Stephanie Gandomi[1], Melissa Parra[1], Jill Kelsay[2] , Chia-Ling Gau[1], Kent McKelvey[2]
1) Ambry Genetics, Aliso Viejo, CA
2) University of Arkansas for Medical Sciences, Little Rock, AR

Clinical Report of Concurrent Trisomy 21 and 22q11.21 Microdeletion Identified Through Array-Based Comparative Genomic Hybridization

BACKGROUND

INTRODUCTION:

- Phenotypic variability, sometimes extensive, is the rule rather than the exception for even the most common genetic syndromes.
- Even though more than 95% of Down syndrome (DS) is due to full, non-mosaic trisomy 21 there is a wide range of intellectual ability, medical concerns, and psychiatric differences.
- We report an adult woman with a long-standing diagnosis of Trisomy 21 by karyotype who presented to a DS clinic at the University of Arkansas for Medical Sciences with uncharacteristic psychiatric and facial features, which led us to seek array comparative genomic hybridization (aCGH).
- Literature review revealed only one additional case of an infant with a concurrent diagnosis of mosaic trisomy 21 and a larger 22q deletion in the DG critical region, leading to a more classic VCFS/DG presentation with congenital cardiac defects, which were absent in our proband (Balci *et al.,* 2009).
- Individuals with Down syndrome demonstrate about 50% reduced risk in developing psychopathology compared to individuals with other forms of mental retardation (reviewed in Fidler *et al.,* 2008)

PRODH (OMIM: 606810)

- ***PRODH*** is located in the VCFS/DiGeorge critical region, and has been associated with hyperprolinemia type 1 (HP1) and additional neurological features.
- Heterozygous and homozygous mutations in ***PRODH*** are associated with schizophrenia, early psychomotor developmental delay, cognitive defects, autistic features, and epilepsy (Afenjar *et al.,* 2007).

NEUROLOGICAL PHENOTYPES IN DOWN SYNDROME (Reviewed in Lott *et al*., 2012)

- Phenotype results from genetic expression and environmental influences
- Changes across the life span and ranges among individuals with Down syndrome.
- Brain morphology contributes to overall phenotype expression.
- Various genes located on Chromosome 21 (i.e. *DYRK1A*) can contribute to deregulation of neural pathways.
- Researchers implicate various factors to the contribution of neurological phenotype including: abnormally developed neural circuitry, age-specific onset of epilepsy, amyloid deposition, and gene-to-gene interactions on Chromosome 21.

METHODS

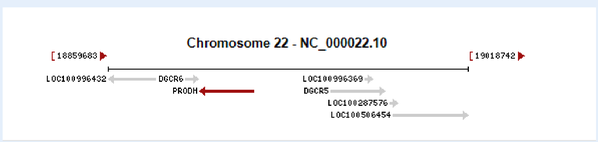
- Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's specimen using a standardized kit and quantified by Nanodrop. The aCGH method is based on the hybridization of fluorescently labeled patient genomic DNA (Cy-5) with fluorescently labeled reference DNA (Cy-3) to a 180K oligonucleotide array. Genomic patient DNA relative to the reference DNA are represented as fluorescent ratios (Cy5/Cy3) that are further quantified by image analysis software and analytical software. Quantified results indicate each targeted-DNA sequence as loss of copy number (deletion), gain of copy number (duplication) or normal copy number. This technology has been validated using patients with known microdeletions/duplications and other unbalanced karyotypes detected by traditional cytogenetic methods.

- The Ambry CMA: 180K Oligo array V2 contains 180,000 probes covering > 400 genetic disorders. Exon level resolution is achieved in ~ 680 disease causing genes. The backbone spacing of the probes is set at an average of 13 Kb throughout the entire human genome and at 5 Kb on the X chromosome. The array also includes probes for the pericentromeric and subtelomeric regions with dense probe coverage spanning 10 Mb at each subtelomere. There is no probe coverage of the pseudoautosomal regions 1 and 2 (PAR1 and PAR2) on the X and Y chromosomes.

FIGURE 1: Neurological Phenotypes in Down Syndrome and *PRODH* Alterations:

Neurological/Behavioral Features of Down Syndrome	*PRODH*-associated Neurological/Behavioral Phenotype
Intellectual disability (range in IQ levels between mild to severe disability)	Hyperprolinaemia type II
Cognitive development delays/slows down over time	Seizures/epilepsy
Long-term memory challenges	Mental retardation
Challenges with receptive and/or expressive language skills	Schizophrenia
Express more "approach behaviors" (i.e. outgoing, cheerful, sociable)	Severe psychomotor delay
More pro-social behaviors (i.e. sharing, participation in group activities, etc.)	Severe hyperactivity
Motor development difficulties due to muscular hypotonia	Sleep disturbances with bruxism
Psychopathology features	
Psychopathology Reported in Down Syndrome (reviewed by Fidler et al., 2008)	
Hyperactivity	
Aggression	
Stubborness	
Disobedience	
Inattention	
Impulsivity	

FIGURE 2: *PRODH on* Chromosome 22 Map



From NCBI Gene Page: http://www.ncbi.nlm.nih.gov/gene/5625

RESULTS/ DISCUSSION

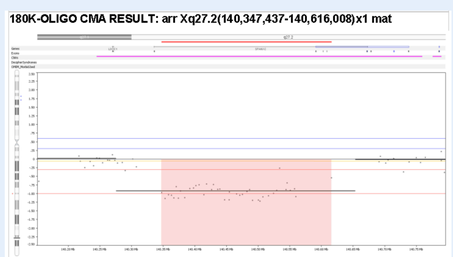
Microarray Results:

- The proband was found to carry a copy number **GAIN of the entire chromosome 21**, which is consistent with her previously identified diagnosis of Down Syndrome (Trisomy 21).
- The proband also carried two additional copy number LOSSES (deletions) on chromosome Xq27.2 and chromosome 22q11.21.
- The **Xq27.2 deletion** encompassed 2 genes including *SPANXA2-OT1, SPANXC*. This CNV was found to be maternally inherited on parental FISH analysis. Since the proband's mother was unaffected, this deletion is considered benign.
- The **22q11.21 deletion** encompassed 4 genes including *DGCR5, DGCR6, DQ585141,* ***PRODH*****.** This loss partially overlaps the 22q11.2 microdeletion syndrome region (also known as Velocardiofacial /DiGeorge syndrome), however this alteration is significantly smaller than the typical 22q11.2 microdeletion of approximately 1.5-3Mb.
- The clinical presentation of each individual varies and is dependent upon the breakpoints and the genes within the deleted region. The 22q11.2 microdeletion syndrome is characterized by dysmorphic facial features, cardiac anomalies (including Tetralogy of Fallot, severe aortic coarctation with hypoplastic left aortic arch, ventricular septal defects+/- pulmonary atresia, and truncus arteriosus), absence or hypoplasia of the thymus and parathyroid gland, mental retardation, psychiatric problems including schizophrenia, velopharyngeal insufficiency, submucosal cleft palate, hypotonia, and occasional findings of renal anomalies and seizures (Ryan *et al.,* 1997 and Tan *et al.* 2010)
- Missense and gross deletion mutations in the ***PRODH*** gene (OMIM:606810) are associated with autosomal recessive type I hyperprolinaemia, characterized by psychomotor delays, cognitive impairment, behavioral abnormalities (specifically hyperactivity), and increased risk for epilepsy and schizophrenia (Guilmatre *et al.* 2010; Jacquet *et al.* 2002 and Jacquet *et al.* 2003).

Interpretation of Identified CNVs:

- Our proband's phenotype is significant for schizophrenia and alterations in ***PRODH*** have been reported in association with these psychiatric findings.
- Results revealed trisomy 21 along with a 0.1-0.3 Mb deletion in 22q11.21 encompassing four genes: *DGCR5, DGCR6, DQ585141*, and ***PRODH,*** partially overlapping the VCFS/DiGeorge (DG) critical region. Literature review revealed only one additional case of an infant with a concurrent diagnosis of mosaic trisomy 21 and a larger 22q deletion in the DG critical region, leading to a more classic VCFS/DG presentation.

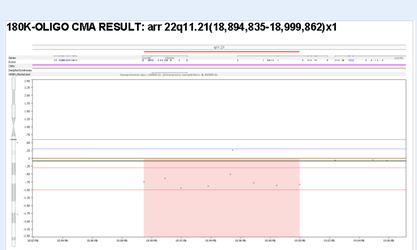
FIGURE 3: Patient's Copy Number Variations on 180K Microarray Analysis

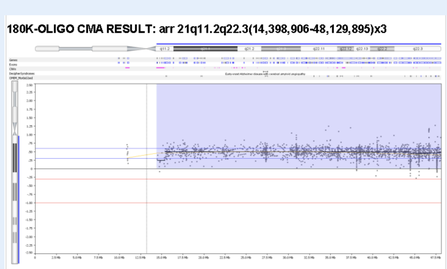


180K Oligo — Result: LOSS (Xq27.2, Suspected benign; GAIN (21q11.2q22.3), Pathogenic; LOSS (22q11.21), Unknown Significance

Change	Chromosome Region	Min Interval	Min Size (Mb)	Max Size (Mb)
LOSS	Xq27.2	140,347,437-140,616,008	0.269	0.383
GAIN	21q11.2q22.3	14,398,906-48,129,895	33.623	37.096
LOSS	22q11.21	18,894,835-18,999,862	0.105	0.318

arr Xq27.2(140,347,437-140,616,008)x1, 21q11.2q22.3(14,398,906-48,129,895)x3, 22q11.21(18,894,835-18,999,862)x1





Copy Number Variation	Number of Genes Involved in CNV	Genes Involved in CNV	CNV Origin
Xq27.2	2 genes	*SPANXA2-OT1, SPANXC*	maternally inherited
21q11.2q22.3	348 genes	Entire chromosome 21--Trisomy 21	*de novo*
22q11.21	4 genes	*DGCR5, DGCR6, DQ585141, PRODH*	*likely de novo*

CONCLUSIONS

- Phenotypic variance in common syndromes may be explained by aCGH testing .
- We conclude that based on our proband's clinical phenotype , her additional 22q11.21 deletion contributed to the uncharacteristic psychiatric and facial features detected in the clinical evaluation.
- This case supports the utility of aCGH as a first-tier test for diagnosis of individuals with multiple anomalies and as a repeat test in cases where clinical suspicion is high.
- Our case also highlights the importance of clinical correlation and the possibility that multiple chromosome abnormalities must be considered when common syndromes present with atypical findings.
- The resolution of aCGH may have profound implications not only for the proband but also for other family members and presents an additional consideration for counselors in the prenatal, pediatric, and adult settings.

References

Afenjar et al. (2007) Brain Dev 28(11):1098-1107.
Balci et al. (2009) Genet Counsel 20(4):373-377.
Fidler et al., (2008) Down Synd Res Pract 37-48; RF: www.down-syndrome.org/research
Guilmatre et al. (2010 Hum Mut 31(8):961-965.
Jacquet et al. (2002) Hum Mol Genet 11(9):2243-2249.
Jacquet et al. (2003) J Med Genet 40(1):e7.
Lott et al. (2012) Prog Brain Res 197:101-121.
Ryan et al. (1997) J Med Genet 34(10):798-804.
Tan et al. (2010) Clin Genet 78(3):201-218.