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Ministero dell'Università e della Ricerca

Italiadomani
PIANO NAZIONALE DI RIPRESA E RESILIENZA

HEAL ITALIA ROADSHOW
TECNOLOGIE E INNOVAZIONE
INNOVATION ON THE ROAD

PRIMO CONVEGNO NAZIONALE DEL CENTRO DI MEDICINA DI PRECISIONE – HEAL ITALIA PER LE MALATTIE RARE

www.ancona.centridimedicinadiprecisione.it

Responsabile scientifico
Prof. **Gianluca Moroncini**

UnivPM – Ancona
Aula Montessori
Facoltà di Medicina
e Chirurgia

venerdì 28 febbraio
14:30 → 18:30
sabato 1 marzo
09:00 → 13:00

Progetto "Health Extended ALliance for Innovative Therapies, Advanced Lab-research, and Integrated Approaches of Precision Medicine (HEAL ITALIA) Codice PE00000019, CUP I33C22006900006 – finanziato dal PNRR M4C2 I1.3 – DD MUR 341 del 15/03/2022

HEAL ITALIA

CENTRO DI MEDICINA DI PRECISIONE™

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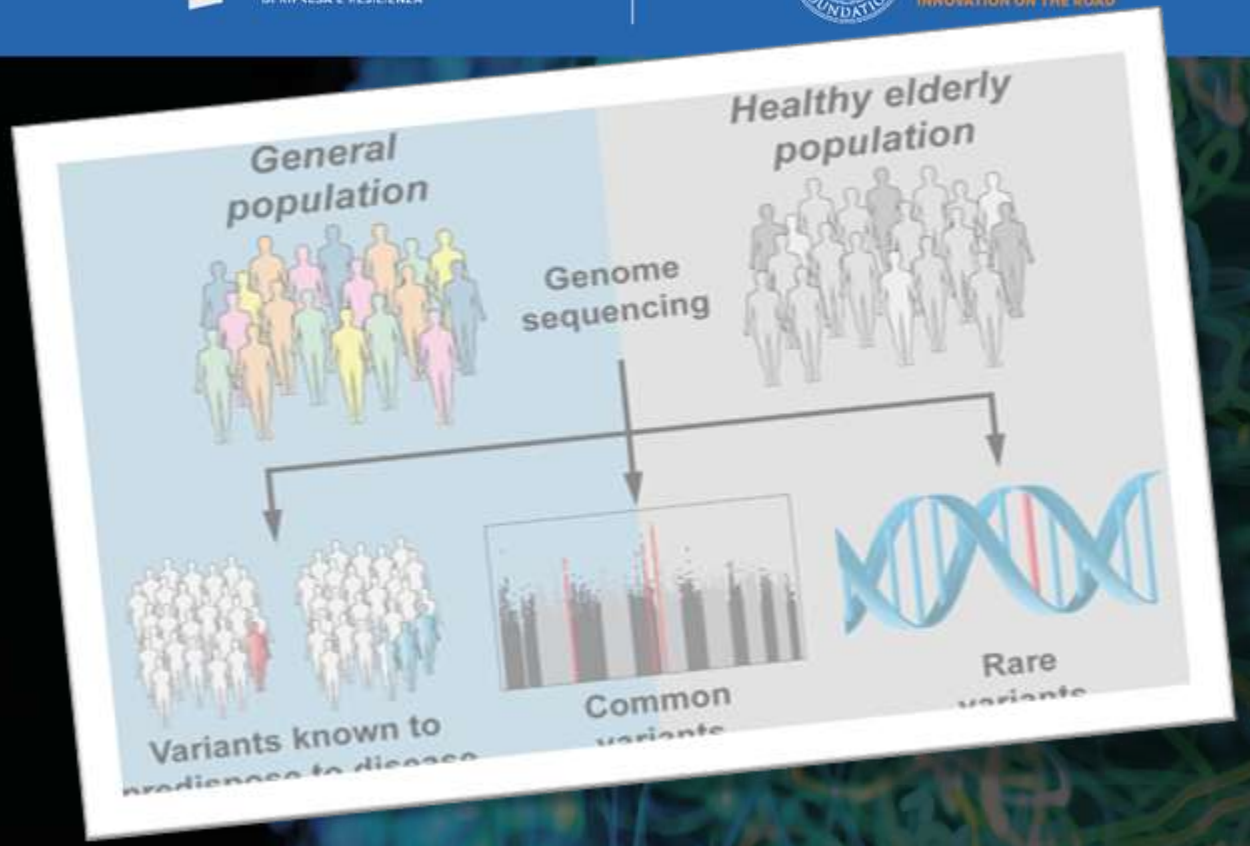
Repository UniCa della variabilità genetica nei Sardi -

Rare genetic variants in common tumors



Sabrina Giglio & **Andrea Perra**

Medical Genetics Unit - Department of Medical Sciences and Public Health

Oncology and Molecular Pathology Unit - Department of Biomedical Sciences

UNICA UNIVERSITÀ DEGLI STUDI DI CAGLIARI

Knowing the primary cause of a disease is essential to understanding its mechanisms and for its proper classification, prognosis, and treatment.

Understanding a pathogenic variant in a monogenic disease serves as one of the most robust diagnostic examples of "personalised and precision medicine," as this variant carries an almost 100% risk of developing the disease by a certain age.

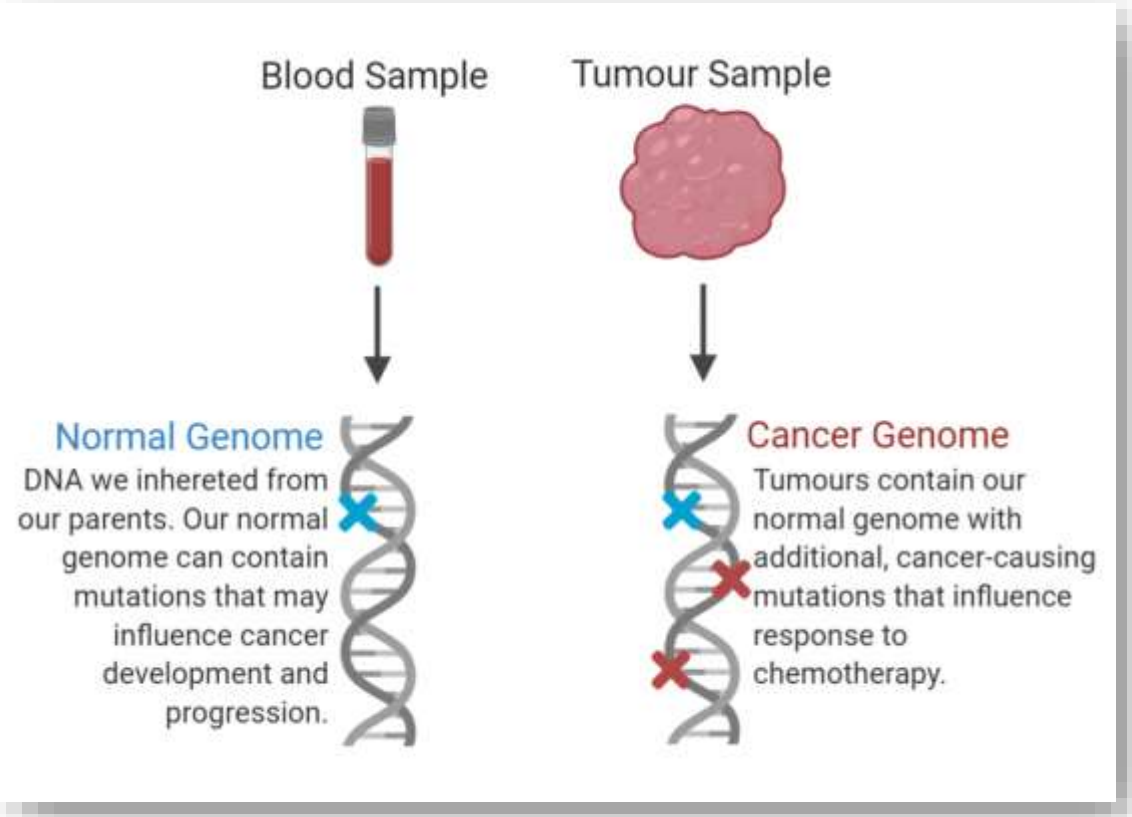
Can we think of precision medicine starting from genomic information in complex diseases, such as tumours?

Tumor Genomics and Precision Oncology

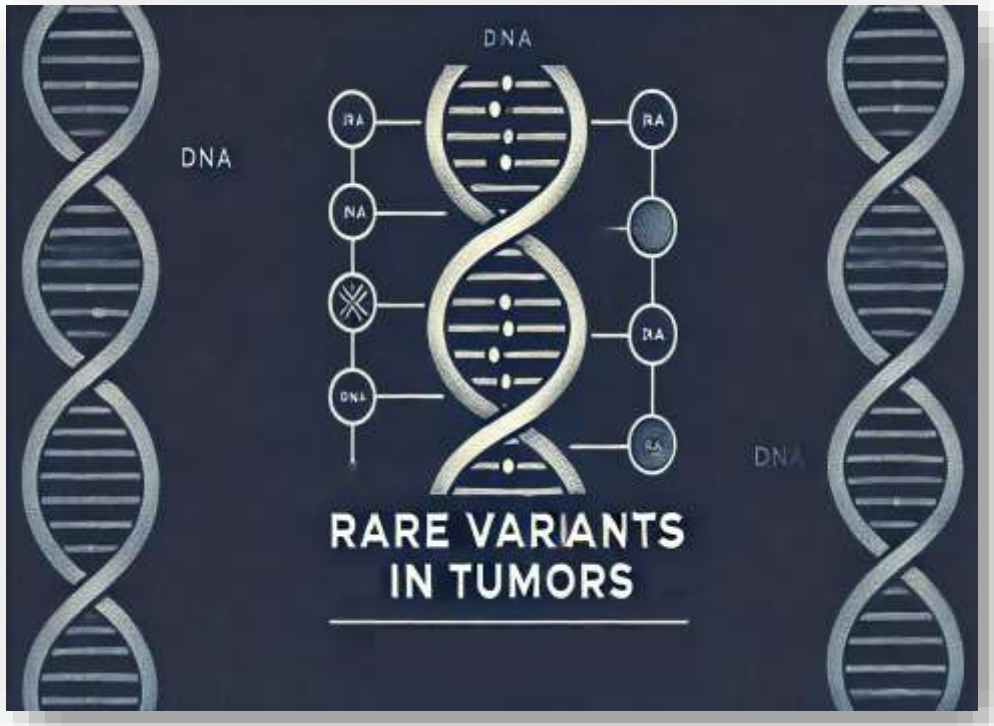
Typically, tumours exhibit around 50,000 somatic variants; however, **germline variants** can now be recognised as **predisposing individuals to disease**.

- Hereditary familial tumours
- Sporadic tumours

After filtering, between **20 and 15 variants** are generally considered suitable for further evaluation.



Hereditary familial tumours

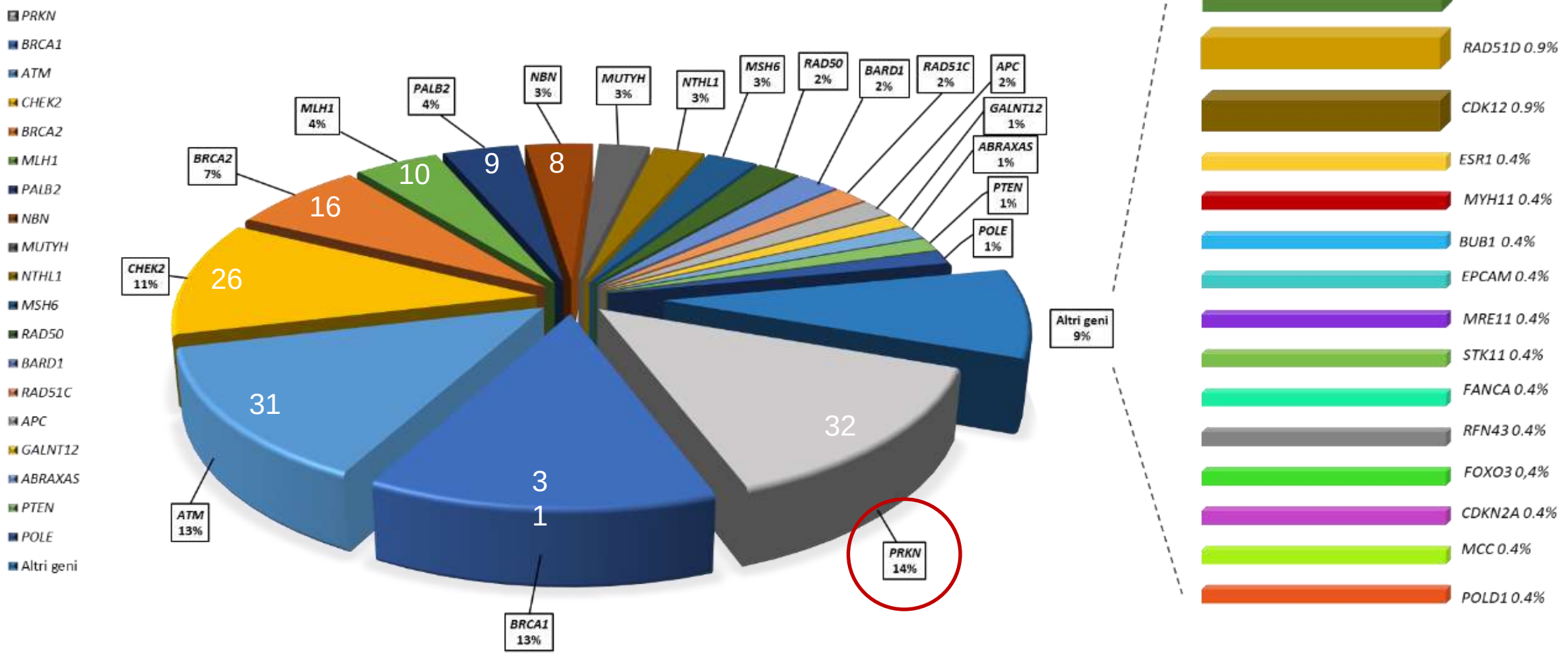


Family members face a heightened risk of developing a particular type of cancer.

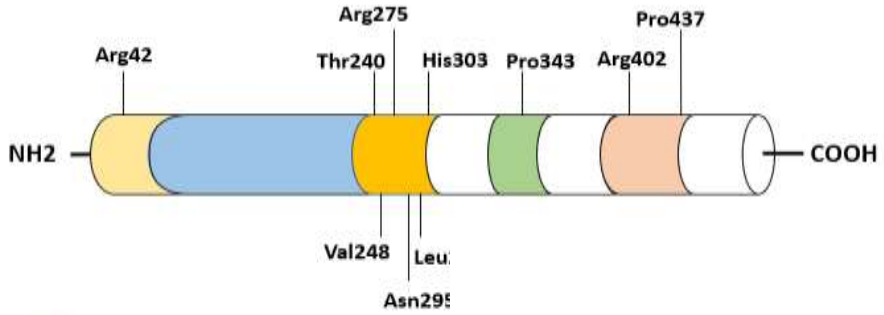
Familial adenomatous polyposis (FAP)
Lynch syndrome (HNPCC, Hereditary Non-Polyposis Colorectal Cancer) **involve *mismatch repair genes*** *MLH1, MSH2, MSH6, and PMS2*.

Hereditary breast and ovarian cancers **are associated with *BRCA1* and *BRCA2*, as well as, more rarely, other genes such as** *TP53, PALB2, CDH1, ATM, BARD1, BRIP1, CHEK2, RAD51C, and RAD51D*.

Gruppo K MAMMELLA

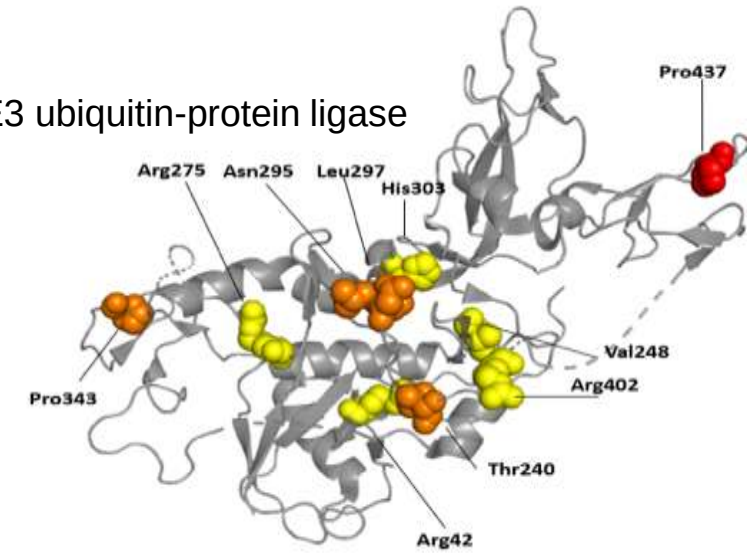


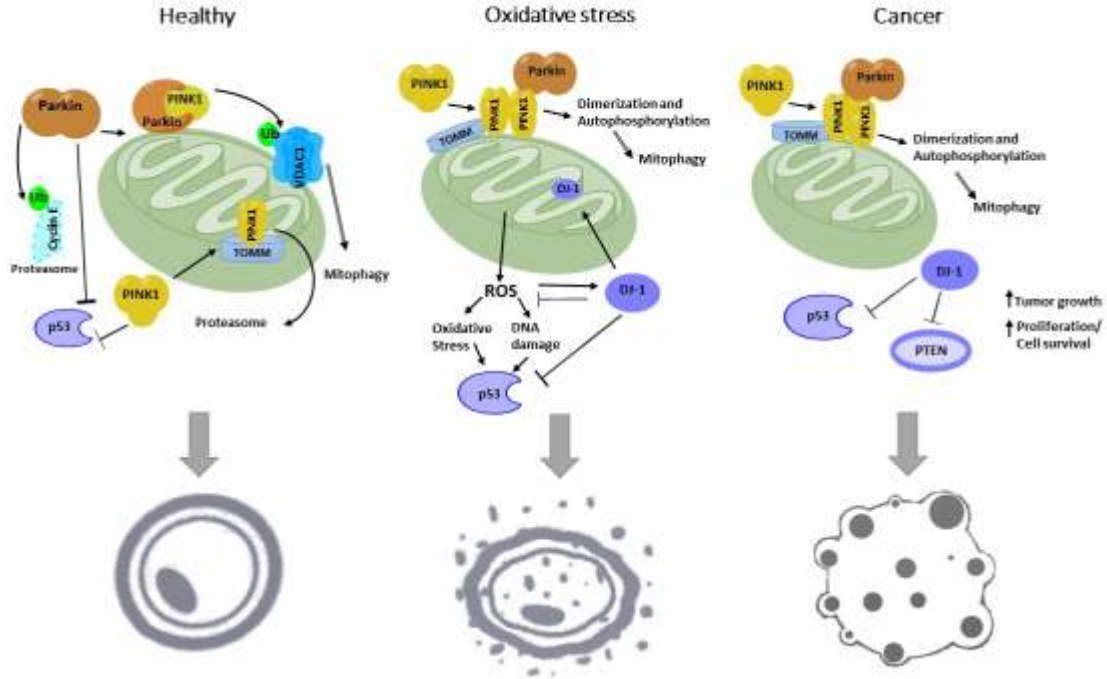
PRKN



- UBL domain →1
- SH2-like
- RING1 →5
- In Between Ring fingers (IBR)
- RING2 →2

E3 ubiquitin-protein ligase





Functional interplay has been reported between the Parkin and p53, a well-established tumor suppressor

Parkin protein expression was **found to be absent in 68% cases of breast cancer**.

PARTNER AND LOCALIZER OF BRCA2_*Cytogenetic location: 16p12.2 Genomic coordinates (GRCh38): 16:23,603,165-23,641,310*
MutY DNA GLYCOSYLASE_*Cytogenetic location: 1p34.1 Genomic coordinates (GRCh38): 1:45,329,242-45,340,440*
ENDONUCLEASE III-LIKE 1_*Cytogenetic location: 16p13.3 Genomic coordinates (GRCh38): 16:2,039,820-2,047,834*

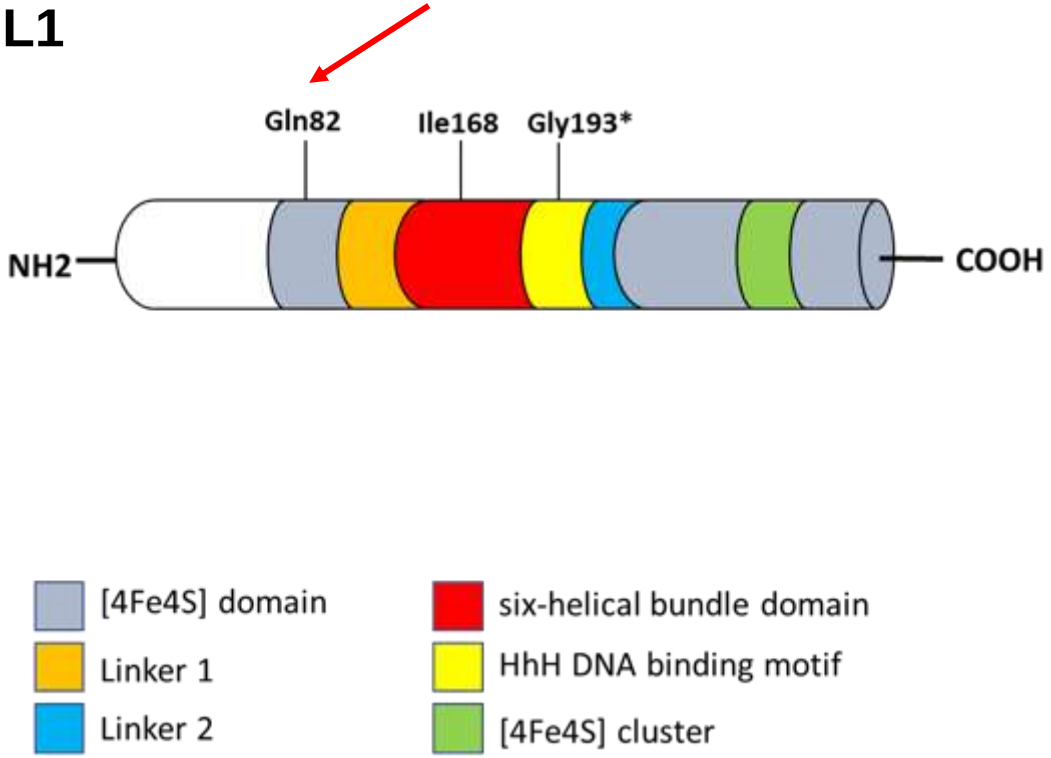
CASO	ETA'	DIAGNOSI	ISTOLOGIA	GENE	VARIANTE	
E014	49	TUMORI MULTIPLI	NST	*PALB2 (NM_024675.4)*	ex3	c.127A>G (p.Lys43Glu)
123.19	57	K MAMMELLA	DUTTALE		ex3	c.127A>G (p.Lys43Glu)
1146.22	42	K MAMMELLA	INFILTRANTE NST			
3256.22	48	K MAMMELLA	INFILTRANTE NST		ex4	**c.420del (P.Lys140fs)**
493.2	47	K MAMMELLA	DUTTALE			
285.19	37	K MAMMELLA	DUTTALE		ex4	c.813T>A (p.Ser271Arg)
3028.22	75	K MAMMELLA MASCHILE	DUTTALE		ex4	c.813T>A (p.Ser271Arg)
1346.21	71	K OVAIO	SIEROSO ALTO GRADO		ex11	c.(3113+1_3114-1)_(3201+1_3202-1)dup (p.?)
2469.22	67	K MAMMELLA	NST		ex11	c.(3113+1_3114-1)_(3201+1_3202-1)dup (p.?)
81.21	54	K MAMMELLA	INFILTRANTE NST		ex12_13	c.3201+1_3202-1 (p.*297_?)
2802.22	48	K MAMMELLA	DUTTALE		ex13	c.3428T>A; p.(Leu1143His)
1896.22	42	K MAMMELLA	DUTTALE	*MUTYH (NM_001048174.2)*	ex7	c.452A>G (p.Tyr151Cys)
1803.21	42	K MAMMELLA	MUCINOSO		ex12	c.1063del (p.Ala357fs)
757.21	50	K MAMMELLA	DUTTALE		ex13	c.1174C>A (p.Leu392Met)
1423.21	41/48	K MAMMELLA BILATERALE	DUTTALE/ INFILTRANTE NST			
1469.21	28	K MAMMELLA	INFILTRANTE NST			
1071.22	42	K MAMMELLA	LOBULARE		ex13	**c.1103G>A p.(Gly368Asp)**
3568.22	48	K MAMMELLA	LOBULARE			
3635.22	51	K MAMMELLA	INFILTRANTE NST			
2864.22	50	K OVAIO	ENDOMETRIALE DIFFERENZIATO			
2468.22	62	K MAMMELLA	NST		ex13	c.1258C>A (p.Leu420Met)
230.21	51	K OVAIO	NST		ex14	c.1437_1439del (p.Glu480del)
840.22	38	K MAMMELLA	DUTTALE	*NTHL1 (NM_002528.7)*		
3417.22	54	K MAMMELLA	NST INFILTRANTE			
3161.22	47	K MAMMELLA	DUTTALE		ex2	**c.244C>T(p.Gln82Ter)**
2704.22	37	K MAMMELLA	INFILTRANTE TRIPLO MEGATIVO			
1442.21	55	K MAMMELLA	DUTTALE			
633.21	46	K MAMMELLA	INFILTRANTE TRIPLO NEGATIVO			
46.18	29	K MAMMELLA	DUTTALE TRIPLO NEGATIVO		ex3	c.503T>C)p.Ile168Thr)
477.21	54	K MAMMELLA	INFILTRANTE NST		ex3	c.503T>C)p.Ile168Thr)
1641.22	50/58	K MAMMELLA BILATERALE	DUTTALE/TUBULARE		ex6	c.578_584del (p.Gly193fs)

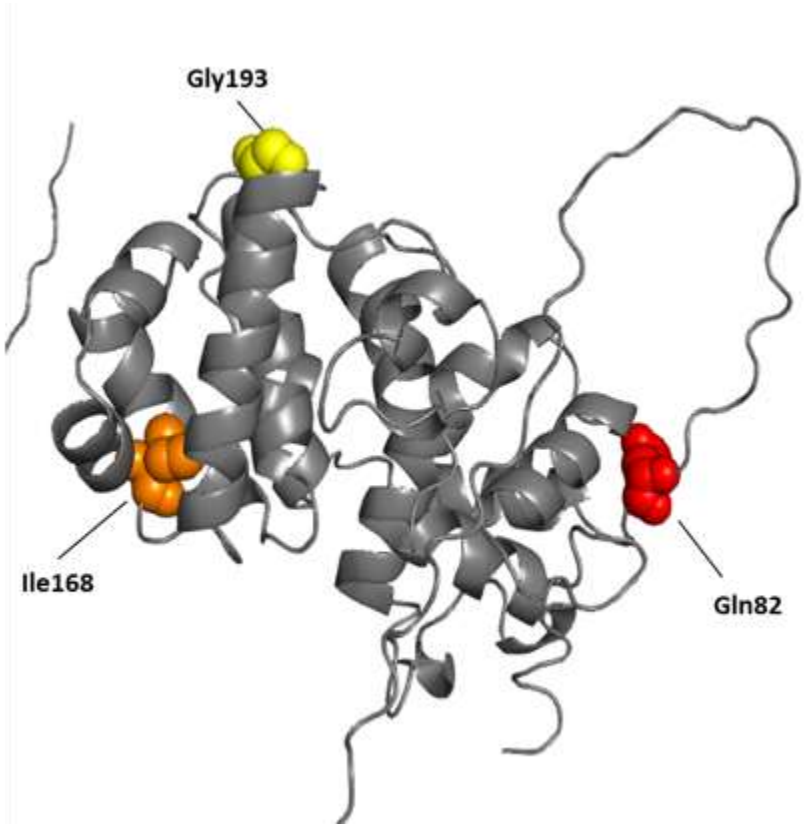
c.420del (P.Lys140fs) →3/320
Allele Frequency : **0.3%**
(6/941)
Allele Frequency ENF: **ND**

c.1103G>A p.(Gly368Asp)
6/320
Allele Frequency : **0.37%**
(7/941)
Allele Frequency ENF: **0.5%**

c.244C>T(p.Gln82Ter)→8/320
Allele Frequency : **0.7%**
(13/941)
Allele Frequency ENF: **0.19%**

NTHL1

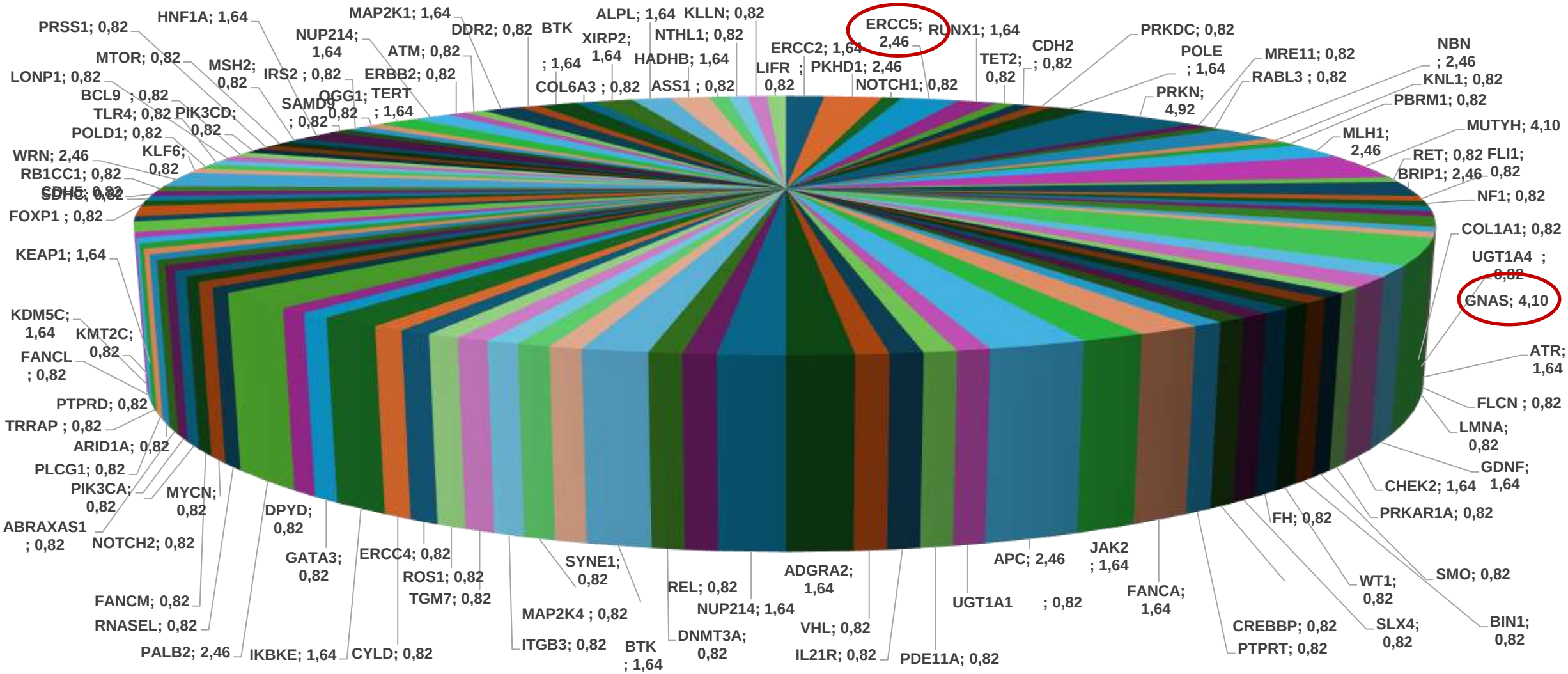




- The variant falls within the *iron-sulfur binding cluster domain* [4Fe4S]
- It is described in the literature as associated with *NTHL1-associated syndrome* (Molecular Signature 30)
- A recent international and multicenter study on women with breast cancer reveals a **reduced expression of the protein even in heterozygous carriers**, which could, therefore, be associated with a low to moderate risk of developing breast cancer, which increases when combined with risk factors

Li N., et al. NPJ Breast Cancer. 2021

Uncommon variants in 122 exomes exhibiting strong tumour association.



Regarding results on tumoral tissue, ***somatic pathogenic variants*** *of the TP53 gene were found in* ***72.16%*** *of patients (compared to literature data, our percentage is approximately* ***16% higher).***

BRCA1 and BRCA2 were mutated in ***13.40% and 9.28%*** *of patients*

In addition, 30 cases had additional pathogenic variants in different genes such as CHEK2, ATM, PTEN, PIK3CA, MSH2, MSH6, MLH1, PALB2, STK11, APC, FBN1, BUB1, CDHR1, FGFR2, ARID1A, RAD50 and RAD51C.

Germline variants: ***POLD1, PLK3, ROS1, PRKN, PCK2, XRCC4, OGG1, PLEKHM1, POLG, LIFR*** *(also breast),* ***MCCC2, EGF****.*

OVARIAN CANCER CASES

Pathway	Gene	Germline variants	Somatic variants
DNA damage repair: Homologous recombination repair (HRR)	***BRCA1***	4.12 %	13,40 %
	BRCA2	6.19 %	9,28 %
Other HRR-related genes	***BARD1***	2,06 %	1,03%
	ATM	2,06 %	2,06%
	CHEK2	2,06 %	2,06%
	PALB2	/	2,06%
Cell cycle	***TP53***	/	72,16%
DNA damage repair: Mismatch repair	***MSH2***	1,03 %	1,03%
	MSH6	1,03 %	1,03%
	MLH1	/	1,2%
Phosphoinositide 3-kinase	***PTEN***	/	7,6%
	PIK3CA	/	6,3%
Other	*		

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Fresh Tissue and Germinal DNA HDR and exome sequencing

CASE	AGE	DIAGNOSIS	Histopathology of OC	SNP array	HDR/ lc-WGS	SOMATIC VARIANTS	GERMLINE VARIANTS
2569.22	65	Ovarian Cancer	Ovarian Cancer - Serous, high grade	arr(X,1-22)cx	+		

There is ample evidence that **activating EGF variants** drive cellular processes related to ovarian cancer development, tumour cell survival, and metastasis.

EGF stimulates EGFR, which **is overexpressed in up to 60% of ovarian epithelial tumours**.

EGFR regulates complex cellular events and several signalling pathways.

In ovarian cancer, **EGFR activation is associated with an increased malignant tumour phenotype and a worse prognosis for patients**. However, unlike other EGFR-positive solid tumours, treatment of ovarian tumours with anti-EGFR agents has induced a response and could be a **target for therapy**.

EXOMES

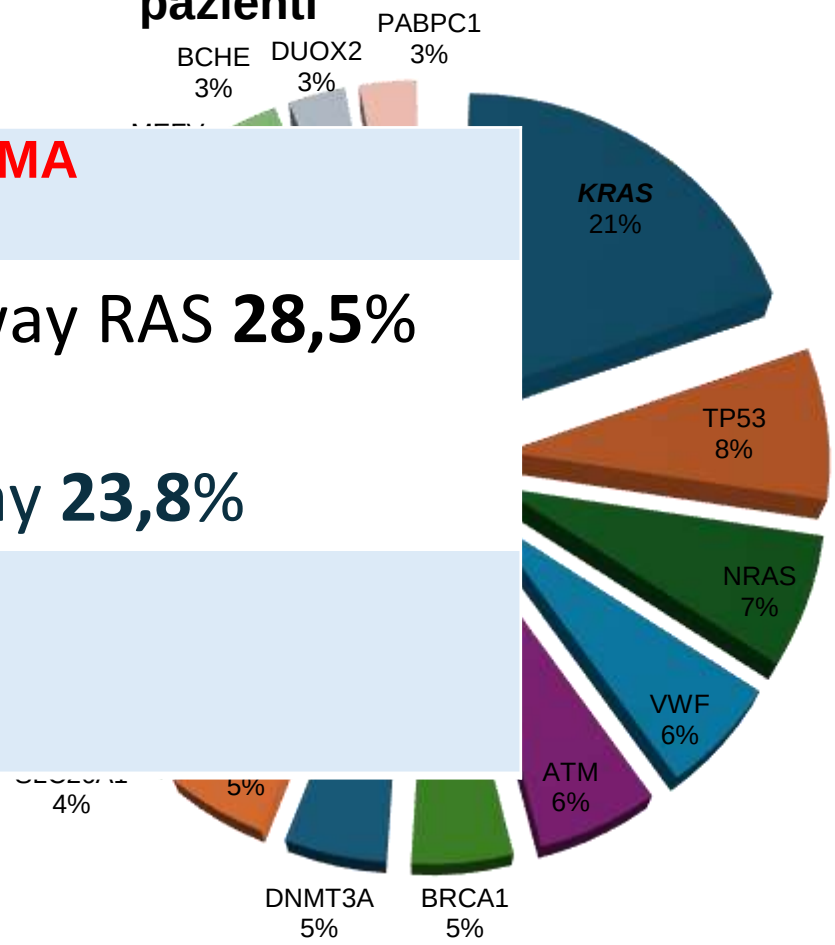
Multiple myeloma

	CASE number	Subtypes	FISH/KARYO	Other Genes
1				
	ARR411	MM ESORDIO	1q21-22x4; 1p32.3x1; t(4;14), 13q14.3x1; 17p13.1x1	***BRCA1*** (NM_007299.4):c.43A>C (p.Ile15Leu) (VUS)
	SNP713	MM RICADUTA	1q21-22x4; 1p32.3x1; t(4;14); 13q14x1; 17p13.1x1	***KRAS*** (NM_004985.5):c.183A>C (p.Gln61His) (PAT) ***BRCA1*** NM_007294.4:c.43A>C (p.Ile15Leu) (VUS)
	SNP830	MM RICADUTA	1q21-22x5; 1q25x6; 1p32.3x3; t(4;14); 11x4; 13q14.3x1; 17p13.1x1; 17x4	***KRAS*** (NM_004985.5):c.183A>C (p.Gln61His) (PAT) ***BRCA1*** NM_007294.4:c.43A>C (p.Ile15Leu) (VUS)
2	ARR422	MM RECIDIVA	1q21-22x3; 11x3; 13x1; 17p13.1x1	***CHEK2*** NM_007194.4:c.1312G>T p.Asp438Tyr (VUS) SMARCA4 NM_003072.5:c.802G>A p.Val268Met (VUS) ***TP53:*** c.818G>A p.Arg273His (PAT) MISSENSE
3	SNP525	MM	1qx3; 4x3; 11x3; 13x1	***ATM*** (NM_000051.4):c.5071A>C (p.Ser1691Arg) (VUS)
4	SNP528	MM ESORDIO	11x3;13q14x1; 14q32.3x1; 17p13.1 x2; 17x3	APC NM_000038.6:c.4435G>T p.Val1479Phe (VUS)
5	SNP533	SMOLDERING	t(11;14)	***BRCA2*** (NM_000059.4):c.1283T>G (p.Leu428Arg) (VUS)
6	SNP539	MM RICADUTA	1p32.3x1; 11x3; 17p13.1x1	PMS2 (NM_000535.7):c.1253C>T (p.Ser418Phe) (VUS)
7	SNP574	MM ESORDIO	11x3; 13x1	NO
8	SNP591	MM RICADUTA	4x3; 11q13.3x3; t(11;14); 13q14.3x1; 17x3	DDX11 c.1403dup(PAT); MLH1 c.1190T>A(VUS); MLH1 c.512T>A(VUS)
9	SNP594	MM RICADUTA	1p32.3x1; t(11;14)	MSH2 (NM_000251.3):c.1748A>G (p.Asn583Ser) (VUS) SMAD4 (NM_005359.6):c.851A>G (p.Gln284Arg) (VUS)
10	SNP600	MM MICROMOLECOLARE SOSPETTO	11x3; 13x1; 14x1; 17p13.1x1	SMARCA4 (NM_003072.5):c.704_705insGCCTGG (p.Gly243_Pro244dup) (VUS)
11				

Multiple myeloma

GENE	n°	TOT	%	
KRAS	20	95	21,05	c.183A>C (p.Gln61His) in 4 pz (PAT); c.35G>C (p.Gly12Ala) in 5 pz (PAT); c.35G>T (p.Gly12Val) in 2 pz (PAT)
TP53	8	95	8,42	c.517G>T (p.Val173Leu) ; c.538G>A; p.(Glu180Lys) (LP); c.817C>T (p.Arg273Cys) (PAT); c.818G>A (p.Arg273His) (PAT); c.782+2T>G (p.?) (PAT); c.591_601del (p.Glu198AlafsTer7) (LP); c.991C>T; (p.Gln331Ter) (PAT); c.626_627del; (p.Arg209fs) (PAT);
NRAS	7	95	7,37	c.182A>G (p.Gln61Arg) (LP) x4; c.181C>A (p.Gln61Lys) in 2 pz
VWF	6	95	6,32	c.4517C>T (p.Ser1506Leu) (LP) in 5 pz
ATM	6	95		
BRCA1	5	95		
DNMT3A	5	95		
BRAF	5	95		
SLC26A1	4	95		
ABCA4	4	95		
CHEK2	4	95		
PTPN11	4	95		
NTHL1	3	95		
CD36	3	95		
MSH2	3	95		
MEFV	3	95		
GLB1	3	95		
BCHE	3	95		
DUOX2	3	95		
PABPC1	3	95		
CHD2	2	95		
PCK2	2	95		
DNAJC19	2	95	2,11	
PEX10	2	95	2,11	
PADI3	2	95	2,11	c.335T>A p.(Leu112His) (LP) in 2 pz
AFG2A	2	95	2,11	
WNT10A	2	95	2,11	c.682T>A (p.Phe228Ile) (LP) in 2 pz
BRCA2	2	95	2,11	
SMARCA4	2	95	2,11	
PMS2	2	95	2,11	

varianti geniche rilevate in almeno 3 pazienti



CASI SEQUENZIATI PER ESOMA

KRAS: **20,6%** e geni del pathway RAS **28,5%**

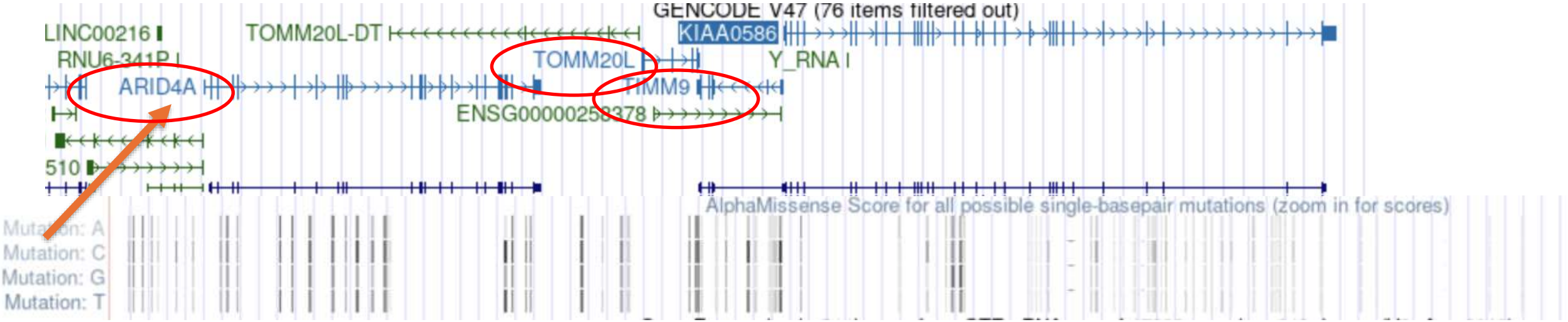
TP53: 7,8%

BRCA1/2: 9,5% e geni pathway **23,8%**

Sardinian haplotype and Multiple myeloma

Variant	Observed Frequency	NFE Frequency (GnomAD)
KIAA0586 *(rs534542684)*	0.021093	0.004433

The variant in the ***KIAA0586*** gene (*rs534542684*), known for its role in ciliopathies, has a significantly higher frequency in Sardinia (**2.1% vs. 0.4433%).**



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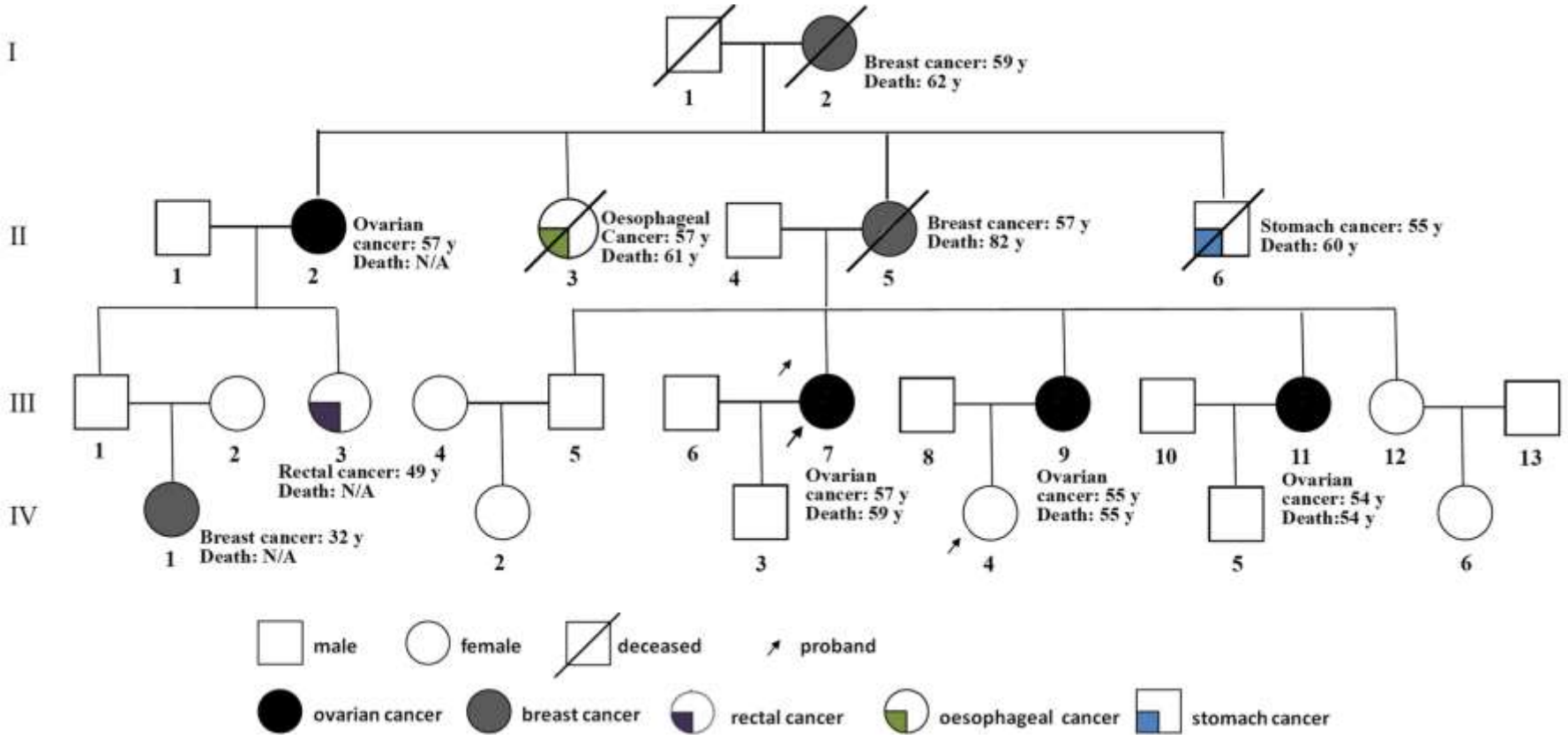
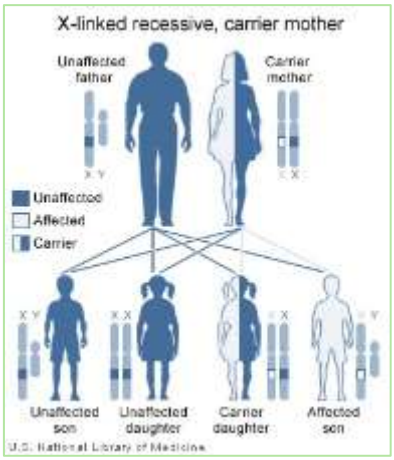
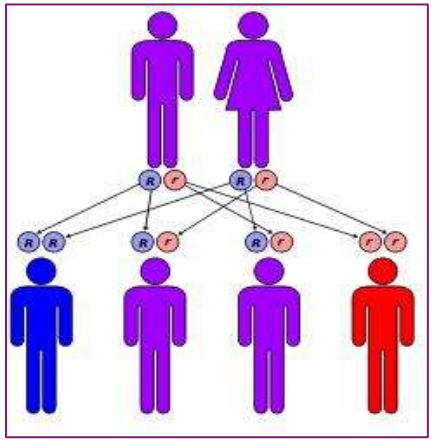
Despite exome sequencing, at least 40% of familial cases remain unexplained

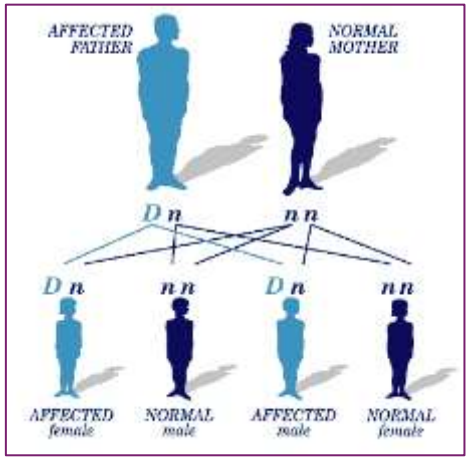


No germinal variants.

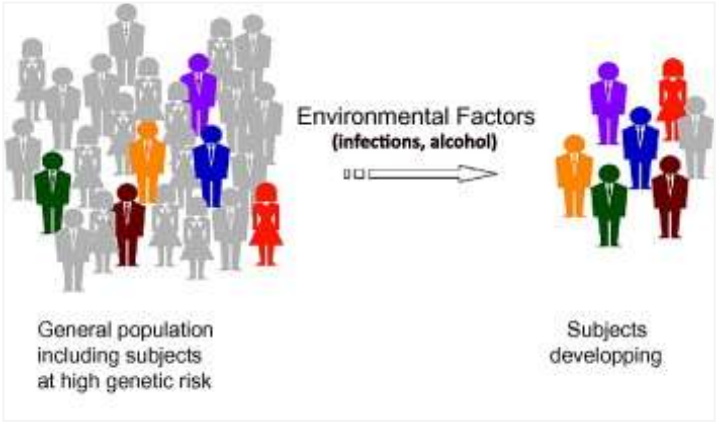
The extent of "genetic causality" differs according to the mode of inheritance







Although monogenic diseases are rare, polygenic risk alleles (polygenic risk scores) are present in common adult-onset diseases, where variants among multiple genes are necessary to cause illness.



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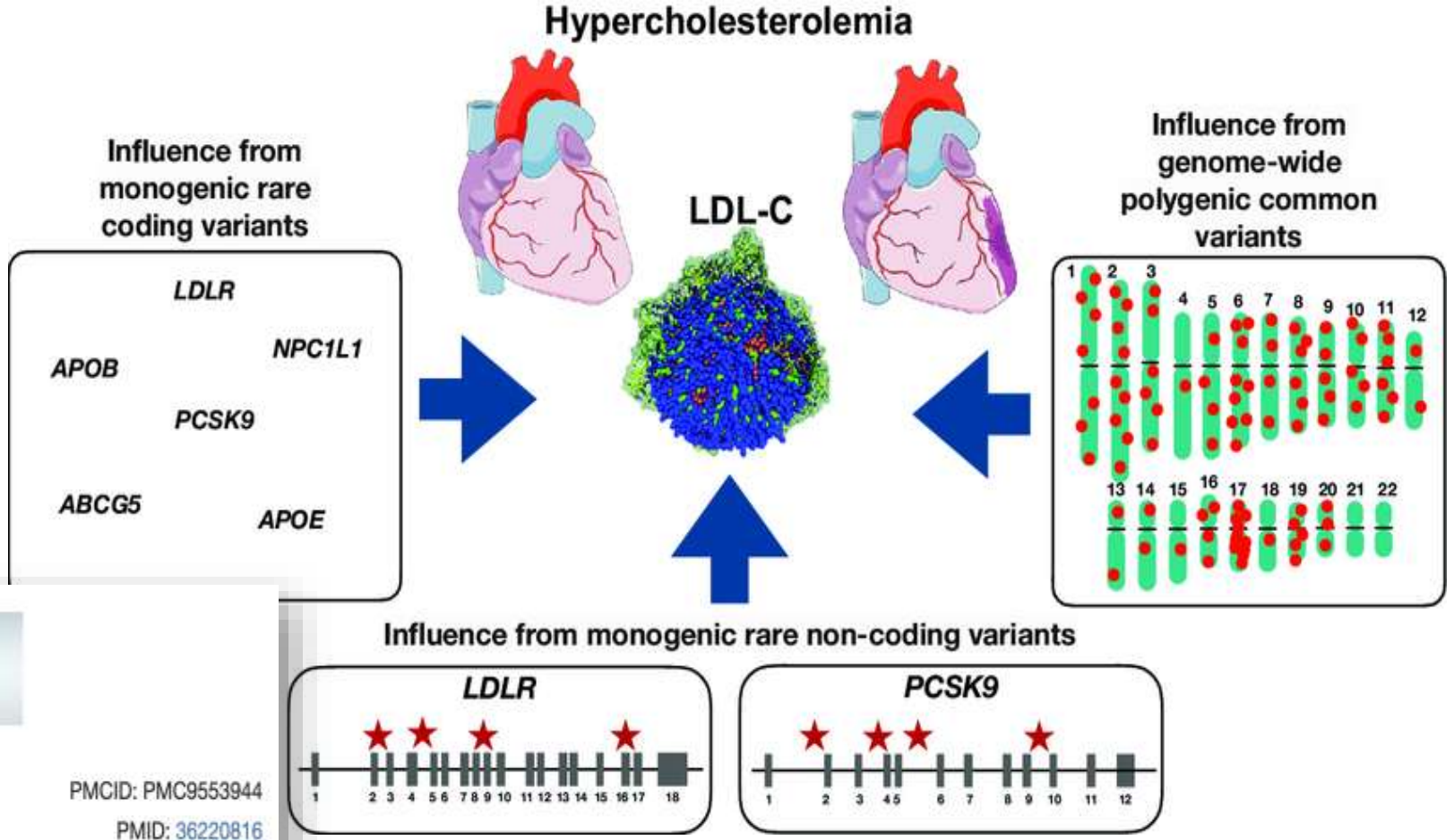
WGS captures the majority of genomic variation by identifying both common and rare variants that may contribute to the genetic composition of a disease.



nature COMMUNICATIONS

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PMCID: PMC9553944
PMID: 36220816

Whole genome sequence analysis of blood lipid levels in >66,000 individuals

Margaret Sunitha Selvaraj,[1,2,3] Xihao Li,[4] Zilin Li,[4] Akhil Pampana,[2] David Y. Zhang,[5,6] Joseph Park,[5,6]

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Article

Polygenic architecture of rare coding variation across 394,783 exomes

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Check for updates

Daniel J. Weiner[1,2,3,6]✉, Ajay Nadig[1,2,3,6]✉, Karthik A. Jagadeesh[1,4], Kushal K. Dey[1,4], Benjamin M. Neale[1,2,3], Elise B. Robinson[2,3,5], Konrad J. Karczewski[1,2,3] & Luke J. O'Connor[1]✉

Both common and rare genetic variants influence complex traits and common diseases. Genome-wide association studies have identified thousands of common-variant associations, and more recently, large-scale exome sequencing studies have identified rare-variant associations in hundreds of genes[1–3]. However, rare-variant genetic architecture is not well characterized, and the relationship between common-variant and rare-variant architecture is unclear[4]. He… the heritability explained by the gene-wise burden of rar… common traits and diseases in 394,783 UK Bi… (allele frequency < 1 × 10^{-3}) exp… average—much l… by … …bility for … Our results indicate that … number of large-effect genes, that … are mechanistically convergent, and that rare coding … bute only modestly to missing heritability and population risk … ification.

… 614 | 16 February 2023

Common and rare variants converge mechanistically, **and rare coding variants** will contribute to the population's missing heritability and risk stratification.

Both **common and rare genetic variants influence complex traits** and **common diseases**.

Genome-wide association studies (GWAS) h… ve identified thousands of co… t associations, and m… exome se…

This paper quantifies the heritability explained by **the gene burden of rare coding variants in 22 common traits** and diseases in **394,783 exomes from the UK Biobank**.

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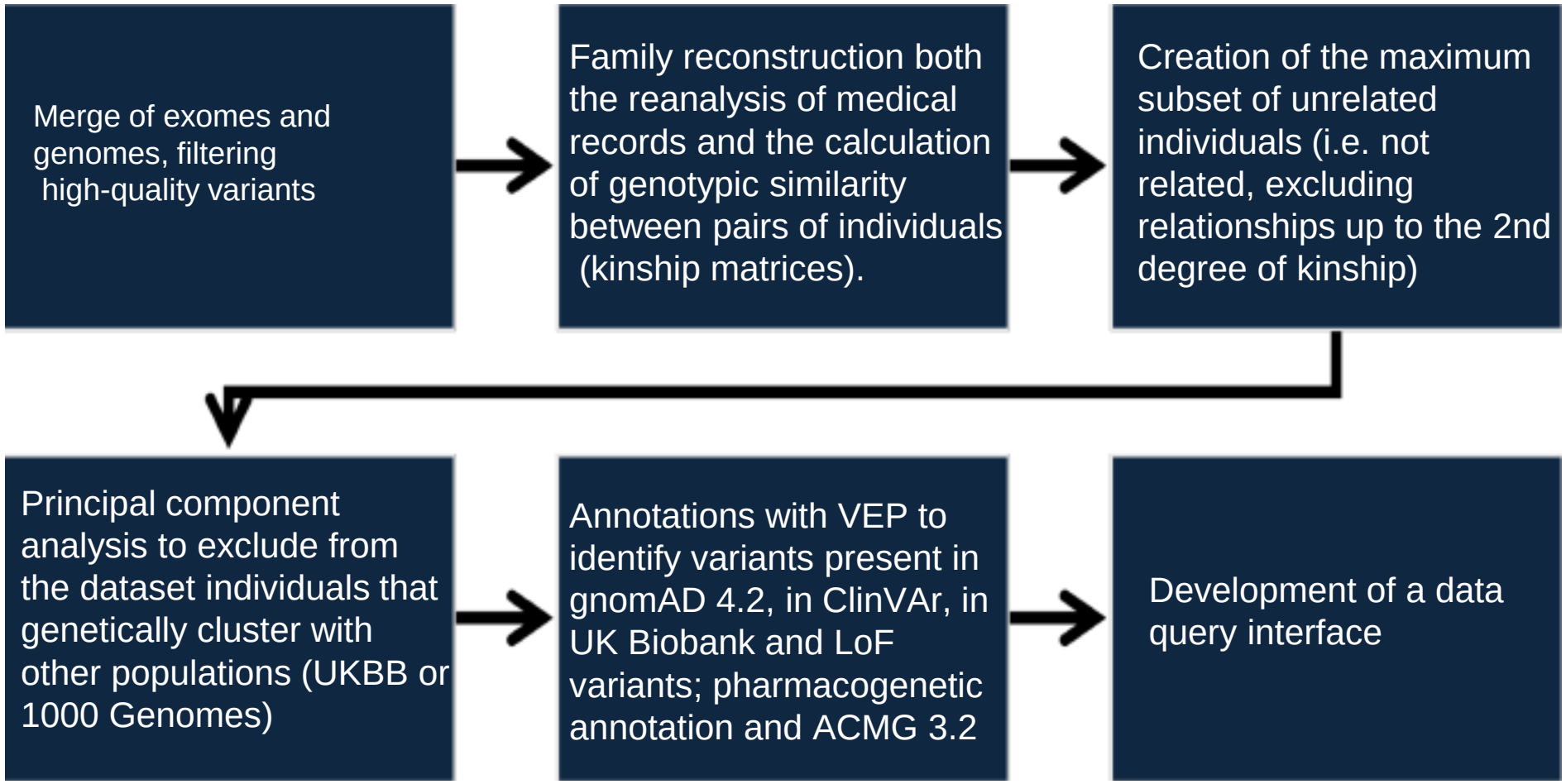
The Genome of the Sardinian Population

The study of a population with **unique genetic characteristics**, such as the Sardinian one, offers the opportunity to discover **new functionally significant genetic variants** that have not been observed in large-scale sequencing studies and to **open new avenues for understanding the impact of known variants related to diseases** (tumours and other complex diseases).

An essential step in valorising this data is reorganising it by creating a **single repository of sequences** that enables controlled-access queries (for instance, by researchers or clinicians wanting to determine if a suspected variant has already been identified in other patients or healthy individuals).



Informatics pipeline workflow for a single repository of exomic and genomic sequences and high-quality variant annotation.

To:

- Study the allelic frequency of pathogenic variants among Sardinians;

- Identify variants that differ in Sardinians compared to other populations, using the UK Biobank, GnomAD, and 1000 Genomes datasets as references (data *in house*), with particular emphasis on variants that are deterministically involved in the etiopathogenesis of genetic diseases, including tumours;

- Identify clinically relevant variants for incidental findings (according to ACMG 3.2 guidelines) and for drug response (in accordance with DPWG and CPIC guidelines);

- Evaluate the existence of human knockouts within the examined population, i.e. healthy individuals with loss of function in both alleles of a given gene.

Preliminary results

Pilot analysis of the first 2,000 exomes, **1,430 of which belonged to unrelated Sardinian individuals**, enabled us to observe several points of interest (which will soon be explored following the inclusion of an additional 1,000 already sequenced samples in the dataset).

We identified around **2.2 million genetic variants in canonical transcripts**, with **40.74% of these absent from gnomAD 4.1**.

Among these, **nearly 35,000 loss-of-function variants (LoF)** were detected, including those causing premature stops, affecting essential splice donor/acceptor sites, or resulting in frameshifts. Of these LoF variants, **78.64% were observed as singletons**.

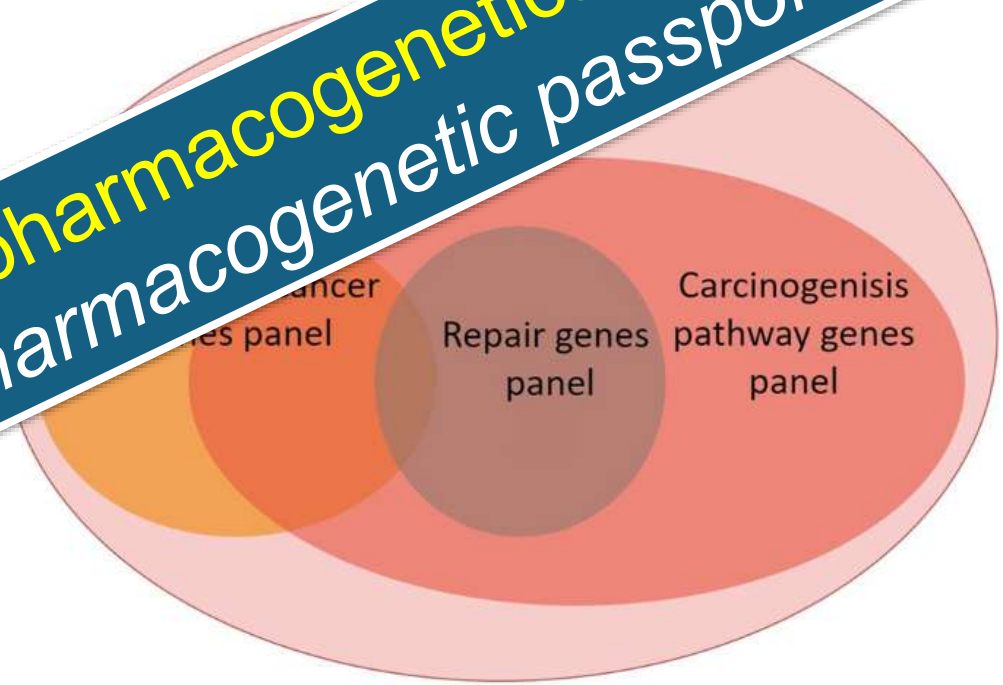
From the analysis of pathogenic and likely pathogenic variants, we identified a subset of these **with a higher frequency in Sardinia than in global populations**, particularly those located in genes associated with non-syndromic genetic hearing loss (***GJB2***), in addition to those already documented (for example, in HBB and G6PD).

We also estimated that approximately **29% of the sequenced individuals carry a clinically actionable genetic variant** from the ACMG SF 3.2 gene list.

Tumori: studio dellaTumors: Study of the **Polygen**
Component in Risk Stratification in Tumo

- Oncogenes, which promote the proliferation of tumour cells
- Tumour suppressor genes, which inhibit this growth/proliferation
- Anti-mutational genes
  the stability of de
  (DNA)
- m
  tran
  a role in post-
  processing

Implementation of a "germinal" pharmacogenetics program through the creation of a *pharmacogenetic passport*



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Grazie a tutti !

Sabrina Giglio & Andrea Perra