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Atypical Allele Dosage Patterns and Mechanisms in the Diagnosis of Whole and Segmental UPD

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I. Introduction

Single nucleotide polymorphism (SNP) microarray technology has been established as a high resolution tool to detect the presence of small regions of genomic imbalance. About 115,000 postnatal and 51,000 prenatal (11,000 POC) cases have been studied to date in our lab. The patterns and positions of allele homozygosity in chromosome homologues provide insight into potential regions of risk for autosomal recessive disorders and uniparental disomy (UPD). UPD was detected by the observation of extended homozygotic allele cues (whole chromosome: 230 cases confirmed, 100 isoUPD) or in a limited terminal segment by similar cues (segUPD: 62 cases) and has been found in a mosaic state in either category. In addition, two separate cell line corrections of a trisomy zygote to the normal diploid state can result in characteristic mosaic allele ratio patterns that indicate the presence of two distinct biparental cell lines or a UPD/biparental cell line mixture (9 cases). Those cases involving imprinted chromosomes and a UPD/biparental cell line can result in modified imprinting syndromes that are not detected by standard methylation assays (one case with 80% UPD15 mat). Although most segUPD has been associated with an emerging homozygotic cell line originating from a single mitotic recombination initiation site, multiple sites are common in cancer evolution and were also seen in constitutional studies in two patients (see also Choate et al). We have detected segmental UPD associated with correction of deletions, translocation derivatives, inverted duplication-deletions, and somatic selective proliferative advantage. We have also observed non-mosaic segUPD in blood or amniocyte analysis that was in the process of correcting in the placenta. Additionally, in rare cases the skewing of allele dosage ratios across the whole genome indicates the presence of admixtures that are associated with chimerism. Genome wide isoUPD chimeras mixed with a biparental population have been seen in 13 cases, 5 of which share a haplotype with the biparental population and 8 that display a third haplotype. The apparent mechanism behind these observations and their potential clinical significance are the focus of this report.

II. Materials and Methods

Postnatal DNA samples were obtained from blood or buccal cells while prenatal samples were either amniocyte or fetal tissue/placental derived.

Either Affymetrix version 6.0 Genechip or Affymetrix® CytoScan® HD array [Affymetrix® and CytoScan® are Registered Trademarks of Affymetrix, Inc.] was used for these studies. UPD testing was recommended for run of homozygosity (ROH) thresholds in a single chromosome >20 Mb, interstitially, or >10 Mb, telomerically (15 and 8 Mb for an imprinted chromosome). In symptomatic cases UPD testing was recommended without ROH.

Either methylation specific PCR, microsatellite trio comparisons or pyrosequencing were used for UPD testing.

III. Results

Figure 1. Whole chromosome UPD

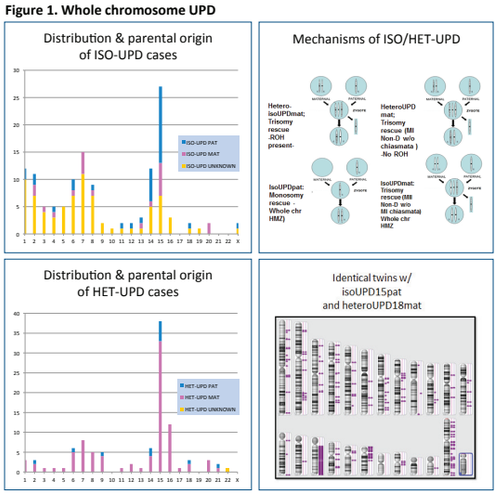
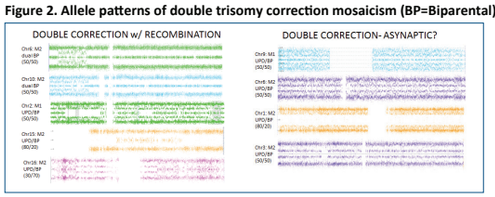


Figure 2. Allele patterns of double trisomy correction mosaicism (BP=Biparental)



Segmental UPD tables

Table 1: Non-mosaic segmental UPD

Case	UPD Interval	ROH (Mb)	Origin	Age	Indication
1*	1pter → p36.13	16.3	mat	AF	NIPT: Terminal del(1)(p36.23) and dup(1)(p36.23p36.22)
2	1pter→ p36.22	9.4	mat	9.3yo	Multiple congenital anomalies; der(1)t(1;17) in amnio analysis
3	5pter → p15.1	16.4	mat	51yo	Cardiomyopathy; hypertension; mental retardation
4	7q33→qter	26.21	mat	AF	VSD, small right ventricle; previous mosaic abnormal CVS
5	10q26.13 → qter	11.2	mat	NB	Clinically normal; CVS with del(10)(q26 -> qter)
6	11q13.1 → qter	71.4	mat	12yo	Encephalopathy
7	14q24.3 → qter	32.3	pat	AF	Scalp & leg edema; short femurs
8	15q15.3 → qter	58	pat	42yo	Recurrent pregnancy loss
9	Xq25→ qter	30	mat	NB	Fragile X full mutation x2; no paternal repeat
10	Xq13.1->qter	85.05	pat	2yo	Congenital anomalies of face and neck

* Mosaic in post-delivery POC array analysis

Table 2: Mosaic segmental UPD

Case	UPD Interval	ROH Total (Mb)	Percentage Mosaic	Age	Indication
1	12q13.12->qter	84.4	80-90	1.5yo	Multiple congenital anomalies
2	12q22->qter	80.3	10-20	4mo	DD
3	3pter→p24.3	23.46	30	AF	AV canal defect, 2 vessel cord, co-twin with anencephaly, oligohydramnios
4	11q13.4 → qter	62.3	25	AF	Clinodactyly; bright bowel
5	12q13.13 → qter	88	25 (PB); 15 (buccal); 0 (plac.)	NB	Dysmorphic features
6	12q13.13 → qter	81.45	80-90%	1.4yo	Peg teeth, dry skin, DD, Speech delay, slight gross motor delay, MCA
7	13q12.3 → qter	85.3	20	5.3yo	Atrial septal defect
8	14q12 → qter	74	25	8.9yo	DD
9	14q22.1 → qter	55.5	27	3.2yo	Autism; multiple congenital anomalies
10	15q13.3 → q15.2 15q15.q22.31 15q22.31->qter	71.2	15 45 70 [30% del(15)(q25.1q25.3)]	12yo	Short stature; DD
11	19q13.2 19q13.2->qter	2.75 17.36	25 37	35yo	MCA
12	21q21.1->qter	24.38	40	29yo	Possible trisomy; developmental disorder of scholastic skills

Table 3: Segmental UPD with contiguous triplications

Case	UPD Interval	ROH (Mb)	Triplication Interval	Trip (Mb)	Age	Indication
1	1pter → p36.21	11.6	1p36.21 → p36.22	1.32	4.8yo	None given
2	1pter → p36.13	19.4	1p36.13 → p36.12	3.6	7.6yo	None given
3	1pter→ p36.33	1.5	1p36.33 → p36.32	2.1	2.8yo	DD
4	1q43->qter	10.07	1q42.3->q43	4.23	NB	Multiple congenital anomalies
5	2pter→ p24.2	17.5	2p24.2 → p23.3	8.2	CVS	Cystic hygroma
6	3pter→ p25.1	13.7	3p25.1 → p24.1	14.7	4.9yo	DD
7	4pter → p15.2	26	4p15.2 → p14	13.4	NB	None given
8	8pter→ p12 (80%, 20% del)	31.3	8p12 → p11.21 (3.5 copy)	11	POC	Cystic hygroma
9	8pter → p21.1	24.4	8p21.21 → p12	5.2	25yo	Brain deformity, DD, hearing loss
10	8p24.3 -> qter	0.19	8q24.12->q24.3	24.3	Amnio	Thick NT, suspected CHD, cleft palate
11	9pter → p22.3	15.5	9p22.3 → p21.3	9.7	5.9yo	DD
12	17pter → p13.3	1.9	17p13.3 → p13.2	3.8	1.3yo	DD
13	17q25.3 → qter	2.1	17q25.3 → 25.3	3.1	2.6yo	Delayed milestones
14	21q21.1 → qter (70%, 30% del)	28.1	21q11.2 → q21.1 (3.4 copy)	5	POC	Advanced maternal age
15	22q13.31 → qter	3.45	22q13.2 → q13.31	5.2	1.1yo	Microcephaly

Figure 3. SegUPD subgroup percentages

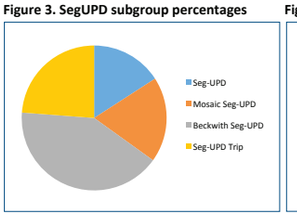


Figure 4. Mechanism of seg-UPD

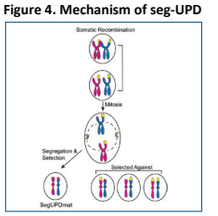


Figure 5. Seg-UPD associated with correction of genomic imbalance

Table 1 Case 2

AMNIOCYTE DER(1)T(1;17)

SEGUPD OF 1P IN BLOOD OF 9YO AFTER AF ANALYSIS OF DER(1)T(1;17)(P36.3;Q21)[48]/46,XX[2]

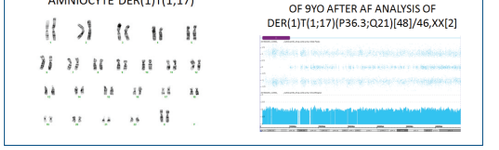


Table 1 Case 8

42 yo w/ Sustained Infertility SegUPD15q: Deleted Ova?

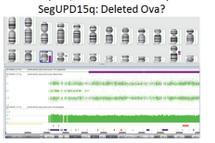


Table 3 Case 8

POC W/ INVERTED DUP-TERMINAL DEL 8P WITH INCOMPLETE CORRECTION BY MITOTIC RECOMBINATION (20% DEL, 3.5 COPY GAIN)

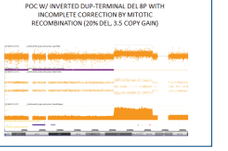


Figure 6. Mosaic Seg-UPD

Table 2 Case 3

AF WITH ANCEPHALIC TWIN INDICATION; PZ DELETION & REPAIR OR 3P "DRIVER" GENE SKEWING?

Table 2 Case 5

Segmental UPD12q13.13-qter: Three DNA sources

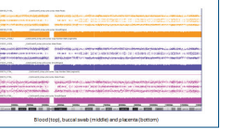


Table 2 Case 6

16 mo infant with dry skin, DD and two zone segUPD12q

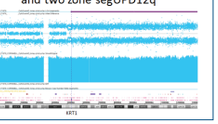


Table 2 Case 10

Constitutional 3-region seg UPD; 12 yo with growth delay

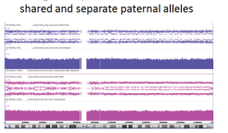
EVIDENCE OF MULTI-LINE DELETION REPAIR?

Figure 7. Whole genome chimeras and mechanisms

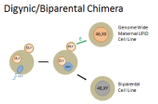
case #	hmz origin	haplo #	hmz %	specimen
1	androgenic	2	40	AF
2	androgenic	2	33	AF
3	androgenic	2	60	AF
4	androgenic	3	25	AF
5	androgenic	3	70	POC
6	androgenic	3	50	CVS
7	androgenic	3	50	CVS+/AF-
8	androgenic	2	50	BL
9	?	2	50	POC
10	androgenic	3	50	POC
11	digenic	3	25	AF
12	digenic	3	25	AF
13	digenic	3	30	POC
14	NA	4	50	BL*
15	NA	4	50	BL*

* Restricted hematologic chimera

Androgenic-Biparental Chimeras: Chr 2 shared and separate paternal alleles

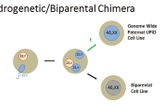


Digynic/Biparental Chimera



Possible mechanism for ~50% matching maternal alleles

Androgenetic/Biparental Chimera



Possible mechanism for identical paternal alleles in H_2C

IV. Conclusion

Altered allele dosage ratios provide characteristic patterns in the analysis of SNP microarrays. The specific characteristics and frequency of various genetic subgroups detected and the associated risks are as follows:

- **Whole chromosome UPD:** heteroUPD is more common (130/230) and primarily maternal (90%) while isoUPD, thought to be largely monosomy rescue based, had only a 70% paternal origin. Lack of a higher percentage is apparently due to high maternal nondisjunction rates that increases with asynaptic meiosis preceding trisomy rescue (particularly for chromosomes 15 & 21). Risks of UPD include AR disorders, imprinting syndromes, and transient trisomy effects.
- **"Double correction":** 9 cases of trisomy corrected by 2 different cell lines were detected with distinct allele ratio patterns. The correction resulted in either two biparental cell lines or a biparental/uniparental mix. Quantitative measures are necessary to confirm chr 15 UPD since the presence of the biparental line can result in a false negative methylation PCR assay.
- Three cases of UPD that occurred simultaneously for two separate homologues were seen, a 13yo with both isoUPD 1 and 15 and identical twins that each had paternal isoUPD15 and maternal heteroUPD18. Although both twins had AS, one twin had symptoms of +18, suggestive of a later gestational correction.
- Twelve cases of UPD with sustained presence of trisomy were seen, three of which were isoUPD, again indicative of absence of recombination.
- **SegUPD:** SegUPD is associated with correction of deletions, apparent translocation derivatives, and invdup/del rearrangements. In the case of invdup/del rearrangements correction can either normalize gene dosage or result in a contiguous triplication. Invdup/del rearrangements are *de novo*, but corrected translocation derivatives can correlate with a familial translocation. In addition, corrected deletions can reoccur in families due to a deletion present in a parent that corrects in most cells, but not in the gametes (see Johnson et al).
- **Mosaic segUPD:** 12 non-BWS cases show segUPD occurring in a cell population that originated from an apparent single abnormal cell. Case 10 (**Table 2**) with three different mitotic recombination initiation sites appears to be associated with interstitial deletion repair for which a 30% dosage deficit still remains. The absence of a dosage deficit in other cases raises the possibility that segUPD is largely based on mutational drive (see Choate et al).
- **Whole genome chimeras:** 13 consisted of a genome wide isoUPD and biparental mix. Eight showed 3 haplotypes and 5 showed 2. Androgenic origin predominated (9) with characteristic cystic placentas. In addition, two hematological chimeras from shared twin circulation had 4 haplotypes. One of these patients was almost 40yo, indicative of the sustained stem cell survival.

V. References

Choate et al. *Science* 2010; 330(6000):94–97.
Choate et al. *J Clin Invest* 2015 Apr; 125(4):1703-7
Johnson et al. *Clin Genet* 2014; 85:376–380

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