Optical Genome Mapping Improves Genetic Diagnosis in Chronic Granulomatous Diseases

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Research Article

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Abstract

Purpose: Chronic granulomatous disease(CGD) is mainly caused by defects in genes encoding subunits of NADPH oxidase complex (*CYBB*, *CYBA*, *NCF1*, *NCF2*, *NCF4*), and its chaperone (*CYBC1*). Next generation sequencing has successfully identified pathogenic variants in 131 clinically confirmed CGD patients in the single center, left 4 cases negative. This study sought to explore new technology to resolve these diagnostic dilemmas at whole genome level.

Methods: The clinical data were collected. Optical genome mapping (OGM) was performed to investigate clinically-relevant structural variants greater than 500 bp in size. Targeted long-range PCR followed by next generation sequencing and Sanger sequencing were utilized for confirming breakpoints junctions.

Results: OGM find a novel pathogenic copy number variant in *NCF2* (1506 bp ~ 1526 bp in length) in P3. The exact downstream breakpoints of this deletion located in a thymine (T) rich region (18 T in reference and 58 T in P3), which remain difficult to detect with current short-read and long-read sequencing. Together with a second pathogenic variant in *NCF2 in trans* (c.1130_1135del) detected by exome sequencing, P3 was genetically diagnosed.

Conclusion: OGM improves genetic diagnostic yield (1/3) in challenging CGD patients. Structural variants in *NCF2* and other genes should be considered in undiagnosed CGD disease. Further research in undiagnosed population with OGM is warranted.

1. Introduction

Chronic granulomatous disease (CGD) is a rare and potentially fatal disorder of neutrophil function. It is characterized by severe recurrent lift-threatening bacterial, fungal, mycobacteria infections, and dysregulated inflammatory response resulting in granuloma formation and other inflammatory disorders such as colitis [1]. The molecular etiology of CGD is defective nicotinamide adenine dinucleotide (NADPH) oxidase [2]. Deficiency in genes encoding subunits of NADPH and / or its chaperone have been identified in CGD patients: 1)X-linked gene *CYBB* (OMIM *300481), the dominant form in European countries, America (~ 67% of CGD patients) [3, 4], and China (~ 89% of CGD patients [5, 6]; and 2) autosomal genes *CYBA* (OMIM *608508), *NCF1* (OMIM *608512), *NCF2* (OMIM *608515), *NCF4* (OMIM *601488), and *CYBC1/Eros* (OMIM*618334) (acts as a chaperone). In contrast to situation in non-consanguineous countries, *CYBA*, *NCF1* and *NCF2* deficiency are the most frequent form in countries like Jordan [7, 8], Iran [9], Turkish [10, 11] and Israel [12, 13]. *CYBC1* and *NCF4* deficiency are rare globally: biallelic pathogenic variants in *CYBC1* have been identified in 8 Icelanders [14] and one Saudi-Arabian [15]; while that in *NCF4* has only one case reported [16].

In some patients clinically diagnosed with CGD disease, initial genetic Sanger sequencing and / or whole exome sequencing (WES) identifies only one demonstratedly or predictedly pathogenic variant (PPV) in the above genes. Bionano Optical Genomic Mapping (OGM), an emerging technology with the potential to resolve diagnostic dilemmas, has shown its power to interrogate >500bp variants in single molecular

level and panoramic view point of whole genome, including large deletion / duplication [17, 18], translocation [19], inversion [20], and other complicated rearrangement [21, 22]. This study thus subjected to OGM patients with clinically but not genetically diagnosed CGD disease to learn if OGM could detect CGD-related variants that theretofore had eluded identification.

2. Materials And Methods

2.1 Subjects

From 131 cases underwent next generation sequencing (panel and whole exome sequencing), excluded those genetically identified as harboring biallelic PPV in target genes (*CYBB*, *CYBA*, *NCF1*, *NCF2, NCF4, CYBC1*), leaving 4 patients with either a single demonstrated PPV in *NCF2* (1 case) or none PPV in target genes. DNA was available from 3 patients and were used for OGM.

Clinical diagnosis was made based on 1) typical clinical features, characterized by recurrent lift-threatening infections of bacteria, fungus, mycobacteria and excessive inflammatory responses leading to granuloma formation in multiple organs; and 2) result of defective respiratory burst detected by dihydrorhodamine-1,2,3 (DHR) test, and decreased protein level of gp91 protein levels (flow-cytometry-based extracellular staining with Moab 7D5) [5].

2.2 Neutrophil Evaluation

Respiratory burst of neutrophil was measured by flow cytometry-based DHR test as described before [5]. In brief, whole blood samples were incubated with DHR and stimulated with phorbol-12-myrismte-14-acetate (PMA) for 20 min. Then, red blood cells were lysed with hemolysin. After stimulation and lysis, samples were analyzed using a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA). Stimulation index (SI) was defined as geometric mean of DHR from stimulated samples/geometric mean of DHR from unstimulated samples of the same individual.

2.3 Whole-exome Sequencing

Genomic DNA(gDNA) was extracted from ethylenediaminetetraacetic acid (EDTA)-treated peripheral blood using QIAamp DNA Blood Mini Kit (51106; Qiagen, Germany). gDNA was enriched with the xGen Exome Research Panel v2.0 (Integrated DNA Technologies, Coralville, Iowa) and sequenced on HiSeq 2000/2500 (Illumina, San Diego, CA) to generate 150 bp paired end reads at a target depth of 100 ×. Average sequencing coverage was >=100-fold with >96% of the region-of-interest covered at least 20-fold. The quality of raw data was checked by FASTQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). After removing the low-quality reads, the adaptor reads were mapped to the reference genome (GRCh37/hg19) with Burrows-Wheeler Aligner (BWA). GATK was used for insertion and deletion realignment, quality recalibration, and variant calling.

Detected variants were annotated using ANNOVAR. Variants with minor allele frequencies (MAFs) of >0.5% were filtered based on frequency in healthy population databases including 1000 Genome Project (1000G, http://www.internationalgenome.org/data), the Exome Aggregation Consortium Browser (ExAC, http://exac.broadinstitute.org), and the Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org/).

Novel and rare variants (MAF<0.5%) were classified and analyzed for pathogenicity according to records in Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php), ClinVar database (https://www.ncbi.nlm. nih.gov/clinvar) and the American College of Medical Genetics and Genomics (ACMG) guidelines [23].

2.4 Optical Genomic Mapping

High molecular weight DNA was extracted from fresh whole blood using Bionano Prep Blood and Cell Culture DNA Isolation Kit (Bionano Genomics #30033, San Diego, CA). Optical genomic mapping and annotation of gDNA from the patients were outsourced to WeHealth Biomedical Technology Co., Ltd (Shanghai, China). DNA labeling was processed with Bionano Prep DLS DNA Labeling kit (Bionano Genomics #30071) according to the kit protocol. In brief, DNA was labeled with DLGreen fluorophores using DLE-1 enzyme at 37°C for 2 h, digested with proteinase K at 50 °C for 30 min, and cleaned up with 1× DLE-1 buffer. Subsequently, DNA backbone was stained with DNA stain, 5× DTT, and 4× flow buffer for 1 h and homogenized overnight at 4 °C. Labeled DNA was loaded on a Saphyr chip (Bionano Genomics) and run on a Saphyr instrument (Bionano Genomics). De novo genome map assembly was performed using Bionano Solve version 3.4 (Bionano Genomics) and structural variants (SVs) - (based on assembled maps) and CNV - (based on molecule coverage) were called against the human reference genome (GRCh37/hg19). Data were analyzed with Bionano Access and Bionano Tools on Saphyr Compute Servers (Bionano Genomics).

2.5 Targeted next-generation sequencing (NGS) and Sanger sequencing

Targeted-NGS was performed to validate the sequences of SVs acquiring by OGM. Polymerase chain reaction (PCR) amplification regions and primers were designed according to breakpoint regions of SVs (Table 1). PCR was carried out using a LongAmp Taq PCR Kit (E5200S; NEB, Ipswish, MA). 50-100 ng gDNA was used as PCR template (conditions: 96 °C for 5 min followed by 35 cycles of 96 °C for 30 sec, 60 °C for 30 sec, and 72 °C for 4 min, with a final extension at 72 °C for 4 min). PCR products purification, library construction, sequencing, and data analysis were the same as described in WES. Sanger sequencing was used to validate the breakpoint junctions. Primer sequences and coordinates are listed in Table 1.

Table 1
Primers used for targeted-long PCR and Sanger sequencing

	Amplified region	Sequence of oligonucleotide(5'-3')		Length (control / patient)	
Long-PCR	chr1:183588277-183596046	FL	TAAAAAAGATAGGGAAATAGGAGAAGC	7770	6270
		RL	TGAACACACATTAGGAGAGGTAGAGAG		
Sanger sequencing	chr1:183589798-183591851	FS	TGTAAAGCGCTGGGATAGATAGAG	2054	1000
		RS	ATTTTGACATTTGCAGGAAATTGG		

3. Results

3.1 Clinical characteristics

Three patients (1 male, 2 females) were enrolled. The onset of CGD for all was in the neonatal period. The clinical diagnosis of CGD was confirmed based on typical clinical features and defective respiratory burst detected by DHR test. The sites of infection included lung (P1, P2, P3), lymph node (P1, P3), skin (P1, P3), soft tissue (P1), abdominal cavity (P3) and central nervous system (P3).

Infectious pathogens included bacteria (P1, P2, P3), fungus (P3) and mycobacteria (P3). Pulmonary granuloma formation was found by pulmonary CT in 2 patients (P2, P3). P3 was diagnosed with bacillus Calmette-Guérin (BCG) infection, lymphatic tuberculosis, tuberculosis and abdominal tuberculosis at the age of 3y, and recovered from anti-tuberculosis treatment at the age of 5y. The clinical characteristics were shown in Table 2.

Table 2
Clinical characteristics of the enrolled three patients

	Patient 1	Patient 2	Patient 3
Gender	M	F	F
Age of onset	1 m	0.5 m	0.3 m
Age of last follow up	2 y	17 m	12 y
Onset of symptoms	perianal abscess	lung infection	lung infection
Sinopulmonary infections/pathogen	Yes/ Haemophilus influenzae	Yes/ Respiratory syncytial virus	Yes/ Staphylococcus aureus, Streptococcus pneumoniae, Candida
Lymphnoditis	Yes	No	Yes
Skin infection	Yes	No	Yes
Soft tissue infection	Yes	No	No
Abdominal infection	No	No	Yes
Central nerve system	No	No	Yes
Granulomatous formation	No	lungs	lungs
Mycobacterium	No	No	BCG infection; lymphatic tuberculosis, tuberculosis and abdominal tuberculosis
Fungal	No	No	Yes
SI	1.3	10.2	2.3
M: male; F: female; Y: year; M: month; BCG: bacillus Calmette-Guérin			
Reference range of SI: >100			

3.2 Genetic Findings On Exome Sequencing

A single variant in *NCF2* (NM_000433:c.1130_1135del) was identified in P3 (Figure 1), and it was not recorded in ExAC, 1000G nor gnomAD. This variant leads to an in-frame deletion (NP_000424.2:p.D377_M378). It was designated as pathogenic according to the criteria above and to American College of Medical Genetics and Genomics (ACMG) guidelines [23]. Sanger sequencing of parent-child trio found this variant is maternal (Figure 1).

No pathogenic variants in known CGD-related genes (including *CYBB*, *CYBA*, *NCF1*, *NCF2*, *NCF4*, *CYBC1*) were identified in P1 and P2.

3.3 Genetic Findings On Optical Genomic Mapping

A 1,457 bp deletion in *NCF2* (chr1:183,545,180-183,564,069) was indicated in P3, through manual investigation of unaligned labels to the label pattern on reference genome (Figure 2A). This region encompasses 5'-untranslated regions (5'-UTRs), exon 1-3, and partial intron 3. The start codon of *NCF2* translation is located in exon 1, and loss of exon1 is predicted to a complete loss of NCF2 synthesis (NP_000424.2). It has not been reported, and was absent from gnomAD and Database of Genome Variants (DGV). Therefore, this deletion was classified as pathogenic according to ACMG guidelines [23, 24].

No CGD-related CNV (including *CYBB*, *CYBA*, *NCF1*, *NCF2*, *NCF4*, *CYBC1*) were identified in P1 and P2.

3.4 Targeted NGS and Sanger sequencing revealed breakpoints region of the deletion

To validate the deletion and define the breakpoints at single nucleotide level in P3, long range PCR following targeted next generation sequencing, and Sanger sequencing were performed. To validate the origin of paternal (inferred from the inheritance pattern and result of WES), same experiment was done in parallel on her father.

Sanger sequencing revealed the deleted region was 1506 bp ~ 1526bp, with upstream breakpoint within chr1: 183,559,109-183,559,112 (reference sequence is TTTT), and downstream within chr1:183,560,617-183,560,634 (reference sequence is $T_1T_2T_{3\ldots}.T_{17}T_{18}$), respectively (Figure 2B). Same results obtained from his father confirmed its paternal origin.

This precise deletion, involves the region of 5'-untranslated region (5'-UTR), exon 1 and partial intron 1. Loss of start codon of *NCF2* translation lead to a complete loss of NCF2 synthesis.

4. Discussion

OGM allowed us genetically to identify a novel ~1500 bp pathogenic NCF2 variant, and thus diagnosed a NCF2 deficient CGD out of three WES-negative patients (33% diagnostic yield). This case representing the first use of OGM to solve an undiagnosed case within the CGD.

The ~1500 bp deletion in P3 had been missed by prior WES. Retrospective manual inspection of the WES data demonstrated a deletion of 449 bp encompassing exon1 of *NCF2* (NC_000001.10:g.183,559,290-183,559,739). It was missed because WES primarily focused on detection of SNVs. CNV analysis of WES data is believed to be capable of finding exon1 loss within *NCF2*, yet WES for P3 was done far before extended bioinformatics analysis for CNV has been developed. Further, OGM performed better accuracy in suggesting variant size (1500 bp vs 449 bp) than WES. Sanger sequencing based on OGM results

helped final detection of the exact breakpoints: one located in intron with 4 thymine (T) in reference and the other in 5'-untranslated regions with 18 T in reference. Of note, the resulted junction region contains 58 T, which may due to DNA demage repair after twice break of genome. This is outside of the capacity for short-reads-matching based WES. WGS may cover this variant completely, yet reads matching quality is believed to be too low at ploy T region.

Copy number variants in *NCF2* have been reported in CGD patients: 1) a large homozygous deletion encompassing exon2 detected by Sanger sequencing, with exact size and breakpoint unknown [3]; 2) a 1.1 kb homozygous duplication encompassing exons 8-9 (NC_000001.10:g (?_183536035)_(183536500_?)dup) identified by reverse-transcription PCR [25]. 3) a 1,380 bp homozygous deletion encompassing exon5 (c.366+2401_502-527del, p.V123_W167del) identified by reverse-transcription PCR [26]. These discoveries warrant a necessary of considering structural variants in undiagnosed CGD cases.

Limitation of OGM is that, the breakpoint junctions obtained must be verified by sanger sequencing (for large copy number variation, targeted long-PCR is a priority). Lucky for P3 that, the deleted region was confirmed to be located surrounding exon 1 of *NCF2* gene and its promoter region, thus suspended NCF2 synthesis. Loss of NCF2 in P3 was a result in accordance with that of OGM, which gave a larger deletion region including exon1, exon2, exon3 and promoter. However, for one case in internal study, the exact breakpoint was confirmed to be far outside of a target gene, thus did not disturb its expression and function in fact (data not shown).

For OGM-negative cases in this study, reasons were speculated as bellow: 1) the exact variant maybe SNV and located in deep-intron or other regions outrange of WES, thus RNA-sequencing or WGS maybe an alternative; 2) the exact variant is copy number variant but in a size less than 500 bp, which is outrange of OGM. This speculation is based on a 149bp pathogenic variant recorded in Clinvar: NC_0000001.11: g (?_183577579)_(183577727_?) dup, accession number VCV000832066.1.

5. Conclusion

This study demonstrates that OGM, to a certain extent, improves genetic diagnosis in WES-negative Chronic Granulomatous Disease. This result expands the spectrum of genetic variation in *NCF2* disease and detail a novel genomic deletion in *NCF2* associated with Chronic Granulomatous Disease. For patients, it helps in genetic counseling and make hematopoietic stem cell transplantation for P3 promising. However, instead of replacing, OGM still needs to be applied in combination with WES and / or other genetic testing methods.

Declarations

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Availability of data and materials

Regarding data and privacy protection, the dataset supporting the conclusions of the article is available upon individual request directed to the corresponding author.

Authorship Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Xiaoying Hui, Jingmin Yang, Wenjie Wang, Jing Zhang, Jia Hou, Wenjing Ying, bijun Sun, Lipin Liu, Danru Liu, Qinhua Zhou. Xiaochuan Wang and Jinqiao Sun drafted the work and revised it critically for important intellectual content. The first draft of the manuscript was written by Xiaoying Hui and Jingmin Yang and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Disclosure of Conflicts of Interest

The authors declare that they have no conflict of interest.

Ethics approval

The study was approved by the Ethics Committee of the Children’s Hospital of Fudan University.

Consent to participate

The patient and his parents provided written informed consent for enrollment in this study.

Consent for publication

Not applicable

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References

[1] Leiding JW, Holland SM. Chronic Granulomatous Disease. GeneReviews®[Internet]. https://www.ncbi.nlm.nih.gov/books/NBK99496/. Accessed 9 Aug 2012 [Updated 11 Feb 2016].

[2] Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A, et al. Human Inborn Errors of Immunity: 2019 Update on the Classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol. 2020;40(1):24-64.

[3] Badalzadeh M, Fattahi F, Fazlollahi MR, Tajik S, Bemanian MH, Behmanesh F, et al. Molecular analysis of four cases of chronic granulomatous disease caused by defects in NCF-2: the gene encoding the p67-phox. Iran J Allergy Asthma Immunol. 2012;11(4):340-4.

[4] Roos D. Chronic Granulomatous Disease. Methods Mol Biol. 2019;1982:531-42.

[5] Zhou Q, Hui X, Ying W, Hou J, Wang W, Liu D, et al. A Cohort of 169 Chronic Granulomatous Disease Patients Exposed to BCG Vaccination: a Retrospective Study from a Single Center in Shanghai, China (2004-2017). J Clin Immunol. 2018;38(3):260-72.

[6] Gao LW, Yin QQ, Tong YJ, Gui JG, Liu XY, Feng XL, et al. Clinical and genetic characteristics of Chinese pediatric patients with chronic granulomatous disease. Pediatr Allergy Immunol. 2019;30(3):378-86.

[7] Bakri FG, Mollin M, Beaumel S, Vigne B, Roux-Buisson N, Al-Wahadneh AM, et al. Second Report of Chronic Granulomatous Disease in Jordan: Clinical and Genetic Description of 31 Patients From 21 Different Families, Including Families From Lybia and Iraq. Front Immunol. 2021;12:639226.

[8] Bakri FG, Martel C, Khuri-Bulos N, Mahafzah A, El-Khateeb MS, Al-Wahadneh AM, et al. First report of clinical, functional, and molecular investigation of chronic granulomatous disease in nine Jordanian families. J Clin Immunol. 2009;29(2):215-30.

[9] Fattahi F, Badalzadeh M, Sedighipour L, Movahedi M, Fazlollahi MR, Mansouri SD, et al. Inheritance pattern and clinical aspects of 93 Iranian patients with chronic granulomatous disease. J Clin Immunol. 2011;31(5):792-801.

[10] Koker MY, Sanal O, De Boer M, Tezcan I, Metin A, Ersoy F, et al. Mutations of chronic granulomatous disease in Turkish families. Eur J Clin Invest. 2007;37(7):589-95.

[11] Koker MY, Camcioglu Y, van Leeuwen K, Kilic SS, Barlan I, Yilmaz M, et al. Clinical, functional, and genetic characterization of chronic granulomatous disease in 89 Turkish patients. J Allergy Clin Immunol. 2013;132(5):1156-63 e5.

[12] Wolach B, Gavrieli R, de Boer M, Gottesman G, Ben-Ari J, Rottem M, et al. Chronic granulomatous disease in Israel: clinical, functional and molecular studies of 38 patients. Clin Immunol. 2008;129(1):103-14.

[13] Wolach B, Gavrieli R, de Boer M, van Leeuwen K, Berger-Achituv S, Stauber T, et al. Chronic granulomatous disease: Clinical, functional, molecular, and genetic studies. The Israeli experience with 84 patients. Am J Hematol. 2017;92(1):28-36.

[14] Arnadottir GA, Norddahl GL, Gudmundsdottir S, Agustsdottir AB, Sigurdsson S, Jensson BO, et al. A homozygous loss-of-function mutation leading to CYBC1 deficiency causes chronic granulomatous disease. Nat Commun. 2018;9(1):4447.

[15] Thomas DC, Charbonnier LM, Schejtman A, Aldhekri H, Coomber EL, Dufficy ER, et al. EROS/CYBC1 mutations: Decreased NADPH oxidase function and chronic granulomatous disease. J Allergy Clin Immunol. 2019;143(2):782-5 e1.

[16] Matute JD, Arias AA, Wright NA, Wrobel I, Waterhouse CC, Li XJ, et al. A new genetic subgroup of chronic granulomatous disease with autosomal recessive mutations in p40 phox and selective defects in neutrophil NADPH oxidase activity. Blood. 2009;114(15):3309-15.

[17] Cope H, Barseghyan H, Bhattacharya S, Fu Y, Hoppman N, Marcou C, et al. Detection of a mosaic CDKL5 deletion and inversion by optical genome mapping ends an exhaustive diagnostic odyssey. Mol Genet Genomic Med. 2021;9(7):e1665.

[18] Chen M, Zhang M, Qian Y, Yang Y, Sun Y, Liu B, et al. Identification of a likely pathogenic structural variation in the LAMA1 gene by Bionano optical mapping. NPJ Genom Med. 2020;5:31.

[19] Barseghyan H, Tang W, Wang RT, Almalvez M, Segura E, Bramble MS, et al. Next-generation mapping: a novel approach for detection of pathogenic structural variants with a potential utility in clinical diagnosis. Genome Med. 2017;9(1):90.

[20] Wang H, Jia Z, Mao A, Xu B, Wang S, Wang L, et al. Analysis of balanced reciprocal translocations in patients with subfertility using single-molecule optical mapping. J Assist Reprod Genet. 2020;37(3):509-16.

[21] Pastor S, Tran O, Jin A, Carrado D, Silva BA, Uppuluri L, et al. Optical mapping of the 22q11.2DS region reveals complex repeat structures and preferred locations for non-allelic homologous recombination (NAHR). Sci Rep. 2020;10(1):12235.

[22] Zheng Y, Kong L, Xu H, Lu Y, Zhao X, Yang Y, et al. Rapid prenatal diagnosis of Facioscapulohumeral Muscular Dystrophy 1 by combined Bionano optical mapping and karyomapping. Prenat Diagn. 2020;40(3):317-23.

[23] Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-24.

[24] Brandt T, Sack LM, Arjona D, Tan D, Mei H, Cui H, et al. Adapting ACMG/AMP sequence variant classification guidelines for single-gene copy number variants. Genet Med. 2020;22(2):336-44.

[25] Borgato L, Bonizzato A, Lunardi C, Dusi S, Andrioli G, Scarperi A, et al. A 1.1-kb duplication in the p67-phox gene causes chronic granulomatous disease. Hum Genet. 2001;108(6):504-10.

[26] Gentsch M, Kaczmarczyk A, van Leeuwen K, de Boer M, Kaus-Drobek M, Dagher MC, et al. Alu-repeat-induced deletions within the NCF2 gene causing p67-phox-deficient chronic granulomatous disease

(CGD). Hum Mutat. 2010;31(2):151-8.

Figures

Figure 1

A heterozygous *NCF2* variant (NM_000433:c.1130_1135del) identified in P3 by exome sequencing.

Variant c.1130_1135 del was confirmed by Sanger sequencing. Hyphen, wild-type status. WT, wildtype; F, father; M, mother.

Figure 2

A heterozygous ~1500 bp *NCF2* deletion identified in P3.

A) Deletion region suggested in optical genome mapping;

B) Breakpoints confirmed by targeted sequencing(red marked). Retrospective analysis of whole exome sequencing suggested a deletion of 449 bp (blue marked).