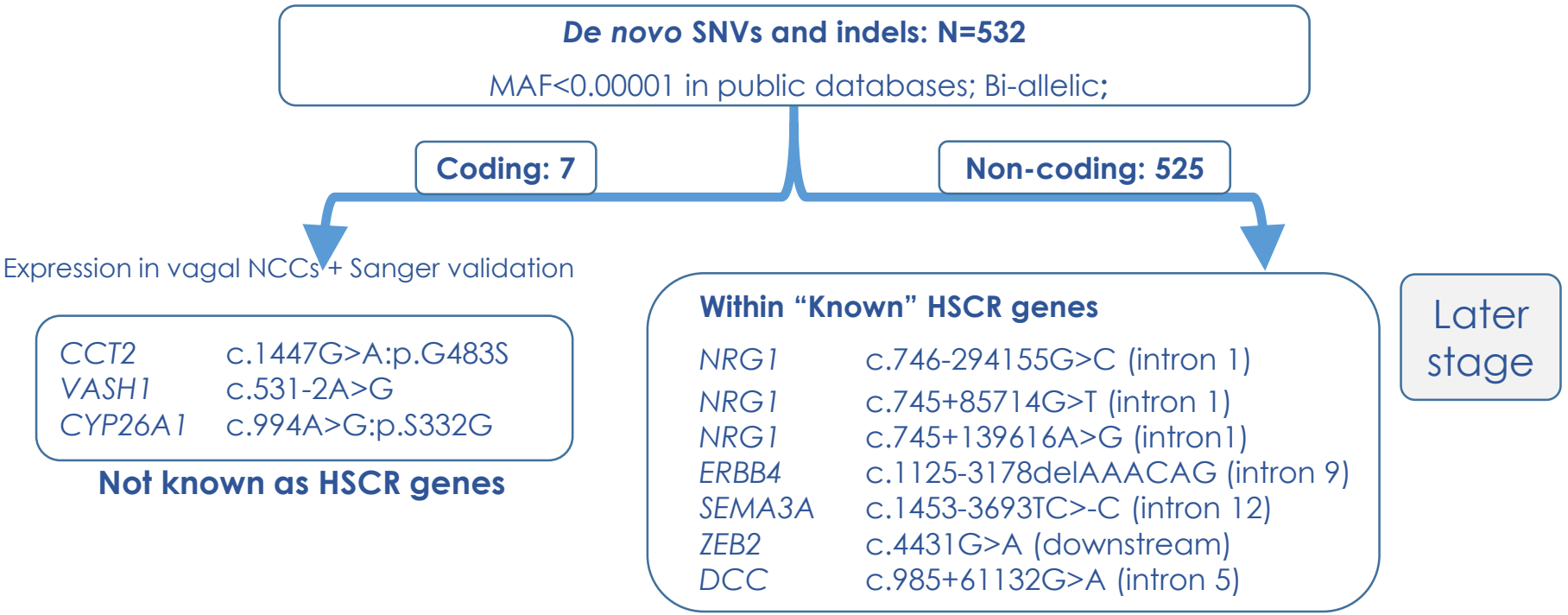


MAF (Minor Allele Frequency)
De novo MAF<0.0001 % or Novel
Recessive: < 0.01% or Novel
Compound heterozygous: < 0.011% or Novel
Digenic : < 1% or Novel



SNV: Single nucleotide variant
Indel: Insertions and deletions <100bp
CH: compound heterozygous
CDS: Coding sequence
NCDS: Non-coding sequence
CNV: copy number variant
GVD: Genome Variation Database

Filtering and selection of *de novo* single nucleotide and indel variants



Amongst the 10-15% genes most intolerant to changes human genes

- *CCT2:* formation of the primary cilia. Bardet-Biedl syndrome –can co-exist with HSCR-
- *VASH1:* secreted protein angiogenesis regulation.
- *CYP26A1:* retinoic acid (RA)-metabolizing enzyme.

Exonic homozygous mutations: none in "HSCR genes"

Patients	Genes	Variant	Gene function
HD09C	*FOCAD*	p.A1709T	Potential tumor suppressor in gliomas
	BRD1	p.A689V	Histones H3 and H4 acetylation
	C5orf42	p.G2168D	Transmembrane protein
	GINS4	p.V171M	Initiation of DNA replication
	GLRX3	p.I330V (cu)	Crucial regulator of cellular iron homeostasis
	HK2	p.A352T	Glycolysis, gluconeogenesis
	ITGB5	p.L94V	Integrin. Extracellular matrix (ECM)
HK164C	*NEK1*	p.R586H	Centrosomal complex. Microtubule assembly
	PLAT	**p.Y425H**	**Direct role in neuronal migration**
	PPP2R3A	p.Y431C	Intracellular signaling
	RRP7A	p.T81	Ribosomal RNA Processing
	STXBP5L	p.R618Q	GTPase activator activity
	USP42	p.D1220G (cu)	Deubiquitinating enzyme
	VRK2	p.R491H	Effector of signaling pathways that regulate apoptosis/tumor cell growth
HK180C	*XRN2*	p.A529T	5'-3' Exoribonuclease 2
HK96C	*BICD2*	p.R398W	Essential for motor neuron physiology
VH105C	*PLEKHA4*	p.R571H	Binds to phosphatidylinositol 3-phosphate
VH108C	*SEMA7A*	p.V320I	Integrin-mediated signaling. Focal adhesion. Neuronal functions

cu: case unique variant

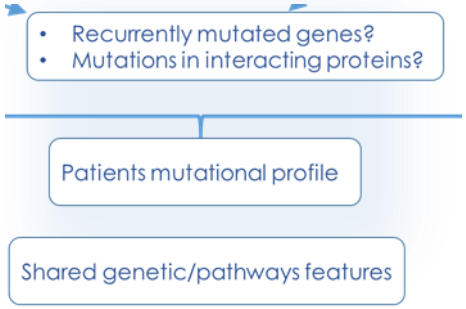
PLAT: **direct role in facilitating neuronal migration**

Compound heterozygous variants (CH)

***RADIL*:** knockdown of *radil* in zebrafish results in multiple defects in pigment cells and enteric neurons

Patients	Gene	Variants (paternal/maternal)	Disorders in humans
HD09C	*BRD4*	c.-7165+1G>A/p.E1326D	Translocation breakpoints in 2 patients with carcinomas
	MAGI3	p.D1059G 9(cu)/p.Y285H	ND
	FGFRL1	p.R241Q/p.A288T	Wolf–Hirschhorn syndrome (WHS)
	SSH2	p.G1378D/p.P1338S(cu)	ND
	TFR2	p.A75V/p.S506G	Hemochromatosis, Type 3
HK164C	*DOCK8*	p.C871W+p.Y2001* (cu)/p.S1177L	Non-syndromic intellectual disability
	CDC14A	p.R582H/p.P125H	Deafness, Autosomal Recessive
	FRAS1	p.N3119S/p.K1873Q	Cryptophthalmos, cutaneous syndactyly and genitourinary anomalies. AR
	SLC24A1	p.E697G/p.S521N	Congenital stationary night blindness (AR)
HK180C	*CUL7*	p.L1710V/p.R707C	3M syndrome (AR). Dubowitz's syndrome
	ACOX2	p.A504T/p.R409H	ND
	ARFGEF3	p.D87Y (cu)/p.E902Q (cu)	ND
	NACAD	p.Q1391E/p.G407R (cu)	ND
HK97C	*PCNT*	p.A1495V / p.R1821W	Microcephalic osteodysplastic primordial dwarfism type 2. Primary autosomal recessive microcephaly type 6. Seckel syndrome type 4
HK9C	*LAMA5*	p.E2378K/p.E2665K	ND
	CMYA5	p.S308F/p.G450D	Cardiomyopathy
	MGAM	p.M1073V/p.D1454G	Intestinal disaccharidase deficiency.
	RADIL	**p.V337M/p.L200P (cu)**	ND
VH105C	*CUL7*	p.S999C (cu)/p.V1691I	3M syndrome (AR). Dubowitz's syndrome
	ZSWIM4	p.R185W (cu)/p.Q267H	ND
VH106C	*SYNE1*	p.S2126F/p.R5617*	SYNE1-Related Autosomal Recessive Cerebellar Ataxia. Emery-Dreifuss muscular dystrophy 4, autosomal dominant
VH108C	*RFC2*	p.E207K/p.P22S	Gene in the Williams-Beuren Syndrome critical region. Infantile hypercalcemia. Aortic stenosis syndrome

Genes recurrently mutated



Considered genes with rare SNVs and/or CNVs

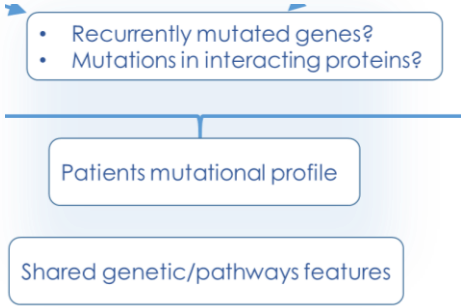
De novo
Homozygous
Compound heterozygous

Identified in this study
-**seed genes**-

HSCR / ENS related genes (117 genes)

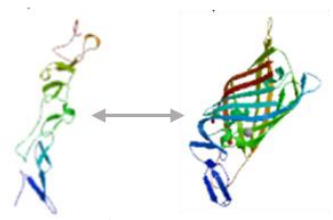
21 genes have different rare variants **in more than one patient**

Functional overlap among genes



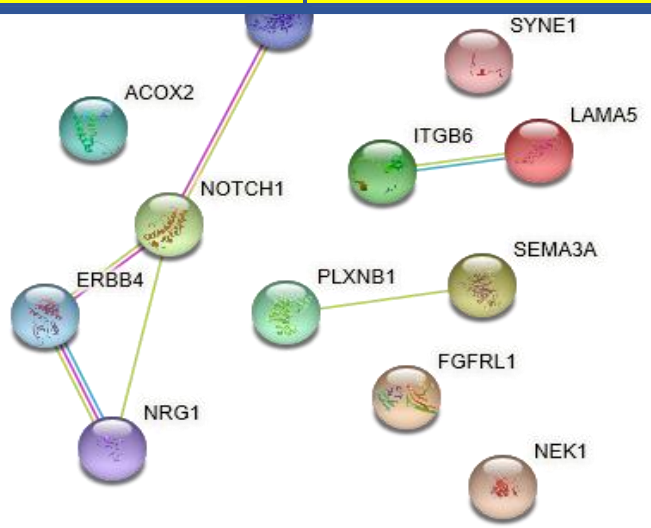
Di/oligo-genic model where variants in two or more interacting genes co-exist in a patient

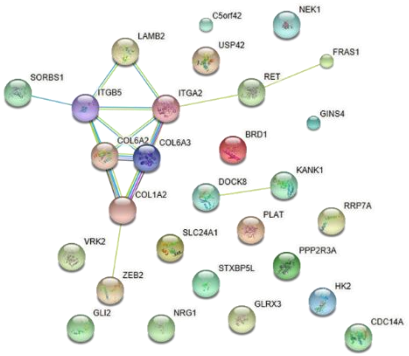
Included genes encoding **interacting partners** to **any of the seed genes** / ENS genes

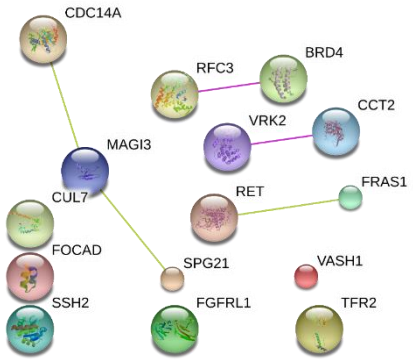


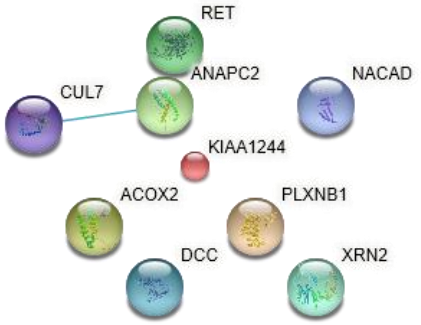
Genetic profile of each patient

Patients	Gene	Variant	Reason for gene selection	Type
VH106C	*SYNE1*	p.S2126F/p.R5617*	Seed	CH
	ERBB4	c.1125-3178delAAACAG (intron 9)	Seed and ENS-gene	NCDS *de novo*
	NRG1	c.746-294155G>C (intron 1)	Seed and ENS-gene	NCDS *de novo*
	NOTCH1	p.R1330H (cu)	ENS-gene	Paternally inherited
	ACOX2	p.R88Q	Seed -CH in patient HK180-	Maternally inherited
	FGFRL1	p.V98L	Seed -CH in patient HD9-	Paternally inherited
	NEK1	p.R355G	Seed -Homo in patient HK164-	Paternally inherited
	LAMA5	p.L2185F	Seed - **CH in patient HK9C**-	Paternally inherited
	ITGB6	p.R499H	**Interacting partner of *LAMA5***	Maternally inherited
	PLXNB1	p.L1686M	ENS-gene	Maternally inherited
	SEMA3A	c.1453-3693TC>-C (intron 12)	Seed and ENS-gene	NCDS *de novo*




C5orf42
NEK1
LAMB2
USP42
FRAS1
SORBS1
RET
ITGB5
ITGA2
GINS4
BRD1
COL6A2
COL6A3
KANK1
DOCK8
COL1A2
RRP7A
PLAT
SLC24A1
VRK2
PPP2R3A
STXBP5L
ZEB2
HK2
GLI2
NRG1
GLRX3
CDC14A


CDC14A
RFC3
BRD4
CCT2
VRK2
MAGI3
CUL7
RET
FRAS1
FOCAD
SPG21
VASH1
SSH2
FGFRL1
TFR2


RET
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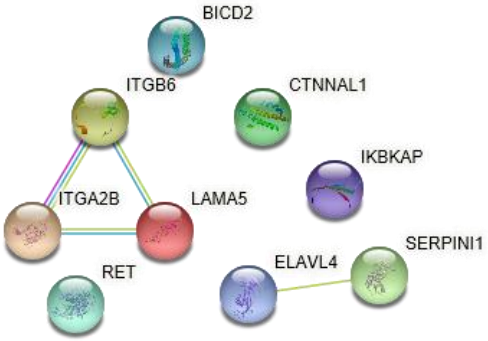
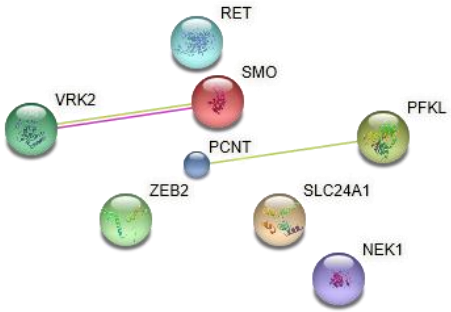
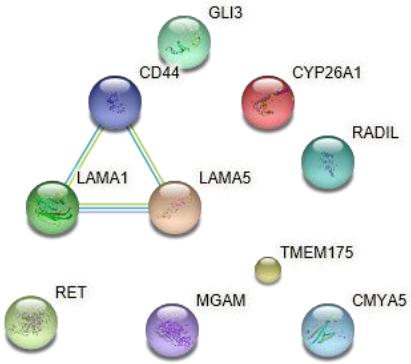
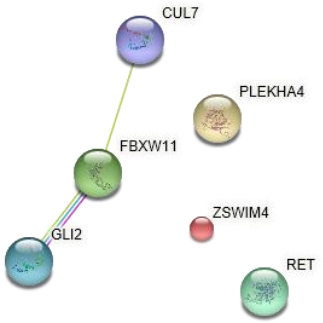
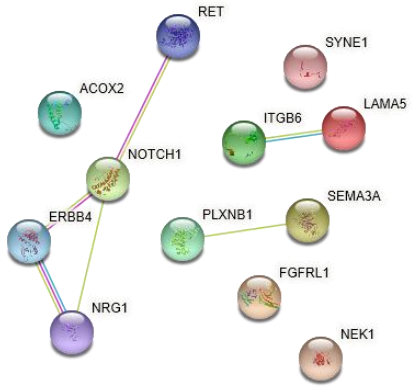
NACAD
CUL7
KIAA1244
ACOX2
PLXNB1
DCC
XRN2

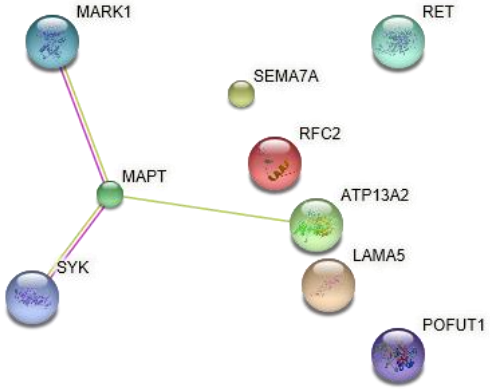

BICD2
ITGB6
CTNNAL1
IKBKAP
ITGA2B
LAMA5
SERPINI1
ELAVL4
RET


RET
SMO
VRK2
PFKL
PCNT
ZEB2
SLC24A1
NEK1


GLI3
CD44
CYP26A1
RADIL
LAMA1
LAMA5
TMEM175
RET
MGAM
CMYA5


CUL7
PLEKHA4
FBXW11
ZSWIM4
GLI2
RET


RET
SYNE1
ACOX2
LAMA5
ITGB6
NOTCH1
SEMA3A
ERBB4
PLXNB1
FGFRL1
NRG1
NEK1


MARK1
RET
SEMA7A
RFC2
MAPT
ATP13A2
LAMA5
SYK
POFUT1

Is there anything in common? Any shared genetic component?

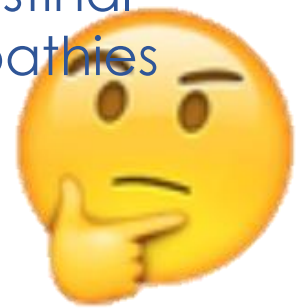
Each patient has variants in at least two interacting and biologically plausible genes

Extra Cellular Matrix-receptor interaction pathway (ECM-receptor) has significantly more interactions than expected ($p=1.5x10^{-11}$)

- *Could any EMC molecule be used as a target?*

Detected variants in **schizophrenia, autism** (*NRG1, NRG3, ERBB4, SEMA3A, PLXNB1, DOCK8, CALN1, NBPF*) and **ciliopathies** (*CCT2*) genes

- Frequently observed association between intestinal dysmotility and psychiatric disorders and ciliopathies (Bardet-Biedl; Joubert syndromes)



Pathological alterations affecting pathway(s) **shared by more than one disorder may underlay apparently unrelated diseases**

- How can we make use of this?
- Predictable phenotypic pattern?

Pilot study

First WGS in HSCR

Very instrumental in setting a pipeline at HKU

Human Medical Research Fund 01121516

The University of Hong Kong

Department of Surgery

Clara Sze-man Tang
Sunny Xuehan Zhuang
Michelle Yu
Man-ting So
Paul Tam

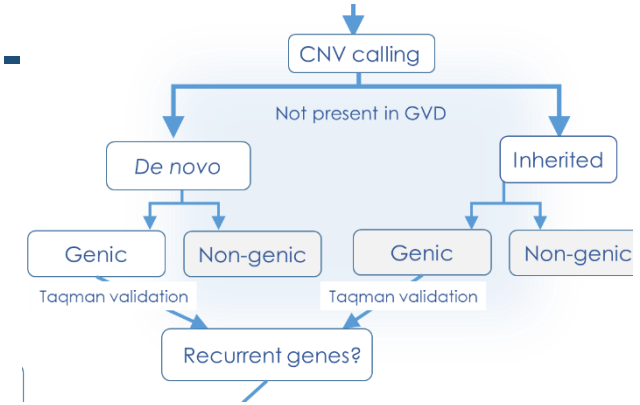
Clara Tang

Centre for Genomic Sciences

Pak Sham
Stacey Cherny

Sunny Zhuang

Preliminary CNV data –genetic profile-



	De novo		Inherited	
	Genes deleted	Genes duplicated	Genes deleted	Genes duplicated
HD09C		CALN1 (6K; intronic) No in 1K project CALN1 (29K; intronic)	GFRA2 (36K; exonic)	NOTCH2 (5K; exonic)
HK164C	NBPF6(3K, exonic)	NBPF8, NBPF9 (9K; exonic)	GFRA2 (36K; exonic)	
Recurrent gene. Duplicated in another HSCR patient	***PPM1L (3.5Kb, intronic) No in 1K project***		***KIAA1279 (5K; exonic)***	
			MGAM (28K; intronic)	
HK180C	NRG3 (2K; intron 1 NM_001010848.3)		GFRA2 (36K; exonic) NRG3 (2K; intron 2; NM_001010848.3) MGAM (28K; intronic)	NOTCH2 (12K; exonic)
HK96C	KLRC3 (6Kb, exonic)		GFRA2 (36K; exonic)	NOTCH2 (12K; exonic)
HK97C	KIR2DL3, KIR3DL3 (38K, exonic) KIR2DL1, KIR2DL4, KIR3DL1 (37K, exonic)			NOTCH2 (12K; exonic)
HK9C	NBPF20 (116K; exonic)		GFRA2 (36K; exonic)	
VH105C	NBPF20 (8K; exonic)		GFRA2 (36K; exonic)	NOTCH2 (12K; exonic)
Recurrent. In different patient	***KIR2DL1,KIR2DL4,KIR3DL1(37K, exonic)***			
Recurrent. In different patient. Duplicated.	LILRA6(2Kb, exonic)immunity?		MGAM (28K; intronic)	
VH106C			GFRA2 (36K; exonic)	NOTCH2 (12K; exonic)
			MGAM (28K; intronic)	
VH108C	KLRC3 (4Kb, upstream)		GFRA2 (36K; exonic)	NOTCH2 (12K; exonic)

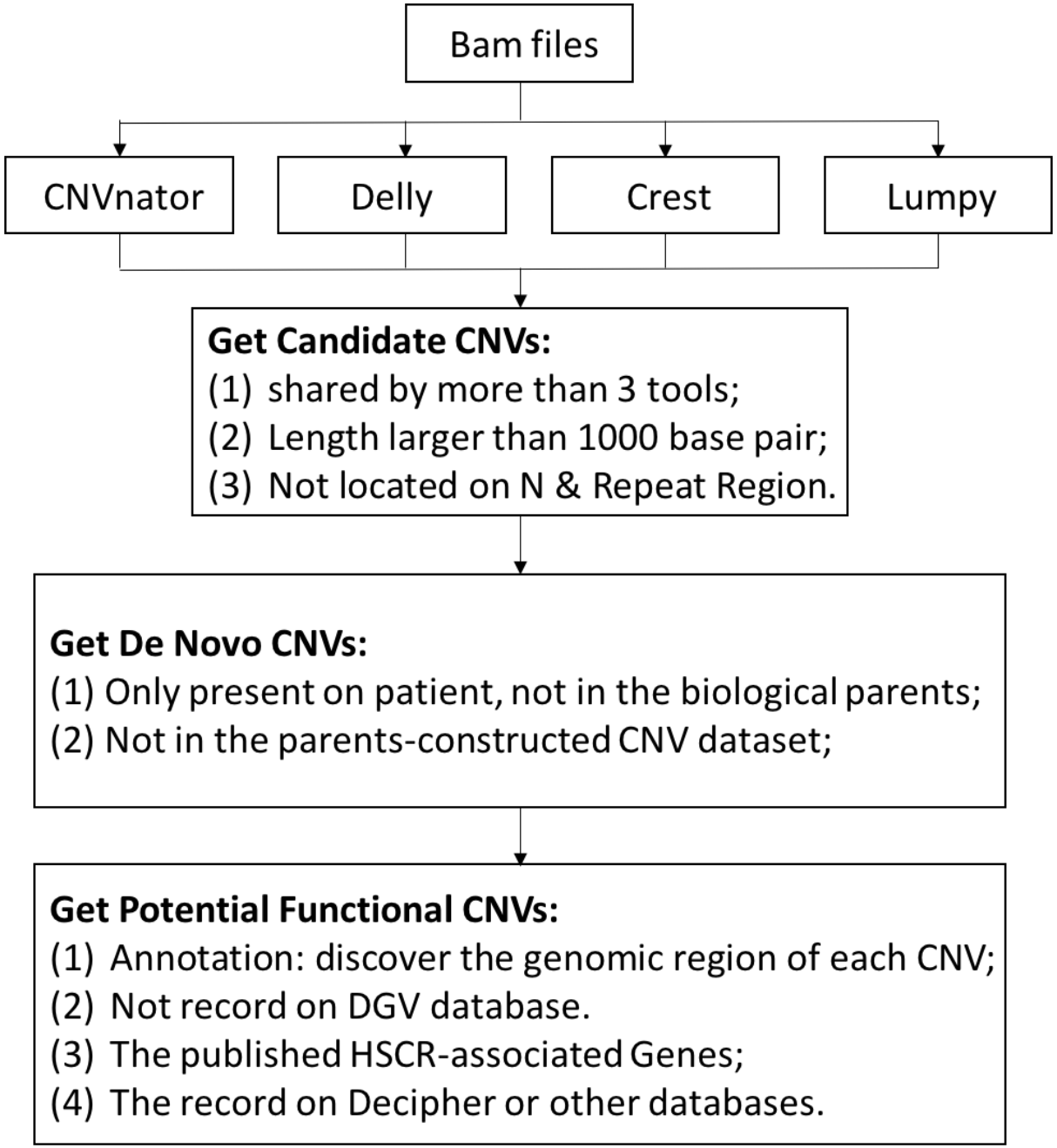
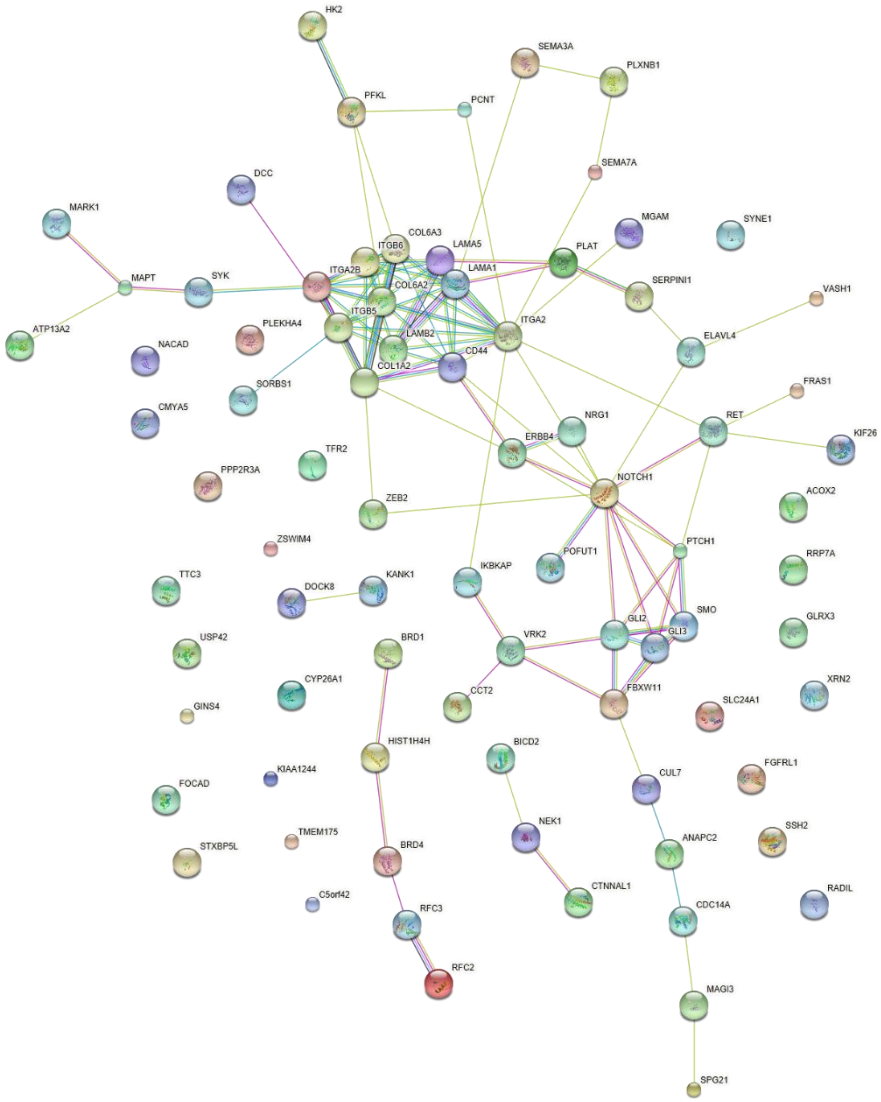

Bam files
CNVnator
Delly
Crest
Lumpy
Get Candidate CNVs:
(1) shared by more than 3 tools;
(2) Length larger than 1000 base pair;
(3) Not located on N & Repeat Region.
Get De Novo CNVs:
(1) Only present on patient, not in the biological parents;
(2) Not in the parents-constructed CNV dataset;
Get Potential Functional CNVs:
(1) Annotation: discover the genomic region of each CNV;
(2) Not record on DGV database.
(3) The published HSCR-associated Genes;
(4) The record on Decipher or other databases.

Table S1: Characteristics of the HSCR patients included in the study

Samples	Relationship	Gender	Aganglionosis leng	Associated anomalies/others	Ethnicity
HK9C	**Proband**	**M**	**Long**	**Necrotizing enterocolitis;** asthma	Chinese
HK9A	Father	M			
HK9B	Mother	F			
HK96C	**Proband**	**M**	**Total**	Parathyroid nodules; bilateral hydrocele; **necrotizing enterocolitis**	Chinese
HK96A	Father	M			
HK96B	Mother	F			
HK97C	**Proband**	**M**	**Long**	Congenital central hypoventilation syndrome (CCHS); **necrotizing enterocolitis**; mild mental retardation; epilepsy	Chinese
HK97A	Father	M			
HK97B	Mother	F			
HK164C	**Proband**	**M**	**Long**	None; consanguineous parents	South-east Asian
HK164A	Father	M			
HK164B	Mother	F			
HD09C	**Proband**	**M**	**Long**	None	Chinese
HD09A	Father	M			
HD09B	Mother	F			
VH105C	**Proband**	**M**	**Long**	None	Vietnamese
VH105A	Father	M			
VH105B	Mother	F			
VH106C	**Proband**	**M**	**Long**	None	Vietnamese
VH106A	Father	M			
VH106B	Mother	F			
VH108C	**Proband**	**M**	**Long**	None	Vietnamese
VH108A	Father	M			
VH108B	Mother	F			
HK180C	**Proband**	**M**	**Long**	None	Chinese
HK180A	Father	M			
HK180B	Mother	F			

All genes



Network Stats

number of nodes:	83	expected number of edges:	35
number of edges:	110	PPI enrichment p-value:	0
average node degree:	2.65	*your network has significantly more interactions than expected (what does that mean?)*	
clustering coefficient:	0.647		

Functional enrichments in your network

Biological Process (GO)

pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
GO:0040011	locomotion	25	8.7e-09
GO:0006928	movement of cell or subcellular component	25	4.3e-08
GO:0009653	anatomical structure morphogenesis	29	5.73e-07
GO:0016477	cell migration	18	1.02e-06
GO:0000902	cell morphogenesis	19	2.73e-06
			(more ...)

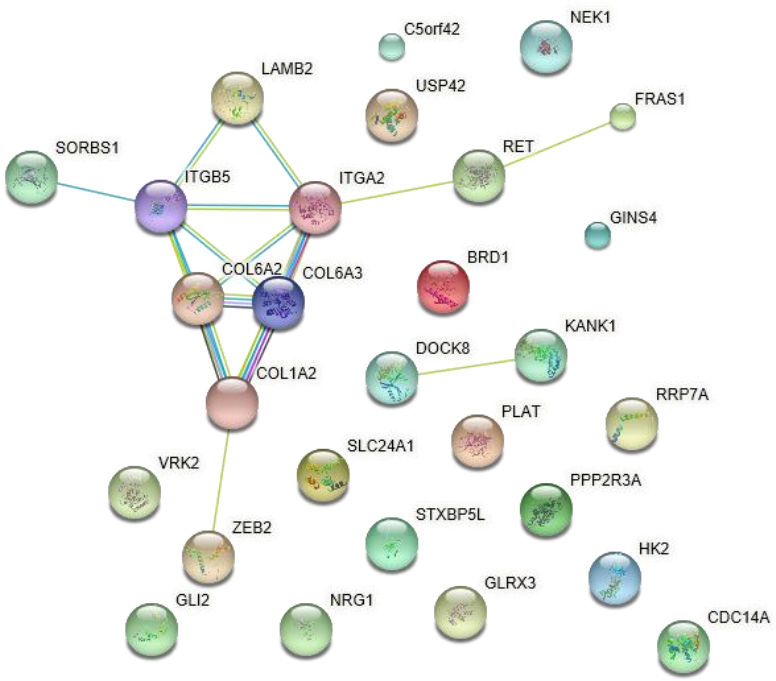
Cellular Component (GO)

pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
GO:0043234	protein complex	35	0.000142
GO:0043235	receptor complex	10	0.000142
GO:0008305	integrin complex	4	0.00135
GO:0043256	laminin complex	3	0.00135
GO:0044420	extracellular matrix component	6	0.0018
			(more ...)

KEGG Pathways

pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
04512	ECM-receptor interaction	11	1.19e-11
04151	PI3K-Akt signaling pathway	12	1.03e-06
04510	Focal adhesion	10	1.03e-06
05200	Pathways in cancer	11	4.92e-06
04340	Hedgehog signaling pathway	5	0.000103
			(more ...)

Patient HK164C



Network Stats

number of nodes:	28	expected number of edges:	4
number of edges:	17	PPI enrichment p-value:	4.61e-07
average node degree:	1.21	*your network has significantly more interactions than expected (what does that mean?)*	
clustering coefficient:	0.912		

Functional enrichments in your network

Biological Process (GO)

pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
GO:0032501	multicellular organismal process	21	0.000422
GO:0044707	single-multicellular organism process	20	0.000862
GO:0040011	locomotion	10	0.00446
GO:0006928	movement of cell or subcellular component	10	0.00863
GO:0007275	multicellular organismal development	16	0.0106
			(more ...)

Cellular Component (GO)

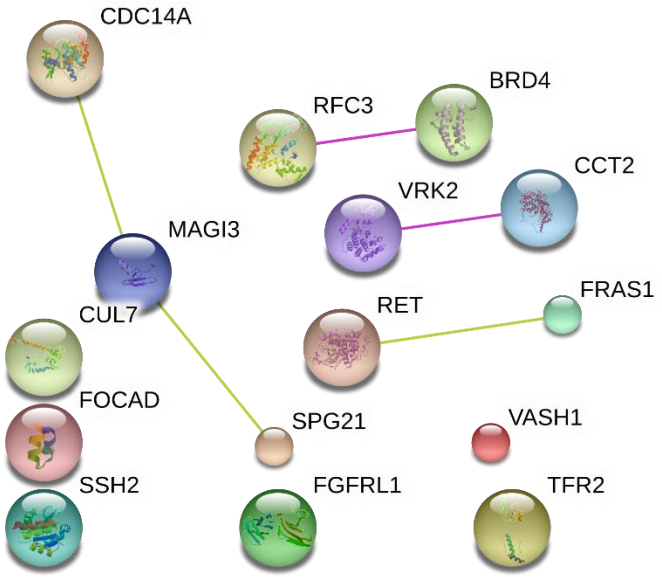
pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
GO:0044420	extracellular matrix component	4	0.0296

KEGG Pathways

pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
04512	ECM-receptor interaction	6	4.32e-07
04151	PI3K-Akt signaling pathway	7	2.59e-05
04510	Focal adhesion	6	2.59e-05
04974	Protein digestion and absorption	3	0.0146

Would any individual of the control population have this constellation of mutations?

Patient HD9

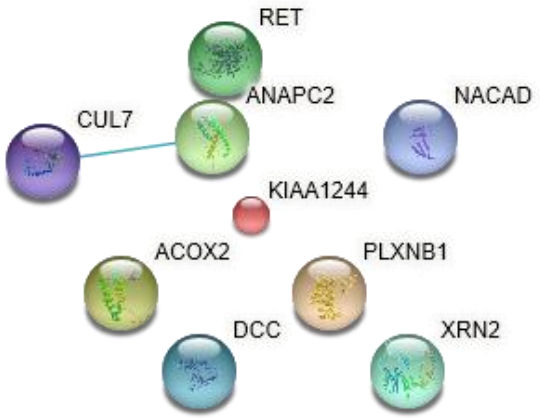


number of nodes:	15	expected number of edges:	1
number of edges:	5	PPI enrichment p-value:	0.00144
average node degree:	0.667	*your network has significantly more interactions than expected (what does that mean?)*	
clustering coefficient:	0.933		

Functional enrichments in your network

- no significant enrichment detected -

Patient HK180C



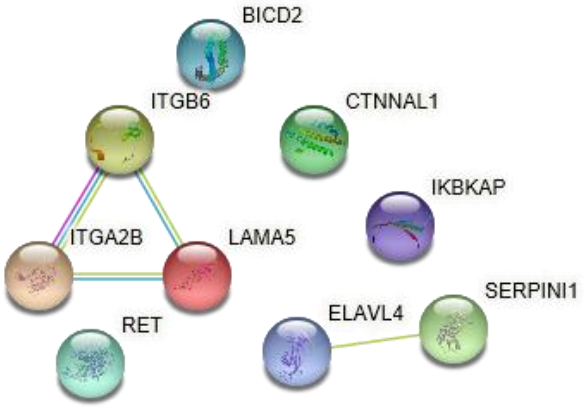
Network Stats

number of nodes:	9	expected number of edges:	0
number of edges:	1	PPI enrichment p-value:	0.286
average node degree:	0.222	*your network does **not** have significantly more interactions than expected (what does that mean?)*	
clustering coefficient:	1		

Functional enrichments in your network

Biological Process (GO)			
pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
GO:0010769	regulation of cell morphogenesis involved in differentiation	5	0.000693
GO:0010975	regulation of neuron projection development	5	0.000693
GO:0050770	regulation of axonogenesis	4	0.000776
GO:0010976	positive regulation of neuron projection development	4	0.00233
GO:0050773	regulation of dendrite development	3	0.0107
GO:0008361	regulation of cell size	3	0.0218
GO:0022604	regulation of cell morphogenesis	4	0.0239
GO:0010770	positive regulation of cell morphogenesis involved in differentiation	3	0.026
GO:0048799	organ maturation	2	0.0264
GO:0050775	positive regulation of dendrite morphogenesis	2	0.046
			(less ...)

Patient HK96C



Network Stats

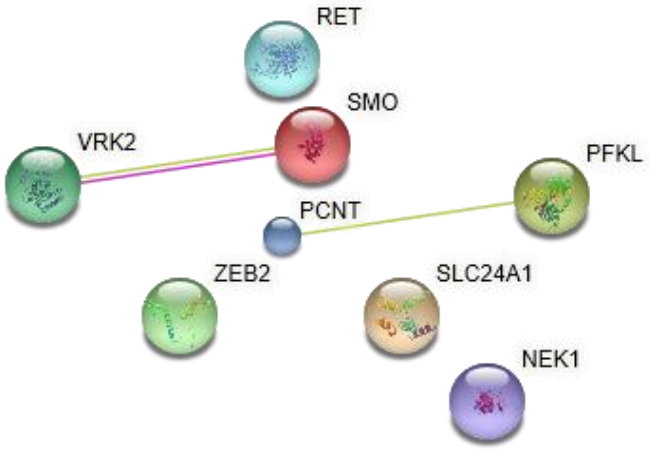
number of nodes:	9	expected number of edges:	0
number of edges:	4	PPI enrichment p-value:	0.000412
average node degree:	0.889	*your network has significantly more interactions than expected (what does that mean?)*	
clustering coefficient:	1		

Functional enrichments in your network

Cellular Component (GO)			
pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
GO:0008305	integrin complex	2	0.0491

KEGG Pathways			
pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
04512	ECM-receptor interaction	3	0.00164
04510	Focal adhesion	3	0.0109
04151	PI3K-Akt signaling pathway	3	0.0232
05200	Pathways in cancer	3	0.0232
05222	Small cell lung cancer	2	0.0232
05410	Hypertrophic cardiomyopathy (HCM)	2	0.0232
05412	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	2	0.0232
05414	Dilated cardiomyopathy	2	0.0232
			(less ...)

Patient HK97C



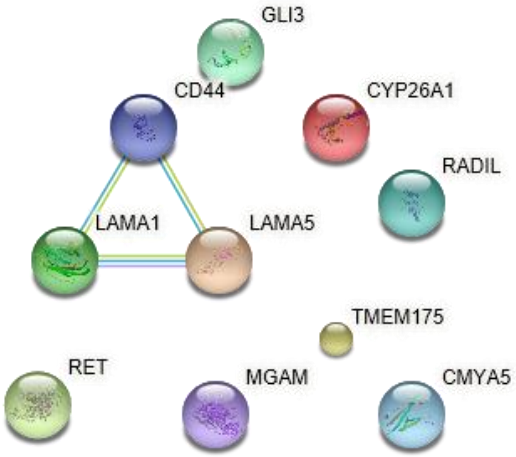
Network Stats

number of nodes:	8	expected number of edges:	0
number of edges:	2	PPI enrichment p-value:	0.0211
average node degree:	0.5	*your network has significantly more interactions than expected (what does that mean?)*	
clustering coefficient:	1		

Functional enrichments in your network

- no significant enrichment detected -

Patient HK9C



Network Stats

number of nodes:	10	expected number of edges:	0
number of edges:	3	PPI enrichment p-value:	0.014
average node degree:	0.6	*your network has significantly more interactions than expected (what does that mean?)*	
clustering coefficient:	1		

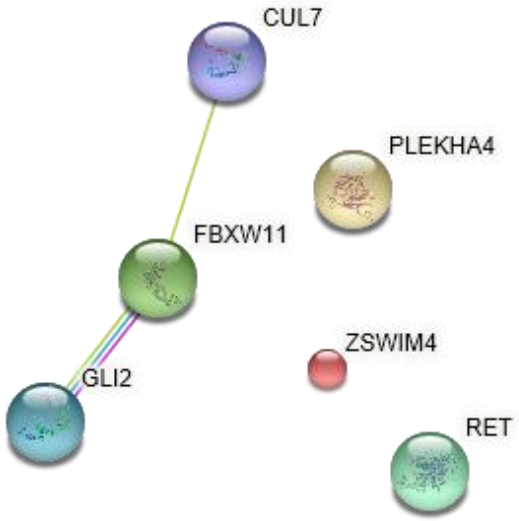
Functional enrichments in your network

Biological Process (GO)			
pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
GO:0001657	ureteric bud development	4	0.000272
GO:0001823	mesonephros development	4	0.000272
GO:0022612	gland morphogenesis	4	0.000371
GO:0060562	epithelial tube morphogenesis	5	0.000376
GO:0048754	branching morphogenesis of an epithelial tube	4	0.000754
			(more ...)

Cellular Component (GO)			
pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
GO:0043256	laminin complex	2	0.00984
GO:0005605	basal lamina	2	0.0404

KEGG Pathways			
pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
04512	ECM-receptor interaction	3	0.00164
05200	Pathways in cancer	4	0.00164

Patient VH105C



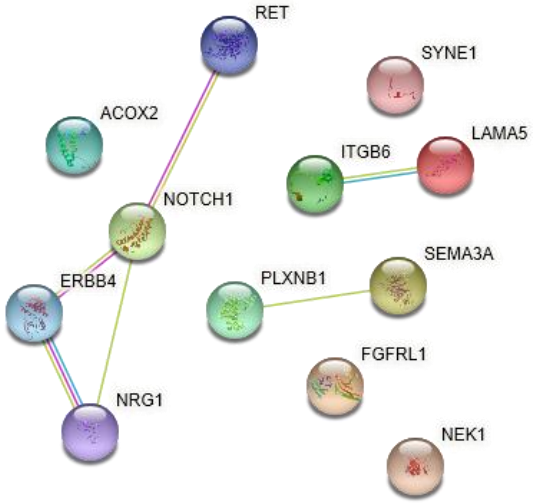
Network Stats

number of nodes:	6	expected number of edges:	0
number of edges:	2	PPI enrichment p-value:	0.00828
average node degree:	0.667	*your network has significantly more interactions than expected (what does that mean?)*	
clustering coefficient:	0.833		

Functional enrichments in your network

KEGG Pathways			
pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
04340	Hedgehog signaling pathway	2	0.025

Patient VH106C



Network Stats

number of nodes: 12
number of edges: 6
average node degree: 1
clustering coefficient: 0.944

expected number of edges: 1
PPI enrichment p-value: 0.00185
your network has significantly more interactions than expected (what does that mean?)

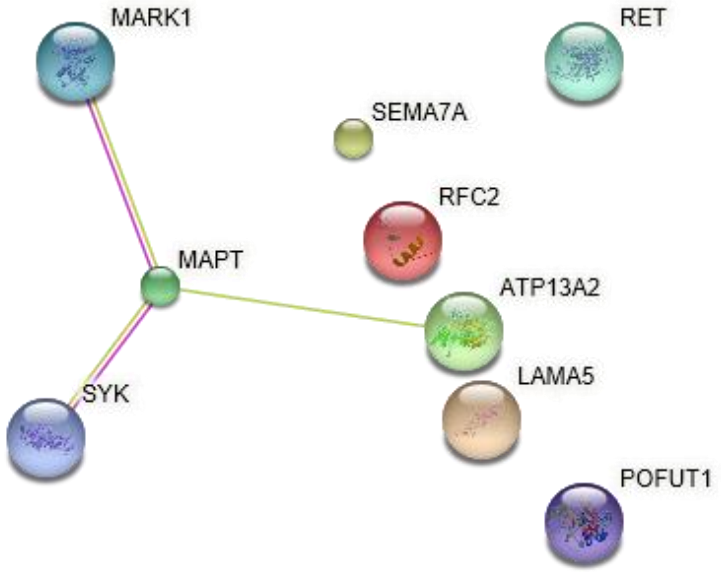
Functional enrichments in your network

Biological Process (GO)			
pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
GO:0014031	mesenchymal cell development	6	1.46e-07
GO:0048762	mesenchymal cell differentiation	6	1.46e-07
GO:0014032	neural crest cell development	5	3.13e-07
GO:0014033	neural crest cell differentiation	5	4.92e-07
GO:0060485	mesenchyme development	6	7.11e-07
			(more ...)

Molecular Function (GO)			
pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
GO:0004714	transmembrane receptor protein tyrosine kinase activity	3	0.0164
GO:0005102	receptor binding	6	0.0164
GO:0004713	protein tyrosine kinase activity	3	0.0456
GO:0004872	receptor activity	6	0.0456

Cellular Component (GO)			
pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
GO:0043235	receptor complex	5	0.000508

Patient VH108C



Network Stats

number of nodes:	9	expected number of edges:	0
number of edges:	3	PPI enrichment p-value:	0.0114
average node degree:	0.667	*your network has significantly more interactions than expected (what does that mean?)*	
clustering coefficient:	0.889		

Functional enrichments in your network

- no significant enrichment detected -

Patients	Gene	Variant	Reason for gene selection	Type	Gene function	Human	Mouse
VH106C	*SYNE1*	p.S2126F/p.R5617*	Seed	CH	Spectrin family r	SYNE1-Relat	SYNE2 is required fo
	ERBB4	c.1125-3178delAAACAG (intron 9)	ENS-gene	**NCDS de novo**	NRG1 receptor.	Erbb4-Relat	Abnormal multiorg
	NRG1	c.746-294155G>C (intron 1)	ENS-gene	**NCDS de novo**	Direct ligand for	Associated	Abnormal NCC mig
	NOTCH1	p.R1330H (cu)	ENS-gene	Paternally inherited	Transmembranc	Adams-Oliv	Abnormal embryon
	ACOX2	p.R88Q	Interacting partner; Seed -**CH in patient HK180-**	Maternally inherited	Acyl-CoA oxida	ND	ND
	FGFRL1	p.V98L	Seed -**CH in patient HD9-**	Paternally inherited	Fibroblast Grow	Wolf-Hirschh	Development dela
	NEK1	p.R355G	Seed -**Homo in patient HK164-**	Paternally inherited	Cell cycle regul	Short-rib tho	Abnormal renal tub
	LAMA5	p.L2185F	Seed - CH in patient HK9C-	Paternally inherited	Extracellular mc	ND	Arrest of hair devel
	ITGB6	p.R499H	Interacting partner of *LAMA5*	Maternally inherited	Integrin. Attach	Amelogene	ND
	PLXNB1	p.L1686M	ENS-gene	Maternally inherited	Semaphorin rec	ND	ND
	SEMA3A	c.1453-3693TC>-C (intron 12)	Seed + ENS gene	**NCDS de novo**	Involved in the	Hypogonad	Abnormal ENS morp