Highly precise breakpoint detection of chromosome balanced translocation in a Chronic Myelogenous Leukemia patient

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Abstract

Chronic Myelogenous Leukemia (CML) has a special phenomenon of chromosome translocation, which is called Philadelphia chromosome translocation. However, the detailed connection of this structure is troublesome and expensive to be identified. Low-coverage whole genome sequencing (LCWGS) could not only detect the chromosomal translocation which does not be known in advance, but also provide the breakpoint candidate small region (with an accuracy of ±200 bases). Importantly, the sequencing cost of LCWGS is about US$300. Then, with the Sanger DNA sequencing, the precise breakpoint can be determined at a single base level. In our project, with LCWGS, BCR and ABL1 are successfully identified and were disrupted at chr22:23,632,356 and chr9:133,590,450, respectively. Due to the reconnection after chromosome breakage, classical fusion gene (BCR-ABL1) was found in bone marrow and peripheral blood. The precise breakpoints were helpful to study the pathogenic mechanism of CML and could better guide the classification of CML subtypes. This LCWGS method is universal and can be used to detect all diseases related to chromosome variation, such as solid tumors, liquid tumors and birth defects.

1. Introduction

Next Generation Sequencing (NGS) has developed rapidly and was widely used in the field of molecular genetics[1]. LCWGS could conduct a comprehensive detection of abnormal chromosome structure, including deletion, duplication, translocation, inversion and more complex types after their combination [2].

Leukemia had a high mortality rate and Chronic myeloid leukemia (CML) accounts for 15%–20% of all adult's leukemias[3, 4]. About 90% of CML were accompanied by t(9;22)(q34;q11), which formed its iconic Philadelphia chromosome[5], and since DNA structure was damaged, it was often accompanied by abnormal structure of other chromosomes. In CML patients, the subtypes of BCR-ABL1 gene fusion were different. Among them: (1) >90% of patients had breakpoints in the BCR gene in exon 12-16 main break region, the resulting fusion gene protein was p210. (2) The rare BCR breakpoint occurred in the region of exon 17-20, resulting in a p230 fusion protein. (3) In rare patients, the BCR breakpoint occurred in the rare zone of exons 1-2, resulting in the fusion protein p190 [6]. The p190, p210, and p230 had persistently enhanced tyrosine kinase (TK) activity which disturbed downstream signaling pathways, causing enhanced proliferation, differentiation arrest, and resistance to cell death [7, 8]. The most effective drug for treating Philadelphia chromosomal disease were tyrosine kinase inhibitors (TKIs) targeting the BCR-ABL1 fusion gene protein. The biggest obstacle to improving the prognosis of patients with Ph-positive CML was drug resistance and new mutations producing from disease progression[9-11]. Comprehensive and accurate detection of mutations in CML patients (especially BCR-ABL1 kinase domain) in treatment progress may be the key to solving these problems [12].

The higher accuracy of the breakpoints, the more conducive to our subsequent further analysis. LCWGS has been reported as a highly accurate, cost-effective, and robust detection approach to detect all abnormal chromosome structures[2]. In our study, we used LCWGS to characterize the breakpoints of a

CML patient with Philadelphia chromosome. We successfully mapped two breakpoints, which disrupted two known gene, BCR and ABL1.The breakpoints which sites on chr22:23,632,356 and chr9:133,590,450 are located on the 13th intron of BCR and the first intron of ABL1, respectively. In addition, we also found other chromosomal structural variations. Roughly, there is no difference in the main gene fusion of different CML patients. However, at a more refined level, they will have different breakpoints and show different clinical symptoms[13-15]. These have important guiding significance for the precise medication of patients and for doctors formulating follow-up treatment plans. More importantly, this technology could detect all mutations to screen out the patients with early CML and find the risk of CML earlier, so that the doctors and patients could carry out active and effective intervention and treatment[16-17].

2. Materials And Methods

2.1 Case selection and sample collection

We recruited a 75-year-old male who was diagnosed with CML to apply the LCWGS method. He has signed the informed consent and this study was approved by the Ethics Committee of the Peking University Shenzhen Hospital. The peripheral blood (heparin tube) was collected for karyotyping. Additionally, the bone marrow sample and peripheral blood (EDTA tube) sample were collected for genomic DNA (gDNA) extraction after anonymization, respectively.

2.2 Karyotyping

For the analysis of chromosome, Giemsa (GTG) band karyotyping at 550-band lever was performed in accordance with the standard laboratory protocol.

2.3 LCWGS

DNA Isolation Kit for Cells and Tissues and QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) is used for genomic DNA extraction from peripheral blood lymphocytes and bone marrow cells. One library of bone marrow sample was constructed with insert size of ~3kb (mate pair).

The bone marrow library was sequenced on the Illumina NovaSeq with 151-bp paired-end reads and a target mean coverage of >8 folds. After removing reads containing sequencing adapters and low-quality reads, the SOAPaligner sequence alignment software (http://soap.genomics.org.cn/) was used for mapping reads to the NCBI human reference genome (version: GRCh37.1). Then we retained the uniquely mapped reads for the subsequent analysis and the specific analysis method has been previously described in detail. Using this specific analysis method, we could take advantage of uniquely paired reads to find all chromosome copy number variations (CNV) and structure variations (SV), and the corresponding breakpoints on the whole genome, and the accuracy of the breakpoints could be accurate to a small region of ±200 bases.

At last, accurate verification of breakpoints was carried out by Sanger sequence. We designed primers with NCBI Primer-Blast (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) for the 500bp upstream and

500bp downstream of the breakpoint region respectively. By comparing the amplified products of Sanger sequence, we could determine the precise breakpoint easily. Oligonucleotide primer pairs of the translocation were designed with Gene Runner software (version 5.0.69 Beta; Hastings Software). Forward primer: CTAGCCTGAAGGCTGATCCC; Reverse primer: AAGCCACTGGCACACTTCA.

2.4 PCR and Sanger sequencing

With designed primers, the putative fragments were amplified through PCR with general PCR conditions. The products were sequenced on an ABI-A3130 genetic analyzer.

3. Results

Karyotype analysis of his peripheral blood indicated that he was 46XY, t(9;22)(q34;q11.2) (Fig.1a). Due to the occurrence of balanced translocation, two fusion genes (BCR-ABL1 and ABL1-BCR) were identified. In the subsequent RT-PCR experiment, Philadelphia chromosome (Ph) (+) was confirmed to be positive with the resulting fusion gene protein p210.

LCWGS was subsequently performed on the bone marrow sample of the case and two derivative chromosomes (der 9 and der 22) were successfully detected (Fig. 1b), which identified the breakpoint on chromosome 9 in a 400bp region (chr9:133,590,268-133,590,668), the chromosome 22's breakpoint in a 69bp region (chr22: 23,632,287- 23,632,356) in the bone marrow sample. The precise position of the breakpoints was confirmed through PCR and Sanger sequencing in both the bone marrow sample and peripheral blood (EDTA tube) sample (Fig.1c). As shown in Fig. 1d and Fig.1e, two accurate breakpoints of Philadelphia chromosome were the same position, chr22:23,632,356 and chr9:133,590,450 from the two different samples of the case.

LCWGS analysis of this case revealed for us more results (Table 1). In addition to t(9;22)(q34;q11.2), we also found four CNVs: one deletion region which copy number is 1 from chr7:110933409 to chr7:111013054 (involving the IMMP2L gene), two duplication region which copy number is 3 from chr18:63,892,542 to chr18:64,158,074 (not involving the gene) and from chr18:66,308,883 to chr18:66,574,415 (involving the four genes CCDC102B, RNU6-39P, SDHCP1, TMX3), respectively, and one duplication region which copy number is 4 from chr22:25,652,709 to chr18:25,918,145 (involving the 5 genes CRYBB2P1, IGLL3P, IGLVIVOR22-1, LRP5L, and MIR6817). Any gene with CNV reported from the case in those Previous reported studies of Pubmed or in OMIM or in DGV was considered as high confidence for a particular phenotype, and the CNVs were therefore considered to be Benign.

4. Discussion

CML originates from pluripotent hematopoietic stem cells and BCR-ABL was the main driving event in CML [18- 20]. A gene fusion mutation occurred between the BCR and ABL1 genes, however the position of the connection breakpoint changed greatly [21]. According to the different connection breakpoints of the BCR-ABL1 fusion gene, the length of the corresponding expressed protein would be different. According

to this, it could be divided into P190, P210, P230. Among them, P210 is the most common. Because the gene structure was destroyed in CML patients, it was often accompanied by variations in the SVs and CNVs of other chromosomes. Although most of these mutations were not reported in the literature, their clinical significance was unclear. In the process of treatment, after using traditional tyrosine kinase inhibitors (TKIs) for a period, patients would develop drug resistance, and the prognosis of the patient was not good. It may be related to these new chromosomal SVs, or produce new BCR-ABL1 fusion subtypes[22,23].

A lot of laboratories were currently in the process of introducing NGS into their routine diagnostic procedures, because it had proven a robust, reproducible, cost-effective alternative to traditional detection methods[24,25]. In this study, we applied LCWGS method to the detection of a CML patient, successfully detected Ph, and given the candidate region of the breakpoint, and finally combined the results of Sanger sequencing to give the precise breakpoint. Not only that, this method could detect all chromosome SVs and CNVs in the sample. This was of great significance for the early screening of CML patients, the accompanying diagnosis during the treatment process, the discovery of new BCR-ABL1 mutation subtypes, and subsequent intervention and treatment. It had been reported in the literature that the Philadelphia chromosome of CML could be treated by gene editing, which required very high requirements for precise breakpoints of gene fusion and other possible mutations[26]. LCWGS had high accuracy, high resolution and comprehensive detection, which happened to provide a panoramic description of chromosome genome mutations in CML patients. Our results proved that the method of precise breakpoint detection of complex chromosome rearrangement could be employed as a diagnostic tool for CML patients.

Cost is the biggest factor affecting the clinical application of a new technology. LCWGS is highly cost-effective with a lower coverage-depth sequencing. In this case, ~80 million read pairs (~24Gb bases) was obtained and the cost was about US$300 per sample for using our approach.

Next, we will collect more leukemia samples for testing, determine the precise breakpoints of fusion genes, improve typing accuracy, and summarize the breakpoint distribution and rules. We will also continue to improve the detection accuracy and lower limit of the data abundance of the algorithm, so that it can screen out the variation types in early patients and other subtypes that are newly developed during the progression of leukemia. Finally, it will provide guidance for gene editing therapy and combined targeted drugs.

5. Conclusion

LCWGS is a cost-effective and accuracy method to detect chromosome SVs and CNVs including deletion, duplication, inversion and translocation without known karyotyping result. It can play a vital role in solid tumors and liquid tumors. The premise of accurate medical treatment is accurate detection.

Declarations

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Ethical approval and informed consent

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Peking University Shenzhen Hospital. All patients provided written informed consent before participation.

Declaration of Competing Interest

None.

Raw data

The original data of this project can be easily obtained from the author by e-mail.

Consent for publication

Not applicable.

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Author contributions

N.X. managed the project. L.X. and Q.ZH. collected and prepared the samples. Sh.T. and M.ZH. performed the sequencing. C.Y. and X.C. performed the bioinformatic analysis. M.ZH. performed the PCRvalidation. C.Y. wrote the paper. N.X. and C.Y. revised the paper. All authors reviewed the manuscript.

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Tables

Table 1. Chromosome CNVS in this patient

Chr	CNV	Mutation Type	Copy Number	Gene	OMIM ID	Phenotype	DGV
7	110933409-111013054	loss	1	IMMP2L	605977	/	0.0006
18	63892542-64158074	gain	3	/	/	/	0.0003
18	66308883-66574415	gain	3	CCDC102B	/	/	0.00007
				RNU6-39P	/	/	
				SDHCP1	/	/	
				TMX3	616102	/	
22	25652709-25918145	gain	4	CRYBB2P1	/	/	0.0004
				LGLL3P	/	/	
				LGLVIVOR22-1	/	/	
				LRP5L	/	/	
				MIR6817	/	/	

Figures

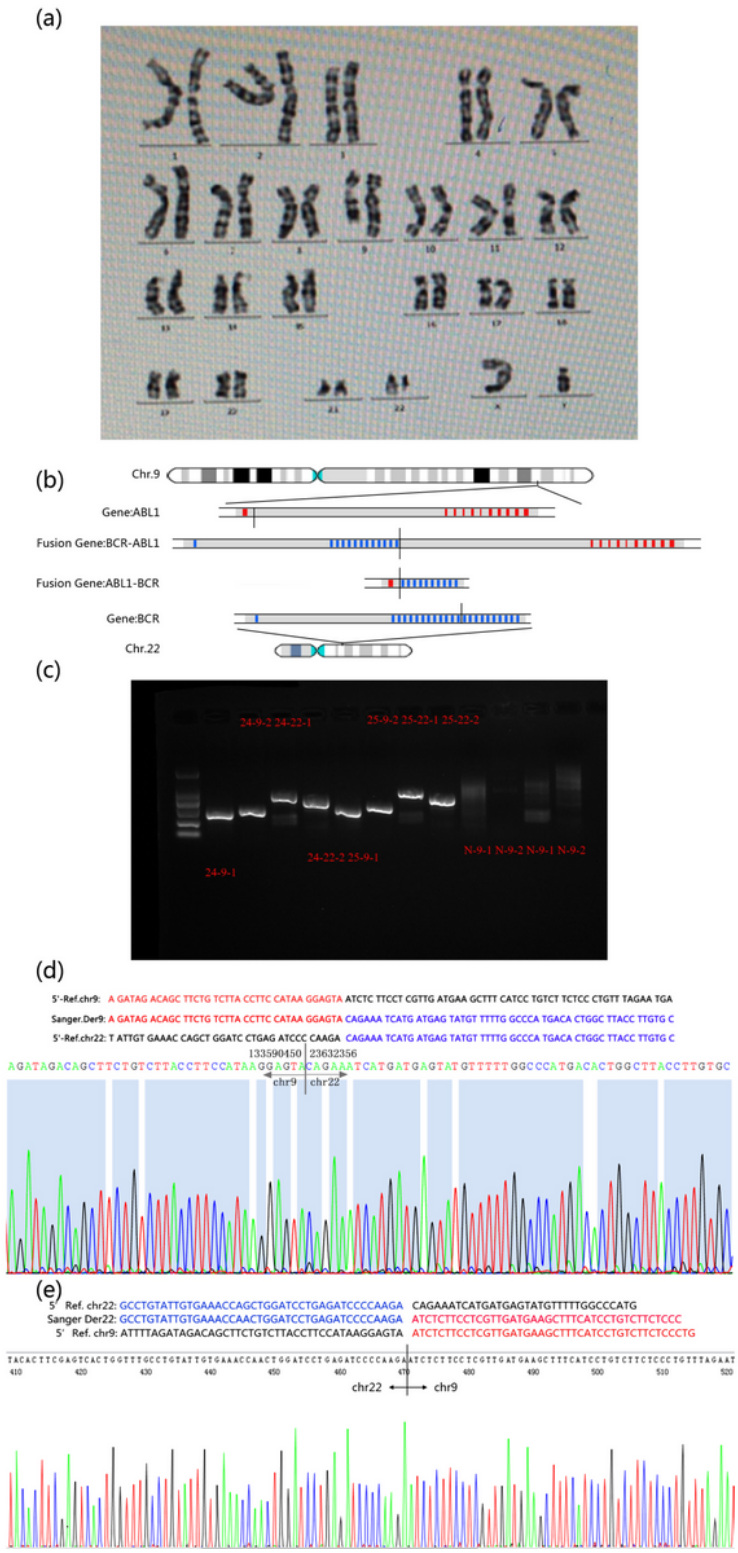

Figure 1

(a) The patient karyotype in peripheral blood. (b) Schematic diagram of chromosome balanced translocation in bone marrow of the patient. The detailed connection mode of the gene fusions is shown in the middle. (c) PCR gel map, Bone marrow: Sample 24 Blood: sample 25 Negative sample: N. Two pair primers (*-9-1 and *-9-2,*-22-1 and *-22-2) were designed for the two gene fusions, BCR-ABL1 and ABL1-BCR. (d) Gene fusion:ABL1-BCR's breakpoint connection diagram of Sanger sequencing in peripheral

blood sample and bone marrow sample.(e) Gene fusion:BCR-ABL1’s breakpoint connection diagram of Sanger sequencing in peripheral blood sample and bone marrow sample.