

Original Article

Genetic diagnosis of one pregnant woman with severe mental retardation and prenatal diagnosis of her fetus

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Abstract: This study aims to investigate the genetic mechanisms of one pregnant woman with severe mental retardation, hypoevolutism, and adverse history of pregnancy and delivery, and to perform the prenatal diagnosis towards the fetus, aiming to provide guidance for her future fertility. High-resolution chromosome assay (HRCA), fluorescence in situ hybridization (FISH), and whole genome DNA microarray (WGDM) were performed for the pregnant woman's testing. Amniocentesis was used to detect the fetal DNA chips. The chromosome of the pregnant woman was 47, XX, del (18) (q21.3), + ish mar (D15Z1 +, SNRPN +) [82]/46, XX, del (18) (q21.3) [18], among which 15q11.2q12 existed the duplication of one 3.044 Mb fragment containing 17 OMIM genes (NIPA1, and SNRPN etc.), which were related to mental retardation, autism, and hypoevolutism. 18q21.33q23 existed the deletion of one 17.992 Mb fragment containing 39 OMIM genes (TNFRSF11A, and PHLPP1, etc.), which were related to mental retardation, overall hypoevolutism, and cleft palate. The fetus inherited maternal 18th abnormal chromosome, namely the fetal 18q21.33q23 existed the deletion of one 17.992 Mb fragment. After genetic counseling, the patient's families strongly demanded the termination of pregnancy, so the pregnancy was terminated at the 21st gestational week. In conclusion, HRCA, FISH, and WGDM had their advantages and disadvantages, respectively. Therefore, the comprehensive application of the above techniques based on patient's conditions could better diagnose the relationships between genetic mechanism and clinical phenotype.

Keywords: Chimera, mental retardation, hypoevolutism, prenatal diagnosis, fluorescence in situ hybridization, whole genome DNA microarray

Introduction

The pathogeneses of mental retardation are complex, which could be caused by the changes of genetic materials or by such non-genetic factors as intrauterine infection during pregnancy, maternal contact with hazardous substances, injury during delivery, or postpartum diseases, among which mental retardation caused by genetic factors would have a certain probability to be inherited to the offspring, thus leading to mental retardation. We comprehensively applied high-resolution chromosome assay (HRCA), fluorescence in situ hybridization (FISH), and whole genome DNA microarray (WGDM) to analyze one pregnant woman with severe mental retardation, overall hypoevolutism, and one-time adverse history of pregnancy and delivery, aiming to explore the relationships between the genetic pathogenesis and clinical manifestations. Meanwhile, the intrauterine fetus was performed prenatal diagnosis, and

the relationships with clinical manifestations were discussed based on the comprehensive analysis of maternal and fetal assay results, aiming to give guidance for her future fertility. The study was reported as follows.

Materials and methods

Patient

Female, about 148 cm, 27 years old, with severe mental retardation and extremely poor cognitive ability, almost entirely depending on her parents' feeding, poorly developed, mild microcephaly, facial central depression, deep orbit, slightly wider eye span, flat nose, low ear position, short neck, non-stretchable slender fingers, feedthrough palmprint in both hands, muscular hypotonia, ataxia, and language delay with occasional pronunciation of "ah". The patient's facial expressions and hands were shown in **Figure 1A, 1B**. The patient's menstruation

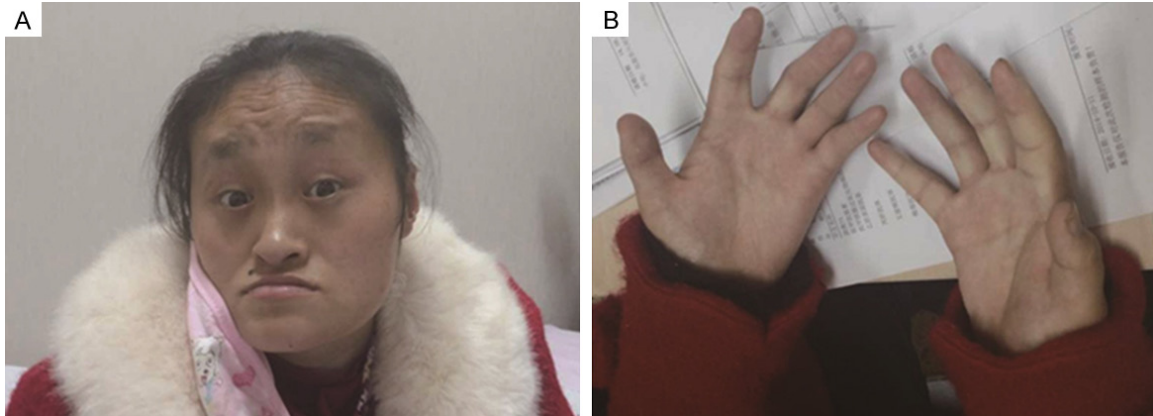


Figure 1. A: Photos of the patient's face; B: Feedthrough palmprint in both hands.

was basically normal, 4 days/30 days; she was married, and no abnormal result was found in gynecological examination, G2P0; she was pregnant for the first time in 2012 but performed induction of labor at the 16th gestational week in a local hospital due to ultrasound-indicated fetal bilateral cheilopalatognathus. In 2014, she was pregnant again, and because of her severe conditions of mental retardation, she was sent to the People's Hospital of Peking University for prenatal diagnosis. Firstly, the maternal peripheral blood was sampled to analyze the reason of mental retardation, and the specific assays included HRCA, FISH, and WGDM. At the 18th gestational week, amniocentesis was performed for WGDM of the fetus. The patient's parents were both farmers without consanguineous relationship or family history of genetic disease. They had two daughters, the patient was their eldest daughter, and the smaller daughter exhibited normal intelligence and phenotype. The parents' fertility ages were 26 years old and 29 years old, respectively; the parents denied the exposure to toxic and hazardous substances before and after the pregnancy; no abnormal karyotype was found in the parents. The patient's husband exhibited mild mental retardation while normal karyotype. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Peking University. Written informed consent was obtained from all participants.

HRCA

Under sterile conditions, 1 ml of EDTA-anticoagulated peripheral blood of the patient was

inoculated into 5 ml of 1640 medium and incubated at 37°C for 48 h; 75 µl of MTX-methotrexate was then added into for 17.5~18 h-co-incubation; after that, the sample was collected and performed G-banding staining (plus C-banding staining if necessary) for preparing the chromosome specimen, with chromosome 15q12 distinguishable in each specimen as the criterion of HRCA. Under oil microscope, 30 splitting phases were counted, among which 7 well-dispersed splitting phases were used for the analysis, when the chimera or abnormal karyotype was found, 100 splitting phases should be covered. The Video Test-Karyo karyotype analysis system was used (Unibiotec, USA).

FISH

The suspension of metaphase cells of the maternal peripheral blood was dropped onto one slide and dried at room temperature; Probe D15Z1 (green), probe SNRPN (red), and probe PML (green) (Vysis, Abbott Molecular Inc., USA) were denaturated in hybridization solution at 76°C for 7 min, respectively, for prehybridization. The chromosome slide specimen was then dehydrated with ethanol and treated with RNase; the denaturation solution was added into BioRed PTC-200 in situ PCR instrument for 2 min denaturation at 70°C. 8 µl of pre-hybridized probes were then dropped into the denatured chromosomal slide for 14~17 h-incubation at 37°C in one wet box. At 41°C, 2 × SSC/50% formamide was used to wash the hybridization slide, flowed by 3-min dehydration by 70%, 90%, and 100% ethanol, respectively; after air-drying, 10 µl of DAPI was added for

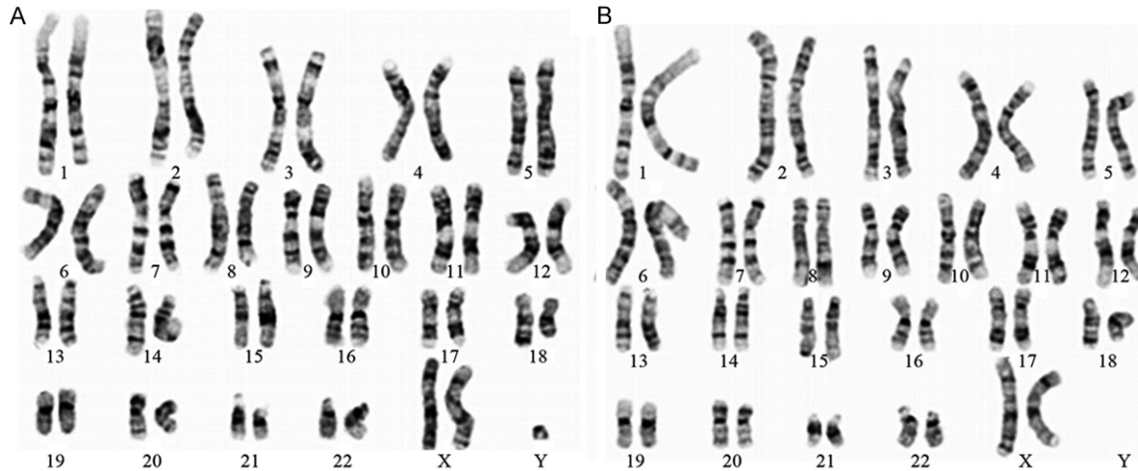


Figure 2. HRCA of G-banding chromosome, the karyotype of the patient was 47, XX, del (18) (q21.3), + mar [82]/46, XX, del (18) (q21.3) [18].

10-min staining. The specific methods referred to the literature [1]. More than 30 interphase nuclear karyotypes should be observed to select those with good dispersion and clear signal for the photography. Leica DM5000B fluorescence microscope was used for the observation, and Leica CW4000 fluorescence analysis system was used for the analysis.

WGDM

2 ml of maternal peripheral intravenous blood was sampled and anticoagulated with EDTA; after obtained the signed informed consent, 10 ml of amniotic fluid was sampled under ultrasound guidance via amniocentesis for WGDM towards the pregnant woman and the fetus, respectively. The genomic DNA was firstly extracted in accordance with the kit instructions (Yuanpinghao (Tianjin) Biotech Co., Ltd.), followed by digestion, ligation, PCR, PCR purification, fragmentation, labeling, hybridization, staining, and scanning. The microarray (Affymetrix CytoScan 750K Array) used for the detection comprised about 750,000 25-mer oligonucleotide probes, covering human whole genome with one probe/4 kb averagely. The hybridized microarray was scanned with one laser scanner (GeneChip® 3000 Scanner with 7G upgrade), and the results were analyzed with the control software (GeneChip® Command Console) and chromosome analysis software (Affymetrix® Chromosome Analysis Suite (ChAS) 2.1). URL of the reference genebanks: <http://omim.org/>; <http://genome.ucsc.edu/>; <https://decipher.sanger.ac.uk/> and <https://decipher.sanger.ac.uk/>.

Results

Analysis of maternal chromosomal karyotypes

The patient's chromosome was of chimera, and the peripheral blood contained two kinds of cell lines (47 and 46 chromosomes, respectively), which both involved in the deletion of 18q-21.2q23 area; furthermore, one contained the marker chromosomes, namely 47, XX, del (18) (q21.3), + mar [82]/46, XX, del (18) (q21.3) [18] (**Figure 2A, 2B**).

FISH

The additionally labeled chromosomes among the cell lines of the 47 maternal chromosomes were further detected, namely the origins of these marker chromosomes were further detected using the FISH technology; Probes D15Z1 (green), SNRPN (red), and PML (green) were used and the hybridized chromosomal loci were 15p11.1-11.2, 15q11-q13, and 15q22, respectively. FISH confirmed this marker chromosome derived from chromosome 15, including the short arm of chromosome 15 and partial long arm containing SNRPN site, namely the chromosomal karyotype of this pregnant woman was: 47, XX, del (18) (q21.3), + ish mar (D15Z1 +, SNRPN +) [82]/46, XX, del (18) (q21.3) [18] (**Figure 3**).

WGDM

arr 15q11.2q12 (22,770,421-25,814,451) × 3, 18q21.33q23 (60,021,425-78,013,728) × 1, (**Figure 4A, 4B**), namely on chromosome 15, 15q11.2q12 existed the duplication of one

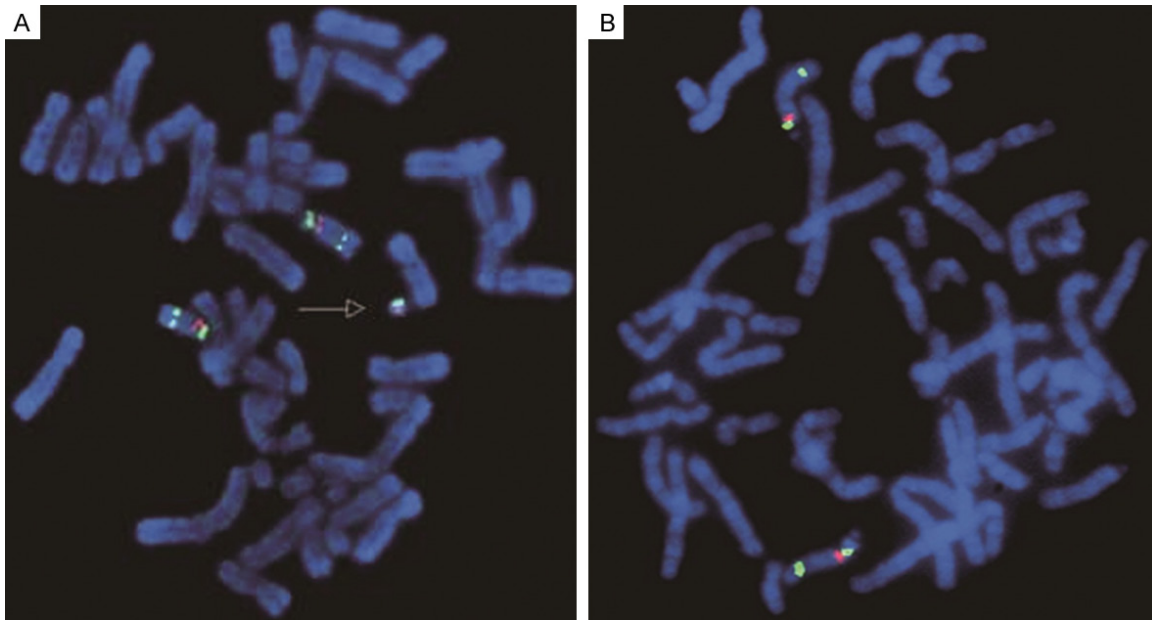


Figure 3. FISH using D15Z1 (green), SNRPN (red), and PML (green) (A: 47 chromosomes; B: 46 chromosomes), the results suggested that the two chromosome 15 both existed the fluorescence of D15Z1 (green), SNRPN (red), and PML (green), mar chromosome exhibited D15Z1 (green)- and SNRPN (red)-positive while PML (green)-negative, confirming the additional chromosome of the 47-chromosomes cell line was from chromosome 15, including the short arm of chromosome 15 and partial SNRPN site-containing long arm, namely 47, XX, del (18) (q21.3), + ish mar (D15Z1 +, SNRPN +) [82]/46, XX, del (18) (q21.3) [18].

3.044 Mb fragment; this fragment was located in the region of Prader-Willi 15q11.2q13.1 deletion syndrome, containing such 17 OMIM genes as NIPA1 and SNRPN, etc. The duplication of this fragment was reported to be related with the clinical manifestations such as mental retardation, autism, or overall hypoevolutism [2, 3]. Meanwhile, this patient existed the deletion of one 17.992-Mb fragment in 18q21.33q23, containing such 39 OMIM genes as TNFRSF11A and PHLPP1 etc. the deletion of this fragment was reported to be related with such clinical manifestations as mental retardation or overall hypoevolutism [4, 5].

Fetal prenatal diagnosis

arr 18q21.33q23 (60,021,425-78,013,728) × 1 (**Figure 5**), the fetal WGD result was existed the deletion of one 17.992 Mb fragment in 18q21.33q23, containing such 39 OMIM genes as TSHZ1, RTTN, or CTDLP1, which were involved in the region of chromosome 18 long arm deletion syndrome, and the clinical manifestations included mental retardation, overall hypoevolutism, short stature, or cleft palate. The mother was the patient of 18q21.33q23 fragment deletion, so the fetus inherited the mother's abnormal chromosome 18.

Pregnancy outcome and fertility guidance

At the 18 + 3 gestational weeks, ultrasound prompted the fetus was equivalent to the age of 16 + 5 gestational weeks plus bilateral cheilopalatognathus; because the fetus existed the chromosome 18 long arm deletion syndrome, which might result in postnatal mental retardation, overall hypoevolutism, short stature, or other clinical phenotypes, after fully communicated with the family members, the family members decided to terminate the pregnancy, and the induction of labor was performed at the 21st gestational week. The aborted fetus exhibited bilateral cheilopalatognathus, but the family refused the autopsy, so whether the fetal visceral malformations existed or not was unknown. Because the pregnant woman exhibited the chimera, containing two kinds of cell lines (47 and 46 chromosomes, respectively), which both exhibited the end deletion of the chromosome 18 long arm; chromosome 15 of the 47 chromosome cell line had the duplication of 15q11.2q12 fragment, and the proband was the patient with chromosome deletion/duplication syndrome, so the probability of pregnancy with normal fetus would be extremely low; the genetic counseling expert adequately informed

Genetic diagnosis of pregnant woman



Figure 4. Results of single nucleotide polymorphism microarray assay of the patient. arr 15q11.2q12 (22,770,421-25,814,451) × 3,18q21.33q23 (60,021,425-78,013,728) × 1. A: It was the schematic diagram of chromosome 15, the blue downward triangle arrow pointed at the micro-duplication region (i.e., there existed the duplication of one 3.044 Mb fragment in 15q11.2q12 of chromosome 15 of the pregnant woman); B: It was the schematic diagram of chromosome 18, the red arrow downward triangle arrow pointed at the micro-deletion region (i.e., there existed the deletion of one 17.992 Mb fragment in 18q21.33q23 of the pregnant woman simultaneously).

the family members with the disease risk of each offspring, furthermore, in strict compli-

ance with the discussion and comments obtained by the Ethics Committee of People's

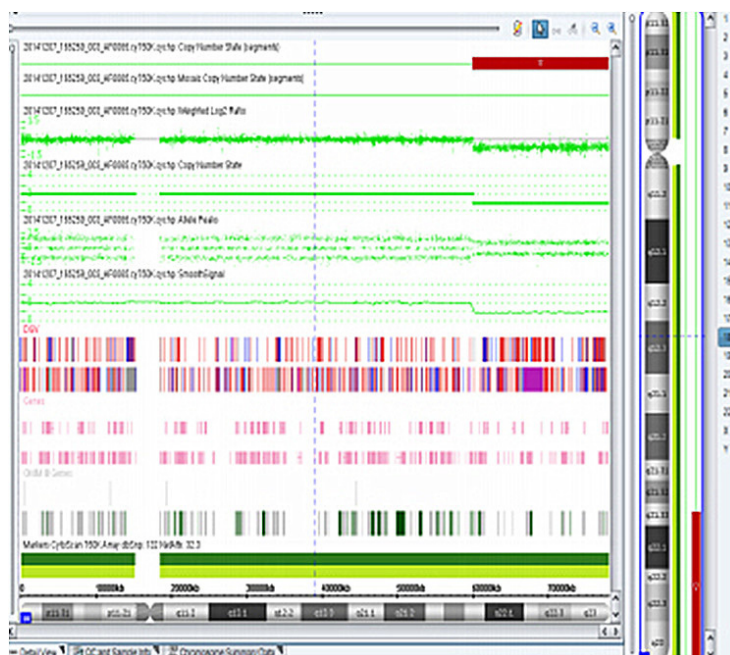


Figure 5. Results of single nucleotide polymorphism microarray assay of the fetus arr 18q21.33q23 (60,021,425-78,013,728) \times 1. **Figure 5** was a schematic diagram of chromosome 18, the red downward triangle arrow pointed at the micro-deletion region (i.e., there existed the deletion of one 17.9 Mb fragment in 18q21.33q23 of the fetus).

Hospital, Peking University, this patient was suggested to take contraceptive measures in future.

Discussion

Chimera could be divided into heterologous and homologous chimera. The heterologous chimera refers to the fact that different cell lines come from different zygotes; the homologous chimera refers to the fact that different cell lines come from the same zygote, and it's mainly caused by the mitosis non-separation or chromosome loss of partial cells when the fertilized egg develops to a certain stage, or by the loss or addition of one chromosome resulted from the delay of chromatid in late mitosis [6]. Chimera would severely affect patient's growth and mental development, and its clinical manifestations depend on the proportion of the abnormal karyotypes [7, 8]. Chromosome deletion refers to one fragment deletion on chromosome. When a normal chromosome occurs deletion, the genes carried in this fragment would also be lost or damaged, so that the corresponding genetic effects would occur.

In this study, the patient had severe mental retardation, overall hypoevolutism, and one

adverse history of pregnancy and delivery; the karyotype analysis of peripheral blood showed her chromosome was of chimera, containing two kinds of cell lines (47 and 46 chromosomes, respectively), which both existed the deletion of q21.2q23 of the chromosome 18 long arm end. Further FISH confirmed that the 47 chromosomes-containing cell line had extra chromosome derived from chromosome 15, containing the short arm of chromosome 15 and partial long arm containing the SNRPN site; furthermore, WGDM confirmed that this patient had the duplication of one 3.044 Mb fragment in the 15q11.2q12 segment of chromosome 15 (containing 17 OMIM genes such as NIPA1 and SNRPN etc.), and the duplication of this region was reported to be related with mental retardation, autism, or overall hypoevolutism [9-19]. Meanwhile, the patients

also had the deletion of one 17.992 Mb fragment in 18q21.33q23, containing 39 OMIM genes such as TNFRSF11A and PHLPP1 etc. The deletion of this fragment was reported to be related with mental retardation, overall hypoevolutism, short stature, or cleft palate [20-24]. The above test results confirmed that the reason of a variety of clinical manifestations in this patient such as mental retardation and hypoevolutism was the simultaneous deletion and duplication of chromosomes, namely she was one patient with chromosome deletion/duplication syndrome. Further prenatal diagnosis showed that the fetus inherited the abnormal maternal chromosome 18, resulting in the deletion of one 17.9 Mb fragment in 18q21.33q23, which contained 39 OMIM genes (TSHZ1, RTTN, and CTD1, etc.) and was involved in the 18q deletion syndrome region. The ultrasound at the age of 18 + 3 gestational weeks prompted delayed intrauterine fetal growth and bilateral cheilopalatognathus; moreover, with the increasing of gestational weeks, other organ malformations might gradually appear because fetus with 18q deletion might occur such clinical manifestations as severe mental retardation, overall hypoevolutism, short stature, or cleft palate in future. This

study successfully diagnosed and confirmed the reason of one pregnant woman with severe mental retardation, and the prenatal diagnosis also confirmed the existence of chromosome deletion syndrome in the fetus; therefore, the pregnancy was timely terminated to avoid the birth of infant with defects. During her first pregnancy, ultrasound at the age of 16 gestational weeks suggested fetal bilateral cheilopalatognathus, so it could not exclude the reason of chromosomal abnormality in the fetus. Because this pregnant woman was one patient with chromosome deletion/duplication syndrome, the probability of fertilizing normal fetus would be very low, so this patient was advised to take contraceptive measures in future.

The pregnant woman reported in this study exhibited the corresponding clinical manifestations such as mental retardation and hypoevolutism because of the partial duplication of chromosome 15 and partial deletion of chromosome 18. Her parents had normal intelligence and phenotypes and no abnormal karyotype; furthermore, they had one normal daughter, so it was analyzed that the pregnant woman chromosomal abnormalities reported in this study were *de novo* mutations. Because the formation of chromosomal abnormality is caused by external factors that acting on the chromosomal DNA molecules, thus causing chromosomal breakage, displacement, or reclosing, men and women of childbearing age should take protective measures towards their gonads when contacting with large-dose ionizing radiation or rays. Meanwhile, we also recommended that women of childbearing age should avoid the exposure to toxic and hazardous substances before pregnancy and in early pregnancy. In addition, elderly parents also might lead to aged sperms or eggs, which could be another factor of chromosome breakage, variation, or number abnormality. Therefore, 25~35 years old is recommended as the best fertility age, elderly pregnant women and persons with high-risk factors should be performed timely prenatal diagnosis to avoid the infant with birth defects.

Disclosure of conflict of interest

None.

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