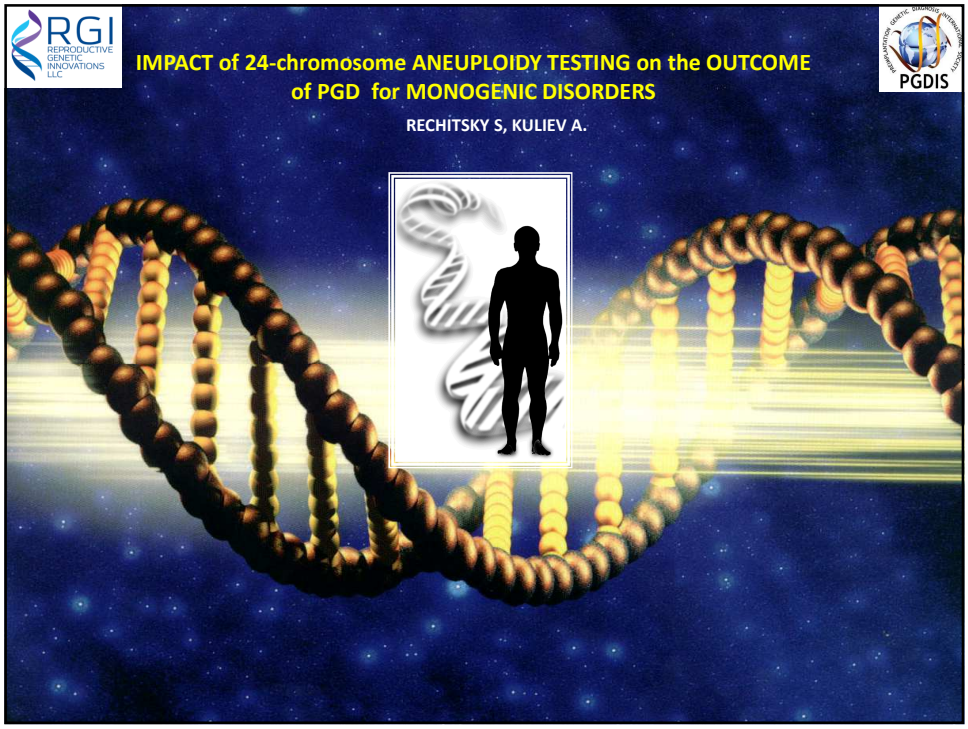
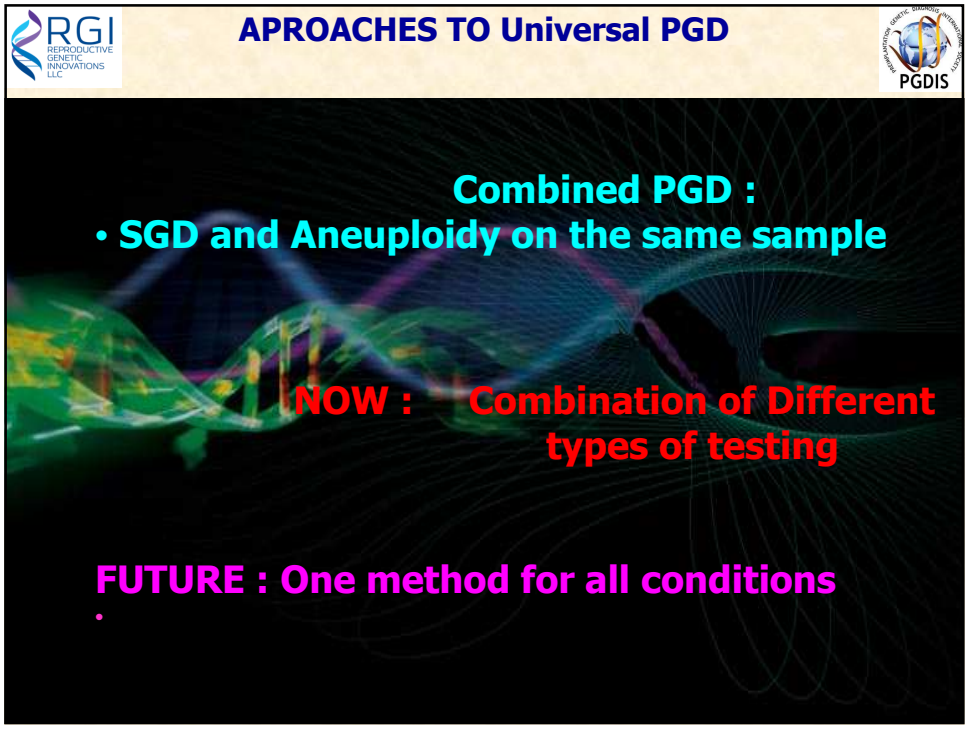

RGI
REPRODUCTIVE GENETIC INNOVATIONS LLC
IMPACT of 24-chromosome ANEUPLOIDY TESTING on the OUTCOME of PGD for MONOGENIC DISORDERS
RECHITSKY S, KULIEV A.
PGDIS


RGI
REPRODUCTIVE GENETIC INNOVATIONS LLC
APROACHES TO Universal PGD
PGDIS
Combined PGD :
• SGD and Aneuploidy on the same sample
NOW : Combination of Different types of testing
FUTURE : One method for all conditions

PRESENT RGI EXPERIENCE

TOTAL : Over 12,500 CASES

4501 PGD CYCLES

FOR 426 MENDELIAN CONDITIONS

1479 Single Gene cases + Aneuploidy

762 Single Gene cases + 24 AT

< 900 TRANSLOCATIONS

FLOW CHART FOR COMBINED TESTING

A single cell is placed in lysis buffer and spanned

Mutation & Linkage (Haplotyping):
PCR Mix with multiple outside primers,

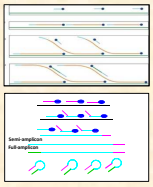
Second Round started as a separate PCR reaction for each locus

Combined testing: SGD+AT-24

WGA:
aCGH+Mutation –: PEP,DOP

Karyomapping : MDA

NGS: DOP,MDA or MALBAC



Single Gene Analysis:

RFLP | Real Time PCR with TaqMan probe | Fluorescent sequencing | Mini-sequencing | NGS | STRs

Aneuploidy testing

Karyomapping

aCGH | NGS | NGS

WGA APPROACHES :

1. PCR BASED: **PEP-PCR** (15 MER DEGENERATE PRIMER)
   DOP-PCR (QUASI-RENDOM ANNEALING OF HYBRID OLIGOS)
   LA-PCR (ADAPTORS WITH UNIVERSAL SEQUENCE LIGATED TO DNA ENDS)
2. ISOTERMAL: **MDA** (RANDOM PRIMING AND STRAND DISPLACEMENT)
1. COMBINATION OF BOTH: **MALBAC** (DISPLACEMETNT PRE-AMPLIFICATION and LOOP CREATION)

MICROARRAY or NGS limitations due to WGA nature:

BREDTH OF GENOMIC COVERAGE;

AMPLIFICATION BIAS DUE TO %GC CONTENT;

ALLELE DROP OUT (ADO)

PREFERENTIAL ALLILIC AMPLIFICATION (PA)

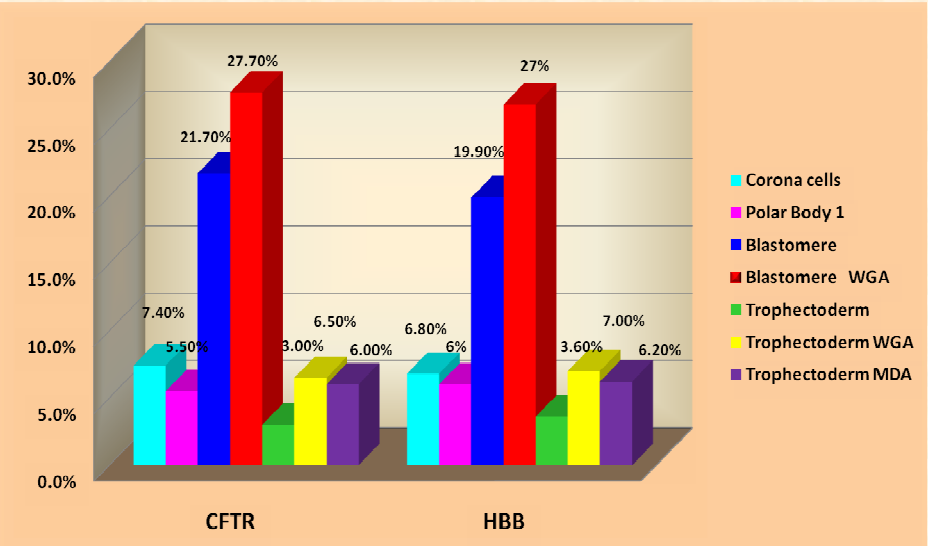
CHIMERIC DNA MOLECULES;

NUCLEOTIDE COPY ERRORS

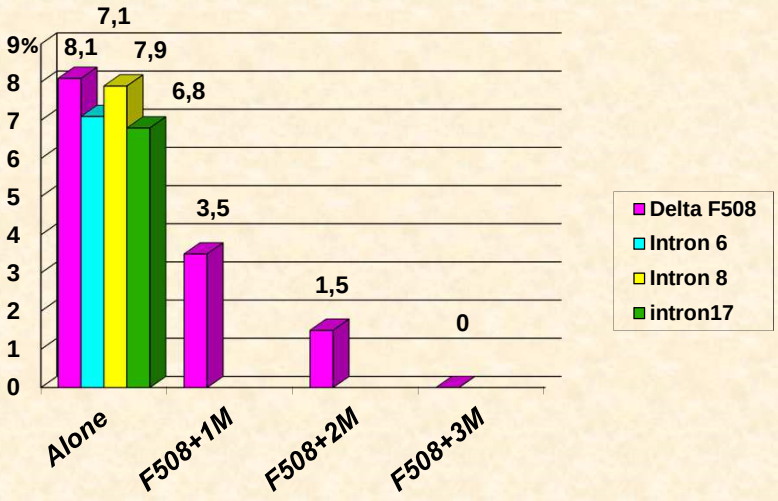
CAN VARY SIGNIFICANTLY FOR DIFFERENT WGA APPROACHES
AND CAN AFFECT INTERPRETATION

A ONE-FIT- ALL WGA METHOD REMAINS TO BE ESTABLISHED

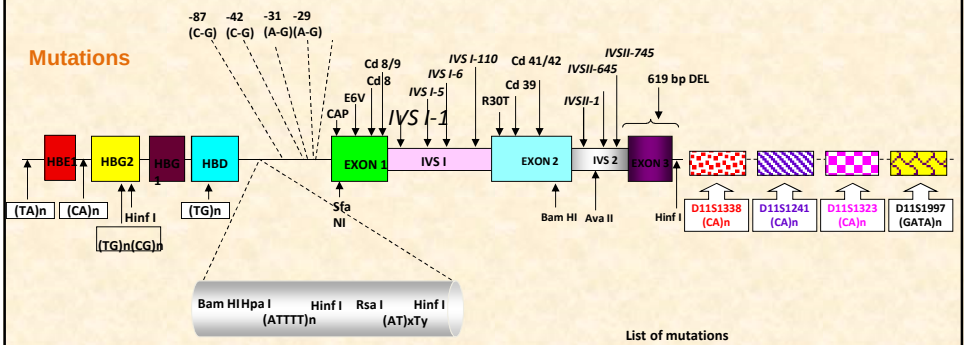
ADO RATE IN DIFFERENT TYPE OF CELLS with or without WGA OR MDA



ADO detection by linked markers



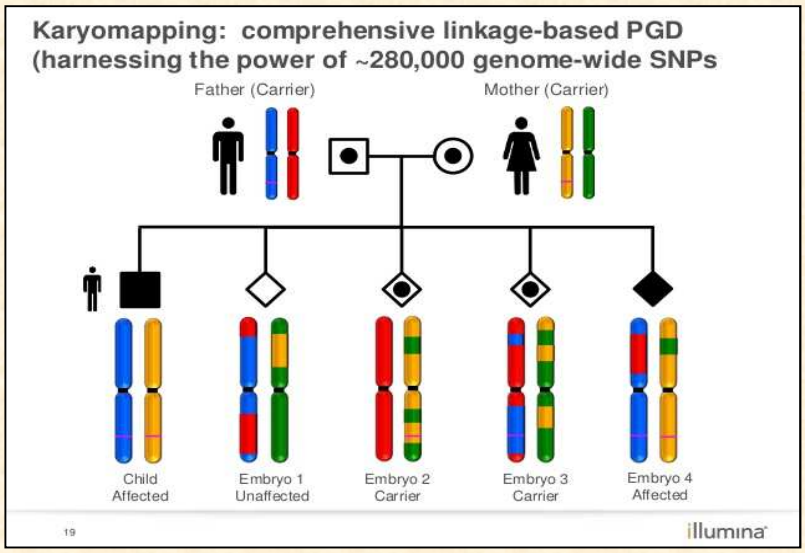
Mutations in beta-globin gene and polymorphic markers used in multiplex PCR analysis



List of mutations

RNA Processing mutations	Transcriptional mutations	Nonfunctional mRNA
IVS I – 1 (G – A)	87 (C–G)	Codon 8 (-AA)
IVS I – 1 (G–T)	42 (C–G)	Codon 39 (C–T)
IVS I – 5 (G–C)	31 (A–G)	Codon 41/42 (-CTTT)
IVS I –5 (G–T)	29 (A–G)	
IVS I – 5 (G–A)	28 (A–G)	
IVS I – 6 (T–C)	E 6V (Sickle cell anemia)	
IVS I – 110 (G–A)	R30T	
IVS 2 – 1 (G–A)	Deletion:	Cap site:
IVS 2 – 654 (C–T)	619 bp	+1 (A – C)
IVS 2 – 745 (C–G)	45 Kb	+2 (T – C)

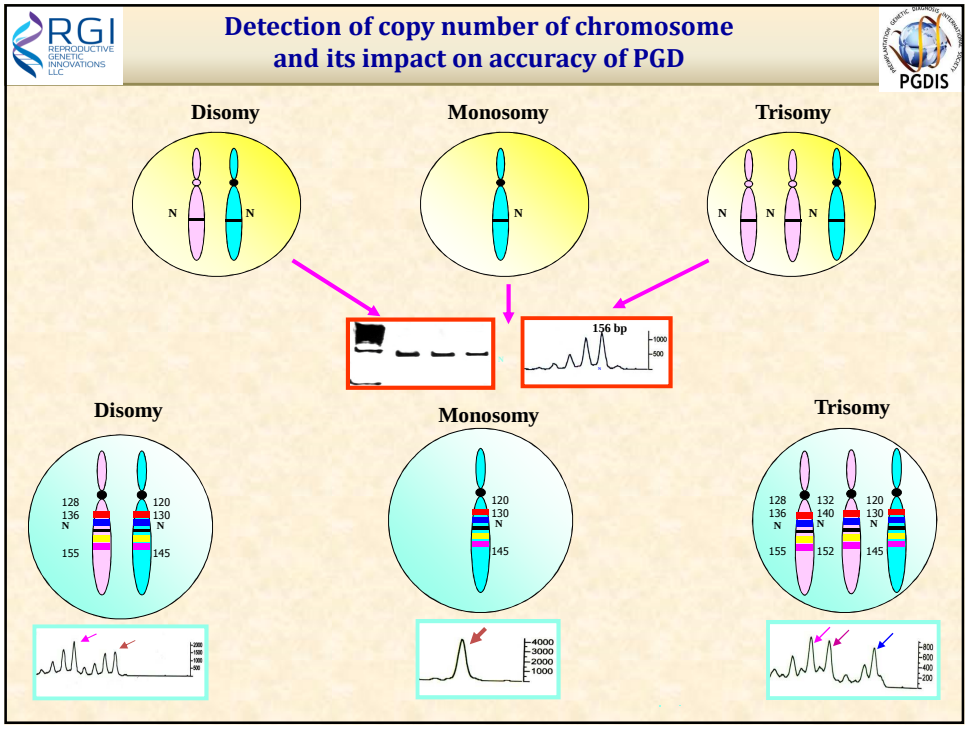
Linkage analysis by Karyomapping

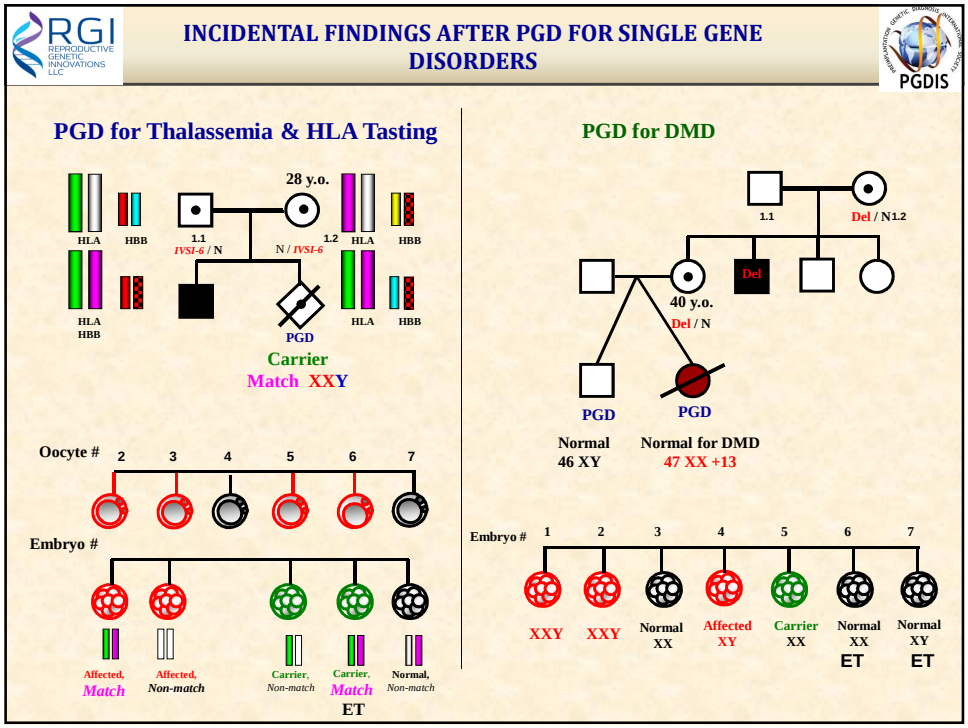


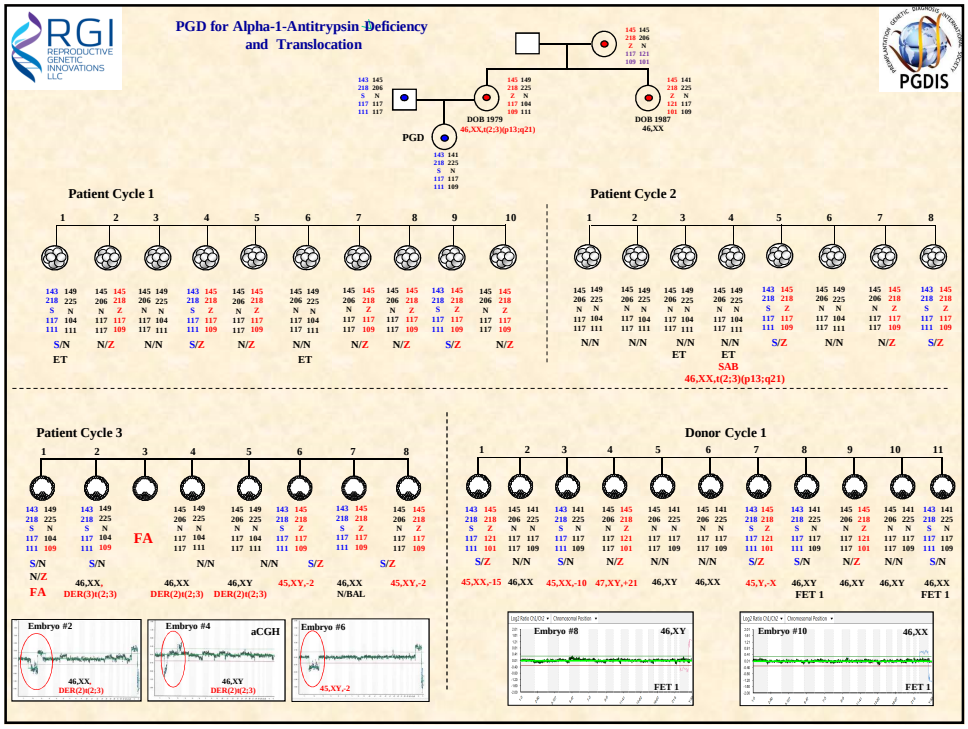
HAPLOTYPING limitations without mutation analysis

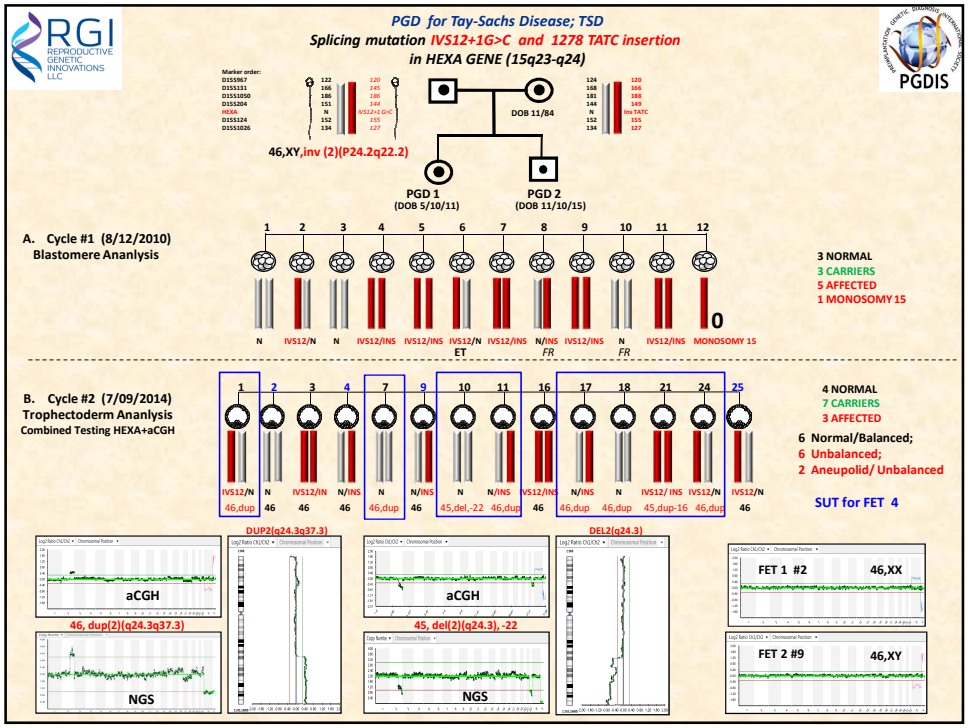
Test type	Total # families	Relatives were Not available for haplotype prediction	Same mutation (including offspring)	De novo mutations	Recombinant baby
SGD	2460	354 12.73%	41 1.7%	179 7.2%	8 0.3%
HLA Typing	320	NA	NA	NA	17 5.3%
TOTAL	2780	354 (12.73)	41 (1.7%)	179 (7.2%)	25 (0.89%)

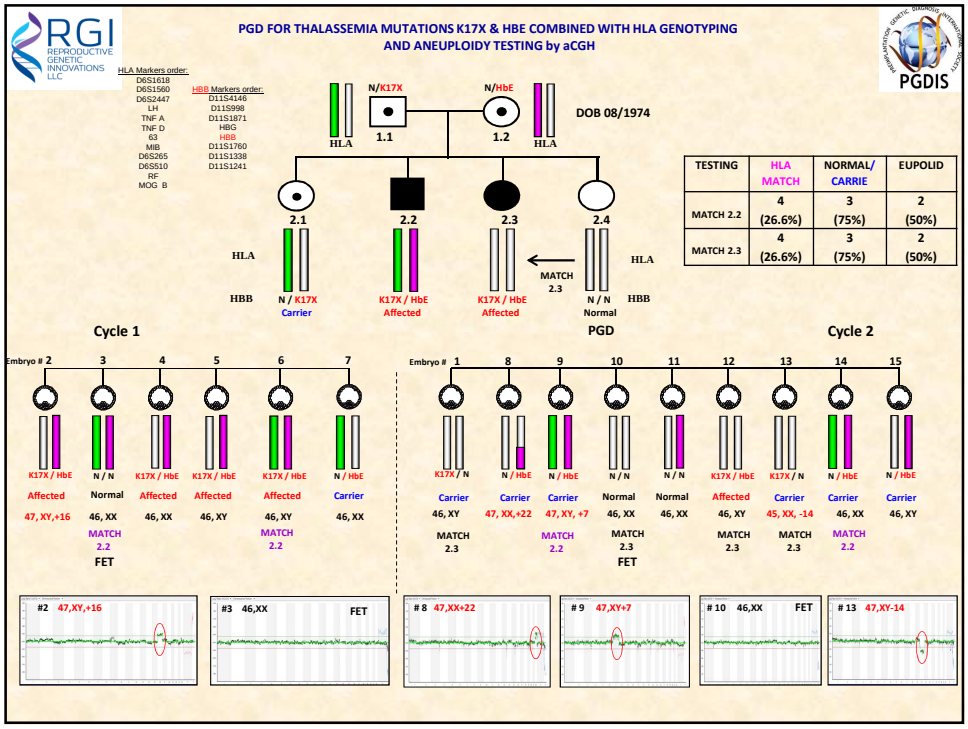
Total : 22.52%

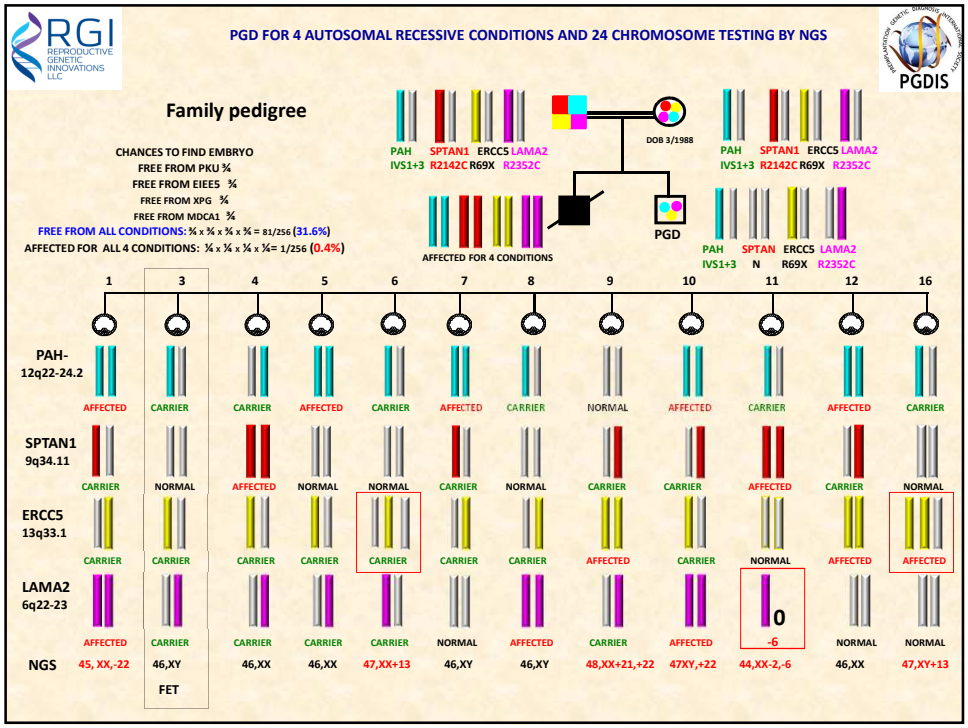

Detection of copy number of chromosome and its impact on accuracy of PGD
PGDIS
Disomy
Monosomy
Trisomy
N
N
N
N
N
156 bp
1000
500
Disomy
Monosomy
Trisomy
128
136
N
155
120
130
N
145
120
130
N
145
128
136
N
155
132
140
N
152
120
130
N
145
4000
3000
2000
1000

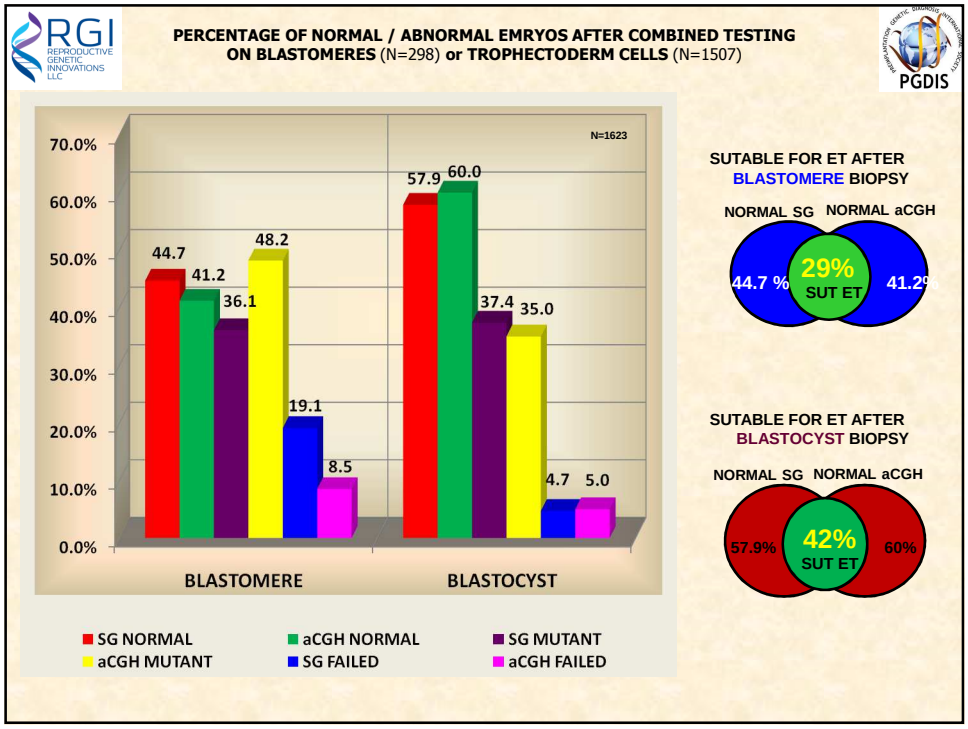

INCIDENTAL FINDINGS AFTER PGD FOR SINGLE GENE DISORDERS
PGDIS
PGD for Thalassemia & HLA Testing
HLA
HBB
1.1
IVSI-6 / N
28 y.o.
1.2
HLA
HBB
N / IVSI-6
HLA
HBB
HLA
HBB
PGD
Carrier
Match XXY
Oocyte #
2
3
4
5
6
7
Embryo #
Affected, Match
Affected, Non-match
Carrier, Non-match
Carrier, Match
Normal, Non-match
ET
PGD for DMD
1.1
Del / N1.2
Del
40 y.o.
Del / N
PGD
PGD
Normal 46 XY
Normal for DMD 47 XX +13
Embryo #
1
2
3
4
5
6
7
XXY
XXY
Normal XX
Affected XY
Carrier XX
Normal XX
Normal XY
ET
ET

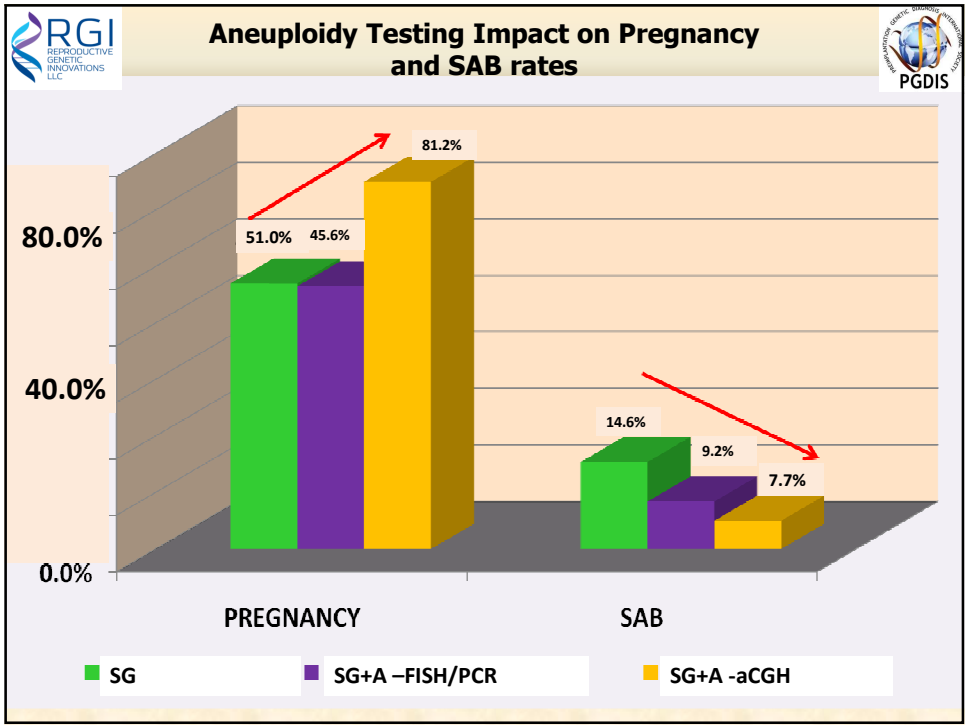

PGD for Alpha-1-Antitrypsin Deficiency and Translocation
PGDIS
DOB 1979
46,X,t(2;3)(p13;q21)
PGD
DOB 1987
46,XX
Patient Cycle 1
S/N
ET
N/Z
N/N
S/Z
N/N
ET
N/Z
N/Z
S/Z
N/Z
Patient Cycle 2
N/N
N/N
N/N
ET
N/N
ET
SAB
46,XX,t(2;3)(p13;q21)
S/Z
N/N
N/Z
S/Z
Patient Cycle 3
S/N
N/Z
FA
S/N
FA
46,XX,
DER(3)t(2;3)
N/N
46,XX
DER(2)t(2;3)
N/N
46,XY
DER(2)t(2;3)
S/Z
45,XY,-2
S/Z
46,XX
N/BAL
45,XY,-2
Donor Cycle 1
S/Z
45,XX,-15
N/N
46,XX
S/N
45,XX,-10
N/Z
47,XY,+21
N/N
46,XY
N/N
46,XX
S/Z
45,Y,-X
S/N
46,XY
FET 1
N/Z
46,XY
N/N
46,XY
S/N
46,XX
FET 1
Embryo #2
46,XX,
DER(2)t(2;3)
Embryo #4
aCGH
46,XY
DER(2)t(2;3)
Embryo #6
45,XY,-2
Embryo #8
46,XY
FET 1
Embryo #10
46,XX
FET 1


PGD for Tay-Sachs Disease; TSD
Splicing mutation IVS12+1G>C and 1278 TATC insertion
in HEXA GENE (15q23-q24)
PGDIS
DOB 11/84
46,XY,inv (2)(P24.2q22.2)
PGD 1
(DOB 5/10/11)
PGD 2
(DOB 11/10/15)
A. Cycle #1 (8/12/2010)
Blastomere Ananlysis
N
IVS12/N
N
IVS12/INS
IVS12/INS
IVS12/N
ET
IVS12/INS
N/INS
FR
IVS12/INS
FR
N
IVS12/INS
MONOSOMY 15
3 NORMAL
3 CARRIERS
5 AFFECTED
1 MONOSOMY 15
B. Cycle #2 (7/09/2014)
Trophectoderm Ananlysis
Combined Testing HEXA+aCGH
IVS12/N
46,dup
N
46
IVS12/IN
46
N/INS
46
N
46,dup
N/INS
46
N
45,del,-22
N/INS
46,dup
IVS12/INS
46
N/INS
46,dup
N
46,dup
IVS12/ INS
45,dup-16
IVS12/N
46,dup
IVS12/N
46
4 NORMAL
7 CARRIERS
3 AFFECTED
6 Normal/Balanced;
6 Unbalanced;
2 Aneupolid/ Unbalanced
SUT for FET 4
DUP2(q24.3q37.3)
DEL2(q24.3)
aCGH
46, dup(2)(q24.3q37.3)
NGS
aCGH
45, del(2)(q24.3), -22
NGS
FET 1 #2
46,XX
FET 2 #9
46,XY


PGD FOR THALASSEMIA MUTATIONS K17X & HBE COMBINED WITH HLA GENOTYPING
AND ANEUPLOIDY TESTING by aCGH
PGDIS
HLA Markers order:
D6S1618
D6S1560
D6S2447
LH
TNF A
TNF D
63
MIB
D6S265
D6S510
RF
MOG B
HBB Markers order:
D11S4146
D11S998
D11S1871
HBG
HBB
D11S1760
D11S1338
D11S1241
N/K17X
N/HbE
HLA
1.1
1.2
DOB 08/1974
2.1
2.2
2.3
2.4
MATCH 2.3
N / K17X Carrier
K17X / HbE Affected
K17X / HbE Affected
N / N Normal
TESTING
HLA MATCH
NORMAL/ CARRIE
EUPOLID
MATCH 2.2
4 (26.6%)
3 (75%)
2 (50%)
MATCH 2.3
4 (26.6%)
3 (75%)
2 (50%)
Cycle 1
PGD
Cycle 2
Embryo # 2 3 4 5 6 7
K17X / HbE Affected 47, XY,+16
N / N Normal 46, XX MATCH 2.2 FET
K17X / HbE Affected 46, XX
K17X / HbE Affected 46, XY
K17X / HbE Affected 46, XY MATCH 2.2
N / HbE Carrier 46, XX
Embryo # 1 8 9 10 11 12 13 14 15
K17X / N Carrier 46, XY
N / HbE Carrier 47, XX,+22 MATCH 2.3
N / HbE Carrier 47, XY, +7 MATCH 2.2
N / N Normal 46, XX MATCH 2.3 FET
N / N Normal 46, XX
K17X / HbE Affected 46, XY MATCH 2.3
K17X / N Carrier 45, XX, -14 MATCH 2.3
N / HbE Carrier 46, XX MATCH 2.2
N / HbE Carrier 46, XY
#2 47,XY,+16
#3 46,XX FET
8 47,XX+22
9 47,XY+7
10 46,XX FET
13 47,XY-14


PGD FOR 4 AUTOSOMAL RECESSIVE CONDITIONS AND 24 CHROMOSOME TESTING BY NGS
PGDIS
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 x ¾ = 81/256 (31.6%)
AFFECTED FOR ALL 4 CONDITIONS: ¼ x ¼ x ¼ x ¼ x ¼= 1/256 (0.4%)
PAH IVS1+3
SPTAN1 R2142C
ERCC5 R69X
LAMA2 R2352C
DOB 3/1988
AFFECTED FOR 4 CONDITIONS
PGD
PAH IVS1+3
SPTAN N
ERCC5 R69X
LAMA2 R2352C
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 -6 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 44,XX-2,-6 46,XX 47,XY+13


PERCENTAGE OF NORMAL / ABNORMAL EMRYOS AFTER COMBINED TESTING
ON BLASTOMERES (N=298) or TROPHECTODERM CELLS (N=1507)
PGDIS
N=1623
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
44.7
41.2
36.1
48.2
19.1
8.5
57.9
60.0
37.4
35.0
4.7
5.0
BLASTOMERE
BLASTOCYST
SG NORMAL
aCGH NORMAL
SG MUTANT
aCGH MUTANT
SG FAILED
aCGH FAILED
SUTABLE FOR ET AFTER
BLASTOMERE BIOPSY
NORMAL SG
NORMAL aCGH
44.7 %
29%
SUT ET
41.2
SUTABLE FOR ET AFTER
BLASTOCYST BIOPSY
NORMAL SG
NORMAL aCGH
57.9%
42%
SUT ET
60%


Aneuploidy Testing Impact on Pregnancy
and SAB rates
PGDIS
80.0%
40.0%
0.0%
51.0%
45.6%
81.2%
14.6%
9.2%
7.7%
PREGNANCY
SAB
SG
SG+A –FISH/PCR
SG+A -aCGH

Overall Results and outcome of PGD for Single Gene Disorders, HLA & Aneuploidy Testing

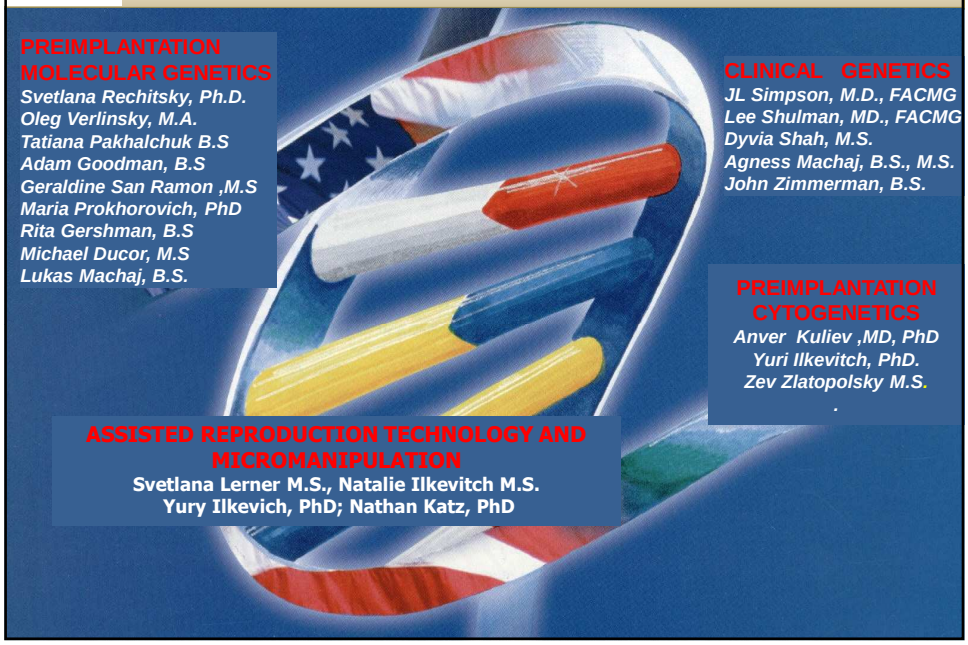
Testing	Patient/ Cycle	# ET	# Embryos	Pregnancy	SAB	Delivery	# Babies
SGD (INCLUDING HLA)	1749/2791	2113	3881 1.8	1078 51.0%	158 14.6%	920	1150
SGD+A by PCR or FISH	492/948	707	1209 1.7	323 45.6%	30 9.2%	293	357
SGD+ 24 AT For 122 conditions (INCLUDING HLA)	485/762	463*	616 1.3	376 81.2% P≤0.0001	29 7.7% P≤0.0027	347 92.3%	382
TOTAL	2726/4501	3283	5706	1777	217	1560	1889

CONCLUSIONS:

- Trophectoderm Biopsy (Day 5&6) has improved amplification efficiency (fewer inconclusive samples) and provide more embryos suitable for transfer
- WGA allowed multiple conditions testing on the same sample and embryo identification

- **Adding aneuploidy testing by aCGH or NGS**
  - Increased pregnancy rates
  - Reduced chance of miscarriage
  - Predicted accuracy is 98%

RGI TEAM



PREIMPLANTATION MOLECULAR GENETICS

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Yury Ilkevich, PhD; Nathan Katz, PhD