PGT-M as a primary tool for avoiding genetic disease at the community level

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DISCLOSURE

PRESIDENT

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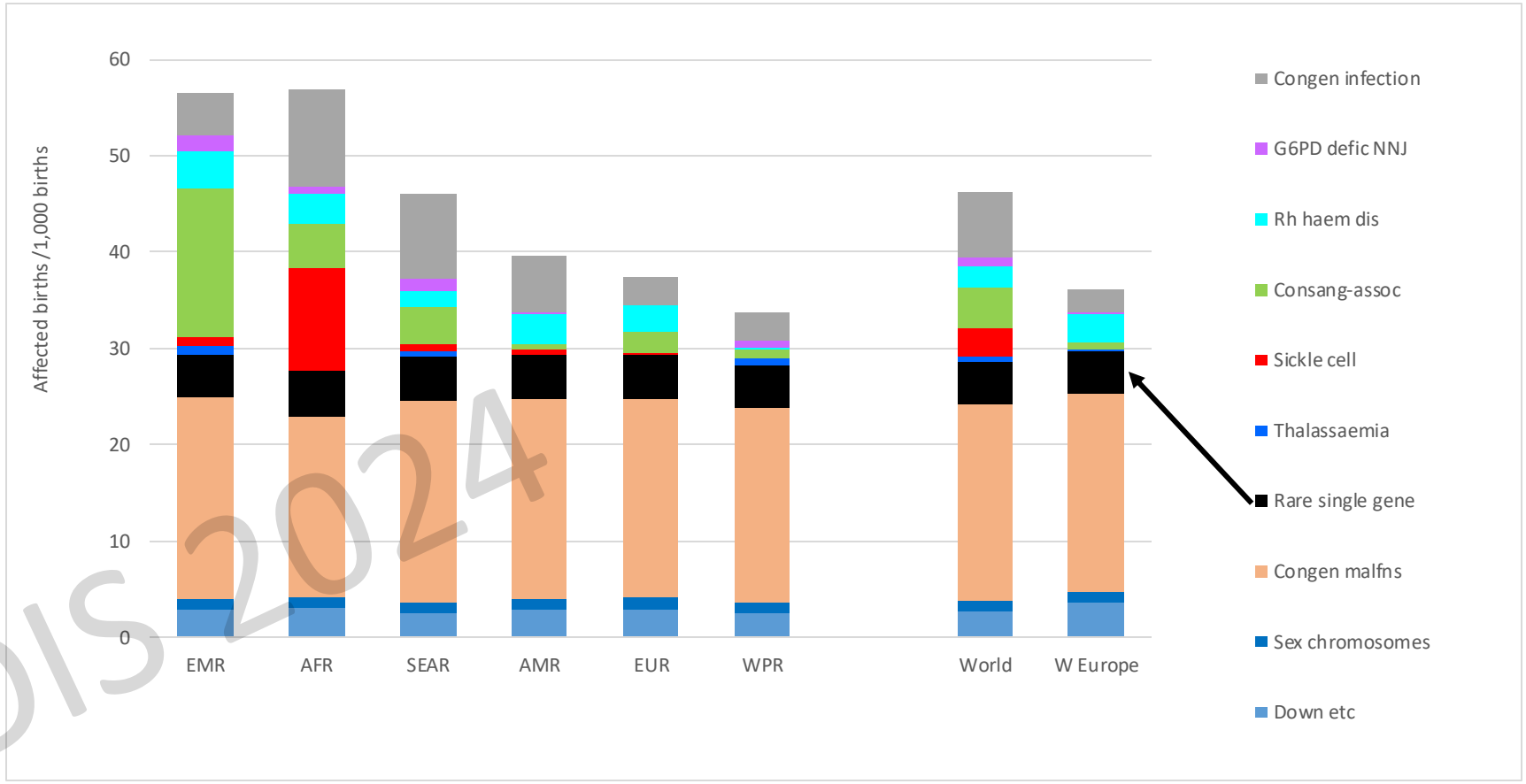
Northbrook, Illinois

RGI offers diagnostic services and

is remunerated for these services

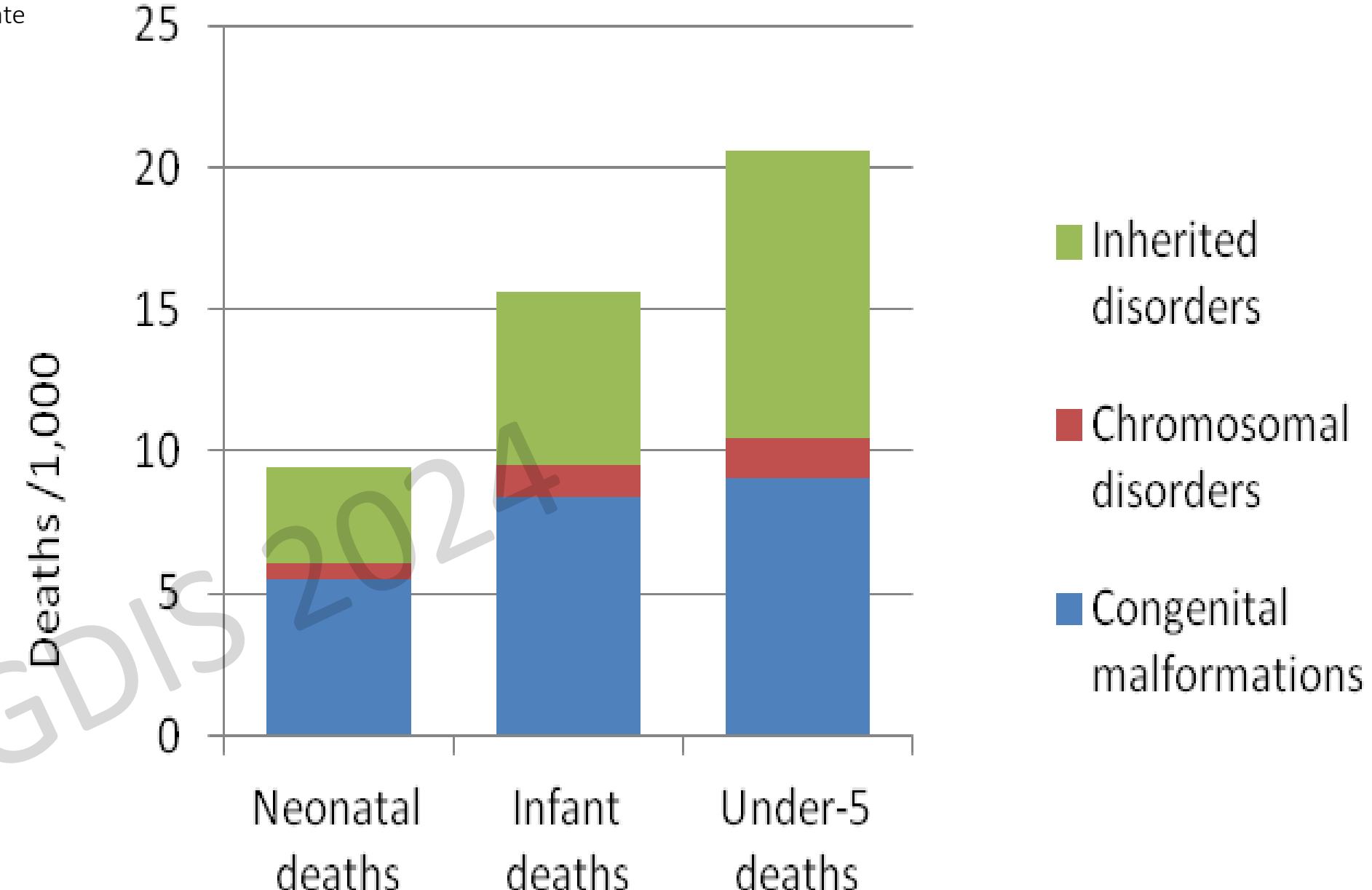
World Birth Prevalence of Congenital disorders



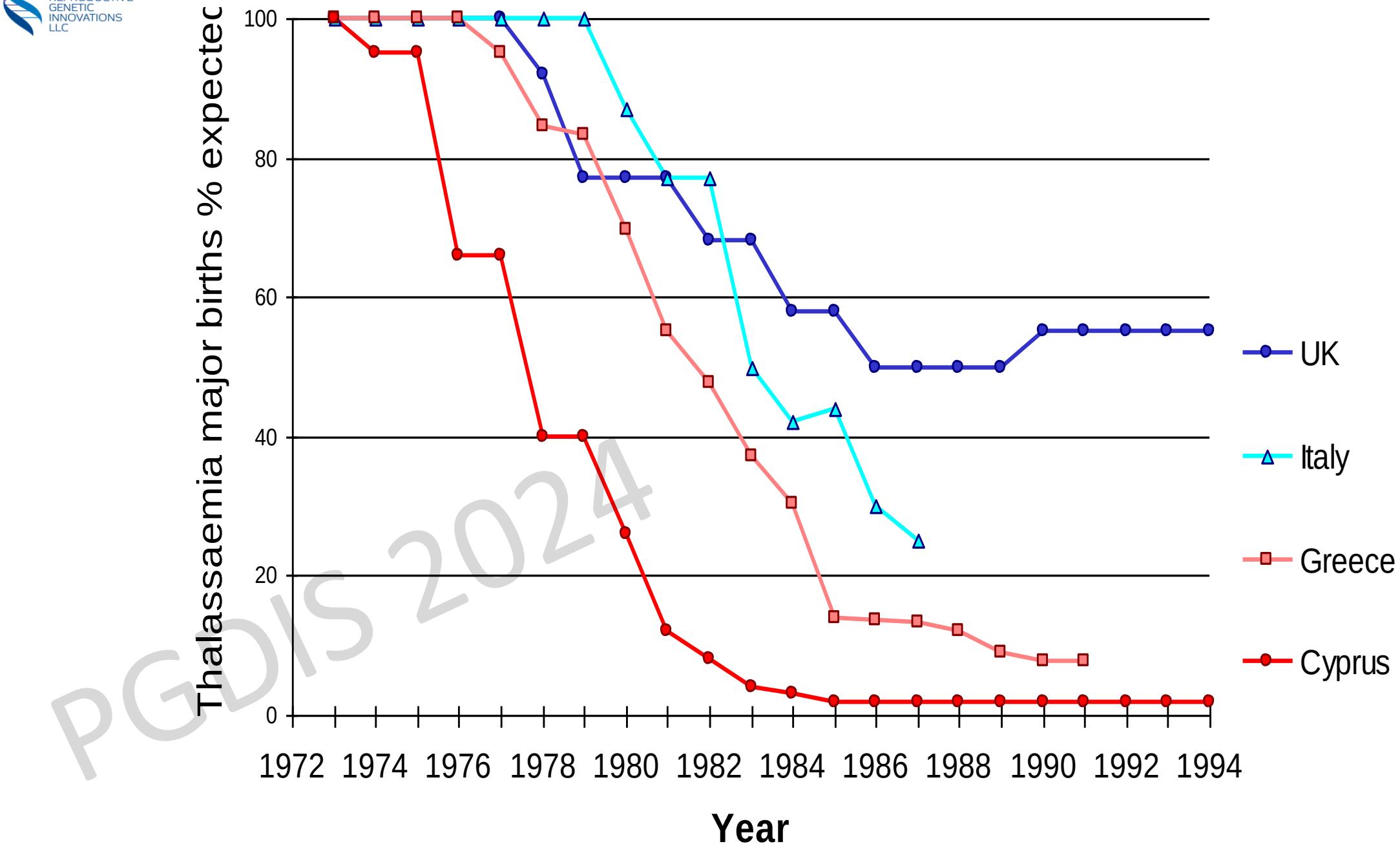
Ethnic oriented -Thal (Blue segments) and Sickle (Red segment; Pan-Ethnic-**Black** segment)

Despite being rare, cumulative impact of single gene disorders to mortality and disability is extremely high

estimate



Prevention of Thalassemia by Prenatal Genetic Screening and Diagnosis



PGT-M: Traditional and Novel Indications

- **Autosomal recessive & Autosomal dominant disorders**
- **X-linked recessive diseases**

- **Late onset diseases:**
  - **Cancer predisposition**
  - **Inherited cardiac diseases**
  - **Neurodegenerative conditions**
  - **HLA genotyping**

HLA, human leukocyte antigen

Impact of Pre-conception Expanded Carrier Screening (ECS) on PGT-M uptake

- **Pre-conception Screening identifies couples at risk**

  (1 affected pregnancy in 300)

- **Embryo selection by IVF and PGT-M provides the option to avoid clinical pregnancy termination**

- **Disease-specific gene panels identify adults with heritable cancers whose offspring learn they are at risk for adult-onset single gene disorders**

Present RGI experience (1990-2023)

Over 36,000 PGT cases performed for 807 conditions

10451 for Mendelian disorders (PGT-M)

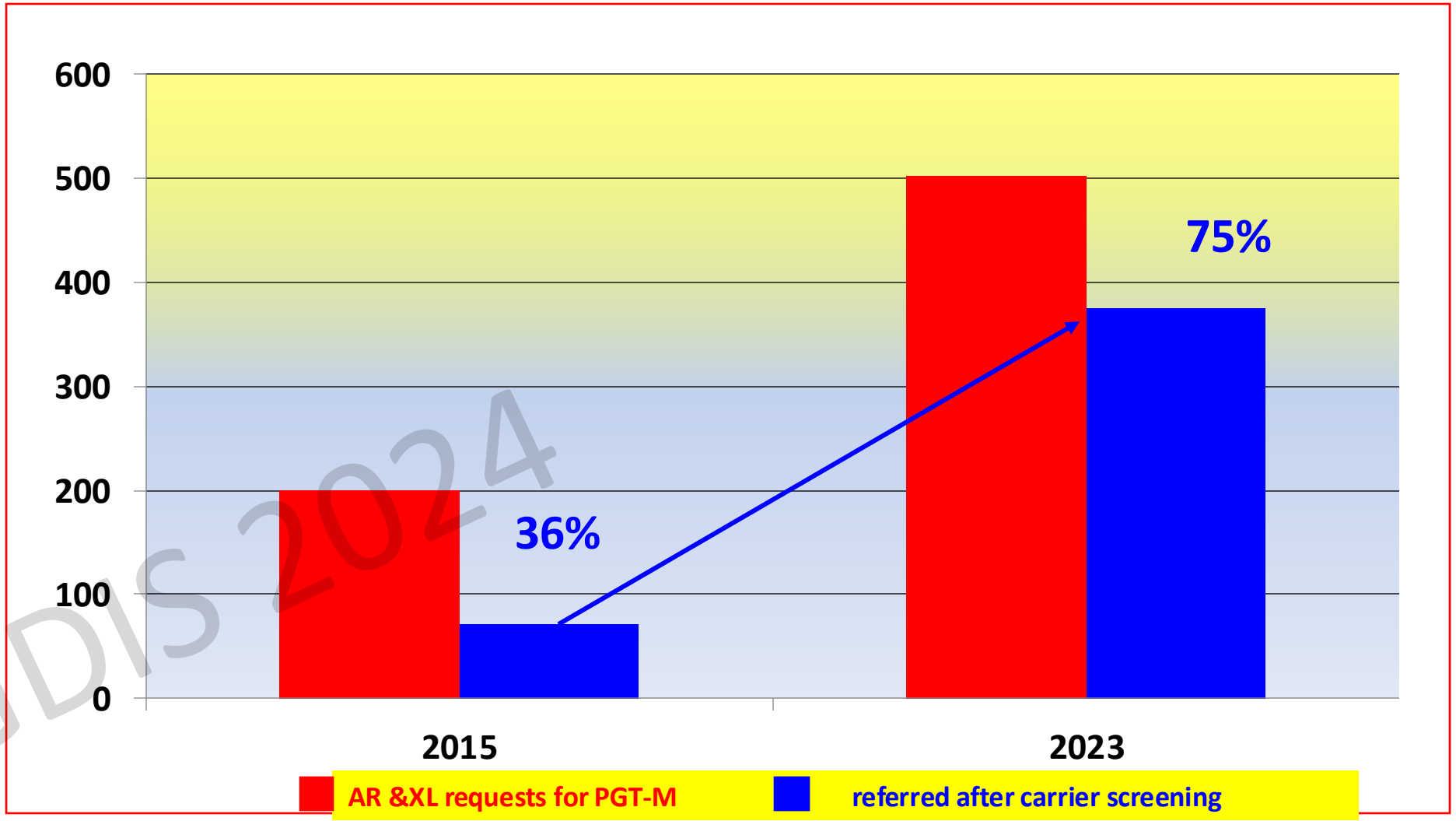
Including:

630 PGT cycles for DE NOVO mutations
1247 for Cancer Predisposition
1197 for Neurodegenerative Conditions
156 for Inherited Cardiac Diseases
569 for HLA genotyping

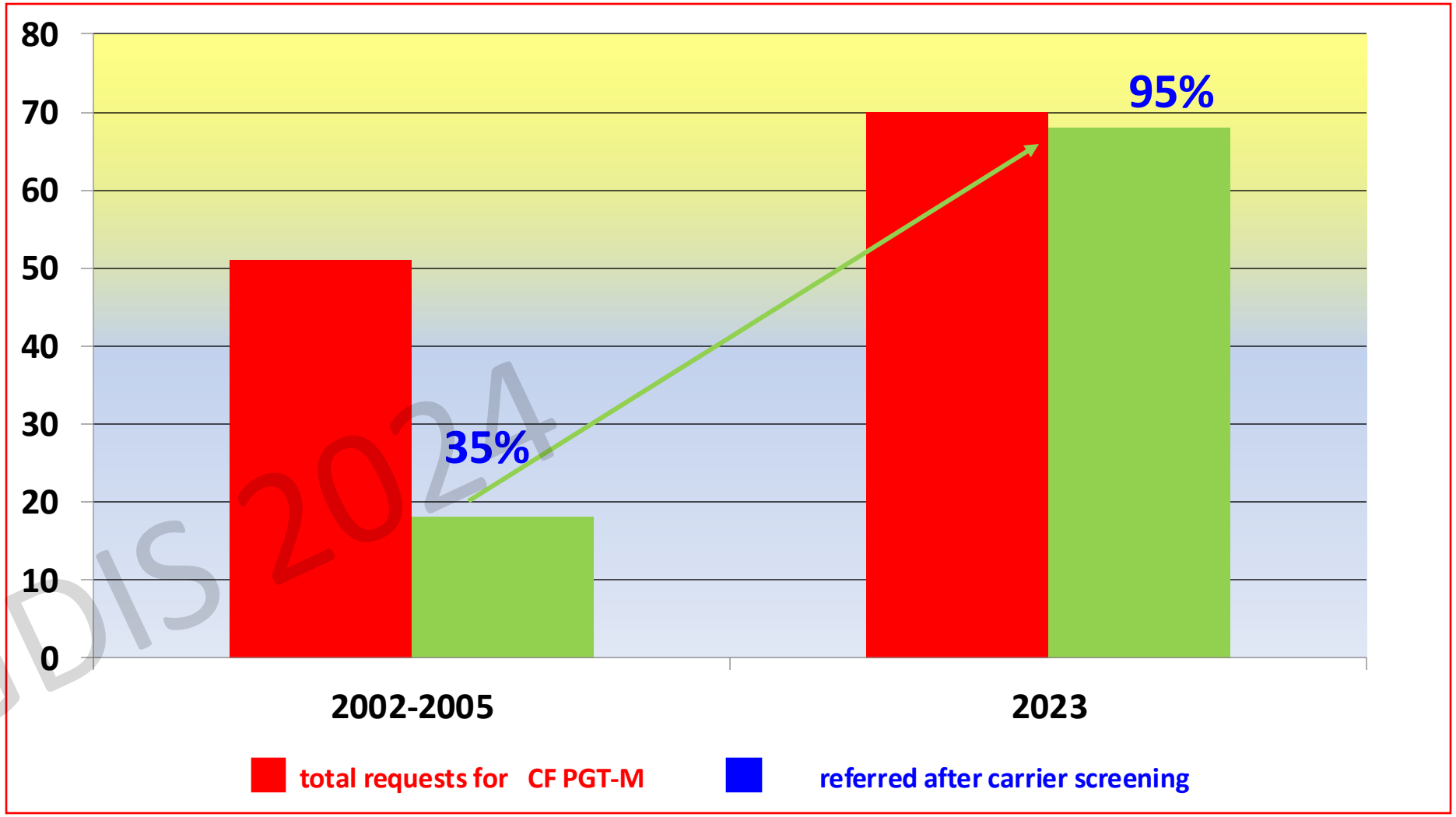
5657 PGT-M combined with PGT-A

25,769 for Aneuploidy (PGT-A)

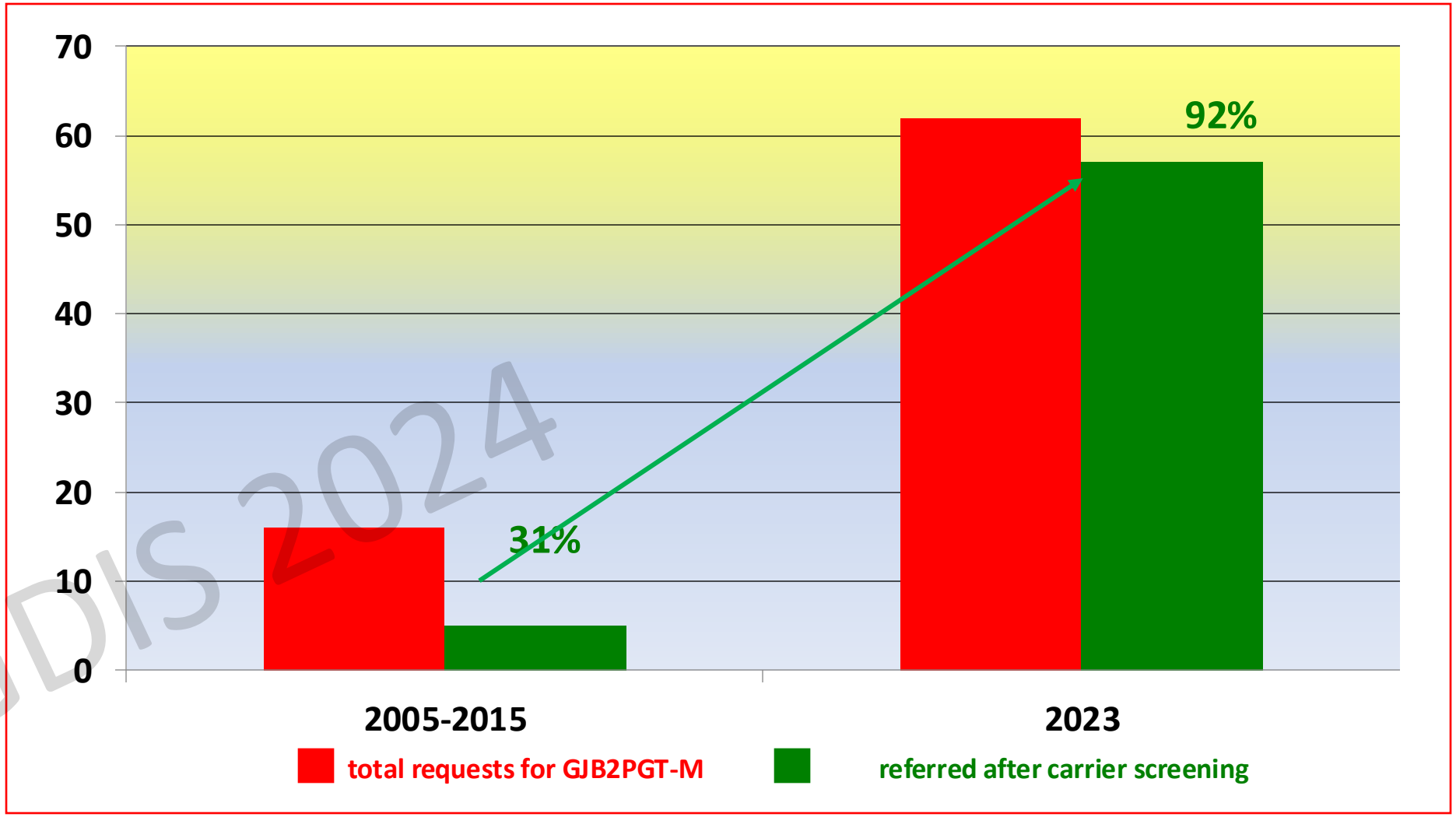
INCREASED UTILIZATION OF PGT-M THROUGH PAN-ETHNIC EXPANDED CARRIER SCREENING



Increase in PGT-M requests for Cystic Fibrosis after carrier screening

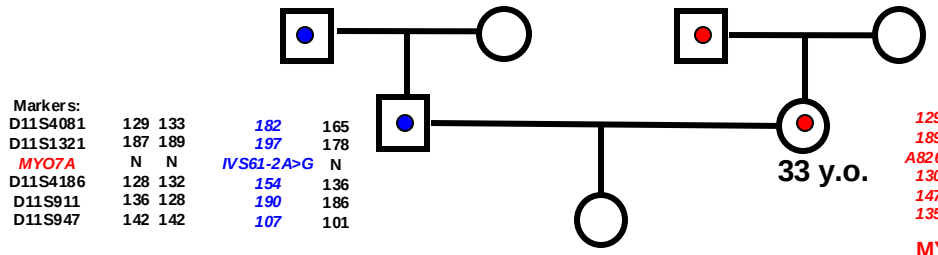


ECS impact on PGT-M requests for Non syndromic hearing loss



PGT for USHER SYNDROME (MYO7A and USH2A genes) and PGT-A by NGS

A. Family pedigree



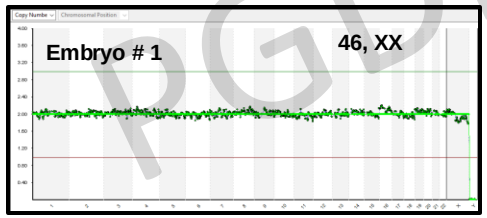
PGT
(embryo #4)
Normal USH2A & MYO7A,
46, XX

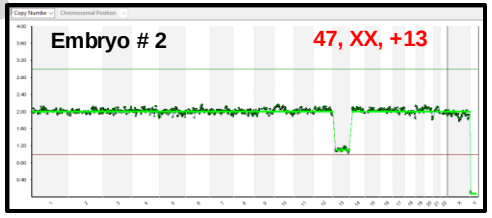
TOTAL -6
NORMAL or CARRIER USH2A -6
NORMAL or carrier MYO7A– 6
EUPLOIUD – 3
For transfer - 3

B. Trophectoderm analysis

Cycle #1

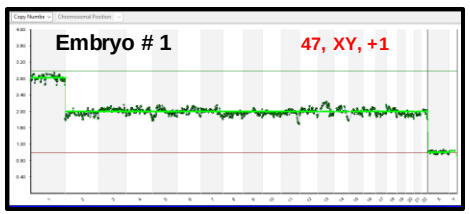
Embryo #	1	2
USH2A	165 182 178 182 N N 136 147 186 184 101 107	165 174 178 182 N N 136 147 186 193 101 105
MYO7A	133 129 189 189 N A826T 132 130 128 147 142 135	129 121 187 195 N N 128 134 136 105 142 148
NGS	46, XX	47, XX, +13
	FET1	

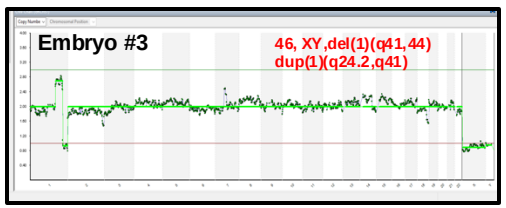


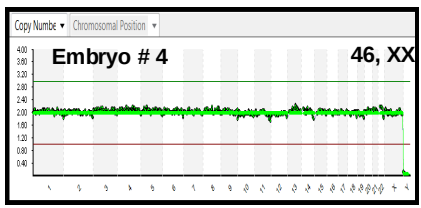


Cycle #2

1	2	3	4
165 174 178 182 N N 136 147 186 193 101 105	165 182 178 182 N N 136 147 186 184 101 107	182 182 197 182 IVS61-2A>G N 154 147 190 184 107 107	165 182 178 182 N N 136 147 186 184 101 107
129 121 187 195 N N 128 134 136 105 142 148	133 121 189 195 N N 132 134 128 105 142 148	133 121 189 195 N N 132 134 128 105 142 148	129 121 187 195 N N 128 134 136 105 142 148
47, XY, +1	46, XY	46, XY,del(1)(q41,44) dup(1)(q24.2,q41)	46, XX
			FET2



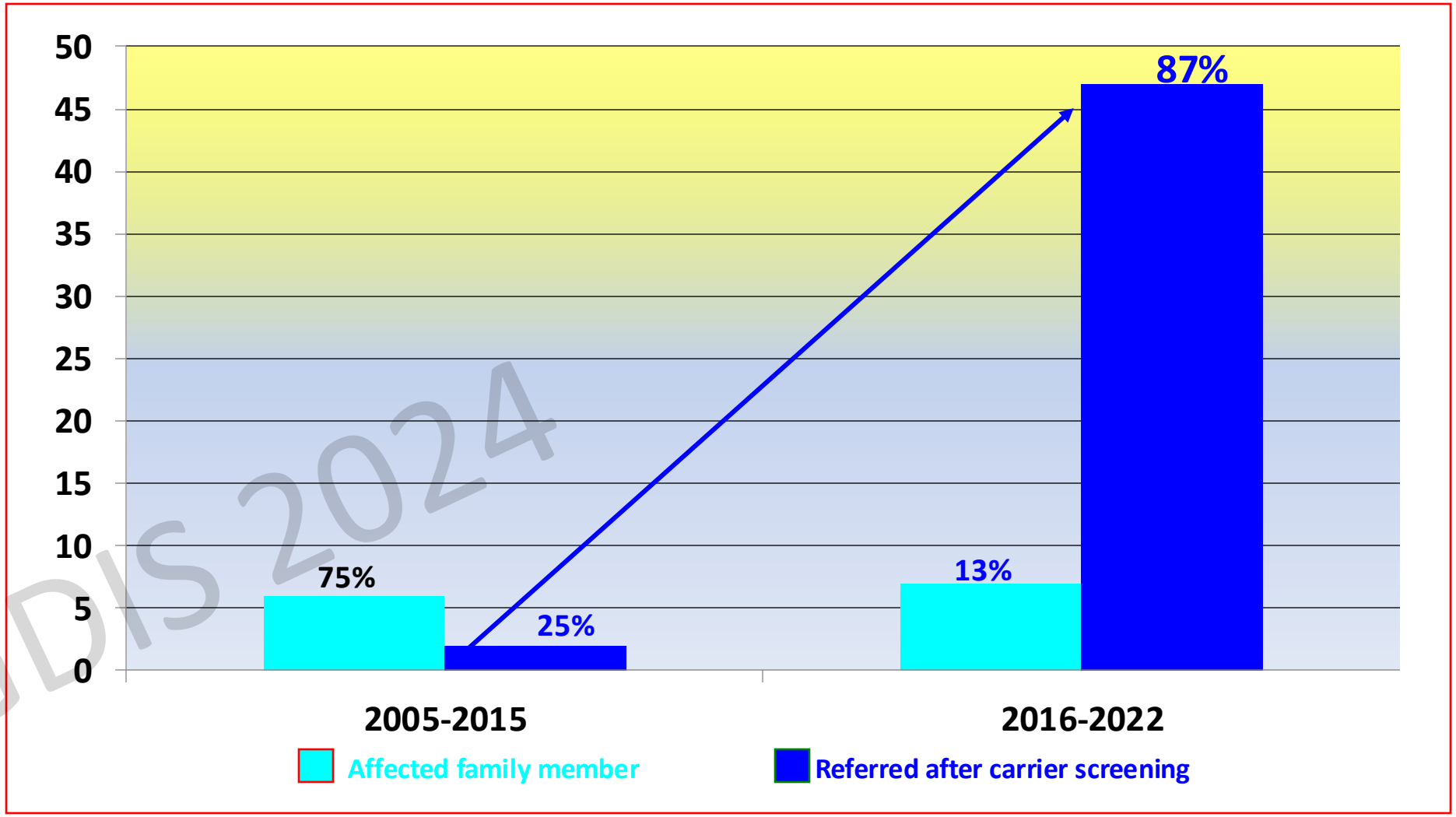




OUTCOMES OF PGT-M for HEREDITARY DEAFNESS (HD)

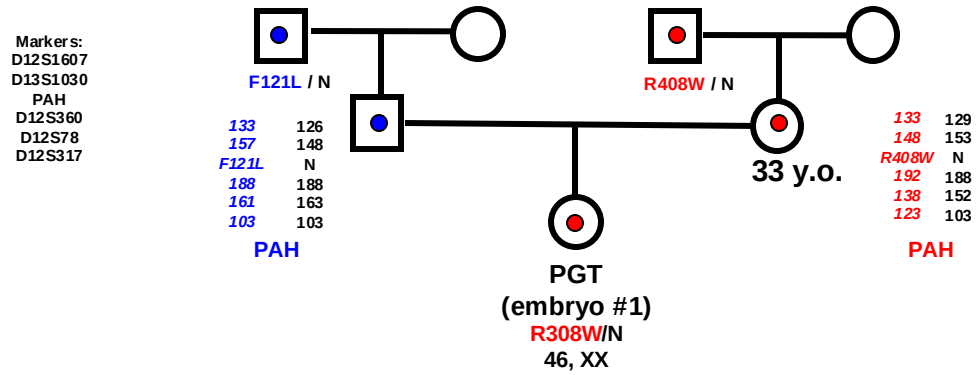
Disease	Gene	Omim	Number of Patients	Number of Cycles	Number of transfers	Number of embryos tranferred	Pegnancy	SAB	Number of Deliveries	Number of Babies
AURICULOCONDYLAR SYNDROME 2; ARCND2	PLCB4	614669	1	3	3	5	2	1	1	1
DEAFNESS, AUTOSOMAL DOMINANT 3B; DFNA3B	GJB6	612643	1	2	2	3	1	0	1	1
DEAFNESS, AUTOSOMAL RECESSIVE 3; DFNB3	MYO15A	600316	1	1	1	1	1	0	1	1
DEAFNESS, AUTOSOMAL RECESSIVE 8; DFNB8	TMPRSS3	601072	2	3	3	3	3	1	2	2
DEAFNESS, NEUROSENSORY, AUTOSOMAL RECESSIVE 1; DFNB1	GJB2	220290	140	201	192	208	126	22	104	104
DEAFNESS, X-LINKED 1; DFNX1	PRPS1	304500	1	1	1	1	1	0	1	1
PENDRED SYNDROME; PDS	SLC26A4	274600	7	10	10	9	4	0	4	4
USHER SYNDROME, TYPE I; USH1	MYO7A	276900	4	4	4	4	2	0	2	2
USHER SYNDROME, TYPE IF; USH1F	PCDH15	602083	3	5	5	7	4	2	2	2
USHER SYNDROME, TYPE IIA; USH2A	USH2A	276901	13	21	27	28	13	3	10	10
USHER SYNDROME, TYPE IIC; USH2C	ADGRV1	605472	1	2	2	3	1	0	1	1
WAARDENBURG SYNDROME, TYPE 2A; WS2A	MITF	193510	3	7	7	9	5	1	4	4
WOLFRAM SYNDROME 1; WFS1	WFS1	222300	1	2	1	1	1	0	1	1
TOTAL	(13 genes)		178	262	258	282 (1.09)	164 (63.5%)	30 (18.2%)	134	134

Increase in PGT-M requests for PHENYLKETONURIA (*PAH* gene) after ECS



PGT-M for PHENYLKETONUREA and PGT-A by NGS after ECS

A. Family pedigree



TOTAL -9
CARRIER PAH- 5
EUPLOIUD – 7
For transfer - 4

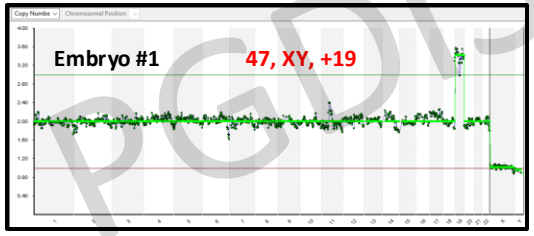
B. Trophectoderm analysis

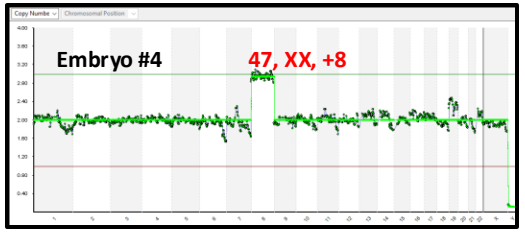
Cycle #1

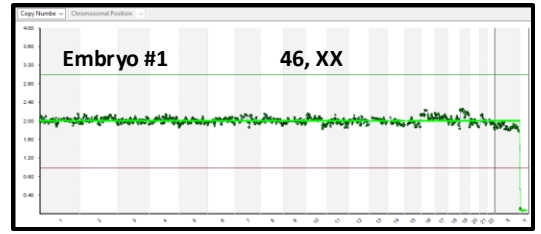
Embryo #	1	2	3	4
PAH	126 133 148 148 N R408W 188 192 163 138 103 123	133 129 157 153 F121L N 188 188 161 152 103 103	133 133 157 148 F121L R408W 188 192 161 138 103 123	133 133 157 148 F121L R408W 188 192 161 138 103 123
NGS	47, XY, +19	46, XX	46, XY	47, XX, +8

Cycle #2

	1	2	3	4	5
	126 133 148 148 N R408W 188 192 163 138 103 123	133 133 157 148 F121L R408W 188 192 161 138 103 123	133 129 157 153 F121L N 188 188 161 152 103 103	133 133 157 148 F121L R408W 188 192 161 138 103 123	126 133 148 148 N R408W 188 192 163 138 103 123
	46, XX	46,XX	46, XX	46, XY	46XY
	FET				

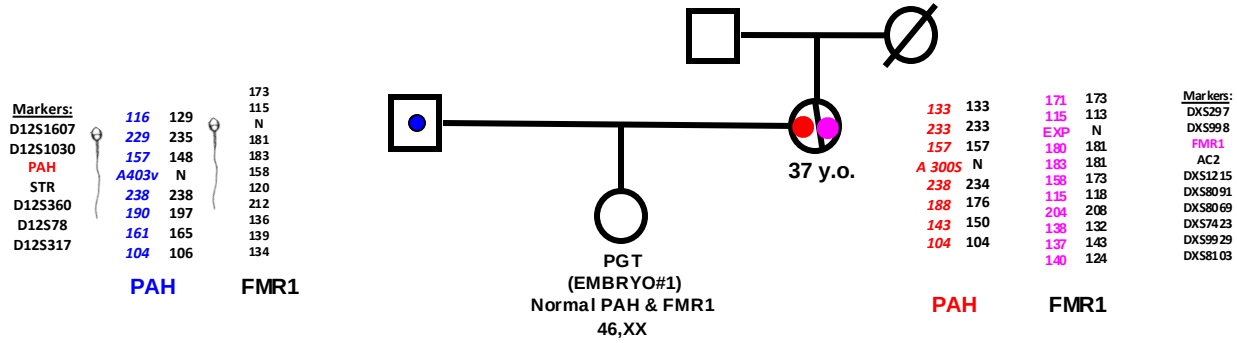




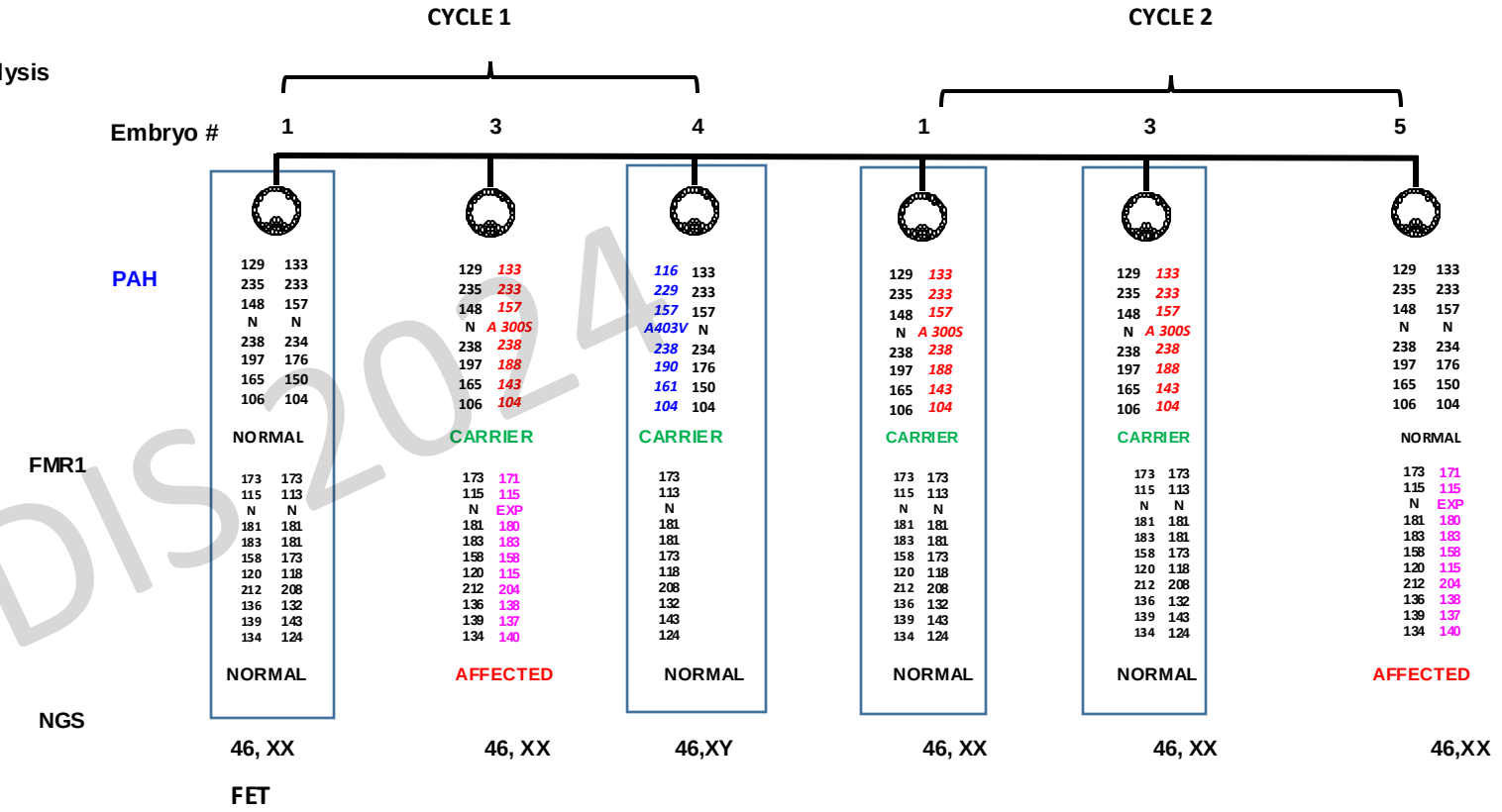


SEQUENTIAL PGT-A by NGS followed by PGT for PHENYLKETONURIA and FRAGILE-X syndrome

A. Family pedigree



B. Trophectoderm analysis

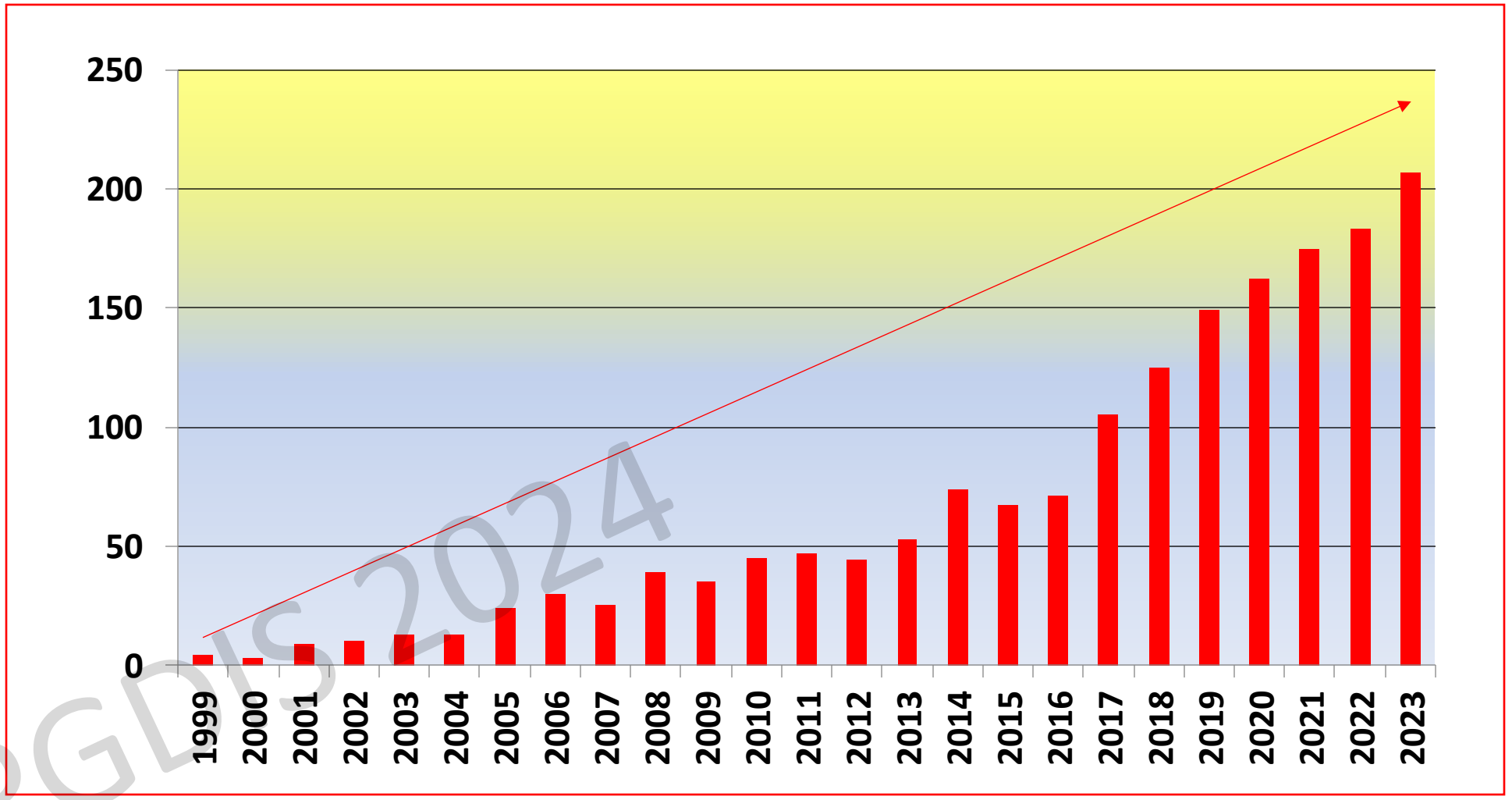


TOTAL 6
CARRIER or NORMAL PAH -6
NORMAL FX 4
For transfer -4

OUTCOMES OF PGT-M for PKU

Disease	Gene	OMIM	Number of Patients	Number of Cycles	Number of transfers	Number of embryos transferred	Pregnancy	SAB	Number of Deliveries	Number of Babies
PHENYLKETONURIA; PKU	PAH	261600	53	91	102	129 (1,12)	53 52%	3 6%	50 94%	54

Steady Increase of PGT-M cycles for cancer predisposition: 1999-20023



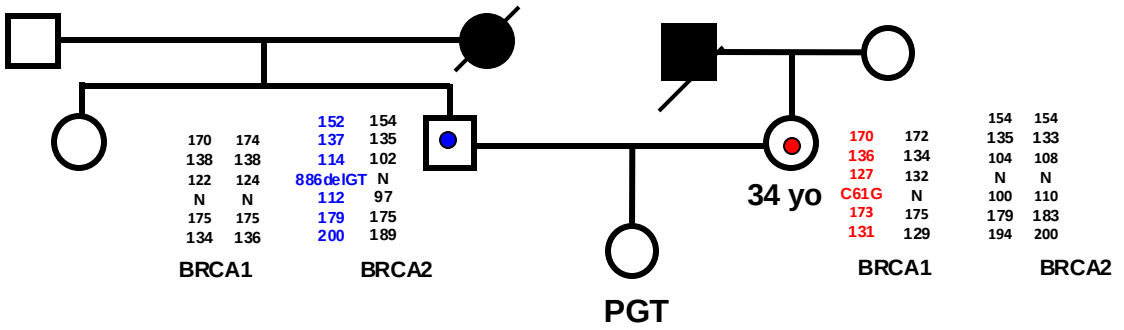
Distribution of most common and unique mutations in BRCA1 and BRCA2 genes

BRCA1 MUTATIONS	MATERNAL	PATERNAL	TOTAL
187 del AG	57	37	94
UNIQUE MUTATIONS	86	13	99
TOTAL	143	50	193
BRCA2 MUTATIONS	MATERNAL	PATERNAL	TOTAL
6174 Del T	44	25	69
UNIQUE MUTATIONS	73	27	100
TOTAL	117	52	169

A.

PGT-M + PGT-A for BRCA1, BRCA2 and Aneuploidy by NGS

Family pedigree

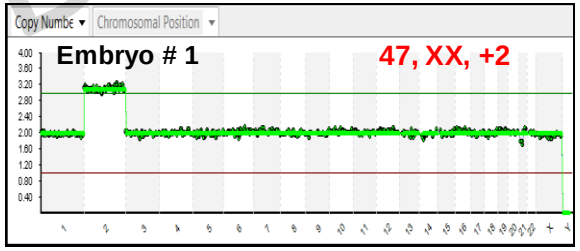


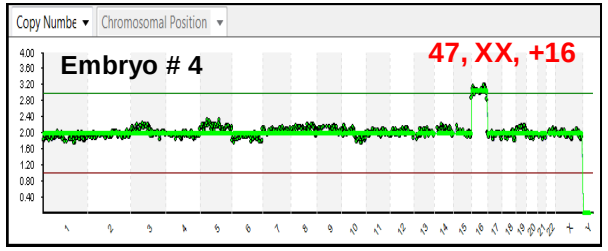
Trophectoderm analysis

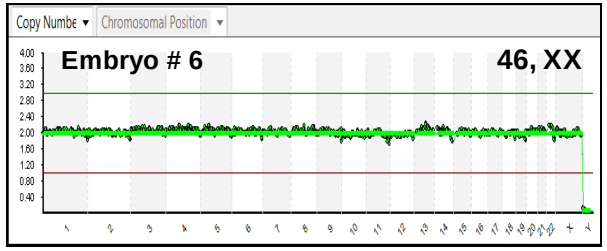
Normal BRCA1 &BRCA2

Embryo #	1	2	3	4	5	6
BRCA 1	170 172 138 134 122 132 N N 175 175 134 129	170 170 138 136 122 127 N C61G 175 173 134 131	170 170 138 136 122 127 N C61G 175 173 134 131	174 172 138 134 124 132 N N 175 175 136 129	170 172 138 134 122 132 N N 175 175 134 129	174 172 138 134 124 132 N N 175 175 136 129
BRCA 2	154 154 135 135 102 104 N N 97 100 175 179 189 194	154 154 135 135 102 104 N N 97 100 175 179 189 194	154 154 135 135 102 104 N N 97 100 175 179 189 194	154 154 135 135 102 104 N N 97 100 175 179 189 194	152 154 137 133 114 108 886delGT N 112 110 179 183 200 200	154 154 135 135 102 104 N N 97 100 175 179 189 194
NGS	47, XX, +2	46, XX	46, XY	47, XX, +16	46, XX	46, XX FET

C.

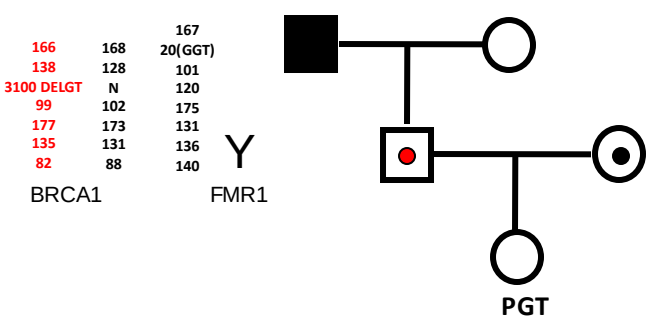






PGT-M for BRCA1, Fragile X and PGT-A by NGS

A. Family pedigree



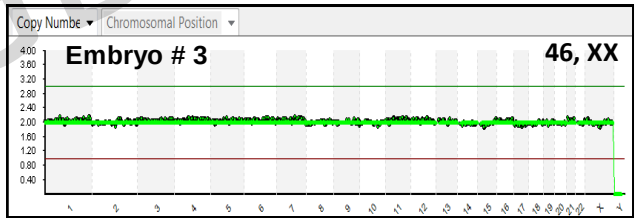
BRCA1		FMR1		Markers order:
168	174	171	173	DXS297
130	138	30(GGT)	71(GGT)	FMR1
N	N	103	105	DXS1193
104	108	117	115	DXS8069
173	175	177	171	DXS8091
131	131	135	131	DXS9929
93	96	140	136	DXS8103
		124	134	DXS1684

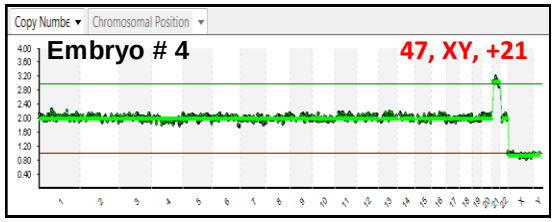
BRCA1		FMR1	
168	174	167	171
128	138	20(GGT)	30(GGT)
N	N	101	103
102	108	120	117
173	175	175	177
131	131	131	135
88	96	136	140
		140	124

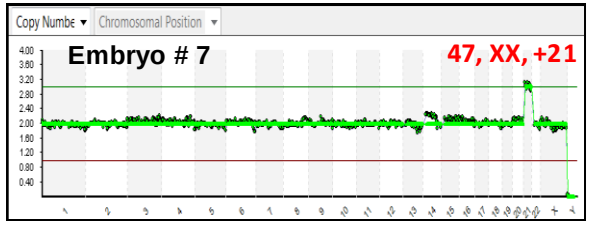
B. Trophectoderm analysis

Embryo #	1	2	3	4	5	6	7	8
	9	10						
BRCA1	166 138 3100DELGT 99 177 135 82 / 174 138 N 108 175 131 96	166 138 3100DELGT 99 177 135 82 / 174 138 N 108 175 131 96	168 128 N 102 173 131 88 / 174 138 N 108 175 131 96	166 138 3100DELGT 99 177 135 82 / 174 138 N 108 175 131 96	166 138 3100DELGT 99 177 135 82 / 168 130 N 104 173 131 93	168 128 N 102 173 131 88 / 168 130 N 104 173 131 93	166 138 3100DELGT 99 177 135 82 / 174 138 N 108 175 131 96	166 138 3100DELGT 99 177 135 82 / 174 138 N 108 175 131 96	166 138 3100DELGT 99 177 135 82 / 168 130 N 104 173 131 93
FMR1	171 30(GGT) 103 117 177 135 140 124	171 30(GGT) 103 117 177 135 140 124	167 171 20(GGT) 30(GGT) 101 103 120 117 175 177 131 135 136 140 140 124	173 71(GGT) 105 115 171 131 136 134	173 71(GGT) 105 115 171 131 136 134	167 171 20(GGT) 30(GGT) 101 103 120 117 175 177 131 135 136 140 140 124	167 173 20(GGT) 71(GGT) 101 105 120 115 175 171 131 131 136 136 140 134	167 171 20(GGT) 30(GGT) 101 103 120 117 175 177 131 135 136 140 140 124	167 171 20(GGT) 30(GGT) 101 103 120 117 175 177 131 135 136 140 140 124
	Y	Y	Y	Y				Y	
NGS	46, XY	46, XY	46, XX	47, XY, +21	46, XY	46, XX	47, XX, +21	46, XX	47, XXY	46, XX

C. FET



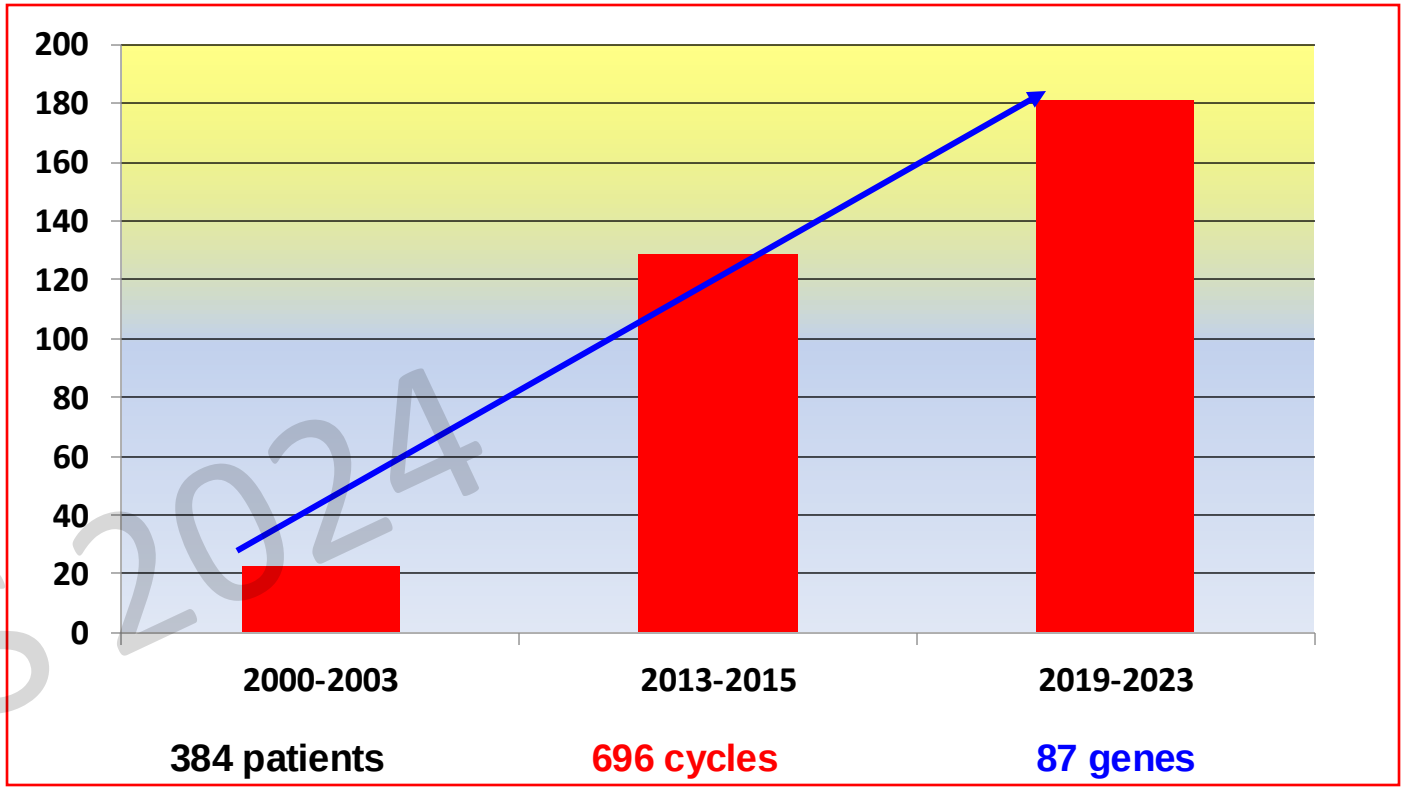




PGT-M Outcome for Cancer Predisposition (48 Conditions, 56 Genes)

# Patient	# Cycle	# Transfers	# Embryo transferred	Pregnancy	SAB	Delivery	Baby
607	1247	885	1275 (1.5)	485 (55%)	46 (9.5%)	439 (90.5%)	443

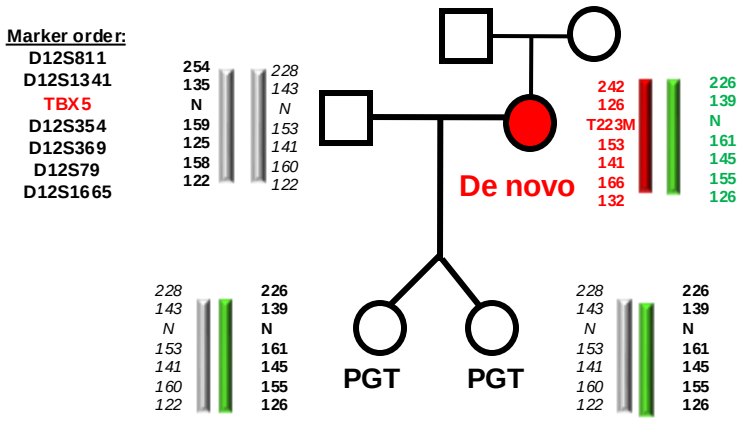
Steady Increase of PGT cycles for De Novo Mutations after Prospective Application of PGT



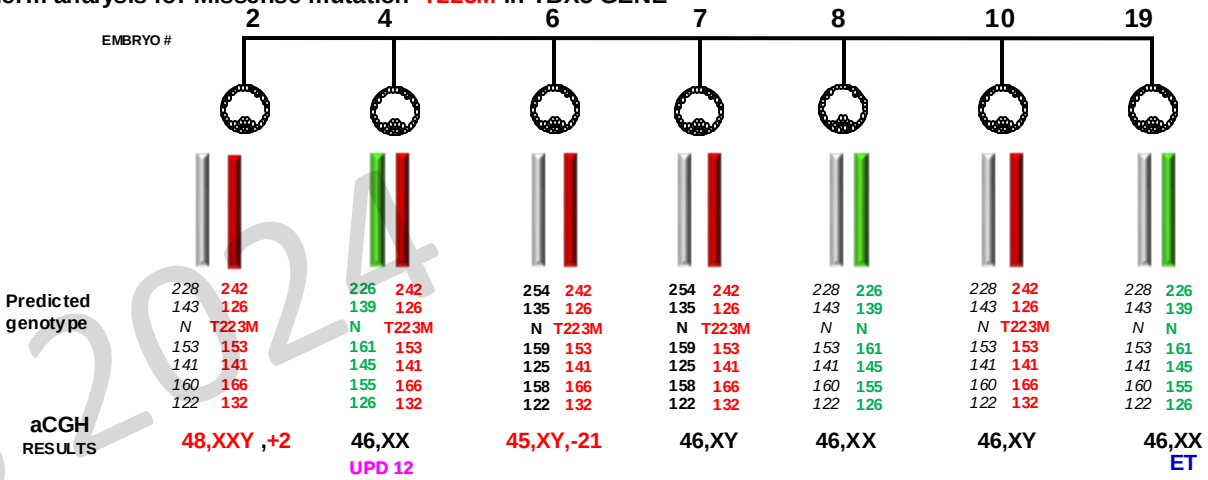
PGT-M for Holt – Oram Syndrome;

Heritable adult-onset cardiac disorders are usually pleiotropic and characterized by structural abnormities in multiple organ systems: thumb anomaly and atrial septal defects

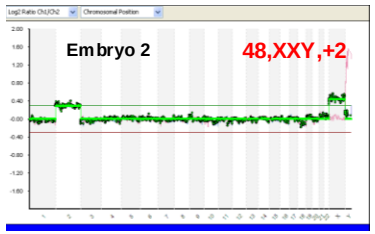
1. Family pedigree

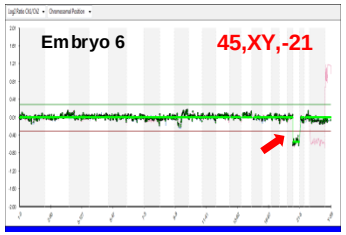


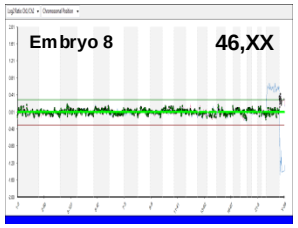
2. Trophectoderm analysis for Missense mutation T223M in TBX5 GENE

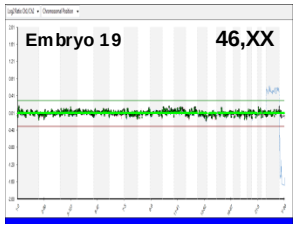


TOTAL – 7
NORMAL – 2
Affected - 5
EUPLOID – 4
UPD – 1
FET - 2

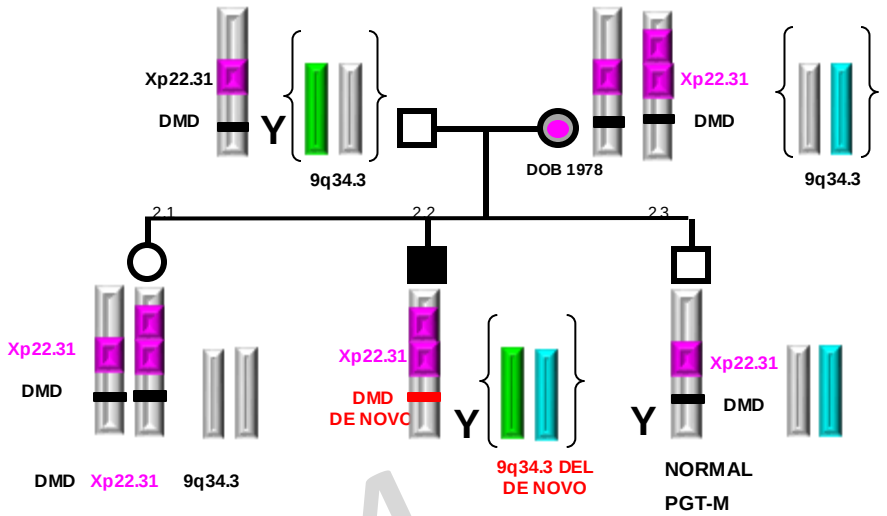






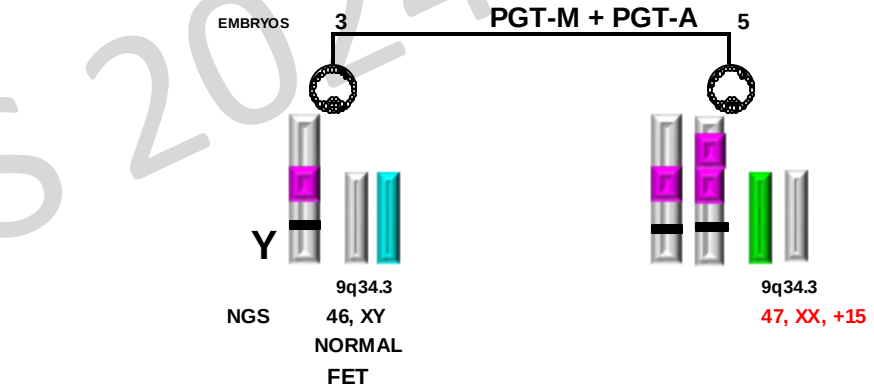


PGT-M for TWO DE NOVO MATERNAL DELETIONS: DMD – exon 8-9 and 9q34.3 (Kleefstra syndrome) and Xp22.31 duplication



Kleefstra syndrome (KS) is a genetic disorder characterized by intellectual disability, childhood hypotonia, severe expressive speech delay and a distinctive facial appearance with a spectrum of additional clinical features.

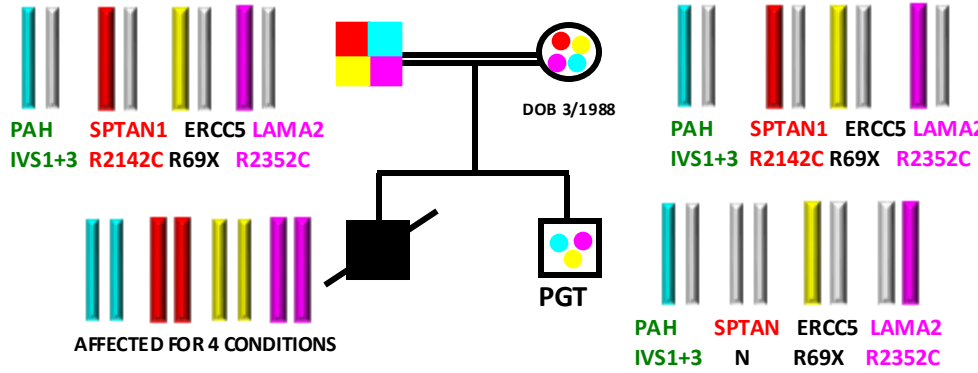
Caused by a microdeletion in the chromosome region 9q34.3 (seen in >85% of cases), leading to the loss of the entire gene. This gene encodes an enzyme that modifies histone function and is essential for normal development.



Combined PGT-M FOR 4 AUTOSOMAL RECESSIVE CONDITIONS AND PGT-A BY NGS

Family pedigree

CHANCES TO FIND EMBRYO
FREE FROM PKU ¾
FREE FROM EIEE5 ¾
FREE FROM XPG ¾
FREE FROM MDCA1 ¾
FREE FROM ALL CONDITIONS: ¾ x ¾ x ¾ x ¾ = 81/256 (31.6%)
AFFECTED FOR ALL 4 CONDITIONS: ¼ x ¼ x ¼ x ¼= 1/256 (0.4%)



	1	3	4	5	6	7	8	9	10	11	12	16
PAH- 12q22-24.2	AFFECTED	CARRIER	CARRIER	AFFECTED	CARRIER	AFFECTED	CARRIER	NORMAL	AFFECTED	CARRIER	AFFECTED	CARRIER
SPTAN1 9q34.11	CARRIER	NORMAL	AFFECTED	NORMAL	NORMAL	CARRIER	NORMAL	CARRIER	CARRIER	AFFECTED	CARRIER	NORMAL
ERCC5 13q33.1	CARRIER	CARRIER	CARRIER	CARRIER	CARRIER	CARRIER	CARRIER	AFFECTED	CARRIER	NORMAL	AFFECTED	AFFECTED
LAMA2 6q22-23	AFFECTED	CARRIER	CARRIER	CARRIER	CARRIER	NORMAL	AFFECTED	CARRIER	AFFECTED	0	NORMAL	NORMAL
NGS	45, XX,-22	46,XY FET	46,XX	46,XX	47,XX+13	46,XY	46,XY	48,XX+21,+22	47XY,+22	-6	44,XX-2,-6	46,XX	47,XY+13

CONCLUSIONS:

PGT-M referral profile is significantly changing with introduction of ECS

Increasing number of patients without family history requested PGT-M, including non-lethal conditions

Prospective referrals more than doubled in the last 5 years overall and even tripled for some selected conditions

ECS has also impacted the spectrum of PGT-M indications with the shift to the late onset conditions with genetic predisposition, such as cancer, cardiac diseases and neurodegenerative disorders

There has been also a shift in spectrum of PGT-M tested BRCA1 and BRCA2 mutations from common to unique mutations

A wider application of ECS will not only improve PGT-M uptake, but also turn it from retrospective tool on family level to a primary genetic disease population level control measure.

THANK YOU!

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An Illustrated Textbook and Reference for Clinicians
An Atlas of
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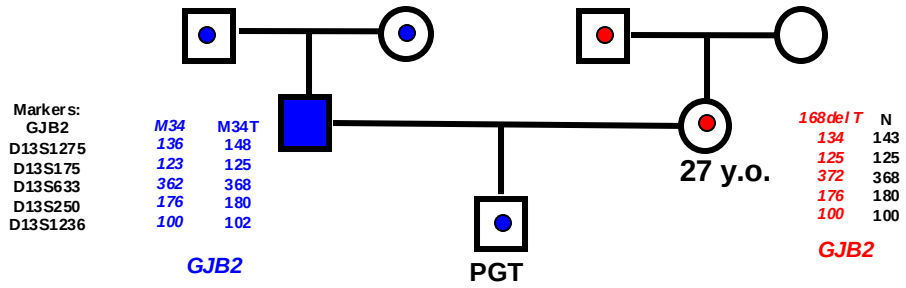
THIRD EDITION
CRC Press
PREIMPLANTATION DIAGNOSIS OF GENETIC DISEASES
A New Technique in Assisted Reproduction
Yury Verlinsky
Anver M. Kuliev
WILEY-LISS

Fig.2 PGT-M for NON-SYNDROMIC HEARING LOSS caused by mutations in *GJB2* gene and sequential PGT-A by NGS

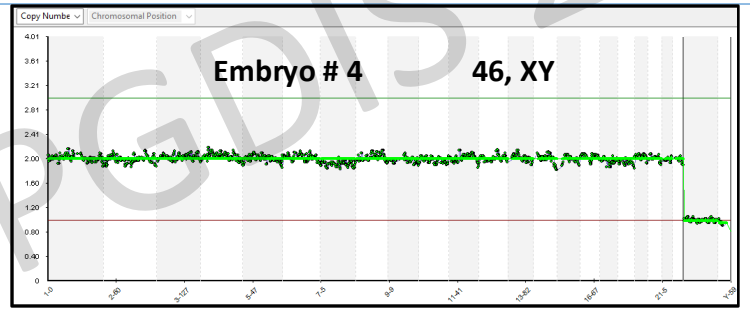
A. Family pedigree

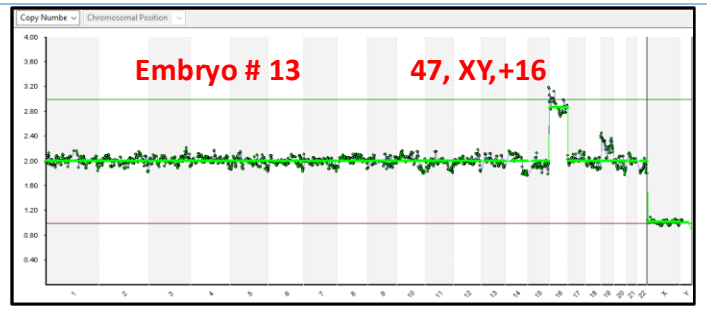


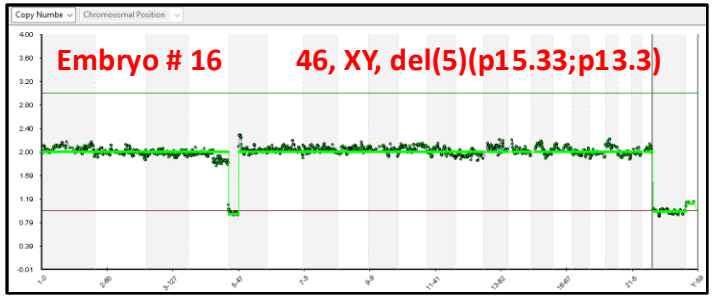
TOTAL -14
CARRIERS M34T – 5
NGS tested - 5
EUPLOIUD – 3
SUTABLE FET - 3

B. Trophectoderm analysis

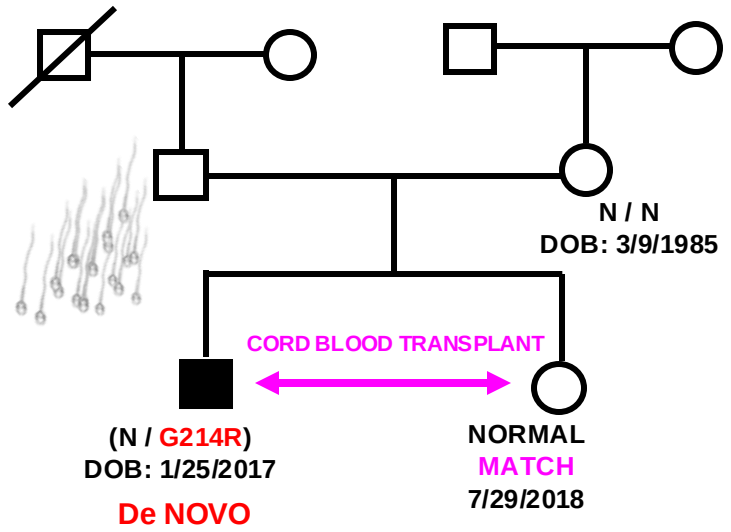
Embryo #	1	2	3	4	5	6	7	8	10	11	12	13	15	16
	M34 168del T	M34 168del T	M34T 168del T	M34T N	M34 168del T	M34 N	M34T 168del T	M34 168del T	M34T 168del T	M34 168del T	M34 N	M34T N	M34 168del T	M34T N
	136 134	136 134	148 134	148 143	136 134	136 143	148 134	136 134	148 134	136 134	136 143	148 143	136 134	148 143
	123 125	123 125	125 125	125 125	123 125	123 125	125 125	123 125	125 125	123 125	123 125	125 125	123 125	125 125
	362 372	362 372	368 372	368 368	362 372	362 368	368 372	362 372	368 372	362 372	362 368	368 368	362 372	368 368
	176 176	176 176	180 176	180 180	176 176	176 180	180 176	176 176	180 176	176 176	176 180	180 180	176 176	180 180
	100 100	100 100	102 100	102 100	100 100	100 100	102 100	100 100	102 100	100 100	100 100	102 100	100 100	102 100
				46, XY FET		46, XY					46, XY	47, XY,+16		46, XY, del(5)(p15.33;p13.3)







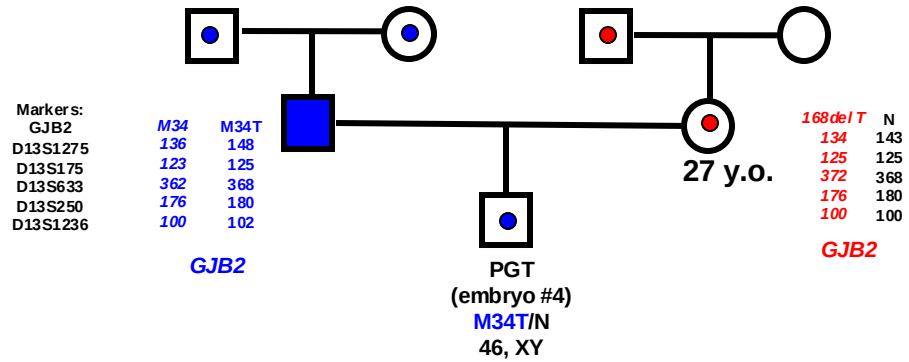
PGT FOR DE NOVO SEVERE CONGENITAL NEUTROPENIA1 (*ELANE* GENE) DETECTED IN CHILD, HLA TYPING AND ANEUPOLIDY BY NGS



Embryo #	GENE Mutation	D19S 878	D19S 565	D19S 424	D19S 209	Embryo #	Predicted Genotype	Predicted HLA Type	ET
1	N	157/152	132/119	110/108	108/106	1	NORMAL*	MATCH	Per PT consent*
2	N	152/146	128/119	110/106	104/98	2	NORMAL*	MATCH	Per PT consent*
3	N	157/152	132/119	110/108	108/106	3	NORMAL*	NON-MATCH	Per PT consent*
4	G214R / N	157/146	132/119	110/106	108/98	4	AFFECTED	NON-MATCH	NO
HLA 1231	N / N	157/152	132/128	110/110	108/104	PARTNER (NORMAL)			
HLA 1232	N / N	152/146	119/119	108/106	106/98	PATIENT: (NORMAL)			
HLA 1236	G214R / N	157/146	132/119	110/106	108/98	CHILD: AFFECTED (de novo)			

PGT-M for NON-SYNDROMIC hereditary Deafness caused by mutations in *GJB2* gene and sequential PGT-A by NGS

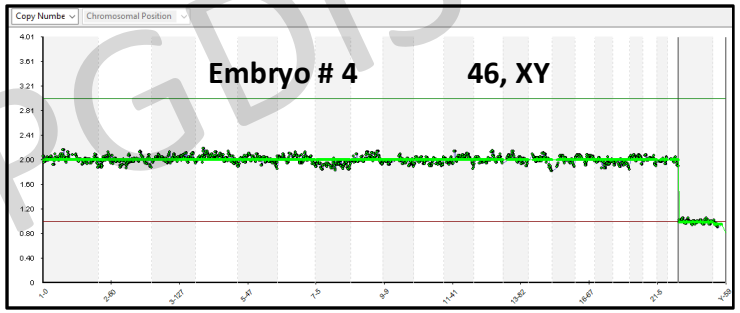
A. Family pedigree

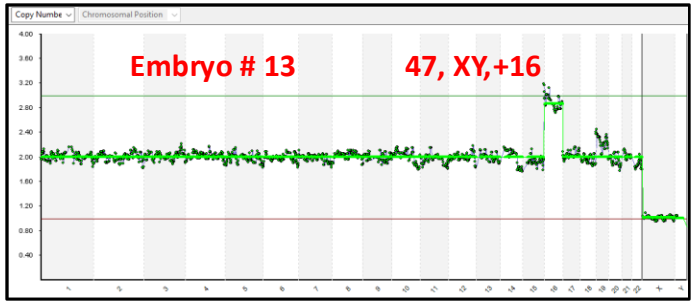


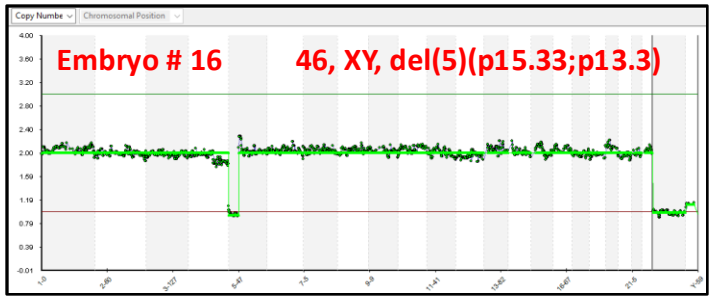
TOTAL -14
CARRIERS M34T – 5
NGS tested - 5
EUPLOIUD – 3
For transfer - 3

B. Trophectoderm analysis

Embryo #	1	2	3	4	5	6	7	8	10	11	12	13	15	16
	M34 168del T 136 134 123 125 362 372 176 176 100 100	M34 168del T 136 134 123 125 362 372 176 176 100 100	M34T 168del T 148 134 125 125 368 372 180 176 102 100	M34T N 148 143 125 125 368 368 180 180 102 100	M34 168del T 136 134 123 125 362 372 176 176 100 100	M34 N 136 143 123 125 362 368 176 180 100 100	M34T 168del T 148 134 125 125 368 372 180 176 102 100	M34 168del T 136 134 123 125 362 372 176 176 100 100	M34T 168del T 148 134 125 125 368 372 180 176 102 100	M34 168del T 136 134 123 125 362 372 176 176 100 100	M34 N 136 143 123 125 362 368 176 180 100 100	M34T N 148 143 125 125 368 368 180 180 102 100	M34 168del T 136 134 123 125 362 372 176 176 100 100	M34T N 148 143 125 125 368 368 180 180 102 100
	AFFECTED	AFFECTED	AFFECTED	CARRIER 46, XY FET	AFFECTED	CARRIER 46, XY	AFFECTED	AFFECTED	AFFECTED	AFFECTED	CARRIER 46, XY	CARRIER 47, XY,+16	AFFECTED	CARRIER 46, XY, del(5) (p15.33;p13.3)

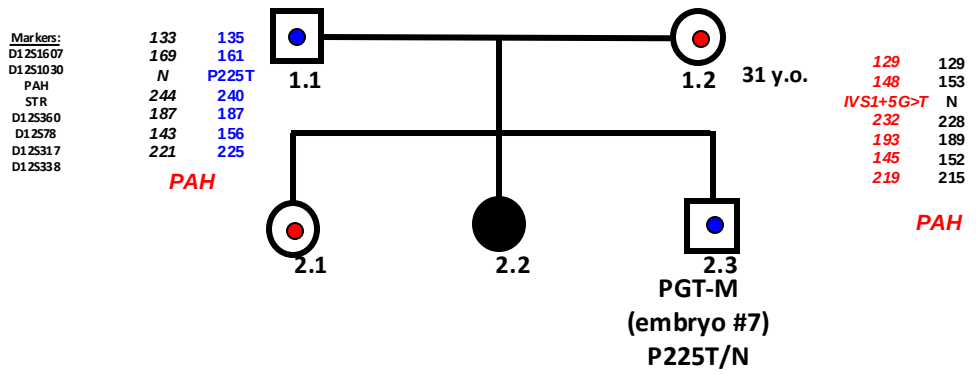






PGT-M for PHENYLKETONURIA after birth of affected child

A. Family pedigree



TOTAL -9
CARRIERS – 6
NORMAL -1
AFFECTED -1
TRISOMY 12 -1
For transfer - 7

B. Trophectoderm analysis