AUCKLAND, NEW ZEALAND

INTERNATIONAL SOCIETY FOR GASTROINTESTINAL HEREDITARY TUMOURS (InSiGHT)

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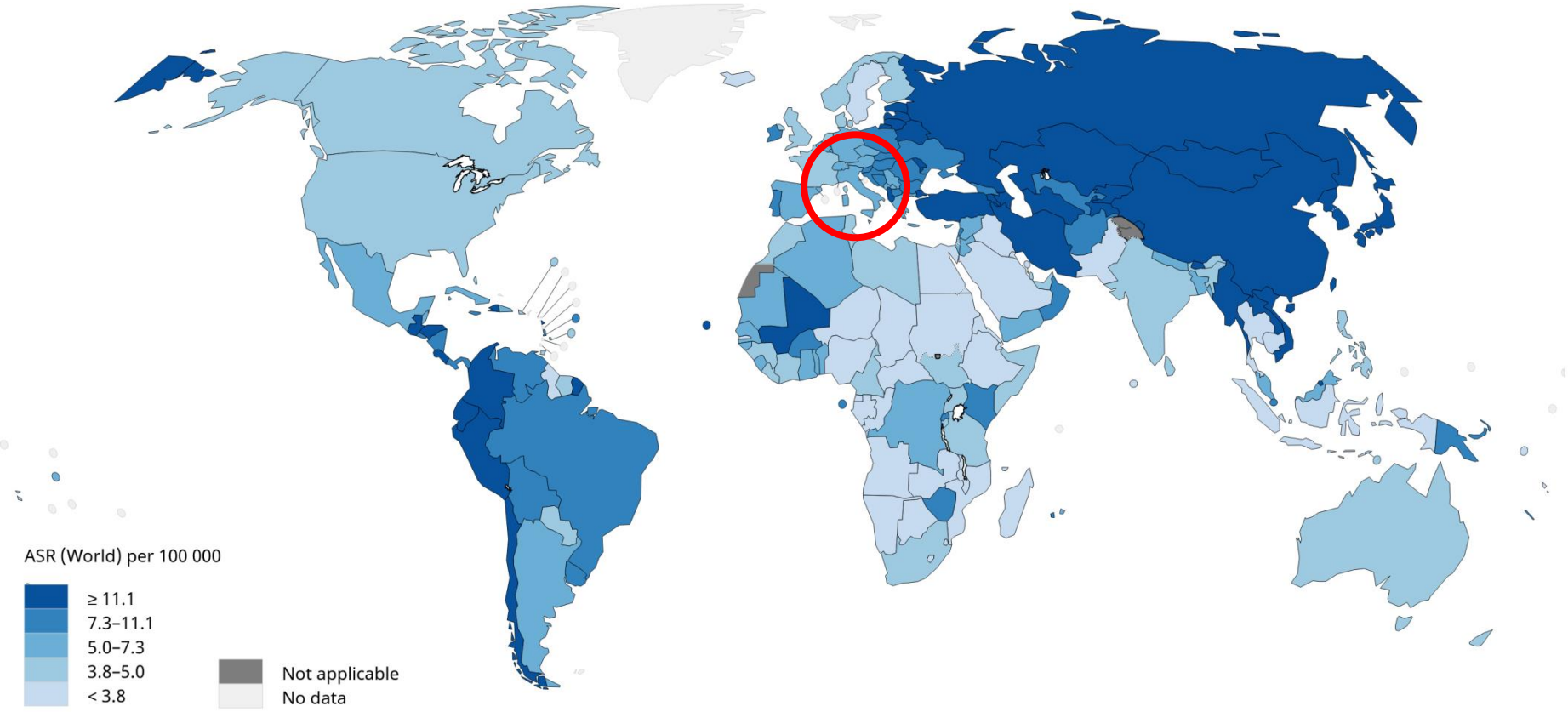
Multiple-gene panel analysis in an Italian cohort of patients with familial gastric cancer

Gianluca Tedaldi

Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS

Gastric cancer (GC)



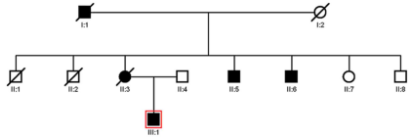
Bray F *et al.* CA Cancer J Clin. 2018

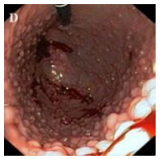
AIM: to study the genetic predisposition to GC in Italy

GC predisposition syndromes:

- **Hereditary Diffuse Gastric Cancer (HDGC): *CDH1*, *CTNNA1***

- **Familial Intestinal Gastric Cancer (FIGC): ?**





- **Gastric Adenocarcinoma and Proximal Polyposis of the Stomach (GAPPS): *APC* promoter 1B**

- Lynch syndrome (HNPCC): *MLH1, MSH2, MSH6, PMS2*
- Li-Fraumeni syndrome (LFS): *TP53*
- Familial Adenomatous Polyposis (FAP): *APC*
- MUTYH-associated polyposis (MAP): *MUTYH*
- Juvenile Polyposis syndrome (JPS): *BMPR1A, SMAD4*
- Peutz-Jeghers syndrome (PJS): *STK11*
- Cowden syndrome (CS): *PTEN*

Next-Generation Sequencing (NGS) approach

Trusight Cancer Panel

Illumina Miseq

Cumulative target region size 255 Kb
Number of target genes 94
Number of target exons > 1,700
Probe size 80-mer
Number of probes ~4,000
Recommended mean coverage 100×
…get minimum coverage 20×
…nt exons covered based on coverage metrics

Colon Cancer

Breast Cancer

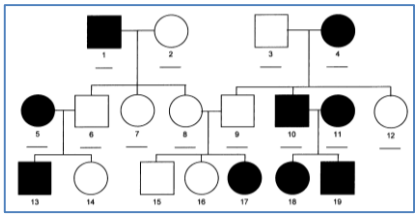
Gastric Cancer

AIP ALK APC ATM BAP1 BLM BMPR1A BRCA1 BRCA2 BRIP1 BUB1B CDC73 CDH1 CDK4 CDKN1C CDKN2A CEBPA CEP57 CHEK2 CYLD DDB2 DICER1 DIS3L2 EGFR EPCAM ERCC2 ERCC3 ERCC4 ERCC5 EXT1 EXT2 EZH2 FANCA FANCB FANCC FANCD2 FANCE FANCF FANCG FANCI FANCL FANCM FH FLCN GATA2 GPC3 HNF1A HRAS KIT MAX MEN1 MET MLH1 MSH2 MSH6 MUTYH NBN NF1 NF2 NSD1 PALB2 PHOX2B PMS1 PMS2 PRF1 PRKAR1A PTCH1 PTEN RAD51C RAD51D RB1 RECQL4 RET RHBDF2 RUNX1 SBDS SDHAF2 SDHB SDHC SDHD SLX4 SMAD4 SMARCB1 STK11 SUFU TMEM127 TP53 TSC1 TSC2 VHL WRN WT1 XPA XPC

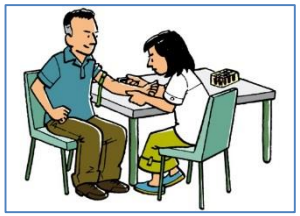
Multiple cancers

Polyposis syndromes

Case series



Genetic counselling



Blood sampling

Molecular analyses

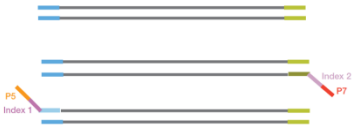
96 patients with a family history of GC:

- 57 patients with Diffuse-type Gastric Cancer (DGC)
- 14 patients with Intestinal-type Gastric Cancer (IGC)
- 8 patients with unspecified Gastric Cancer (GC)
- 14 patients with Lobular Breast Cancer (LBC)
- 3 patients with severe gastric polyposis (GP)

Methods

DNA extraction from peripheral blood

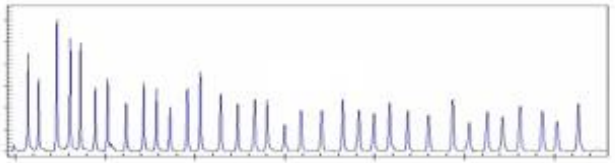
Library preparation
(94 genes)



Sequencing on Miseq

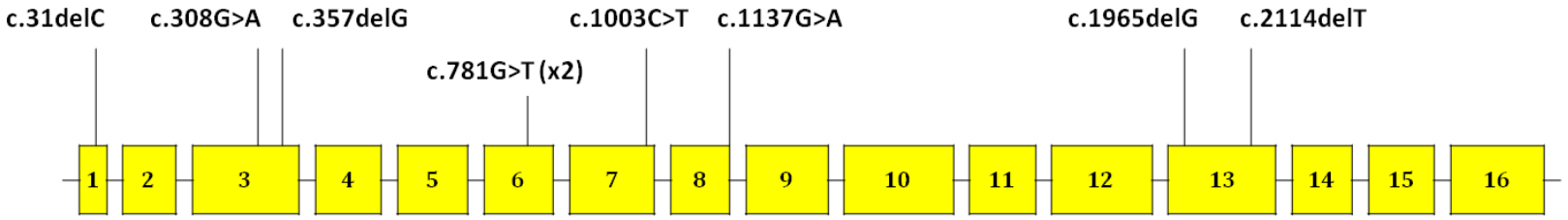
Bioinformatic analysis

Multiplex Ligation-dependent Probe Amplification (MLPA)
to detect large deletions/duplications
(*CDH1* + some selected genes)



CDH1 pathogenic and likely-pathogenic variants

NGS + MLPA



ID	Sex	Selection criteria	Cancer	Age	Other tumours (Age)	Gene mutated	cDNA	Protein	IARC class	dbSNP	Clinvar
BM45	M	I	DGC	47		*CDH1*	c.2114delT	p.L705fs	4	-	
BM74	M	I	DGC	59		*CDH1*	c.1965delG	p.K655fs	4	-	
BM100	M	I	DGC	58		*CDH1*	c.357delG	p.V119fs	4	-	
BM37	F	II	DGC	37		*CDH1*	c.308G>A	p.Trp103Ter	4	rs1555514464	pathogenic
BM60	M	II	DGC	39		*CDH1*	c.1003C>T	p.Arg335Ter	5	rs587780784	pathogenic
BM81	F	II	DGC	18		*CDH1*	c.781G>T	p.Glu261Ter	5	rs121964873	pathogenic
BM115	F	II	DGC	31		*CDH1*	c.781G>T	p.Glu261Ter	5	rs121964873	pathogenic
BM119	M	II	DGC	33		*CDH1*	c.1137G>A	p.Thr379=	5	rs587783050	pathogenic/likely pathogenic
BM73	F	III	LBC	52		*CDH1*	c.31delC	p.L11fs	4	-	
BM112	F	II	DGC	37		*CDH1*	c.1-?_163+?del	p.(?)	5	-	Oliveira C *et al.* 2009

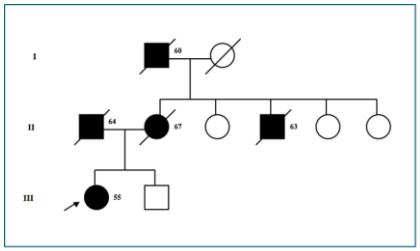
Pathogenic and likely-pathogenic variants in other genes

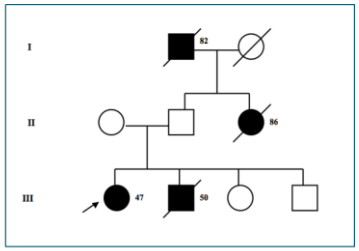
NGS + MLPA

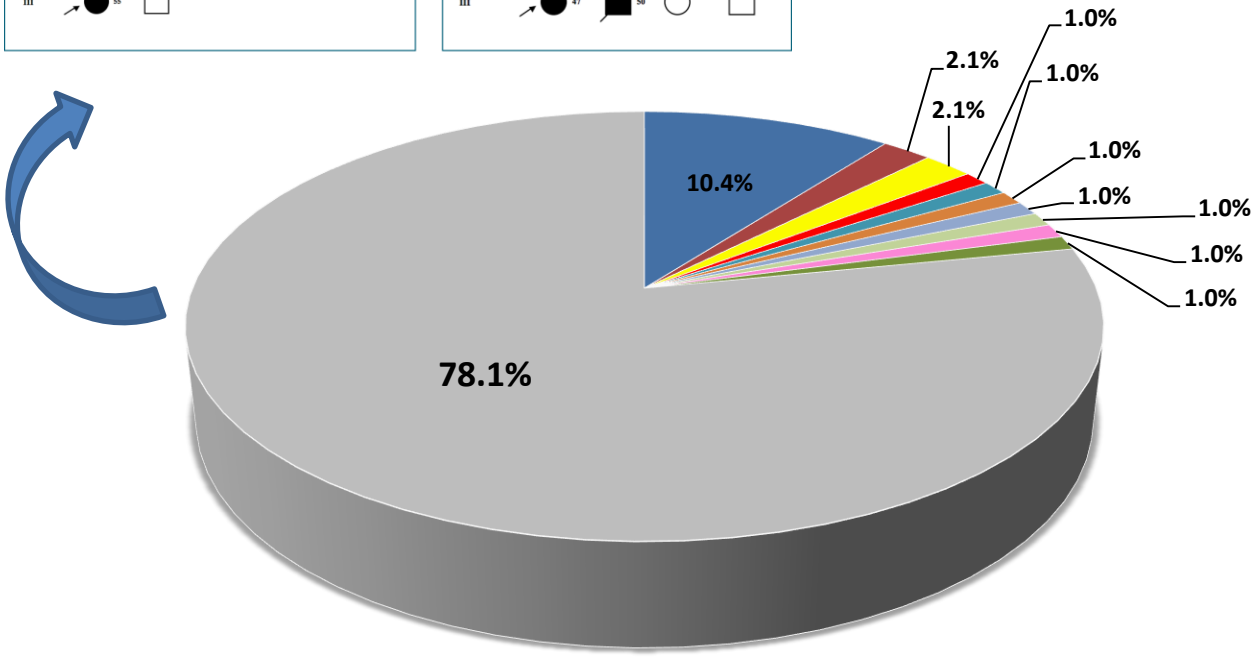
ID	Sex	Selection criteria	Cancer	Age	Other tumours (Age)	Gene mutated	cDNA	Protein	IARC class	dbSNP	Clinvar
BM46	M	I	IGC	54		*ATM*	c.1561_1562del	p.R521fs	4	-	
BM47	F	I	DGC	54	LBC (50y)	*BLM*	c.2394delT	p.H798fs	4	-	
BM89	F	I	DGC	65		*PRF1*	c.1122G>A	p.W374X	5	rs104894176	pathogenic
BM90	M	I	DGC	73		*PMS2*	c.2182_2183del	p.T728fs	4	-	
BM110	F	I	DGC	47		*BRCA1*	c.406delA	p.R136fs	5	rs886040196	pathogenic
BM76	F	II	DGC	32		*ATM*	c.2192dupA	p.Y731_M732delinsX	4	-	
A530	F	IV	LBC	62	LBC (66y)	*PALB2*	c.535C>T	p.Q179X	4	-	
BM10	M	V	IGC	57		*MSH2*	c.367-?_645+?del	p.Ala123_Gln215del	5	-	pathogenic
BM38	M	VI	IGC	60		*BRCA2*	c.6037A>T	p.K2013X	5	rs80358840	pathogenic
BM24	M	VII	GP	52		*BMPR1A*	c.34G>T	p.G12X	4	-	
BM126	F	IV	LBC	62		*PALB2*	c.2718G>A	p.Trp906*	4-5	rs180177122	pathogenic/likely pathogenic

Breast Cancer **Colon Cancer** **Leukemia and Lymphoma**

Summary of the pathogenic and likely-pathogenic variants







- *CDH1 (10)*
- *ATM (2)*
- *PALB2 (2)*
- *BRCA1 (1)*
- *BRCA2 (1)*
- *BMPR1A (1)*
- *MSH2 (1)*
- *PMS2 (1)*
- *BLM (1)*
- *PRF1 (1)*
- ? (75)

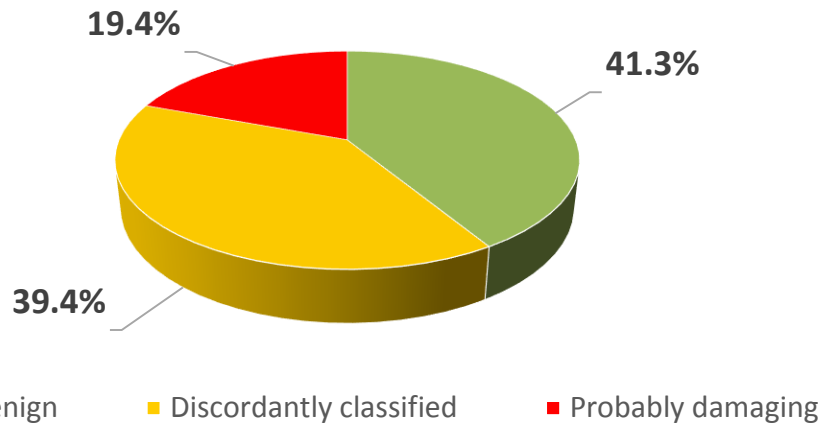
Classification of rare missense variants with prediction tools

Selection of variants with a population frequency <1% or never reported (1000Genomes, Esp6500, Exac03 databases)

- 160 unique missense variants

Prediction of pathogenicity with PolyPhen-2 and SIFT bioinformatics tools

- 66 benign variants (41.3%)
- 63 discordantly classified variants (39.4%)
- 31 probably damaging variants (19.4%) -> 28 patients



Conclusions

The multigene panel approach on 96 cases of FGC allowed us to the detect:

- 10.4% of patients with a deleterious mutation in *CDH1*: low percentage but compatible with a high incidence area;

- 11.4% of patients with functional mutations in other genes involved in predisposition to breast cancer (6.3%), colorectal cancer (3.1%) and leukemia/lymphoma (2.1%);

- 78.1% of patients without functional mutations, 29.2% of whom with missense variants classified as damaging by prediction tools.

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Thank you for your attention

gianluca.tedaldi@irst.emr.it