Genetic profile and clinical application of chromosomal microarray in children with intellectual disability in Hong Kong

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ABSTRACT

Introduction: Chromosomal microarray (CMA) is recommended as a first-tier genetic investigation for intellectual disability (ID), developmental delay, or autism spectrum disorder due to its higher diagnostic yield with respect to conventional karyotyping. The aim of the present study was to investigate the genetic profile and diagnostic yield of CMA in children with moderate, severe and profound ID.

Methods: A pilot cross-sectional study was performed by the Child Assessment Service and the Clinical Genetic Service in Hong Kong from July 2016 to June 2017. Children with unexplained ID were recruited for CMA testing by an expedited referral pathway. Children who were existing clients of the Clinical Genetic Service were also recruited.

Results: Of 225 children included in this study, 68 (30.2%) had genetic diagnoses. Among the 138 children who underwent CMA testing, 53 (38%) children were referred to the Clinical Genetic Service by the expedited referral pathway. The respective diagnostic yields of CMA in moderate, severe, and profound ID were 8.7%, 17.6%, and 23.5% (P<0.05). Children with dysmorphic features demonstrated a much higher yield from CMA (45.8% vs 4.4%, P<0.05).

Conclusion: The overall diagnostic yield (11.6%) of CMA in this cohort is comparable with that of other international cohorts. This further supports the use of CMA as a first-tier genetic investigation for children with ID, developmental delay, or autism spectrum disorder, particularly for those with severe disease.

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New knowledge added by this study

- Approximately one-third of children with more severe forms of intellectual disability exhibited a genetic condition, as determined by chromosomal microarray.
- The diagnostic yield of chromosomal microarray testing increases with the severity of intellectual disability, and with the severity of dysmorphic features.

Implications for clinical practice or policy

- The expedited mechanism, if extended to younger children with developmental delay (with or without autism spectrum disorder), may avoid unnecessary investigations in children, improve the efficiency of service delivery, and reduce societal cost.

Introduction

Intellectual disability (ID) is estimated to affect 1% to 3% of the population in Western societies.[1] It is almost two-fold greater in prevalence in low- and middle-income countries, compared with high-income countries. Importantly, the General Household Survey in 2014 showed the prevalence rate of ID to be approximately 1.0% to 1.4% in Hong Kong.[2]

Intellectual disability is defined as 'significant limitations both in intellectual functioning and in adaptive behaviour, as expressed in conceptual, social, and practical adaptive skills'.[3] These difficulties are evident above the age of 18; ID is indicated by an intelligence quotient (IQ) of approximately two standard deviations (SD) or more below the population mean (IQ ≤70) on the IQ test.

Developmental delay (DD) describes the developmental level of a child, typically <5 years old, who is substantially below the average standard of his peers. Global DD is defined as a significant delay in two or more domains: gross motor, fine motor, language, cognitive, social, or activities of daily living. Significant delay refers to scores >2 SD below the mean on norm-referenced age-appropriate developmental tests.[4]

香港智力障礙兒童之遺傳成因及基因晶片研究

陳英婷、陸浩明、李敏尤、盧輝文

引言：由於基因晶片（CMA）比傳統染色體檢查（karyotype）對人類基因體的數量變化檢測分析更詳盡及有更高的診斷率，CMA因此被建議為智力障礙、發展遲緩或自閉症譜系障礙患者的第一線遺傳驗測方法。本研究旨在檢視中度、嚴重和極嚴重智力障礙的遺傳成因及CMA於兒童智力障礙的臨床應用。

方法：衞生署轄下兒童體能智力測驗服務與醫學遺傳服務於2016年7月至2017年6月期間進行一項先導計劃，對兒童體能智力測驗服務轄下患有智力障礙但原因不明的兒童進行CMA檢測，並對醫學遺傳服務同期合資格的兒童之基因結果數據進行研究。

結果：本研究納入的225名兒童中，68人（30.2%）被診斷出遺傳成因。於接受CMA檢測的138名兒童中，53名（38%）兒童是通過先導計劃的驗測而縮短確診時間。CMA於中度、嚴重和極嚴重智力障礙的確診率分別為8.7%、17.6%和23.5%（P<0.05）。CMA於具有畸形特徵的兒童的確診率較高（45.8%對4.4%，P<0.05）。

結論：CMA的總體診斷率（11.6%）與其他國際研究相若。這進一步支持CMA作為患有智力障礙、發展遲緩或自閉症譜系障礙兒童，尤其是嚴重患者的第一線遺傳驗測方法。

In 2015 and 2016, more than 3000 children per year were diagnosed with DD by the Child Assessment Service (CAS). A study conducted in 2003 to 2004 showed that 80% of children with significant delay and 30% of children with borderline delay were later confirmed to exhibit ID at an older age.[5] Overall, 30% of children with ID had a co-morbid diagnosis of autism spectrum disorder (ASD).

The aetiology of ID is complex. While milder forms of ID are suspected to typically result from the interplay of genetic and environmental factors,[6] biological causes, particularly genetic causes, are often identified in children with significant cognitive delays (IQ <50).[4] Rauch et al[7] studied 670 subjects, generally <6 years of age, with ID: in 39.5%, ID was related to a genetic cause; in 1.3%, it was due to an acquired or environmental cause; and in 50% to 60%, it did not exhibit a known aetiology.

Chromosomal microarray (CMA) or array comparative genomic hybridisation, is recommended by many international professional organisations as a first-tier genetic investigation for children with unexplained DD, ID, or ASD.[4,8,9] Compared with conventional karyotyping, CMA is able to detect copy number variants (CNVs) with much finer resolution and is not reliant on staining and visual resolution limits. In 2010, a review of 33 published studies—involving 21 698 patients with DD, congenital anomalies, or autism—found the diagnostic yield of CMA to be 15% to 20% across all studies, compared with 3% for the standard G-banded karyotype.[9] In a group of 94 patients with no symptoms other than ID, and no clear dysmorphic features, the diagnostic yield was 6.4%.[8] According to the American Academy of Neurology guideline in 2011, CMA testing was abnormal in approximately 7.8% of patients with global DD or ID. The yield was higher (10.6%) in those with syndromic features.[8]

Children with ASD who had co-morbid ID were more likely to yield molecular diagnoses.[10] Approximately 10% of patients with ASD exhibit a de-novo CNV, as detected by CMA.[11] Among ASD children without syndromic features, only 6% received a molecular diagnosis.

In Hong Kong, two studies have investigated the use of CMA in patients with DD, ID, ASD, or multiple congenital anomalies (MCA). Chong et al[12] found clinically significant CMA results in 20 of 105 patients (19%). Tao et al[13] found a diagnostic yield of 11% for pathogenic or likely pathogenic results in 327 children, ages 1 month to >20 years. Excluding patients with MCA, the diagnostic yield of CMA for DD, ID, or ASD was approximately 4.2%.[13]

Chromosomal microarray has high clinical utility. Firstly, it shortens the diagnostic odyssey and may avoid unnecessary investigations, which reduces both individual and societal costs associated with testing and medical care.[8,14] Secondly, it may lead to a clinically actionable recommendation. The prognostic information related to diagnosis from CMA may alert other potential co-morbid conditions that cannot be predicted on the basis of physical examination alone. In a retrospective review of 1792 patients with DD, ID, ASD, or MCA who underwent CMA testing, individuals with a positive diagnosis had a higher rate of clinical actionable recommendations than those with an uncertain result (54% vs 34%, P=0.01).[15] In Hong Kong, a detection rate of 8.6% was reported for clinically actionable CNVs,[13] which was comparable to the reported rates of 3.6% to 7% in Western studies.[15-17] Thirdly, it allows estimation of recurrence risk and informed decisions regarding reproductive options for the parents' future pregnancies.

Children's cognitive development at ≥5 years of age is more stable if the level of ID is known. Children with DD undergo assessment at CAS to facilitate their transition into primary school. Since 2012, the Clinical Genetic Service (CGS) of the Department of Health has provided CMA testing for DD, ID, or ASD. The presence of dysmorphic features, early onset of DD, increased severity of DD, and family history are common features that prompt a genetic referral. Collaboration between CAS and CGS can potentially narrow the service gap for children with DD, ID, or ASD by enabling early access to diagnostic genomic testing, thus facilitating shorter waiting time for genetic and genomic investigation(s) and a more client-friendly turnover time for results.

The aim of this study was to investigate the genetic profile and diagnostic yield of CMA in children with moderate, severe, and profound ID. The data obtained from this study are expected to be useful in future service planning for children with DD or ID. The diagnostic yield of CMA for children with more severe forms of ID is suspected to be higher than the generally quoted figures of CMA (10%) for investigation of ID. This study targeted children with more significant ID, which is more likely to be related to an underlying genetic aetiology.

Methods

This cross-sectional territory-wide study recruited children who attended CAS for developmental assessments, before Primary 1 entry, over a 12-month period from July 2016 to June 2017. All children were at least 5 years of age. Inclusion criteria were children with moderate, severe, or profound ID, with or without ASD. According to the International Statistical Classification of Diseases and Related Health Problems, tenth revision,[18] moderate ID was defined as IQ 35 to 49; severe ID was defined as IQ 20 to 34; and profound IQ was defined as IQ <20. Exclusion criteria included known causes of ID: (i) antenatal causes such as congenital brain malformation or intrauterine infections; (ii) perinatal causes such as prematurity (<34 weeks), birth asphyxia, or hypoxic ischaemic encephalopathy; (iii) postnatal causes such as intracranial bleeding, intracranial infection, or brain trauma; and (iv) other biological causes such as inborn errors of metabolism, brain tumour, neuromuscular disorders, neurodegenerative disorders, or cerebral palsy.

Unexplained ID in this study was defined as children with no identifiable causes for ID who did not meet any of the exclusion criteria. These children were non-syndromic and non-dysmorphic. In addition, they had neither MCA nor family history of ID or ASD among first- and second-degree relatives. The presence of MCA was defined as the involvement of two or more organ systems.

Children were assessed by paediatricians with the Griffiths Mental Developmental Scales,[19] or by clinical psychologists with the Wechsler Preschool and Primary Scale of Intelligence–Revised.[20] The Diagnostic and Statistical Manual of Mental Disorders, 4th edition,[21] ASD diagnostic criteria were used for assessment of ASD.

Genetic profile

For children with genetic diagnoses or who were known clients of CGS, their medical files were retrieved from CAS and CGS for review. Children with syndromic or dysmorphic features, MCA, or significant family history, who had not been previously referred to CGS, were referred for a formal genetic consultation before genetic or genomic investigations were recommended by a clinical geneticist.

An expedited pathway was offered for children with unexplained ID. Pre-genetic counselling was provided by a paediatrician at CAS, followed by direct blood examination for CMA and Fragile X syndrome (FGX) testing at CGS. Consultation with a geneticist was arranged if either CMA or Fragile X testing yielded abnormal outcomes. Otherwise, clients did not consult a geneticist for further counselling.

Chromosomal microarray testing and interpretation

For each patient, 3 mL of blood in ethylenediaminetetraacetic acid was sent to the laboratory at CGS. All samples were tested by PerkinElmer CGX™ v2 60K arrays designed by Agilent SurePrint technology, in accordance with the manufacturer's instructions. The coverage of the array demonstrated an average resolution of 140 kb across the genome, and ≤40 kb in regions of clinical relevance. It evaluated >245 known genetic syndromes and >980 gene regions of functional significance in human development. Data were analysed by Genoglyphix software (Signature Genomics, Spokane [WA], United States). Genomic coordinates were based on genome assembly hg19.

Detected CNVs were systematically evaluated for clinical significance by comparison with information in the proprietary Genoglyphix Chromosome Aberration Database (Signature Genomics), internal laboratory database at CGS and the Department of Health, and public databases (Database of Genomic Variants, International Standards for Cytogenomic Arrays Consortium, and Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources). Categorisation of CNVs was based on available phenotypes and comparison of phenotypes with genes in the region of copy gain or loss. This was performed by searching the following databases: Online Mendelian Inheritance in Man, PubMed, RefSeq, and the University of California Santa Cruz genome browser.[22] Confirmatory fluorescence in situ hybridisation (FISH), multiplex ligation-dependent probe amplification (MLPA), or conventional karyotyping was performed as indicated. Parental testing was offered to aid further interpretation and classification. Copy number variants were classified as pathogenic, likely pathogenic, uncertain clinical significance, or benign, in accordance with the 2011 American College of Medical Genetics practice guidelines.[23] Only pathogenic and likely pathogenic CNVs were regarded as clinically significant.

Sample size calculation

The number of subjects to be recruited was

estimated based on the average number of children with moderate, severe, and profound ID in the CAS database. In 2013 to 2015, the average number of children with moderate ID or worse was approximately 270 children per year. With the assumption that 60% of cases were unexplained,[7] potential cases eligible for CMA were estimated as 160 children per year. Literature showed that the diagnostic yield of CMA was 10% in identifying abnormal cases in similar settings.[13] A 95% confidence interval was desired, with a reliability (d) of 0.05, in obtaining a diagnostic yield ($\hat{p}$) of 10% in this study. The sample size needed was determined following a previously published method[1]:

$$n = \frac{z^2(\hat{p})(1-\hat{p})}{d^2}$$

$$n = \frac{1.96^2(0.1)(1-0.1)}{0.05^2} = 138$$

Hence, a target sample size of 138 was needed.[24]

Statistical analysis

The genetic profile of children in the study was described. The diagnostic yield from CMA was calculated according to the severity of ID. The Freeman-Halton test was used to test associations between the severity of (a) ID and CMA and (b) dysmorphism and CMA findings. The null hypothesis was that there was no association between the severity of ID or dysmorphism and CMA findings. P<0.05 indicated an association between the severity of ID or dysmorphism and CMA findings. The Freeman-Halton test was conducted by using SAS/STAT 9.22.

Results

From July 2016 to June 2017, there were a total of 339 children diagnosed with more severe forms of ID: 241 (71%) children had moderate ID, 49 (14.5%) had severe ID, and 49 (14.5%) had profound ID. Eighty-three children were excluded for the following reasons: (1) they met predefined exclusion criteria; (2) their family could not participate due to geographical reasons (eg, family lived in China); (3) a language barrier affected their understanding of study details (eg, the children or their families spoke primarily Nepalese or Sri Lankan); or (4) their parents could not be contacted for consent. A total of 31 children opted not to participate in the study. In all, 225 (66.4%) of 339 children participated in the study.

Among the 225 children, 116 (51.6%) had a co-morbid diagnosis of ASD. Male (n=151) to female (n=74) ratio was 2:1. The age ranged from 5 to 10 years old with a mean age of 6.6 years old. In all, 71.5% of children had moderate ID, 14.7% had severe ID, and 13.8% had profound ID. Two hundred twenty-one (98%) children had Chinese parents. There were two pairs of consanguineous parents: one Indian couple and one Pakistani couple.

Genetic profile of children with intellectual disability

As shown in Table 1, 68 (30.2%) children were diagnosed with a genetic condition. The percentage of a positive genetic diagnosis increased with the severity of ID. Chromosomal abnormalities comprised 76% (n=52) of the total genetic diagnoses. The most common syndromic diagnosis was Down syndrome (n=22). There were two cases of FGX. Three children had chromosome 22 microdeletion syndromes—one exhibited the more common chromosome 22q11.2 microdeletion syndrome (DiGeorge syndrome), whereas the other two exhibited chromosome 22q13.3 deletion syndrome.

Diagnostic yield of chromosomal microarray in children with intellectual disability

Of the 225 participating children, 138 underwent CMA testing; 53 (38%) children were referred to the Clinical Genetic Service by the expedited referral pathway. Table 2 shows that 16 (11.6%) children demonstrated clinically significant CNVs that explained their ID phenotype and 10 (7.2%) had variants of uncertain significance (VUS). The diagnostic yield of CMA increased with severity of ID: it was 8.7% in moderate ID, 17.6% in severe ID, and 23.5% in profound ID (P<0.05; Table 3). Among the 16 children with clinically significant CNVs, 11 demonstrated copy number loss (deletion), four demonstrated copy number gain (duplication), and one demonstrated an unbalanced translocation between chromosome 7q and 20p (Table 4). One case of Angelman syndrome was detected by CMA and later confirmed with MLPA. One case of Cri du chat syndrome was detected by CMA and later confirmed with FISH. In total, 69% of pathogenic or likely pathogenic CNVs were de novo. Ten children (7.2%) were incidentally identified as carriers of disease: six were alpha thalassemia heterozygous carriers, one was a heterozygous carrier of Joubert syndrome type 4, one was a heterozygous carrier of autosomal recessive disease Joubert syndrome and nephronophthisis, one was a heterozygous carrier of autosomal recessive deafness affecting the *OTOA* gene, and one was a carrier of Klinefelter syndrome.

Discussion

The overall diagnostic yield of CMA among children with ID (11.6%) was consistent with studies performed in other regions of the world. The diagnostic yield of CMA increased with severity of ID and was much higher in children with dysmorphism (45.8% vs 4.4%, P<0.05).

TABLE 1. Genetic profile of children with ID

Diagnosis	Affected gene	No.	Moderate	Severe	Profound	Remarks
Chromosomal disorder						
Gross chromosomal anomalies (diagnosed by karyotype)						
Down syndrome		22	15	7		Dysmorphic
Cri du chat syndrome		2			2	Dysmorphic
Klinefelter syndrome		2	2			
9p deletion syndrome		2	2			Dysmorphic
Chromosomal 15q duplication		1	1			Dysmorphic
Unbalanced translocation between 1p and 9q		1		1		
6q15q22 deletion		1			1	
Pallister-Killian syndrome		1			1	Severe low vision, epilepsy, streaks of hyperpigmentation distributed over skin surface
Microdeletion/microduplication syndrome (diagnosed by karyotype)						
DiGeorge syndrome		1	1			Dysmorphic
Prader-Willi syndrome		1	1			Dysmorphic
Angelman syndrome		2			2	Dysmorphic
Microdeletion/microduplication syndrome (diagnosed by CMA, see Table 4)						
Microdeletion		11	6	2	3	
Microduplication		4	3		1	
Unbalanced translocation		1		1		
Single gene disorder						
Rett syndrome	*MECP2*	3			3	All females
Fragile X syndrome	*FMR1*	2		2		Both with ASD
ATRX syndrome	*ATRX*	1			1	Dysmorphic, hypotonic, squint
Syndromic X-linked mental retardation (Christianson syndrome)	*SLC9A6*	1			1	Infantile-onset epileptic encephalopathy, central hypotonia, non-paralytic squint, autistic features
Cardiofaciocutaneous syndrome	*BRAF*	2		2		Dysmorphic, Noonan-like
Tuberous sclerosis (AD)	*TSC1/TSC2*	2	1		1	
Neurofibromatosis type 1 (AD)	*NF-1*	1	1			
Costello syndrome (AD)	*HRAS*	1	1			
GRIN 1-related intellectual disability syndrome	*GRIN 1*	1			1	Ptosis, severe low vision, dystonia and dyskinesia
Mowat-Wilson syndrome (AD)	*ZEB2*	1		1		Cystic hygroma, severe microcephaly, congenital heart disease, Hirschsprung disease, epilepsy
Coffin-Siris syndrome (AD)	*ARID1B*	1	1			Dysmorphic
Total (a)		68	35	16	17	
Total No. in cohort based on ID severity (b)		225	161	33	31	
Proportion of children with genetic diagnoses (a/b)		30.2%	21.7%	48.5%	54.8%	

Abbreviations: AD = autosomal dominant; ASD = autism spectrum disorder; CMA = chromosomal microarray; ID = intellectual disability

TABLE 2. Chromosomal microarray diagnostic yields in unexplained ID*

	Dysmorphic ID (n=24)	Non-dysmorphic ID (n=114)	Total (n=138)	P value
Positive	11 (45.8)	5 (4.4)	16 (11.6)	<0.05
VUS	1 (4.2)	9 (7.9)	10 (7.2)	
Normal	12 (50.0)	90 (78.9)	102 (74.0)	
Incidental finding	0	10 (8.8)	10 (7.2)	

Abbreviations: ID = intellectual disability; VUS = variants of uncertain significance

* Data are shown as No. (%)

TABLE 3. Chromosomal microarray diagnostic yields based on severity of ID*

	Moderate ID (n=104)	Severe ID (n=17)	Profound ID (n=17)	Total (n=138)	P value
Positive	9 (8.7)	3 (17.6)	4 (23.5)	16 (11.6)	<0.05
VUS	5 (4.8)	4 (23.5)	1 (5.9)	10 (7.2)	
Normal	81 (77.9)	9 (52.9)	12 (70.6)	102 (73.9)	
Incidental finding	9 (8.7)	1 (5.9)	0	10 (7.2)	

Abbreviations: ID = intellectual disability; VUS = variants of uncertain significance

* Data are shown as No. (%)

TABLE 4. Cases with positive chromosomal microarray results

Patient No. / sex	Chromosome	Diagnosis	Genomic coordinates (hg18) of CNVs	Pathogenic CNV size and type	ID ± ASD	Parental testing
1 / F	2	Chromosome 2q11 deletion	arr[GRCh37]2q11.1q11.2(96740437_98017975) × 1	1.28 Mb interstitial deletion at 2q11.1q11.2	Moderate	De novo
2 / M	3	Chromosome 3q13.31 microdeletion syndrome	arr[hg19]3q13.31(113,525,219-115,303,006) × 1	1.78 Mb interstitial deletion at 3q13.31	Moderate	De novo
3 / F	5	Chromosome 5p15.2 microdeletion syndrome (Cri du chat syndrome)	arr[hg18]5p15.2(9,029,310-11,382,195) × 1, arr[hg18]3p26.1(7,550,447-7,682,734) × 0	2.35 Mb interstitial deletion at 5p15.2 132.29 Kb interstitial deletion at 3p26.1	Profound*	De novo
4 / M	6	Chromosome 6q21q22.31 deletion	arr[hg19]6q21q22.31(113,282,465-119,803,338) × 1	6.52 Mb interstitial deletion at 6q21q22.3	Moderate	N
5 / M	7	Unbalanced translocation between 7q and 20p	arr[hg19]7q36.1q36.3(150,115,362-159,123,167) × 1, 20p13p12.3(71,023-5,101,414) × 3	9.01 Mb terminal copy loss at 7q36.1q36.3 5.03 Mb terminal copy gain at 20p13-p12.3	Severe*	De novo
6 / M	7	Chromosome 7q35q36.2 deletion	arr[hg19]7q35q36.2(145,379,142-153,653,280) × 1	8.27 Mb interstitial deletion at 7q35q36.2	Moderate*	De novo
7 / F	11	Chromosome 11p11.2 deletion	arr[hg19]11p11.2(45,108,173-48,388,756) × 1	3.28 Mb interstitial deletion at 11p11.2	Profound*	De novo
8 / M	12	Chromosome 12q23 deletion, alpha thal carrier	arr[hg19]12q23.1q23.3(98,094,511-104,810,413) × 1, 16p13.3(215,499-232,686) × 1	6.72 Mb interstitial deletion at 12q23.1q23.3 17.19 Kb interstitial deletion at 16p13.3	Moderate	De novo
9 / F	14	14q11.2 Microdeletion syndrome	arr[hg19]14q11.2(21,821,702-21,916,153) × 1	94.45 Kb interstitial deletion at 14q11.2	Severe + ASD*	N
10 / M	15	15q11.2q13.1 Microdeletion (Angelman syndrome)	arr[hg19]15q11.2q13.1(22,822,019-28,513,166) × 1	5.69 Mb interstitial deletion at 15q11.2-q13.1	Profound*	De novo
11 / F	15	15q11.2-13.3 Duplication syndrome	arr[hg19]15q11.2q13.3(22,822,019-32,427,979) × 3, 15q13.2q13.3(31,140,606-32,427,979) × 3	9.61 Mb copy gain at 15q11.2-q13.3 1.29Mb copy gain at 15q13.2-13.3	Moderate*	De novo
12 / M	16	16p13.11 Microduplication syndrome	arr16p13.11(15,033,259-16,195,404) × 3	1.16 Mb interstitial duplication at 16p13.11	Moderate*	Mat
13 / F	16	16p13.11 Microduplication syndrome	arr[hg19]16p13.11(15,125,829-16,287,900) × 3	1.16 Mb interstitial duplication at 16p13.11	Profound*	Mat
14 / M	19	19p13.3 Duplication	arr19p13.3(1,044,712-1,972,214) × 3	Interstitial duplication at 19p13.3	Moderate*	De novo
15 / F	22	Chromosome 22q13.31q13.33 deletion	arr[hg18]22q13.31q13.33(45,355,784-49,522,658) × 1, X × 2	4.17 Mb terminal deletion at 22q13.31q13.33	Moderate	De novo
16 / F	22	Chromosome 22q13.31q13.33 deletion	arr[GRCh37]22q13.31q13.33(47807636-51178150) × 1	3.37 Mb terminal deletion at 22q13.31q13.33	Severe*	N

Abbreviations: ASD = autism spectrum disorder; CNV = copy number variant; ID = intellectual disability; Mat = maternal inheritance; N = not tested

* Dysmorphism

Variants of uncertain significance are not uncommon. In all, 7.2% of children in this cohort had VUS (Table 5). Regular follow-up and reassessment by a clinical geneticist is necessary for these children, because VUS may eventually be re-classified as pathogenic or benign as clinical and genomic data accumulate in the literature.

The paradigm shift in the medical genetic and genomic field from the phenotype-first approach to the genotype-first approach is revolutionary. Traditionally, a phenotype-first approach was used to guide the investigation of possible genetic diagnoses, eg, karyotyping for Down syndrome, or specific assays, such as FISH, for DiGeorge syndrome. In the past decade, CMA has allowed more comprehensive unbiased discovery of microdeletion and microduplication syndromes throughout the human genome. Since the 1980s, it has been well-known that certain chromosomal microdeletion and microduplication syndromes are associated with recognisable forms of ID and DD. Classical examples include 15q11-q13 deletion, associated with Prader-Willi and Angelman syndromes, and 22q11.2 deletion, associated with DiGeorge syndrome (velocardiofacial syndrome). Thus far, approximately 50 to 60 recurrent microdeletion or duplication syndromes have been identified in children with DD or ID.

Although CMA is robust, it cannot replace a formal genetic consultation for children with clinically suspected genetic conditions. As an example, in Prader-Willi syndrome, 70% to 75% of cases can be detected by CMA, as they are due to a paternal 15q microdeletion subtype; 20% to 25% of cases require a more specific methodology for genetic confirmation. Therefore, clinical correlation and expert assessment remain necessary.

Males are more susceptible to ID than females; more than 100 X-linked genes are associated with ID.[25] X-linked ID constitutes 5% to 10% of ID in males. One of the best-known causative genes for ID is *FMR1*; mutations of *FMR1* result in FGX. The estimated incidence of FGX is approximately 1 in 4000 males and 1 in 5000 to 1 in 8000 females (approximately 0.5% of cases of ID) in Western countries. Peprah[26] reported that the incidence of FGX in countries/regions with significant Asian populations, such as Canada, Estonia, Japan, and Taiwan, was significantly lower than in Western countries. In a study of 553 male children between the ages of 6 months and 18 years, Chen et al[27] estimated the prevalence of FGX in mainland China

TABLE 5. Cases with variants of uncertain significance

Patient No. / sex	Chromosomal microarray result	Genomic coordinates of CNVs	OMIM gene	ID ± ASD	Parental testing
1 / M	Interstitial deletion at 1q31.2	arr[hg19] 1q31.2(192,211,309-192,842,253) × 1	*RGS21, RGS1, RGS13, RGS2*	Moderate + ASD	De novo
2 / F	Interstitial deletion at 2p22.1 copy number gain at 8q13.3 interstitial deletion at 16q22.1	arr[hg19] 2p22.1(39,741,176-40,107,142) × 1, 8q13.3(72,922,285-73,414,414) × 3, 16q22.1(69,937,248-70,302,476) × 1	*THUMPD2, TRPA1, WWP2, MIR140, CLEC18A, CLEC18C, EXOSC6, AARS*	Severe + ASD	De novo
3 / M	Interstitial deletion at 2q24.3	arr[GRCh37] 2q24.3(165494643_166207407) × 1	*COBLL1, SLC38A11, SCN3A, SCN2A*	Moderate + ASD	De novo
4 / M	Copy number gain at 2q31.1	arr[hg19] 2q31.1(176,947,511-177,055,079) × 3	*EVX2, HOXD13, HOXD12, HOXD11, HOXD10, HOXD9, HOXD8, MIR10B, HOXD4, HOXD3, HOXD1*	Moderate + ASD	De novo
5 / M	Interstitial deletion at 4q32.1	arr[GRCh37] 4q32.1(158045745_159876096) × 1	*GLRB, GRIA2, RXFP1, C4orf46, ETFDH, PPID, FNIP2*	Moderate + ASD	N
6 / M	Interstitial deletion at 4q22.1	arr[GRCh37] 4q22.1(91161691_91618811) × 1	*CCSER1*	Severe	N
7 / M	Copy number gain at 5p13.33	arr[hg19] 5p15.33(445,144-990,819) × 3	*EXOC3, SLC9A3, CEP72, TPPP, TRIP13*	Severe + ASD	De novo
8 / M	Interstitial deletion at 6p21.1p12.3	arr[GRCh37] 6p21.1p12.3(45972996_46745796) × 1	*CLIC5, ENPP4, ENPP5, RCAN2, CYP39A1, SLC25A27, TDRD6, PLA2G7*	Moderate + ASD	De novo
9 / M	Copy number gain at 7p21.2-p21.3	arr[hg18] 7p21.3p21.2(12,155,874-13,472,985) × 3	*TMEM106B, SCIN, ARL4A*	Profound*	Pat
10 / F	Copy number gain at 20q11.21	arr[hg18] 20q11.21(29,542,526-29,579,598) × 3	*HM13*	Severe	Mat

Abbreviations: ASD = autism spectrum disorder; CNV = copy number variant; ID = intellectual disability; Mat = maternal inheritance; N = not tested; OMIM = Online Mendelian Inheritance in Man; Pat = paternal inheritance

* Dysmorphism

to be approximately 0.93% among children with moderate to severe ID. Among the 225 children in our cohort, only two were diagnosed with FGX. Both exhibited ASD and severe ID. The typical physical characteristics of FGX, such as narrow face, protruding ears, and macro-orchidism, are often less obvious in early childhood; notably, they may become more prominent as the child approaches adolescence. This lack of early physical characteristics increases the diagnostic challenge for clinicians. Fragile X syndrome testing, regarded as first-tier genetic testing for DD and ASD in many international guidelines, has been a standard genetic investigation for ID or ASD in Hong Kong for many years.

Incomplete penetrance of a genomic condition within the same family is not uncommon. Notably, there were two such cases of 16p13.11 microduplication syndrome in this cohort. Patient 12 (Table 4) exhibited subtle dysmorphism comprising downslanting palpebral fissures, prominent ears, and mild right ptosis. Left undescended testes and umbilical hernia were operated in infancy. He exhibited global DD and was later diagnosed with moderate ID. His mother and two sisters had an identical chromosomal defect, but exhibited normal intelligence. Patient 13 demonstrated a more severe phenotype with hirsutism, bushy eyebrows, frontal bossing, hearing loss, visual problems, and profound ID. His mother and elder brother, both carrying the microduplication, exhibited normal intelligence. There likely exist unknown environmental or genetic modifiers to modulate susceptibility to ID caused by this microduplication. Thus, relying on family history to determine whether ID is hereditary can be misleading.

An important aspect with respect to obtaining a genetic diagnosis is patient prognosis. The 16p13.11 duplication syndrome is associated with an aortic root defect. In this study, the two affected children and their affected family members were referred for monitoring by echocardiogram. Similarly, Patient 2 exhibited 3q13.31 microdeletion syndrome, which is associated with diabetes mellitus and deafness; this patient was referred for audiological assessment and counselled on lifestyle management to minimise the risk of diabetes. In this study, three of 16 CMA-positive cases (18.8%) were clinically actionable.

Pre-test genetic counselling is as important as post-test counselling. Coincidental findings of genetic changes that either predict adult-onset conditions or reveal carrier status for recessive or X-linked conditions are common. In the present cohort, 10 children were identified as carriers of genetic conditions, including one child diagnosed with Klinefelter syndrome. He presented with moderate ID and ASD. Chromosomal microarray identified a copy number gain of the entire X chromosome. Klinefelter syndrome can be associated with learning disabilities, as well as delayed speech and language development. While a small, but significant, downward shift in mean overall IQ has been reported, general cognitive abilities of patients with Klinefelter syndrome are not typically in the ID range.[28] An extra X chromosome may have contributed partially, but could not entirely explain the severity of ID. The major implications are that individuals with Klinefelter syndrome have a higher risk of endocrine dysfunction, fertility problems, male breast cancer, and autoimmune disease.

This study provided important information with respect to service planning for children with ID in Hong Kong. It allowed testing of an expedited referral mechanism between CAS and CGS, in which cases with unexplained ID benefitted through a significant reduction of waiting time for both pre-testing genetic counselling and investigation turnover time. This study included 38% of the 138 children who were not referred to CGS. The ideal future approach may be to extend the expedited mechanism for children with early-onset significant DD. It can avoid unnecessary investigations, thus lowering stress for both child and parent; importantly, it may reduce societal costs.

There were several limitations in this study. Firstly, a complete genetic profile of ID was not generated, as this cohort excluded mild ID. Secondly, clients from minority cultural groups in Hong Kong were underrepresented, because the language barrier affected recruitment. More effort must be expended to ensure equal opportunities for children from diverse cultural backgrounds. Thirdly, the duration of the study was of insufficient length for commentary on trends regarding the genetic profile of ID in Hong Kong.

Conclusion

The overall diagnostic yield (11.6%) of CMA is compatible with other international cohorts. Chromosomal microarray yield increases with the severity of ID. These data further support the use of CMA as a first-tier investigation for children with significant unexplained ID in Hong Kong.

Author contributions

All authors have made substantial contributions to the concept or design of the study, acquisition of data, analysis or interpretation of data, drafting of the article, and critical revision for important intellectual content.

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Declaration

The authors have no conflicts of interest to disclose. All authors had full access to the data, contributed to the study, approved the final version for publication, and take responsibility for its accuracy and integrity.

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Ethical approval

Approval was obtained from the Ethics Committee of the Department of Health, Hong Kong Special Administrative Region. Informed consent was obtained from parents or legal guardians. Parents and legal guardians were counselled about the indication for CMA, benefits and limitations of test, methodology, reporting time, and possible outcomes upon recruitment.

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