

Original Article

The Clinical and Molecular Spectrum of 15q Duplication Syndrome in Chinese

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Abstract

15q duplication syndrome (OMIM #608636) is a neurodevelopmental disease that characterised by hypotonia, developmental delay, intellectual disability, epilepsy and distinctive facial gestalt. A territory-wide study of 15q duplication syndrome is performed in Hong Kong with aim to examine its clinical and molecular features among Chinese patients. There are total of 12 cytogenetically and molecularly confirmed individuals between the period of January 2011 and December 2015. Four of them have interstitial duplication and 8 of them have isodicentric chromosome 15. The prevalence of 15q duplication syndrome in our Chinese cohort with intellectual disability and autistic spectrum disease is estimated to be 1.0% and 2.9%, respectively. As compared with western population, epilepsy is less common while squint is more prevalent in our Chinese patients. However, no genotype-phenotype correlation can be demonstrated in this study. **Conclusion:** The prevalence and clinical features of 15q duplication syndrome patients in Hong Kong Chinese are comparable with other western populations. It is hope that by having the better understanding of its underlying pathomechanism and their genotype-phenotype correlation would lead to better management and genetic counselling for patient with 15q duplication syndrome.

Key words

15q duplication syndrome; Chinese; Interstitial duplication 15q11-q13; Isodicentric chromosome 15

Introduction

The chromosome 15q11-q13 region is a complex imprinted region that prone to genomic rearrangement. Within this region, some genes are only expressed on the maternally inherited chromosome 15, like *UBE3A* and *ATP10C*; while other genes are only expressed on the paternally inherited chromosome 15, like *MKRN3*, *MAGEL2*, *NDN*, *C15orf2*, *SNURF-SNRPN*. Moreover, this

region harbours five duplicons or breakpoints (BP) that consisted of large segment low copy repeats (LCR).^{1,2} Through non-allelic homologous recombination and U-type crossover mechanism,³ various forms of deletion, duplications, triplications, translocations, and supernumerary marker chromosomes (SMC) at proximal chromosome 15q region would result. Depend on the parent-of-origin and the size of rearrangement, such genomic rearrangement would lead to Angelman syndrome, Prader-Willi syndrome and 15q duplication syndrome.

15q duplication syndrome (OMIM #608636) is a clinically recognisable neurogenetic syndrome. It is caused by either interstitial duplication of chromosome 15q11.2 or extra isodicentric chromosome 15(idic(15)(p11.2-13.3)).³ The clinical features included hypotonia, developmental delay, intellectual disability, epilepsy and distinctive facial gestalt. Most affected individuals would meet the diagnostic criteria of autism or autism spectrum disease. Apart from that, some would also develop behavioural problems like anxiety disorder and attention deficit hyperactivity

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disorder.^{3,4} The prevalence of 15q duplication syndrome in autism cohort is estimated to be 1-3% in early studies.^{5,6} However, the prevalence, comprehensive clinical spectrum and molecular study of 15q duplication syndrome have never been reported in Chinese.

The aim of this study is to summarise the clinical and genetic findings of all cytogenetically and molecularly confirmed 15q duplication syndrome patients in Hong Kong Chinese.

Patients and Method

The Clinical Genetic Service (CGS) of Department of Health is the only government funded tertiary genetic referral center that provides comprehensive genetic counselling, diagnostic and laboratory service for the whole of Hong Kong population. More than 95% of population in Hong Kong is ethnic Chinese. Patients with suspected genetic or syndromic cause of intellectual disability and autism or autism spectrum disease (ASD) are referred for clinical assessment and genetic testing.

In this study, all records of patients with genetically confirmed 15q duplication syndrome between January 2011 and December 2015 under CGS are retrieved from the computer database system. The clinical and laboratory data of these patients are being analysed. The diagnosis of intellectual disability, autism and autistic spectrum disease are made by paediatricians, developmental paediatricians, clinical psychologists or child psychiatrists.

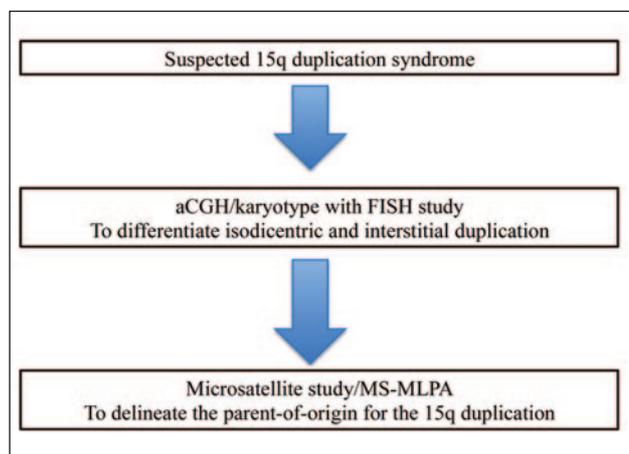


Figure 1 The diagnostic algorithm of 15q duplication syndrome in this study.

Cytogenetic and Molecular Study

The diagnosis of 15q duplication syndrome is confirmed either by standard cytogenetic G banding technique with fluorescence in situ hybridization (FISH) method or array Comparative Genomic Hybridization (aCGH) study. Further molecular studies like microsatellite analysis with parental DNA or methylation-specific multiplex ligation dependent probe amplification (MS-MLPA) is used to delineate the parent-of-origin of the 15q duplication.

MLPA

Patients are screened for rearrangements involving the 15q11-q13 region with the ME028 PWS/AS (MRC-Holland, Amsterdam, The Netherlands). The copy number change and methylation status is detected by methylation-sensitive restriction enzyme. Analysis of the MS-MLPA PCR products is performed on an ABI3500 Genetic Analyser using the GeneMapper software (Applied Biosystems, Foster City, Calif., USA). For copy number analysis, the data generated are being intra-normalised by dividing the peak area of each amplification product by the total area of the reference probes. The ratios are then obtained by dividing the intra-normalised probe ratio in a sample by the average intra-normalised probe ratio of all reference runs. For methylation analysis, the intra-normalised peak area of each MS-MLPA probe from the digested sample is divided by the value obtained for the undigested sample.

Fluorescence In Situ Hybridization (FISH)

FISH is performed on metaphase chromosome spreads preparations from peripheral blood. Three commercial DNA probes that hybridize to the SNRPN locus at 15q11q13 region, PML locus at 15q22 region and centromere of chromosome 15 at 15p11.2 region from Vysis (Abbott) are being used. Ten metaphase cells are being examined to detect cells with loss or gain of specific signals.

Microsatellite Analysis

The microsatellite analysis is studied by using standard protocol. Haplotype analysis is being performed by using eleven polymorphic markers in 15q regions (D15S219, Gabrb3, SC2, 14150, D15S217, M37, SC3, AFM262, AFM323, AFM291 and AFM164). PCR products are visualised with a DNA sequencer and allele sizes are determined by using Genescan and Genotyper software (Applied Biosystems). A positive diagnosis required evidence of unique parental inheritance at \geq informative 2 markers.

Array-CGH

High-resolution whole genome analysis is performed by using PerkinElmer CGXTM 8x60K Human Genome microarrays (Agilent Technologies, Santa Clara, CA). This contains approximately 60 000 sixty-mer probes with resolution of 190Kb in the backbone and 28kb average probe spacing. Labelling is performed by using Agilent Genomic DNA enzymatic labelling kit (Agilent Technologies, Santa Clara, CA, USA) and clean-up of labelled genomic DNA is performed by using Amicon ultra 0.5 ml centrifugal filters according to manufacturers' instructions (Millipore, Billerica, MA, USA). Slides are scanned on an Agilent scanner and processed with Feature Extraction software (v10.7). Each patient DNA is labelled in Cy5 and sex matched reference DNA is labelled in Cy3, which then co-hybridized against the array chip. Results are analysed by using Agilent Genomic Workbench (v6.0 and v6.5) software with the following settings: ADM2 as aberration algorithm, threshold of 6.0, moving average 2 Mb. The results are according to Human Genome build 19 and include imbalances with at least three consecutive probes with abnormal log₂ ratios. All the imbalances are interpreted by consulting the UCSC genome browser (<http://genome.ucsc.edu>), Decipher (Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources - <http://decipher.sanger.ac.uk/>), ClinGen (Clinical Genome Resource - <https://www.clinicalgenome.org/>), OMIM (Online Mendelian Inheritance in Man - <http://www.ncbi.nlm.nih.gov>).

Data Analysis and Statistical Calculation

Epidemiological data, physical characteristics, growth records and molecular findings are then collected for analysis. Clinical photographs are taken during consultation with written consent. For statistical calculation, Fisher's exact test is used for categorical variables. Two-tailed p-values are being computed. Differences are considered to be statistically significant when the p-value is ≤ 0.05 .

Results

During this 5 years' study period, a total of 1,116 patients with intellectual disability and 313 patients with autism or ASD are being referred to our genetic clinic for assessment. Twelve individuals among 10 families with 15q duplication syndrome are diagnosed. All are ethnic Chinese. Their current age in year 2016 are ranged from 3 years 6 months to 34 years old, with a median of

8 years 2 months old. The male to female ratio was 1:2.

Among them, 11 of them have intellectual disability and 9 of them have autism or ASD. Therefore the prevalence of 15q duplication syndrome in our Chinese cohort with intellectual disability and autism or ASD is 1.0% and 2.9%, respectively.

Concerning the underlying genetic mechanism, 4 of them have interstitial duplication and 8 have isodicentric chromosome 15. Two out of these 8 isodicentric cases are in mosaic pattern with abnormal cell lineage ranged from 50% to 93%. The genomic aberration for all symptomatic cases are maternal inherited in origin, that mean the duplication occur in the maternal allele of chromosome 15. Two families with interstitial duplication are identified in this study and the rest of the cases with genomic aberration that arise de novo. The clinical features of our 15q duplication cohort are summarised in Table 1. The facial profiles of patients in this study are shown in Figure 2.

The clinical features of our Chinese patients are compared with western populations in Table 2.⁷⁻¹⁷ It showed that most of clinical features in our Chinese cohort included dysmorphism, joint laxity, hypotonia, intellectual disability and autism are comparable with Caucasian. However, epilepsy is less common while squint is more prevalent in Chinese patients with 15q duplication syndrome.

Discussion

15q duplication syndrome is a neurogenetic syndrome that frequently associated with neurodevelopmental disease. The prevalence of 15q11-q13 duplication in patients with autism is widely assumed to be 1-3% based on two early studies.^{5,6} With the advancement of genomic technology like aCGH and better understanding in the association of copy number variation (CNV) with human disease. It is now known that approximately 10-20% of patients with intellectual disability and 10% of autism or ASD patients have clinical significant CNVs.^{18,19} In the latest reviews and meta-analysis, it has found that 15q11-q13 duplication happened in 1% of patients with autism or ASD and 3% of patient with intellectual disability.^{20,21} The prevalence of 15q duplication in our Chinese cohort with intellectual disability and autism or ASD is 1.0% and 2.9%, respectively.

Concerning on the clinical phenotype, the most consistent features in our study are hypotonia and intellectual disability. About two third of them have facial

dysmorphism and autism. All these are comparable with other studies in the literatures.⁷⁻¹⁷ Epilepsy occurs in 60% of 15q duplication syndrome in the latest survey by Dup 15 alliance with 80% has multiple seizure types and 40% has infantile spasm.²² In our cohort, only 33.3% (4/12) of them have epilepsy with tonic-clonic and focal seizure as the most common seizure semiology. Only one of our patients has infantile spasm with the onset at 3 months of age. Despite multiple anticonvulsant, his epilepsy is refractory that currently evolving into Lennox-Gastaut syndrome. For the rest of the patients, the epilepsy is well controlled by one anticonvulsant. Despite pseudosquint are common among Chinese, true squint happen in more than 90% of our patients that is statistically significant different from other ethnic

groups.

Chromosome 15q11-q13 region imprinting patterns and its long range gene expression are mediated by imprinting control region (ICR) that located upstream of paternal transcription unit *SNRPN* gene through the *UBE3A* gene interaction.²³ The imprinting and parent-of-origin effect has been well demonstrated by our 2 families with interstitial duplication. By methylation specific-MLPA and microsatellite study, the interstitial 15q 11.2 duplication in the mothers of family 6 and family 10 in our study have confirmed to locate at paternal and maternal allele of chromosome 15, respectively. The mother in family 6 with interstitial 15q11.2 duplication in the paternal allele has normal phenotype, while the mother in family 10 with

Table 1 The clinical features of our 15q duplication cohort

Family	Sex/ age	Rearrangement	Origin	Autism	Hypotonia	Intellectual disability	Epilepsy	Dysmorphism	Joint laxity	Squint	Others
1	F/5yr 4m	idic(15) (p11.2-13.3)	M	Yes	Yes	Moderate	No	No	Yes	Yes	
2	F/22yr 5m	idic(15) (p11.2-13.3)	M	Yes	Yes	Mild	Tonic-clonic, focal seizure since 5 yr	Yes	yes	Yes	Behavioural Problems on SSRI
3	F/17yr 1m	mos. idic(15) (p11.2-13.3)(93%)	M	No	Yes	Mild	Tonic-clonic, focal seizure since 4 yr	Yes	Yes	Yes	
4	F/7yr 2m	idic(15) (p11.2-13.3)	M	Yes	Yes	Moderate	Infantile spasm since 3 m, Lennox-Gastaut syndrome now	no	Yes	No	Arachnoid cyst in MRI brain
5	F/27yr 4m	mos. idic(15) (p11.2-13.3)(50%)	M	Yes	Yes	Severe	Tonic-clonic, myoclonic, focal seizure since 15 yr	Yes	Yes	Yes	
6	M/3yr 6m (son)	interstitial duplication 15q11.2q11.2	M	No	Yes	Mild	No	Yes	No	Yes	
	F/38 yr (mother)	interstitial duplication 15q11.2q11.2	P	No	No	No	No	No	No	Yes	
7	F/4yr 9m	idic(15) (p11.2-13.3)	M	Yes	Yes	Moderate	No	Yes	Yes	Yes	
8	M/7yr 11m	interstitial duplication 15q11.2q11.2	M	Yes	Yes	Moderate	No	No	Yes	Yes	
9	M/7yr 1m	idic(15) (p11.2-13.3)	M	Yes	Yes	Moderate	No	Yes	No	Yes	
10	M/8yr 4m (son)	interstitial duplication 15q11.2q11.2	M	yes	yes	Moderate	No	Yes	No	Yes	
	F/34 yr (mother)	interstitial duplication 15q11.2q11.2	M	No	yes	Mild	No	Yes	No	Yes	

M: maternal; P: paternal; mos: mosaic; SSRI: selective serotonin reuptake inhibitors



Figure 2 The facial profile of patients with 15q duplication syndrome in our study. Patients 6, 8 and 10 have interstitial 15q duplication while the rest have isodicentric chromosome 15. The dysmorphic features included high forehead, epicanthic folds, squint, short nose and depressed nasal bridge (Consents have been obtained for publication).

inherited interstitial 15q11.2 duplication in the maternal allele has hypotonia and mild intellectual disability. Maternal duplication of the *UBE3A* gene is the proposed mechanism for such imprinting effect.^{24,25}

For the genotype-phenotype correlation, a dosage effect on 15q11-q13 copies to clinical severity has been demonstrated in previous studies.^{13,16,26} The clinical presentation for isodicentric chromosome 15 with 4 copies of *SNRPN* gene is more severe than interstitial duplication with 3 copies of *SNRPN* gene. The mitigating effect of mosaic 15q duplication has also been reported which depend on the percentage and type of cell line involved. However, such correlation cannot be shown in our Chinese cohort due to small sample size. The phenotypic spectrum and severity among those isodicentric chromosome 15, interstitial duplication and mosaic isodicentric chromosome 15 are similar in this study.

Concerning on recurrence risk and genetic counselling, for isodicentric chromosome 15, it usually arise denovo that recurrence risk in family is negligible. For interstitial duplication, the inheritance risk to offspring from affected

proband is 50%. Due to genomic imprinting, the phenotypic effect depends on the parent-of-origin. Duplication in the maternal allele of chromosome 15 has high risk of developing neurodevelopmental disease and epilepsy, while duplication in the paternal allele usually phenotypically normal.^{15,18}

Table 2 The prevalence of clinical features in our Chinese cohort and comparison with other studies in the literature⁷⁻¹⁷

	Our study (total 12)	Literatures	P value
Autism	9 (75%)	84% (25-100%)	NS
Hypotonia	11 (91.7%)	82% (72-100%)	NS
Intellectual delay	11 (91.7%)	100%	NS
Epilepsy	4 (33.3%)	79% (50-100%)	<0.05
Dysmorphism	8 (66.7%)	73% (20-100%)	NS
Joint laxity	7 (58.3%)	50% (12.5-64%)	NS
Squint	11 (91.7%)	41% (25-44%)	<0.05

NS: not statistically significant

Conclusion

This 5 years' review is the first territory-wide study of 15q duplication syndrome in Chinese. It showed that the incidence and clinical features of Chinese 15q duplication are comparable with other western populations. The prevalence of 15q duplication in our Chinese cohort with intellectual disability and autism or autistic spectrum disease is 1.0% and 2.9%, respectively. Early diagnosis is important for managing 15q duplication patients as they have high risk of developing epilepsy and neurodevelopmental disorder that anticipatory guidance from different specialties is essential. Genetic confirmation on the underlying mechanism for 15q duplication is also important for reproductive risk assessment.

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Conflict of interest

We have no conflict of interest to declare.

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