Advanced Genetic Testing: Clinical Application and Benefits in Real Practice

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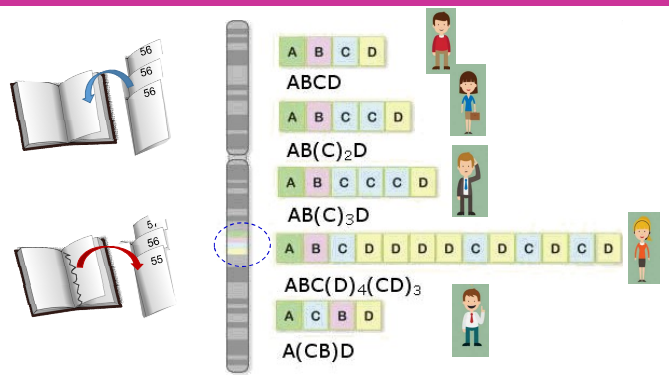
คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล

26 Oct 2019

Genetic Disorders: 4 types

- Chromosomal disorders
  - Multiple anomalies and/or Developmental delay, Intellectual disability, Dysmorphic features
  - Syndrome & Nonsyndrome
- Single gene disorders/mutation
  - Multiples organs - syndromes
  - Specific organs – muscle weakness, kidney etc.
  - Inborn metabolic disorders
- Multifactorial disorders
- Others
  - Epigenetic disorders, imprinting defects
  - Mitochondrial disorders

Chromosome variants: Copy number variation (CNV)



http://lvwarren.blogspot.com/

https://www.freepik.com/free-vector/people-cartoon-characters_761597.htm

CNV: inter-individual genetic polymorphism

- DNA segment size >1 Kb with variable copy number
- Gain or loss; the average size 250 kb
- Found in both healthy and affected individuals
- Contributes to ≈34% of the genome
- **Benign**, **Variant of unknown significant**, **Pathogenic**

  mean number of benign CNVs per person >800

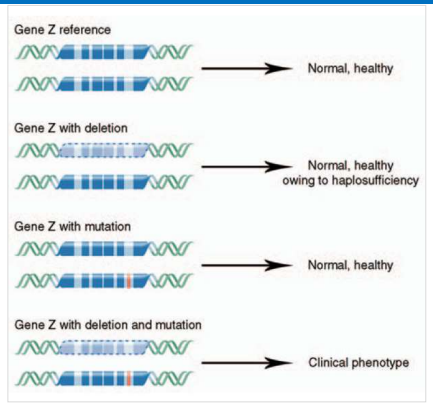
Tyson et al, *Am J Med Genet* 2005
Lee, et all. 2007 Nat Genet

Clinical interpretation of CNV

- 3 categories: Benign, Pathogenic, Unknown significance
- Pathogenic CNVs
  - gene-rich
  - inherited from an affected parent
  - seen in multiple affected individuals
  - large deletion size >3Mb in size, etc.
- Duplications: better tolerated than deletions
- Deletion
  - higher likelihood of being pathogenic
  - benign deletion - average size of 15-20 kb
  - small, gene-rich CNVs are more likely to be pathogenic than larger, gene-poor CNVs

(Lee, et all. 2007 Nat Genet)

Clinical interpretation of CNV



Single-copy deletion can be phenotypically benign,

but it may also be associated with pathogenicity

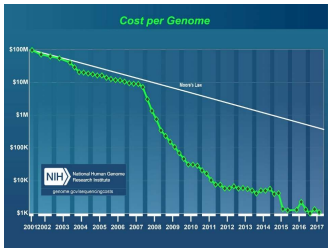
Lee C, et al.Nat Genet. 2007 Jul;39(7 Suppl) :S48-54.

Testing for single gene disorders

- Conventional Sanger sequencing
  - common mutations, familial mutation
  - All exons of one gene
- Next generation sequencing
  - Targeted disease panel
  - Whole exome sequencing
  - Whole genome sequencing
- Exon deletion/duplication
  - MLPA (multiple ligation-dependent probe amplification)
  - Ex: Duchenne muscular dystrophy, Spinal muscular atrophy etc.
- Trinucleotide repeat: PCR, Southern blot

Next generation sequencing

= Multiple gene sequencing in one time



https://www.genome.gov/about-genomics/fact-sheets/DNA-Sequencing-Costs-Data

- **100,000 variants**
- **Clinical history**
- **Known causing genes**
- **Allele frequency**
- **Inheritance pattern**

candidate variants

ACMG

Clinical Application & Benefits

- Disease/disorder based example – Autism
- Individual patient based example – Case 1, 2,3…

Autism: Recurrence Risk

- Chromosome & Single gene dis: 1-50%
- Multifactorial: 4%

Old belief - idiopathic autism = multifactorial

Recent data - high recurrence risk indicating NOT simply multifactorial

Condition	Multi-factorial	Idiopathic autism	Ritvo, 1989, Am J Psychiatry	Constantino, 2010, Am J Psychiatry	Ozonoff S, 2011. Pediatrics
1 affected child; **Next child to be affected**	4%	4% M 7% F 1%	8.6% (Utah Epidemiology Survey)	14.6% (Utah, Autism Registry)	18.7% (Utah, Prospective 3 Yr Longitudinal 664 → 132 ASD) Risk Male x3
2 affected child; **Next child to be affected**	10%	35%			32%

Clinical Utility of CMA

References	Study population	Results
Rauch et al., 2006	Unexplained Dev Delay/Intellectual Disability (DD/ID)	•Missed 0.6% of cases with disease-causing balanced de novo aberration •Highest diagnostic yield of any single test **(28.9%)**
Miller et al.,2010	21,698 patients with DD/ID, MCA and/or ASD	•using CMA as First-line test → diagnostic yield **+12.2%** higher than G-banding
Hochstenbach et al., 2009	DD/ID	•pathogenic CNV in **19%**
Shen et al., 2010	ASD	•abnormal CNV in **18.2%** (abnormal G-banding 2.23%; abnormal FXS test 0.46%)

ACMG Practice Guidelines

Array-based technology and recommendations for utilization in medical genetics practice for detection of chromosomal abnormalities

Melanie Manning, MD, MS, FACMG, and Louanne Hudgins, MD, FACMG, for the Professional Practice and Guidelines Committee

2010
2013

1. Cytogenetic microarray (CMA) test for CNV **Yield 15-30%**
   "**FIRST-line Test**" in initial **postnatal** evaluation
   - Multiple anomalies not specific to well-delineated genetic syndrome
   - Non-syndromic developmental/intellectual disability
   - Autism spectrum disorders (ASD)
2. Growth retardation, speech delay, other less well-studied indication but prospective study required
3. Appropriate F/U when chromosome imbalance is identified
   chromosome/FISH study: patient, parents
   clinical genetic evaluation & counseling

ACMG Practice Guidelines

American College of Medical Genetics

Array-based technology and recommendations for utilization in medical genetics practice for detection of chromosomal abnormalities

2010, con't

- When CMA should **NOT** be ordered
  - Rapid turnaround time is needed -- still true?
  - Suspected specific common aneuploidy
    - T21, T18, sex chromosome aneuploidy (conventional karyotype - more appropriate)
  - FISH with single probe to confirm suspected diagnosis eg, William syndrome, which would be more cost-effective

Experiences @ Ramathibodi Hosp.

Cohort 1: Nonsyndromic Autism

positive 25.4% (29/114)

Cohort 2: DD/ID w/wo dysmorphic features, congenital anomalies, w/wo autism

positive ≈ 30% (30/100)

SCIENTIFIC REPORTS

OPEN

Chromosomal microarray analysis in a cohort of underrepresented population identifies *SERINC2* as a novel candidate gene for autism spectrum disorder

114 Nonsyndromic (idiopathic) ASD

- รามาธิบดี มหิดล
- สงขลานครินทร์
- ธรรมศาสตร์

CMA Diagnostic yield 25.4%

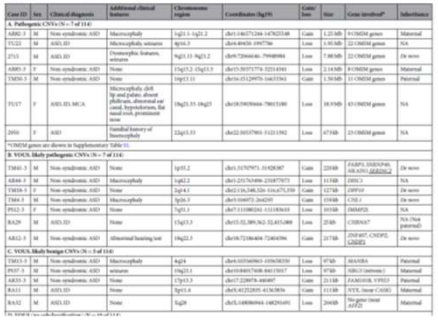
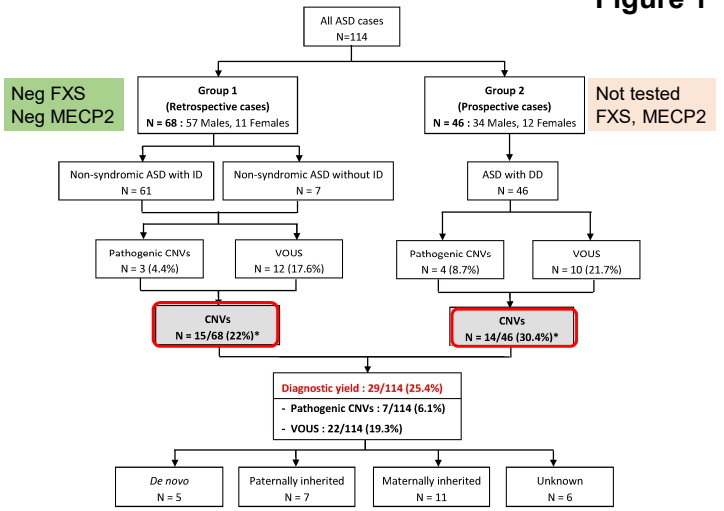


Figure 1



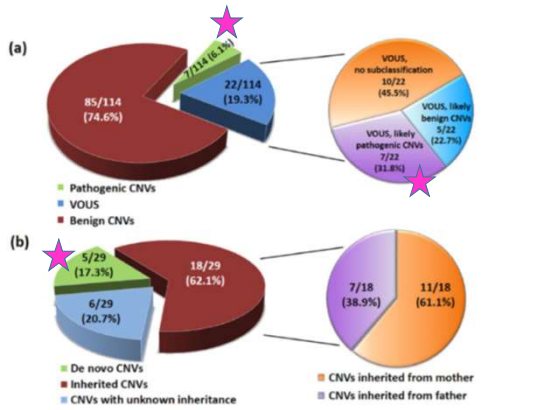
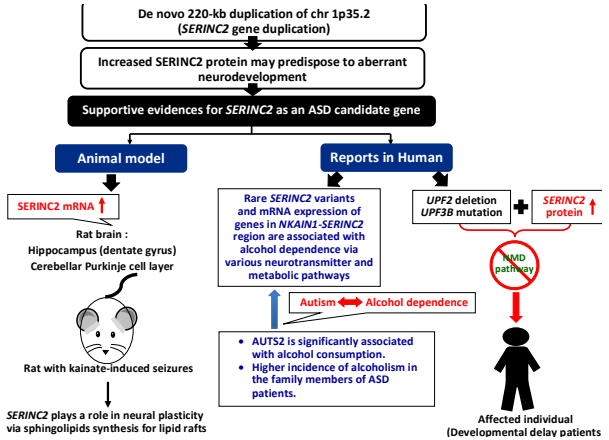


Figure 2. Pie charts summarizing the CNV classifications from the study. (a) Number of pathogenic CNVs and VOUS (likely pathogenic CNVs, likely benign CNVs, and VOUS with no subclassification); (b) Number of paternally or maternally inherited CNVs and *de novo* CNVs.

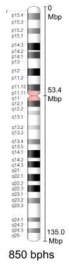
SERINC2 at 1p35.2
A candidate gene for ASD was identified from a *de novo* case



Case 1: A 6 y-old boy
Severe dev delay & autism, blindness, multiple congenital anomalies

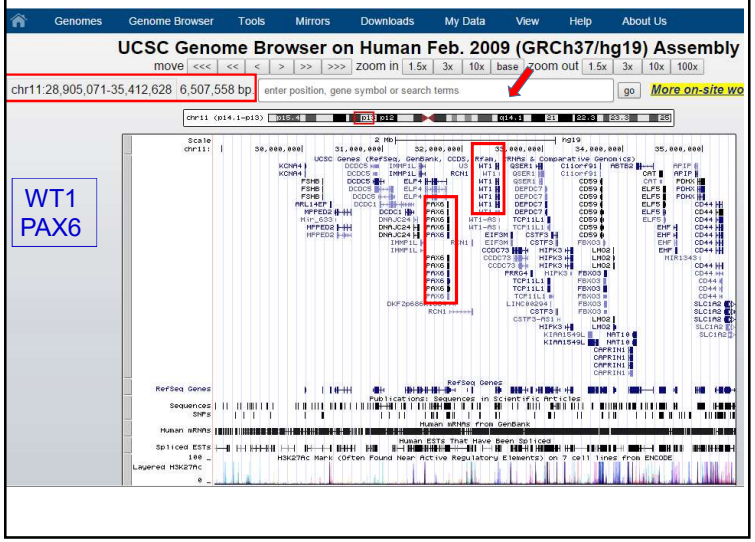
Clinical
- small & cup-shaped ear
- congenital cataract & glaucoma
- autistic-like behavior
- double opening urethra
- KUB U/S: bilateral VUR grade III

Previous Lab test
- 46,XY
- MRI Brain suggestive Lowe syndrome
- Mutation analysis of OCRL gene
  → no mutation identified



aCGH: deletion 11p13-p14.1
chr11:28,905,071-35,412,628
(6.5 Mb deletion)

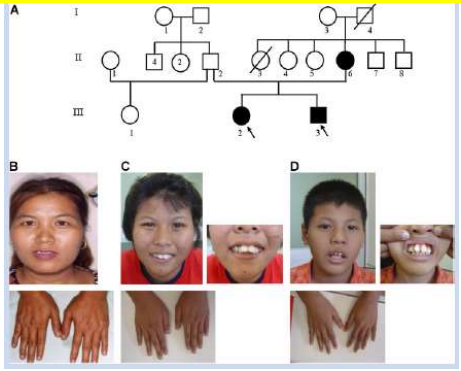
WT1 – tumor suppressor gene
PAX6 – anterior eye segment



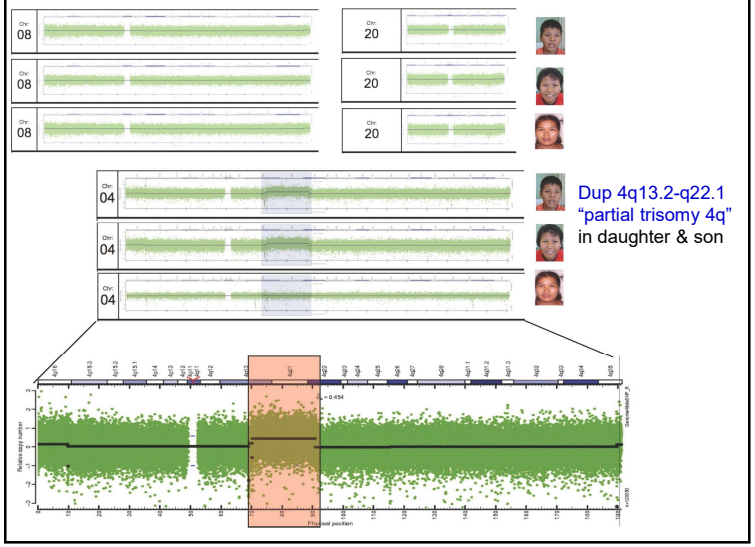
Cases 3-5: Familial MR.

Son : severe MR, prominent maxilla, pulmonic stenosis
Daughter: moderate MR, prominent maxilla.
Mother : mild MR.

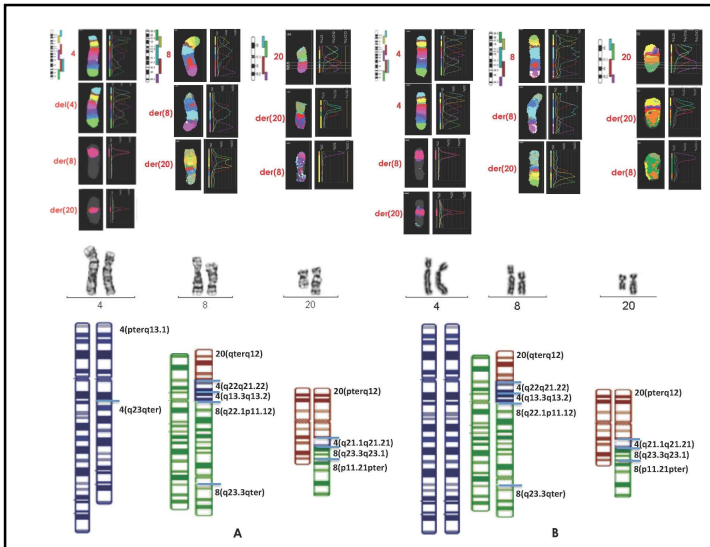
apparently balanced translocation 46,XX or XY, t(8;20)(p12;q12)



Assawamakin A et al., Am J Med Genet 2012



Dup 4q13.2-q22.1
"partial trisomy 4q"
in daughter & son



Cases 6: 4y5m boy

G1P1 GDD at 6m, autism, undescended testes
PE: nondysmorphic face, short uvula, wide based-gait. 46,XY

- karyolite BoB&9 microdeletion
  - NL finding
- At 5y4m
  - atonic seizures
  - declined dev

Cases 6: 4y5m boy

G1P1 GDD at 6m, autism, undescended testes
PE: nondysmorphic face, short uvula, wide based-gait. 46,XY

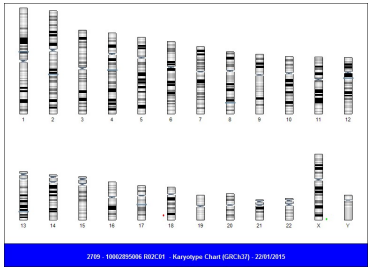
ID 2709: chr X

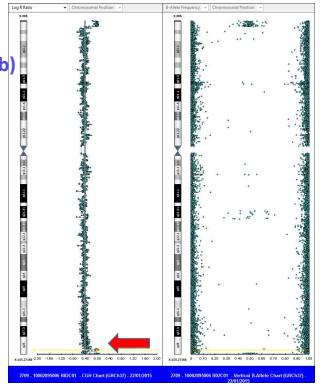
ISCN

arr[GRCh37] Xq28(152,966,348-153,781,876)x2

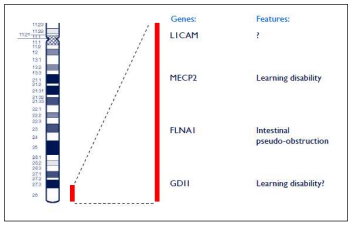
Size (bp)

815,529 (0.85 Mb)





Genes at Xq28



MECP2	Male	Female
Loss/deletion or point mutation	still birth, neonatal encephalopathy	Rett syndrome
Duplication: Xq28 duplication syndrome*	X-linked DD/ID, behavioral and psychiatric phenotypes (ADHD, autism, anxiety, psychotic), dev regression	No clinical symptoms or mild phenotype: LD

*Testing of maternal samples is important in determining recurrence risk.

https://www.ncbi.nlm.nih.gov/books/NBK349624/

http://www.rarechromo.org/

Unique

Support and Information

Rare Chromosome Disorder Support Group,
PO Box 2189,
Caterham,
Surrey CR3 5GN,
UK
Tel/Fax: +44(0)1883 330766
info@rarechromo.org
www.rarechromo.org

This leaflet is not a substitute for personal medical advice. Families should consult a medically qualified clinician in all matters relating to genetic diagnosis, management and health. The information is believed to be the best available at the time of publication. It was compiled by *Unique* and reviewed by Dr Damien Sanlaville, Hospices Civils de Lyon, France; Professor Dian Donnai, University of Manchester, UK and by Professor Maj Hultén BSc, PhD, MD, FRCPath, Professor of Reproductive Genetics, University of Warwick, UK. 2010

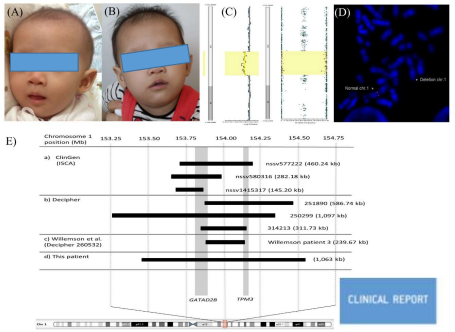
Rare Chromosome Disorder Support Group — Charity Number 1110661

Unique

Xq28 duplications

Recommend Follow-up test following positive CMA
- patient:
- parents: inherited or *de novo*, Recurrence risk, PND
- methods: high resolution chromosome / FISH

Cases 7: 6m girl, Dev delay
G1P1 nondysmorphic, mildly spastic, poor weight gain. 46, XX



CLINICAL REPORT

AMERICAN JOURNAL OF medical genetics A

1q21.3 Deletion Involving *GATAD2B*: An Emerging Recurrent Microdeletion Syndrome

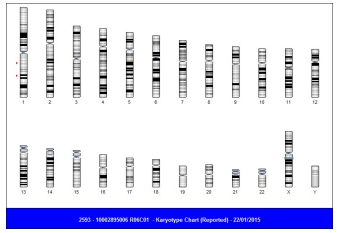
Thipwimol Tim-Aroon,[1] Natini Jinawath,[2] Weerin Thammachote,[2] Praweena Sinpitak,[3] Anchalee Limrungsikul,[4] Chaiyos Khongkhatithum,[5] and Duangrudee Wattanasirichaigoon[1]*

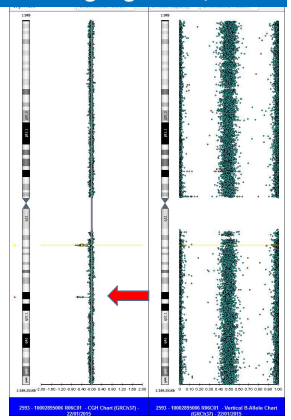
GATAD2B
– point mutation causing DD/ID
- microdeletion involving the gene

Cases 7: 6m girl, Dev delay
G1P1 nondysmorphic, mildly spastic, poor weight gain. 46, XX

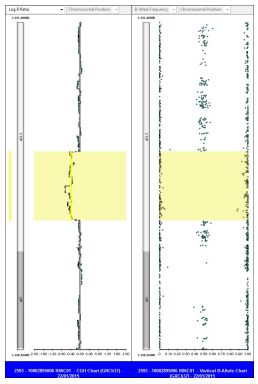
ID 2593: chr 1

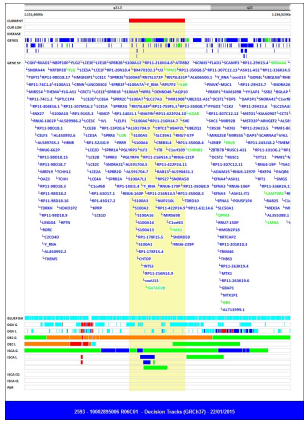
ISCN	Size (bp)
arr[GRCh37] 1q21.3(153,460,453-154,523,588)x1	**1,063,136 (1.06 Mb)**





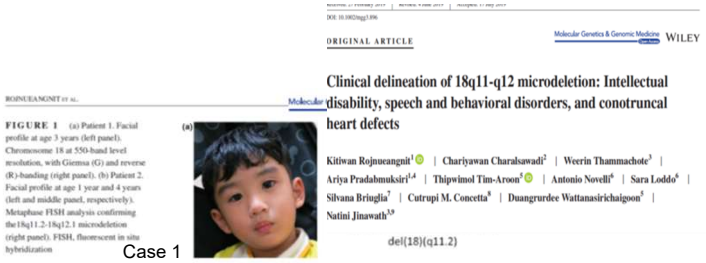
ID 2593: 1q21.3 microdeletion syndrome (?)





GATAD2B

Cases 8: 3y boy
Dev delay, speech delay, autism, conotruncal heart defect, 46, XY



ORIGINAL ARTICLE

Molecular Genetics & Genomic Medicine WILEY

Clinical delineation of 18q11-q12 microdeletion: Intellectual disability, speech and behavioral disorders, and conotruncal heart defects

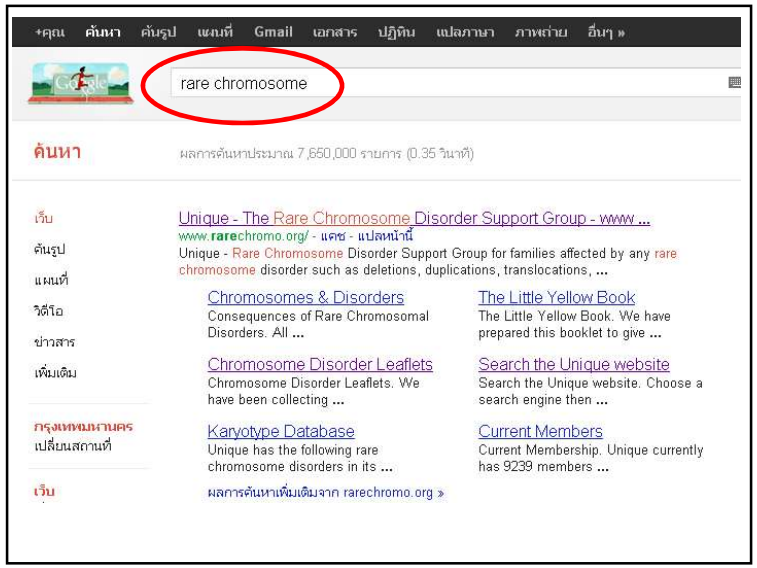
Kitiwan Rojnueangnit[1] | Chariyawan Charalsawadi[2] | Weerin Thammachote[3] | Ariya Pradabmuksiri[1,4] | Thipwimol Tim-Aroon[2] | Antonio Novelli[6] | Sara Loddo[6] | Silvana Briuglia[7] | Cutrupi M. Concetta[8] | Duangrudee Wattanasirichaigoon[5] | Natini Jinawath[3,9]

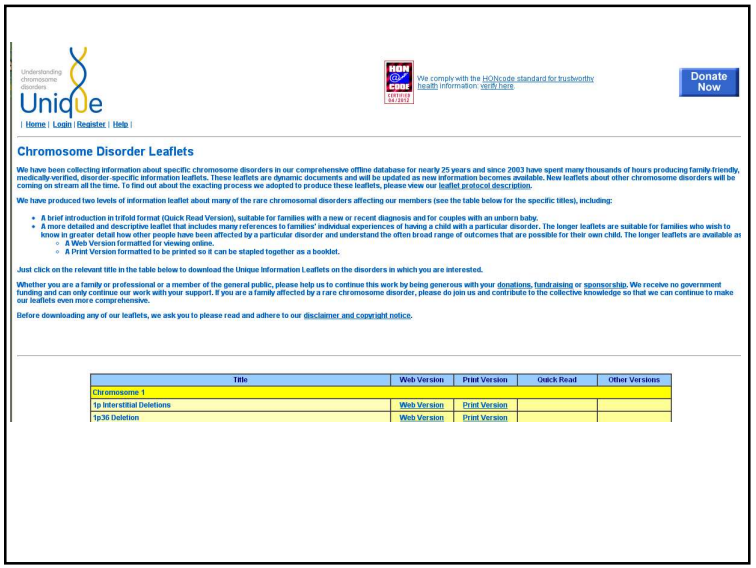
FIGURE 1 (a) Patient 1. Facial profile at age 3 years (left panel). Chromosome 18 at 550-band level resolution, with Giemsa (G) and reverse (R)-banding (right panel). (b) Patient 2. Facial profile at age 1 year and 4 years (left and middle panel, respectively). Metaphase FISH analysis confirming the 18q11.2-18q12.1 microdeletion (right panel). FISH, fluorescent in situ hybridization

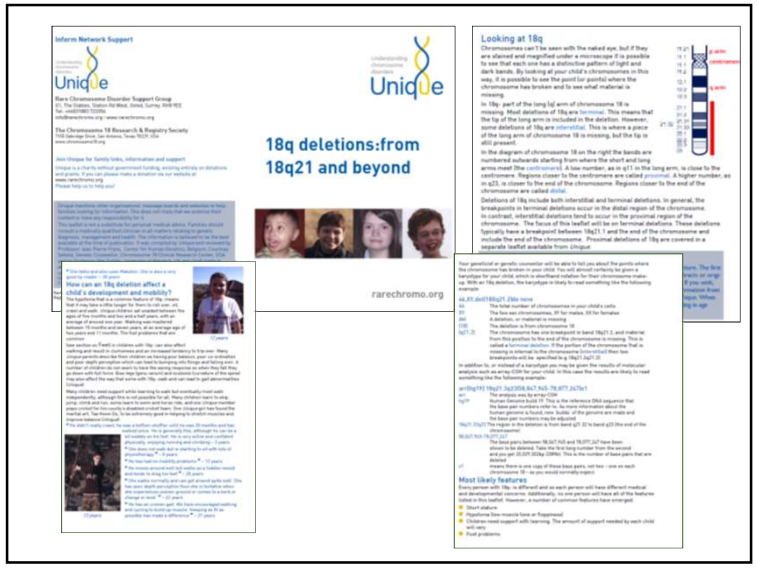
Case 1

del(18)(q11.2)

Case 2







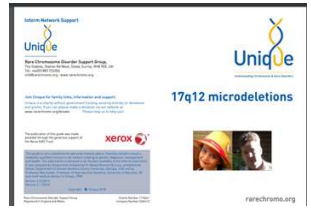
Case 10: A 6.5 y old boy

prenatal diagnosis of multicystic dysplstic kidney, 46,XY
Early childhood: dev delay, autism

METHOD OF ANALYSIS:
Array SNP based analysis (Infinium CytoSNP-850Kv1.1 BeadChip, Illumina) for patient

CYTOGENETIC AND/OR MOLECULAR DIAGNOSIS:
arr[hg19] 17q12(34,466,631-36,307,189)x1

Chromosome band: 17q12
Deleted region – genomic location (hg19): 34,466,631-36,307,189
Size (bp): 1,840,559
Number of SNP markers within deleted region: 551
Normal flanking loci – genome location: 34,465,375-36,450,598



Case 10: A 6.5 y old boy

prenatal diagnosis of multicystic dysplstic kidney, 46,XY
Early childhood: dev delay, autism

NARRATIVE SUMMARY:

SNP array analysis showed a 1.84 Mb interstitial deletion on the long arm of chromosome 17 within band 17q12 from nucleotide 34,466,631-36,307,189, encompassing 2 OMIM genes that are known to be frequently deleted in chromosome 17q12 microdeletion syndrome. These genes are *HNF1B* (OMIM*189907) and *LHX1* (OMIM*601999).

Chromosome 17q12 microdeletion syndrome (OMIM*614527) is characterized by variable combinations of the three following findings: structural or functional abnormalities of the kidney and urinary tract (80% of affected individuals), maturity-onset diabetes of the young type 5 (MODY5) (40%), and neurodevelopmental or neuropsychiatric disorders e.g., global developmental delay, intellectual disability, autism spectrum disorder, schizophrenia, anxiety, and bipolar disorder (50%).

This recurrent deletion has high penetrance, with about 70% occurring *de novo* and 30% inherited from an affected parent who may have variable expressivity. Therefore, clinical correlation and genetic counseling are suggested. If indicated, parental array study is also recommended.

For more information, please refer to https://ghr.nlm.nih.gov/condition/17q12-deletion-syndrome , and https://www.ncbi.nlm.nih.gov/books/NBK401562/.

CLINICAL INTERPRETATION:
17q12 microdeletion syndrome

- Structural or functional defect of KUB system 80%
- ID, ASD, schizophrenia, bipolar 50%
- Maturity onset of diabetes of the young 40%

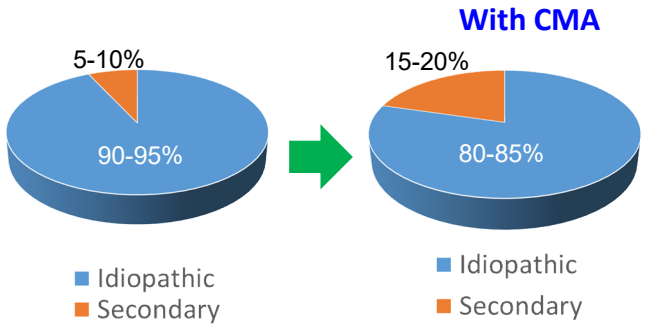
Advantages of CMA

- Identify small deletion/duplication of chromosomal segment
- Identify gain/loss of genetic material in individual with physical/cognitive impairment but with apparently balanced translocation - actually unbalanced rearrangement
  - 20% of individuals with apparently balanced translocation (de novo or familial) have loss/gain of genetic material
- Accurate information on microdeletion of tumor susceptibility gene in the deleted segment
- May identify genes that are disrupted by breakpoint

Benefits of CMA to Patients

- Avoid other unnecessary investigations
- Patient
  - Precise patient care & complication surveillance
- Family
  - Precise genetic counseling
  - Recurrence risk estimation
  - Prevention & PND

Distributive causes of Autism



Advantage of Whole Exome Sequencing (WES) in ASD

- Detect smaller deletion & duplications not identified by microarray in 7% of ASD cases
- Single nucleotide variants (SNV) & very small deletion & duplication of individual gene

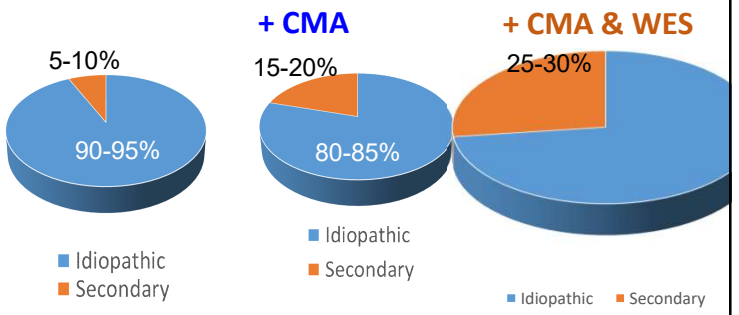
- Trio data using NGS

NGS	germline SNV mutation rate*	# de novo SNVs
WGS	$1.1\text{-}1.2x10^{-8}$	70
WES	$1.3\text{-}2.17x10^{-8}$	0.6-1.3
Most de novo SNVs, CNV, small indels – paternal origin		

*per position per generation

Rubies SD and Buxbaum JD, 2015
Loke YJ et al, 2015

Distributive causes of Autism



Rubies SD and Buxbaum JD, 2015
Loke YJ et al, 2015

Reminders

Disorder	Karyotype	CMA	Other test
Down	√		
Cri du chat	√		
Unexplained DD/MR w/wo malformation	√	√**	
MCA or Autism	√	√ **	?? future: WES
FXS			FMR1**Methylation PCR PCR-Southern
Angelman	FISH 75%	√ 80% (75%del +5% if using SNP array)	SNRPN-methylation PCR (80%)** FISH 75%
Prader-Willi	FISH 75%	√ 75% del	SNRPN-methylation PCR (99%) ** FISH 75%
Rett			DNA sequencing**
Williams/ VCFS	FISH		

Take Home Message

When to order CMA 'If affordable'
- Multiple anomalies not specific to well-delineated genetic syndrome
- Non-syndromic developmental/intellectual disability
- Autism spectrum disorders (ASD)

When not to order CMA
- Suspected specific common aneuploidy / microdeletion syndrome

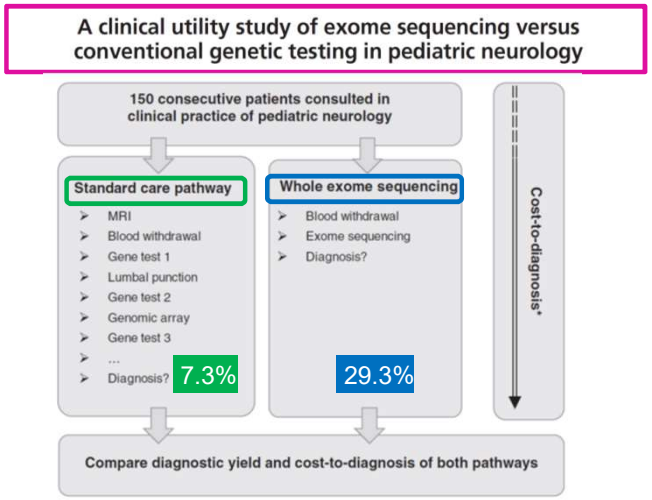
Limitation of CMA
– Clinical interpretation of VUS
– does not include FXS
- partially include PWS, Angelman

NGS

Pretest counseling is IMPORTANT

Use of Clinical WES in Pediatric Practice

- Yang Y et al, (NEJM 2013;369:1502-11)
  Diagnostic yield 25-30% for undiagnosed disorder



Vissers LELM, et al. Gene Med 2017

Unknown diagnosis case 1

- Girl 1y2m
- **Acute Liver failure** **Reye syndrome?**
- Bicytopenia
- Failure to thrive since 9m
- Minor dysmorphic features
- UOA, PAA, eye exam, acylcarnitine: NL
- Liver bx: mixed micro-macrovesicular fatty change

***NBAS* mutations —> infantile liver failure, short stature, and atypical osteogenesis imperfecta.**

Unknown diagnosis case 2

- Familial intellectual disability
- CT brain: Dandy-Walker syndrome