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A rare phenomenon: Double trisomy rescue detected during clinical SNP microarray testing

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Introduction

Trisomy rescue, through loss of a chromosome homologue, restores diploidy, correcting chromosomal imbalance that is detrimental for human development. Rescue generates a cell line with biparental inheritance or a cell line with uniparental disomy (UPD). UPD, the inheritance of both chromosome homologues from only one parent, can lead to clinical consequences if the rescued chromosome is imprinted or if the pairing of identical pathogenic autosomal recessive mutations occurs. UPD can be inferred by the loss of a haplotype visualized as an extended region of homozygosity (ROH) that localizes to a single diploid chromosome detected by SNP microarray testing. We present data from 14 individuals in which SNP array analysis shows an *extra* haplotype at a single chromosome. This rare allele dosage pattern is consistent with the rescue of meiotic trisomy by two events each involving loss of a different homologue rather than the more commonly observed rescue of post-zygotic trisomy by loss of one homologue, a unique phenomenon we call double rescue. Samples from seven individuals exhibit mosaicism for two cell lines with biparental inheritance, each cell line containing a different chromosome homologue from the same parent in combination with the homologue from the other parent, two individuals shown here **(Figures 2 and 3)**. Samples from five individuals display mosaicism for a cell line with biparental inheritance and a cell line with UPD, two of these involving imprinted chromosomes, two individuals shown here **(Figures 3 and 4)**.

Methods

All studies were done utilizing the Affymetrix® Cytoscan® HD array (Affymetrix® and CytoScan® are registered trademarks of ThermoFisher Scientific). The log2 ratio plot and smooth signal graph detects copy number while the B-allele frequency graph displays haplotypes. An allele of a polymorphic SNP located at a certain genomic position is denoted as A or B and the B-allele frequency (BAF) is equal to the number of B-alleles over the total number of alleles at that site. A value of 0 corresponds to an A allele from both parents, 0.5 indicates heterozygosity for an A and B allele and 1 corresponds to a B allele from both parents **(Figure 1)**. If two cell lines are present then the expected B-allele frequency is given by the following equation: (M)(B1)+(1-M)(B2)/(M)(A1+B1)+(1-M)(A2+B2) where B is the number of B alleles in cell line 1 and cell line 2, A is the number of A alleles in cell line 1 and cell line 2, M is the percent mosaicism for cell line 1.

Patient	Sample	Indication	Trisomy	Position	Allele pattern	Cell lines	Ratio	# Crossover
1	POC, villi	40 yr old: Spontaneous abortion	Chr 2, MII	2p25.3p25.1 2p16.2p12 2q12.2q35	7.24 Mb pattern IV 27.99 Mb pattern IV 112.12 Mb pattern IV	2 biparental lines	25:75	5
2	Blood	29 yr old: Repeated pregnancy loss	Chr 4, MII	4p16.3p15.1	4.88 Mb pattern IV	2 biparental lines	30:70	2
3	Blood	Newborn: Polycystic kidneys, multiple congenital anomalies	Chr 6, MII	6p24.3p21.2	28 Mb pattern IV	2 biparental lines	40:60	2
4	Blood	Newborn: Multiple congenital anomalies[b]	Chr 7, MII	7p22.3p11.2 7q32.1q36.3	54.71 Mb pattern IV 30.47 Mb pattern IV	2 biparental lines	10:90	2
5	Blood	11 yr old: Intellectual disability	Chr 9, MI	9pter to qter	pattern IV	unclear	50:50	0
6	Blood	Newborn: Respiratory distress, low birth weight	Chr 9, MI	9p24.3q22.32 9q22.32q34.11 9q34.11q34.3	96.66 Mb pattern IV 35.68 Mb pattern III 8.87 Mb pattern IV	1 biparental and 1 UPD line[a]	50:50	2
7	Buccal	2 yr old: Congenital anomalies, microcephaly, lack of normal development	Chr10, MII	10p15.3p11.23	30.5 Mb pattern IV	2 biparental lines	50:50	1
8	Blood	35 yr old: prior history of cleft palate, venous malformations and clubfoot during early childhood. cfDNA mosaic T13	Chr13: MII	13q12.3q34	85.48 Mb pattern IV	2 biparental lines	30:70	1
9	Blood	5 yr old: Delayed milestones, obesity	Chr15, MII	15q11.2q14 15q14q24.3 15q24.3q26.3	39.16 Mb pattern III 38.99 Mb pattern IV 24.38 Mb pattern III	1 biparental and 1 UPD line	20:80*	2
10	Amnio	40 yr old: AMA, Thickened nuchal fold and placenta	Chr 16, MII	16p13.3p13.12 16p13.12q12.2 16q12.2q24.3	12.91 Mb pattern IV 42.32 Mb pattern III 35.13 Mb pattern IV	1 biparental and 1 UPD line	30*:70	2
11	Amnio	41 yr old: AMA, history of pregnancy loss	Chr 18, MII	18p11.32p11.31 18q12.1q22.1	5.19 Mb pattern IV 33.01 Mb pattern IV	2 biparental lines	35:65	3
12	CVS	38 yr old: AMA	Chr 20, MII	20p13p12.2 20q13.12q13.33	9.99 Mb pattern IV 34.81 Mb pattern III 18.10 Mb pattern IV	1 biparental and 1 UPD line[c]	40*:60	2
13	Amnio	34 year old: cfDNA positive trisomy 22	Chr 22, MI	22q11.21q13.31 22q13.31q13.33	44.8 Mb pattern IV 6.49 Mb pattern III	1 biparental and 1 UPD line	40*:60	1
14	POC, villi	31 year old: Bilateral ventriculomegaly, possible cerebellar hypoplasia, dextrocardia, chest mass	Chr 22, MI	22pter to qter	Pattern IV	Unclear[d]	50:50	0

[a]*Third trisomy 9 cell line in 12% of cells*
[b]*Dysmorphic features, hypertelorism, low set ears, retrognathia, hypotonia, widely spaced nipples, light hydronephrosis, possible left undescended testicle, possible rocker bottom feet, clenched hands, agenesis of corpus callosum. Trisomy 8 also present in 70% of cells, meiosis II non-recombinant or mitotic origin*
[c]*Third trisomy 20 line in 22% of cells in microarray. Karyotype 47,XY,+20[3]/46,XY[47]*
[d]*Third trisomy 22 line in 15% of cells*
**UPD line*

Table 1. Clinical indications and cytogenetic findings in 14 double trisomy rescue patients.

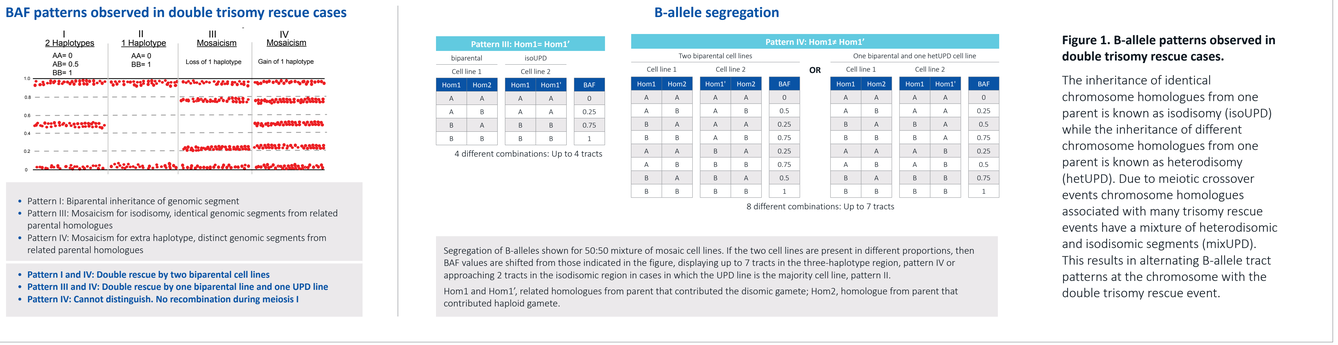

Figure 1. B-allele patterns observed in double trisomy rescue cases.

The inheritance of identical chromosome homologues from one parent is known as isodisomy (isoUPD) while the inheritance of different chromosome homologues from one parent is known as heterodisomy (hetUPD). Due to meiotic crossover events chromosome homologues associated with many trisomy rescue events have a mixture of heterodisomic and isodisomic segments (mixUPD). This results in alternating B-allele tract patterns at the chromosome with the double trisomy rescue event.

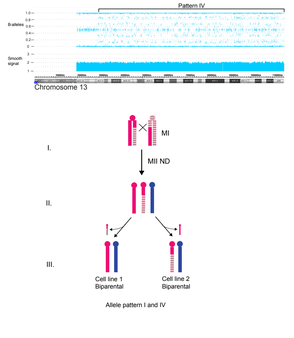

Figure 2. Double rescue of MII derived trisomy 13 in a 35-year-old with a prior history of cleft palate, venous malformations and clubfoot during early childhood (Table 1, patient 8).

Microarray testing of peripheral blood was initiated due to current cfDNA screening indication of mosaic trisomy 13 of suspected maternal origin. Testing reveals a terminal 85.48 Mb seven-tract extra haplotype segment at 13q12.3q34 (pattern IV, bracket) adjacent to a normal three tract segment (pattern I). Double rescue results in two biparental lines in a 30:70 ratio with one crossover site at the junction of the three-tract and seven-tract region at 13q12.3.

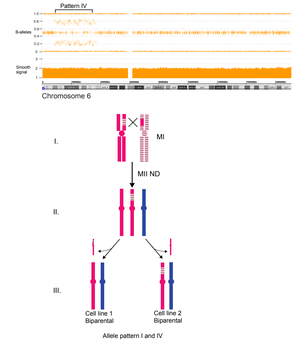

Figure 3. Double rescue of MII derived trisomy 6 in newborn with polycystic kidneys and multiple congenital anomalies, (Table 1, patient 3).

Microarray testing of peripheral blood shows an interstitial 28 Mb five-six tract segment (expansion of the BAF tract values of ~0.2 and 0.8) at 6p24.3p21.2 (extra haplotype pattern IV, bracket) adjacent to two three-tract segments (pattern I). Double rescue results in two biparental cell lines in a 40:60 proportion with two crossover sites at the distal and proximal junctions of the three tract and five-six tract regions.

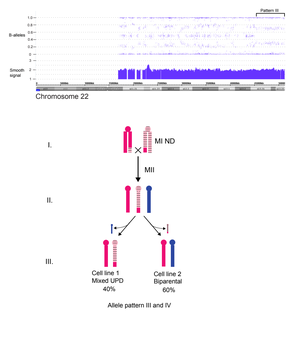

Figure 4. Double rescue of MI derived trisomy 22 in a fetus of a 34-year-old with cfDNA indication of increased trisomy 22 risk (Table 1, patient 13).

Microarray analysis of amniotic fluid indicates a 44.8 Mb proximal five-six tract BAF region at 22q11.1q13.31 (pattern IV, haplotype gain) adjacent to a distal 6.49 Mb four tract BAF region at 22q13.31q13.33 (pattern III, haplotype loss). The juxtaposition of the two segments signifies double rescue by a biparental cell line (60% of cells) and a mixUPD cell line (40% of cells) and one crossover at the junction of the two segments.

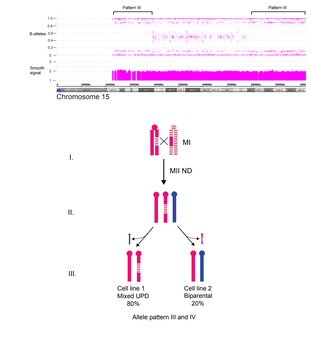

Figure 5. Double rescue of MII derived trisomy 15 in a 5-year-old with delayed milestones, obesity and suspected Prader-Willi syndrome (Table 1, patient 9).

Microarray testing shows three regions with altered BAF patterns. There is a proximal 39.16 Mb four tract segment at 15q11.2q14 (pattern III, haplotype loss), a central skewed BAF 38.99 Mb seven tract segment at 15q14q24.3 (pattern IV, haplotype gain) and a 24.38 Mb distal four tract segment at 15q24.3q26.3 (pattern III, haplotype loss). These patterns signify double rescue by a biparental cell line (20% of cells) and a mixUPD line (80% of cells) with one crossover at the junction of each segment. Notably, SNRPN methylation testing of peripheral blood from this patient resulted in a negative result because the presence of the minority biparental cell line interfered with the ability of non-quantitative SNRPN testing to confer a diagnosis. Subsequent pyrosequencing testing showed UPD15 of maternal origin in a similar percentage as detected by array (~80%).

Conclusion

It is important to be aware of the possibility of multiple trisomy rescue events. Trisomy double rescue results in mosaicism for two diploid cell lines in one individual. SNP microarray testing can reveal an etiology for clinical phenotypes that might result in false negatives with conventional non-quantitative testing methodologies in the case of imprinted chromosomes. Therefore, in these cases it is important to recommend pyrosequencing follow-up testing as conventional microsatellite and methylation-based testing methodologies are not sensitive enough to be able to quantitate the UPD cell line. Interestingly, two of the postnatal samples submitted for microarray testing were from women with prenatal referrals implying that double rescue is compatible with clinically normal or mildly affected individuals (**Table 1**, patients 2 and 8). It is possible that clinical findings of infertility could arise if residual trisomy persists in germ cells due to a lack of proliferative or selective basis for correction.

References

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