Application of an improved targeted next generation sequencing method to diagnose non-syndromic mental retardation in one step: A case report

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Abstract. The genetic basis of congenital mental retardation includes chromosomal anomalies and single gene mutations. In addition to chromosome microarray analysis, next-generation sequencing (NGS) and Sanger sequencing have additionally been applied to identify single gene mutations. However, no methods exist to identify the cause of an anomaly in one step. The present study applied an improved targeted NGS method to diagnose an 8-year-old Chinese Han female with mental retardation in one step. The microdeletion 17p11.2 was successfully detected by the improved targeted NGS and no single gene mutations were identified. The same microdeletion was verified using low coverage whole-genome sequencing. Fertility guidance was also given to the patient's parents. In the present study, an improved targeted NGS method was applied to diagnose non-syndromic mental retardation of unknown cause in one step. This improved method has the potential to be developed into a screening panel for the effective diagnosis of genetic abnormalities in non-syndromic mental retardation and other congenital anomalies.

Introduction

Congenital mental retardation is a neurodevelopmental disease that affects 2-3% of the population worldwide (1,2). Genetic factors may explain >50% of congenital mental retardation cases (3). Chromosomal anomalies, including chromosomal deletion, duplication and trisomy contribute to 4-28% of cases and known monogenetic diseases explain 3-9% of cases (4). Various methods may be used to detect genetic abnormalities, including chromosomal microarray (CMA) and next generation sequencing (NGS) (5-10). However, the absence of any accompanying deformities in non-syndromic mental retardation makes it difficult to select the detection method. Using numerous methods increases cost and reduces efficiency. To the best of our knowledge, no method to detect genetic abnormalities in one step exists at present.

Typically, custom-made capture array-based targeted NGS is used to detect disease-causing mutations in monogenic disease (8). A custom-made capture array that is able to detect various types of mutations, including microdeletion, was previously designed (11). In the present study, this improved targeted NGS method was applied to diagnose a case of non-syndromic mental retardation of unknown cause. This may aid in the detection of genetic abnormalities in one step.

The microdeletion 17p11.2 was successfully detected by improved targeted NGS and no single gene mutations were identified. Subsequently, the same microdeletion was validated using low coverage whole-genome sequencing (LCS). Fertility guidance was additionally given to the parents of the patient.

In the present study, the patient was diagnosed with Smith-Magenis syndrome (SMS). SMS is a rare, congenital syndrome that affects ~1 in every 25,000 individuals. Features include mental retardation, behavioral abnormalities and distinctive facial features, difficulty sleeping, delayed speech and development, resulting from a 17p11.2 deletion encompassing the retinoic acid-induced protein 1 (RAI1) (12-14). It is estimated that 70% of patients with SMS have a common 3.7 Mb deletion; the remaining 30% have larger or smaller deletions. Approximately 90% of SMS patients have a 17p11.2 deletion; the remaining 30% have mutations in the RAI1 gene (15).

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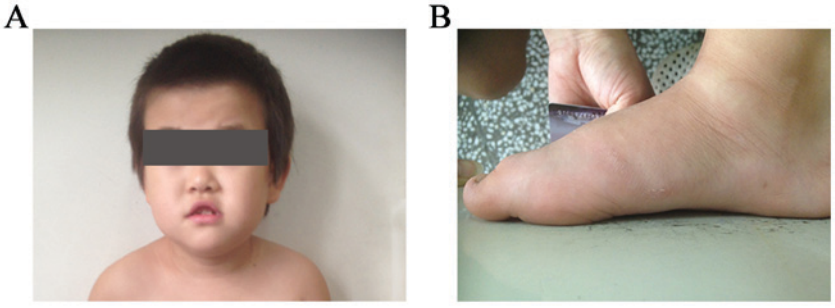

Figure 1. Distinct facial features of the patient. A typical Smith-Magenis syndrome phenotype was observed, with a (A) tented upper lip, depressed nasal bridge and (B) characteristic flat feet.

The present study applied an improved targeted NGS method to diagnose a patient with non-syndromic mental retardation of an unknown cause in an effective and a fast way, without using other methods. The result was validated by LCS. This improved method has the potential to be developed into a screening panel to effectively identify genetic abnormalities in non-syndromic mental retardation and other congenital anomalies.

Case report

Patient information. An 8-year-old Chinese Han female and her parents attended the Wuhan Medical & Health Center for Women and Children hospital due to developmental delay and signs of mental retardation that were present from infancy. The research was prospectively reviewed and approved by the ethics committee of BGI-Shenzhen (approval no. BGI-IRB 15083; Shenzen, China). Written informed consent for participation in the present study was obtained from the patient's parents.

The patient was the first child of unrelated parents who had no family history of inherited diseases. The patient was born by cesarean section with a birth weight of 3.35 kg and a body length of 50 cm. A distinct facial appearance was observed, with short palpebral fissures, a depressed nasal bridge, hypertelorism, and an upper lip with tented morphology and a V-like shape (Fig. 1A and B).

By age 2 years, the patient had sleep difficulties with nocturnal awakenings which gradually increased in frequency. The patient exhibited signs of mental retardation and decreased motor development compared with other children of the same age. The patient was talkative, although failed to convey information effectively.

Giemsa (G)-banded cytogenetic analysis. Routine G-banding chromosome analysis was performed as described (16); a 5 ml peripheral blood sample was collected from the patient and her parents. Chromosomes from cultured lymphocytic cells were treated with 0.25% trypsin (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) for 2 min at 37˚C and stained with 6% Giemsa (Sigma-Aldrich; Merck KGaA) for 10 min at 37˚C. The results revealed that the patient carried the normal karyotype (46 chromosomes; XX; Fig. 3). In addition, the parents of the patient carried the normal karyotype.

Improved targeted NGS. Improved targeted NGS was performed on peripheral blood samples of the patient. The

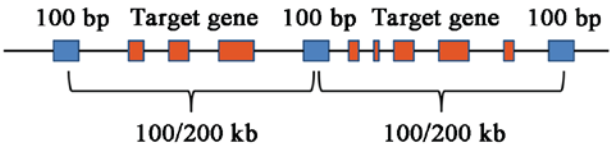

Figure 2. Target spot in the designed capture array for the detection of microdeletions and microduplications. The region that contained known microdeletions and microduplications was covered and a target spot at an interval of 0.1 Mb (length, 100 bp) was selected; for the remaining genome region, a target spot at an interval of 0.2 Mb (length, 100 bp) was selected.

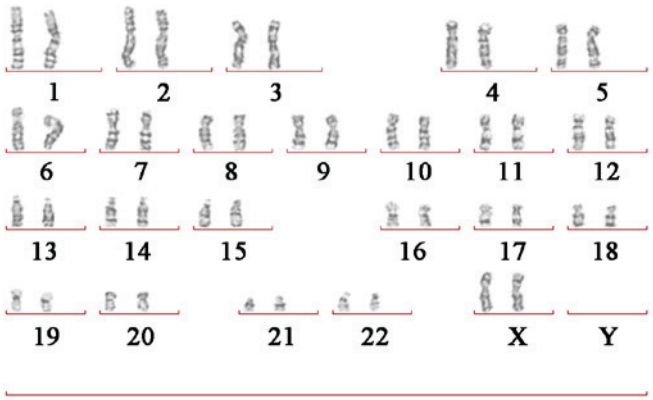

Figure 3. Patient karyotype. G-banding analysis revealed a normal chromosomal karyotype.

designed capture array (NimbleGen; Roche Molecular Diagnostics, Pleasanton, CA, USA) focuses on known associated single gene-coding regions and flanking intronic boundaries 10 bp. The region that contained known microdeletions and microduplications was covered and a target spot at an interval of 0.1 Mb (length, 100 bp) was selected; for the remaining genome region, a target spot at an interval of 0.2 Mb (length, 100 bp) was selected (Fig. 2). Targeted NGS analysis with normalization was performed for the patient (17). The microdeletion and microduplication regions designed on this array were selected from the database of DECIPHER (18). The 45 known microdeletion and microduplication diseases can be identified at present listed in Table I.

Sequence capture, enrichment and elution was performed according to the standard protocol, as previously mentioned (11). Following enrichment, high-throughput sequencing was performed using the Illumina Hiseq2000 analyzer (Illumina,

Table I. Known chromosomal microdeletion or microduplication diseases.

Syndrome	Chromosomal region
12q14 microdeletion syndrome	chr12: 65071919-68645525
15q13.3 microdeletion syndrome	chr15: 30910306-32445407
15q24 recurrent microdeletion syndrome	chr15: 74412643-75972911
15q26 overgrowth syndrome	chr15: 99357970-102521392
16p11.2 microduplication syndrome	chr16: 29606852-30199855
16p11.2-p12.2 microdeletion syndrome	chr16: 21512062-30199854
16p11.2-p12.2 microduplication syndrome	chr16: 21475060-29284077
16p13.11 recurrent microdeletion or microduplication	chr16: 14986684-16486684
17q21.31 recurrent microdeletion syndrome	chr17: 43705166-44294406
1p36 microdeletion syndrome	chr1: 10001-12840259
1q21.1 recurrent microdeletion or microduplication	chr1: 146533376-147883376
1q21.1 susceptibility locus for Thrombocytopenia Absent Radius syndrome	chr1: 145386506-145748067
22q11 deletion or duplication syndrome	chr22: 19009792-21452445
22q13 deletion syndrome	chr22: 51045516-51187844
2p15-16.1 microdeletion syndrome	chr2: 59285696-61819815
2p21 microdeletion syndrome	chr2: 44410451-44589584
2q33.1 deletion syndrome	chr2: 196925121-205206939
2q37 monosomy	chr2: 239969863-240322643
3q29 microdeletion or microduplication syndrome	chr3: 195726835-197344663
7q11.23 duplication syndrome	chr7: 72744455-74142672
8p23.1 deletion or duplication syndrome	chr8: 8100055-11764629
9q subtelomeric deletion syndrome	chr9: 140513443-140730578
Angelman syndrome type 1	chr15: 22749354-28438266
Angelman syndrome type 2	chr15: 23619912-28438266
α-thalassemia-intellectual deficit syndrome	chr16: 60001-834372
Azoospermia factor microdeletion	Chr Y: 14352761-15154862
Charcot-Marie-Tooth syndrome type 1A	chr17: 14097915-15470903
Cri du Chat syndrome	chr5: 10001-12533304
Early-onset Alzheimer disease with cerebral amyloid angiopathy	chr21: 27252860-27543446
Familial Adenomatous Polyposis	chr5: 112043201-112181936
Miller-Dieker syndrome	chr17: 1-2588909
NF1-microdeletion syndrome	chr17: 29107097-30263321
Pelizaeus-Merzbacher disease	chrX: 103031438-103047547
Smith-Magenis syndrome	chr17: 16773072-20222149
Potocki-Shaffer syndrome	chr11: 43994800-46052450
Renal cysts and diabetes	chr17: 34815072-36215917
Rubinstein-Taybi syndrome	chr16: 3775055-3930121
Sotos syndrome	chr5: 175724636-177052116
Steroid sulphatase deficiency	chrX: 6455812-8133195
11p13 deletion syndrome	chr11: 31806339-32457087
Wolf-Hirschhorn syndrome	chr4: 1569197-2110236
Xp11.22-linked intellectual disability	chrX: 53401070-53683275
Xp11.22-p11.23 microduplication	chrX: 48334549-52117661
Xq28 duplication	chrX: 153287263-153363188
Xq28 microduplication	chrX: 153624563-153881853

Chr, chromosome.

Inc., San Diego, CA, USA). Image analysis and base-calling were performed using the Illumina Pipeline version 1.3.4 (Illumina, Inc.).

The depth ratio (patient:control) was used to evaluate the microdeletions and microduplications; the control referred to healthy subjects unrelated to the patient (samples obtained

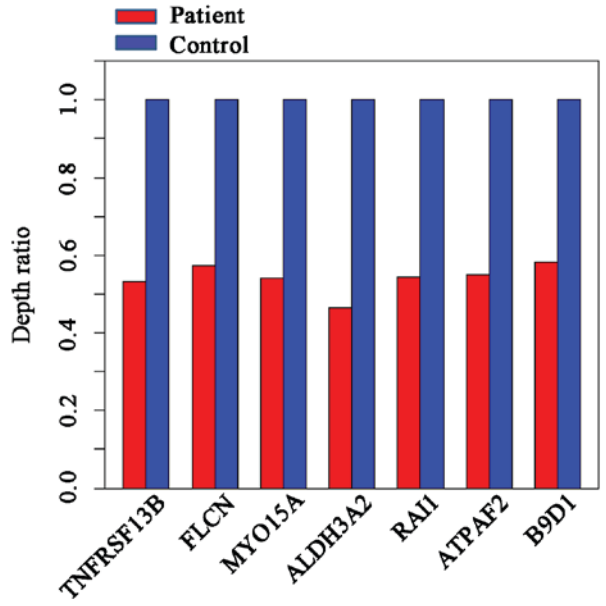

Figure 4. Identification of a heterozygous deletion on 17p11.2 in the patient with targeted next generation sequencing. The depth ratio (patient:control) of seven genes located on 17p11.2 was calculated from the designed capture array. The average depth ratio of the patient was approximately 0.55, indicating a heterozygous deletion. Control, healthy subjects unrelated to the patient.

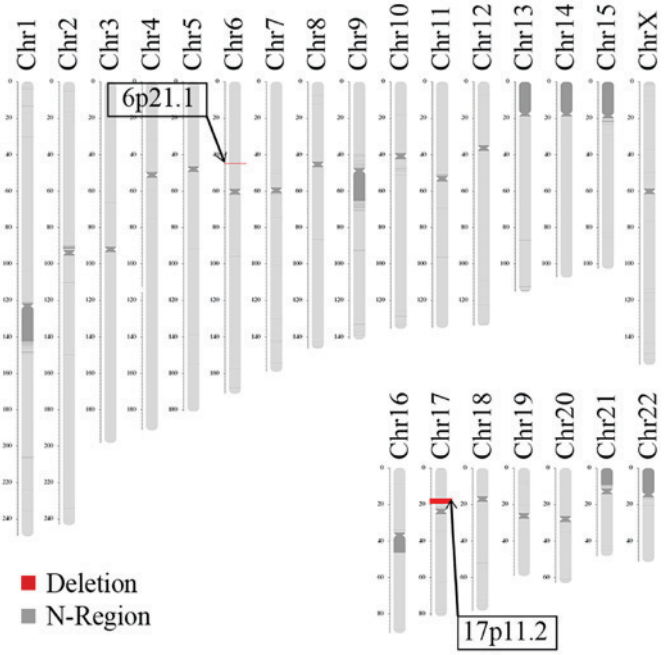

Figure 5. Electrical karyotyping image construction with low coverage whole-genome screening. Red bands indicate the deleted regions. N-region bands indicate the normal regions. Chr, chromosome.

from BGI). If the ratio is 1, this indicates that no deletion has been detected. A ratio of ~0.5 indicates the detection of a heterozygous deletion. A ratio of zero indicates a potential homozygous deletion.

The designed capture array identified a 17p11.2 deletion. No single gene mutations were identified. The depth ratio was obtained for seven genes located on 17p11.2; the average depth ratio of the patient was ~0.55, indicating a heterozygous deletion (Fig. 4). As chromosome 6 was not designed in the capture array, the specific deletion on chromosome 6 was not identified.

LCS. To validate the results obtained from the improved NGS method, LCS was additionally performed on the peripheral blood samples of the patient and parents using the Illumina Hiseq2000 (Illumina, Inc.) platform with the population-scale microdeletions and microduplications calling (PSCC) method, as described previously (19). PSCC is a stable and sensitive method for the detection of copy number variation (CNV). PSCC is able to identify deletions with a resolution in the 100 kb range. It has three modules which include a two-step correction procedure to remove the local GC content bias, a binary segmentation method to locate the candidate microdeletions and microduplications regions and a combined statistics test to estimate the signal reliability. Subsequently, the microdeletions and microduplications are determined.

LCS identified two deletions in the patient, located on 6p21.1 and 17p11.2 (Fig. 5). The deletion size on 6p21.1 was approximately 172.71 kb (chromosome 6, 44895251-45077965). The Database of Genomic Variants (dgv.tcag.ca/dgv/app/home) indicated that the identified deletion was not causative. The deletion size on 17p11.2 was approximately 3.66 Mb (chromosome 17, 16572714-20229256), which is typically observed in patients with SMS. The LCS results revealed the parents were normal, with no disease-causing microdeletions and microduplications or balanced translocation (data not shown).

Discussion

Congenital mental retardation is a neurodevelopmental disease with a variety of causes. Genetic factors have significant involvement in the etiology of congenital mental retardation (3). Numerous methods, including CMA and NGS, may be used to detect genetic abnormalities. However, it is difficult to initially select a method to be performed. Using a number of methods increases cost and reduces efficiency. At present, a method to detect genetic abnormalities in one step does not exist. Herein, an improved targeted NGS method was developed to diagnose chromosomal anomalies and single gene mutations in one step at low cost. In the present study, an unknown cause of non-syndromic mental retardation was successfully diagnosed with the improved targeted NGS method. SMS has similar phenotypic features to other syndromes, including 9q34 deletion syndrome, Prader-Willi syndrome, 22q11.2 syndrome, Sotos syndrome and Williams syndrome. Therefore, it may be easily misdiagnosed (14). In the present study, the improved targeted NGS method detected a deletion located in 17p11.2. Thus, the patient was accurately diagnosed with SMS.

The methods of the present study had certain limitations. Chromosomal microdeletions and microduplications were only detected in the targeted captured region, and the whole genome region was not covered. Therefore, breakpoints of chromosome aberration were unable to be detected. Additionally, there was the potential for false negative errors. A future study will improve the scheme to minimize the false

negative rate, by increasing the targeted captured region and optimizing the information analysis algorithm.

Additionally, fertility guidance was given to the parents of the patient. The origin of the majority of deletions is *de novo*, and are more rarely attributed to an unbalanced segregation of a parental balanced translocation (20). In the present case, the chromosomes of the asymptomatic parents were normal; no disease-causing microdeletions, microduplications or balanced translocations were detected, indicating that the deletion 17p11.2 was *de novo*. The incidence of *de novo* deletions may primarily occur as random chance events during gamete formation or early embryo development. Therefore, the risk of having another affected child may be very low. However, the deletion may be caused by germline mosaicism, a well reported explanation of the possible origin of autosomal dominant and X-linked disorders (21-24). As for *de novo* deletions, limited information is available in the literature. Rothlisberger *et al* (20) reported that germline mosaicism is rare, although it may never be excluded as the origin of *de novo* structural aberrations. Sanchez *et al* (25) reported that a 15q11.2-q13 deletion in dizygotic twins with Angelman syndrome originated from somatic and germline mosaicism of the mother. The present study did not assess the possibility of germline mosaicism, as it is difficult to obtain gametes. However, prenatal testing should be proposed to this family during genetic counselling to minimize recurrence risks in subsequent gestation.

In summary, targeted NGS is typically used to detect variants of monogenic diseases. In the present study, it was investigated if the currently available targeted NGS was already suitable for the molecular diagnosis of microdeletion. Subsequently, the 17p11.2 deletion was identified by the designed capture array and no single gene mutation was identified. It was demonstrated that this method could successfully identify microdeletion and duplication. Therefore, this improved method has the potential to be developed into a screening panel for effective diagnosis of genetic abnormalities in non-syndromic mental retardation and other congenital anomalies.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author's contributions

WW, BM and YY designed the research and produced the first draft of the article. LM and XG performed the experimental studies. XW and DY contributed to drafting and revising the manuscript. HL, YS, XW and DY performed the data analysis.

Ethics approval and consent to participate

The research was prospectively reviewed and approved by the ethics committee of BGI-Shenzhen (approval no. BGI-IRB 15083; Shenzen, China). Written informed consent for participation in the present study was obtained from the patient's parents.

Consent for publication

Written informed consent for participation in the present study was obtained from the patient's parents.

Competing interests

The authors declare that they have no competing interests.

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