Whole Exome Sequencing of Chronic Myeloid Leukemia Patients

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Abstract

Background: Previous studies have shown that leukemogenic chromosomal translocations, including fusions between Break point Cluster Region (BCR) and Abelson (ABL) are present in the peripheral blood of healthy individuals. The aim of this study was to gain insights into the genetic alterations other than BCR-Abl translocation in molecular level, which cause chronic myeloid leukemia (CML).

Methods: We performed whole-exome sequencing on four cases representative of BCR-ABL positive CML in chronic phase of the disease.

Results: We did not identify any pathogenic mutation in all known genes involved in CML or other cancers in our subjects. Nevertheless, we identified polymorphisms in related genes.

Conclusion: It is the first report of exome sequencing in Philadelphia chromosome positive CML patients. We did not identify any pathogenic mutation in known cancer genes in our patients who can be due to CML pathogenesis or technical limitations.

Keywords: CML, Whole exome sequencing, Iran

Introduction

“Human chronic myeloid leukemia (CML) is a myeloproliferative disorder (MPD) caused by the Philadelphia chromosome translocation, a t (9; 22) that generates the BCR/ABL fusion oncoprotein” (1).

The BCR-ABL fusion protein is a constitutively active tyrosine kinase. Normally, this kinase precisely regulates downstream genes, including c-Myc, Akt and Jun, all of which are major players to the proliferation and survival of normal cells. However, the hyperactivity of the BCR-ABL kinase upsets this fine balance and propels cells towards uncontrolled proliferation and survival, both of which provide a growth advantage to the malignant cells bearing this mutation, ultimately leading to CML (2).

Next generation sequencing has proven to be an effective tool to identify recurrent, specific mutations in solid tumors and leukemias. Although the genetic heterogeneity of cancer necessitates some warn in the interpretation and application of the NGS results (3, 4), high-throughput sequencing remains a powerful instrument to refine potentially cancer diagnosis and treatment (5).

The aim of this study was to gain insights into the genetic alterations other than BCR-Abl translocation in molecular level, which finally cause CML. We performed whole-exome sequencing of four
cases representative of BCR-ABL positive CML in chronic phase of the disease.

**Material and Methods**

This study was conducted in Tarbiat Modares University Tehran, Iran in 2014. We used exome sequencing technology to identify mutations in molecular level in four individuals with CML who had given informed consent for sample collection and analysis. CML diagnosis was suspected by the Complete Blood Count (CBC) testing and then confirmed by identifying BCR-Abl translocation by real-time PCR. The selected patients were in chronic phase of CML without any other interfering disease and they received no treatment before sampling. DNA was extracted from peripheral blood using the conventional salting-out method. The qualifying DNA samples were exome sequenced by BGI (Beijing Genomics Institute).

**Exome sequencing procedures and data analysis**

First, genomic DNA was randomly cleaved into a fragment library, purified and subsequently enriched by NimbleGen 2.1M-probe sequence capture array. The enriched library targeting the exome was sequenced on the Illumina HiSeq 2000 platform to acquire paired-end reads with a read length of 90 base pairs. After removing reads containing sequencing adapters and low-quality reads with more than five unknown bases, high-quality reads were aligned with the human genome reference sequence (hg19/GRCh37) using Bowtie2 software 27 with default parameters. The PCR duplicates detected from Alignment files were subsequently removed with Picard (http://picard.sourceforge.net/) to improve alignment accuracy. The Genome Analysis Toolkit (GATK) was then employed for base quality recalibration, local realignment around the potential insertion/deletion (Indel) sites and variant calling. The raw single nucleotide variants were filtered for low mapping quality, low coverage, SNP clusters, etc. Then, the filtered variants were annotated using ANNOVAR for the following parameters: function (exonic or splicing); gene; exonic function (synonymous, nonsynonymous, stop gain, non-frameshift or frameshift indels); amino acid change; conservation; dbSNP (version 135) reference number; allele frequency in 1000 Genomes Project (2012 Feb version).

**Results**

Data characteristics of exome sequencing of four samples are shown in Table 1. Statistics of annotated variants in four samples before and after filtering are listed in Table 2. We also prepared a list of all genes already involved in CML reported in publications summarized in Table 3.

**Table 1: Whole exome sequencing characteristics**

| Items/samples                      | 23022   | 23031   | 23652   | 23878   |
|------------------------------------|---------|---------|---------|---------|
| Total effective reads              | 51161535| 51082069| 50259766| 50395087|
| Total effective yield (Mb)         | 4532.41 | 4522.97 | 4444.54 | 4470.17 |
| Average read length (bp)           | 88.59   | 88.54   | 88.43   | 88.7    |
| Average sequencing depth on target | 56.7    | 56.12   | 56.14   | 56.66   |
| Coverage of target region          | 99.70%  | 99.60%  | 99.70%  | 99.70%  |
| Coverage of flanking region        | 94.20%  | 93.70%  | 93.90%  | 95.50%  |
| Fraction of target covered with at least 20x | 86.10% | 85.10% | 87.10% | 89.10% |
| Fraction of target covered with at least 10x | 95.70% | 94.90% | 96.50% | 96.80% |
| Fraction of target covered with at least 4x | 98.90% | 98.60% | 99.10% | 99.00% |
| Fraction of flanking region covered with at least 20x | 18.70% | 18.70% | 18.50% | 20.40% |
| Fraction of flanking region covered with at least 10x | 41.00% | 40.80% | 40.90% | 44.30% |
| Fraction of flanking region covered with at least 4x | 71.00% | 70.50% | 70.50% | 74.50% |
| Mapping rate                       | 99.49%  | 99.36%  | 99.37%  | 99.54%  |
| Duplicate rate                     | 5.51%   | 5.58%   | 5.38%   | 5.97%   |

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Table 2: Whole exome sequencing data statistics

| Items/samples            | 23022 | 23031 | 23652 | 23878 |
|--------------------------|-------|-------|-------|-------|
| Total variants           | 84385 | 83618 | 85059 | 87055 |
| SNPs variants            | 76627 | 76016 | 77235 | 78637 |
| INDEL variants           | 7758  | 7602  | 7824  | 8418  |
| Novel SNPs variants      | 2751  | 2750  | 2708  | 2778  |
| Novel INDEL variants     | 1951  | 1875  | 1938  | 2155  |
| Novel functional SNPs    | 441   | 434   | 432   | 389   |
| Novel functional INDEL   | 63    | 56    | 59    | 58    |

Table 3: CML candidate genes (known to be involved in CML)

| Gene     | Description                                                                 | Link                                                                 |
|----------|----------------------------------------------------------------------------|----------------------------------------------------------------------|
| JAK 2    | Janus kinase 2                                                              | [http://www.ncbi.nlm.nih.gov/pubmed/25657500](http://www.ncbi.nlm.nih.gov/pubmed/25657500) |
| STAP2    | signal transducing adaptor family member 2                                  | [http://www.ncbi.nlm.nih.gov/pubmed/22231445](http://www.ncbi.nlm.nih.gov/pubmed/22231445) |
| IKZF1    | IKAROS family zinc finger 1                                                 | [http://www.ncbi.nlm.nih.gov/pubmed/18408710](http://www.ncbi.nlm.nih.gov/pubmed/18408710) |
| FANCD2   | Fanconi anemia, complementation group D2                                   | [http://www.ncbi.nlm.nih.gov/pubmed/21203397](http://www.ncbi.nlm.nih.gov/pubmed/21203397) |
| COP9S    | COP9 signalsome subunit 5                                                  | [http://www.ncbi.nlm.nih.gov/pubmed/21935931](http://www.ncbi.nlm.nih.gov/pubmed/21935931) |
| SKP2     | S-phase kinase-associated protein 2, E3 ubiquitin protein ligase            | [http://www.ncbi.nlm.nih.gov/pubmed/20717963](http://www.ncbi.nlm.nih.gov/pubmed/20717963) |
| SHC1     | SHC (Src homology 2 domain containing) transforming protein 1              | [http://www.ncbi.nlm.nih.gov/pubmed/10676600](http://www.ncbi.nlm.nih.gov/pubmed/10676600) |
| GAB2     | GRB2-associated binding protein 2                                           | [http://www.ncbi.nlm.nih.gov/pubmed/12124177](http://www.ncbi.nlm.nih.gov/pubmed/12124177) |
| GRB2     | growth factor receptor-bound protein 2                                     | [http://www.ncbi.nlm.nih.gov/pubmed/10887132](http://www.ncbi.nlm.nih.gov/pubmed/10887132) |
| CRK      | v-crk avian sarcoma virus CT10 oncogene homolog                            | [http://www.ncbi.nlm.nih.gov/pubmed/8632906](http://www.ncbi.nlm.nih.gov/pubmed/8632906) |
| DOK2     | docking protein 2, 56kDa                                                    | [http://www.ncbi.nlm.nih.gov/pubmed/15611294](http://www.ncbi.nlm.nih.gov/pubmed/15611294) |
| DOK1     | docking protein 1, 62kDa (downstream of tyrosine kinase)                    | [http://www.ncbi.nlm.nih.gov/pubmed/15611294](http://www.ncbi.nlm.nih.gov/pubmed/15611294) |
| NEDD9    | neural precursor cell expressed, developmentally down-regulated 9          | [http://www.ncbi.nlm.nih.gov/pubmed/21848808](http://www.ncbi.nlm.nih.gov/pubmed/21848808) |
| SGK223   | homolog of rat pragma of Rnd2                                               | [http://www.ncbi.nlm.nih.gov/pubmed/20697350](http://www.ncbi.nlm.nih.gov/pubmed/20697350) |
| RhoA     | ras homolog family member A                                                 | [http://www.ncbi.nlm.nih.gov/pubmed/22443473](http://www.ncbi.nlm.nih.gov/pubmed/22443473) |
| LRRK1    | leucine-rich repeat kinase 1                                                | [http://www.ncbi.nlm.nih.gov/pubmed/20697350](http://www.ncbi.nlm.nih.gov/pubmed/20697350) |
| CBL      | Cbl proto-oncogene, E3 ubiquitin protein ligase                             | [http://www.ncbi.nlm.nih.gov/pubmed/9195915](http://www.ncbi.nlm.nih.gov/pubmed/9195915) |
| TWIST-1  | twist family BHHL transcription factor 1                                    | [http://www.ncbi.nlm.nih.gov/pubmed/21123820](http://www.ncbi.nlm.nih.gov/pubmed/21123820) |
| PIK3R1   | phosphoinositide-3-kinase, regulatory subunit 1 (alpha)                    | [http://www.ncbi.nlm.nih.gov/pubmed/23292937](http://www.ncbi.nlm.nih.gov/pubmed/23292937) |
| INPPL1   | inositol polyphosphate phosphatase-like 1                                  | [http://www.ncbi.nlm.nih.gov/pubmed/10194451](http://www.ncbi.nlm.nih.gov/pubmed/10194451) |
| HCK      | HCK proto-oncogene, Src family tyrosine kinase                             | [http://www.ncbi.nlm.nih.gov/pubmed/12592324](http://www.ncbi.nlm.nih.gov/pubmed/12592324) |
| LYN      | LYN proto-oncogene, Src family tyrosine kinase                             | [http://www.ncbi.nlm.nih.gov/pubmed/12509383](http://www.ncbi.nlm.nih.gov/pubmed/12509383) |
| HoxA9    | homeobox A9                                                                 | [http://www.ncbi.nlm.nih.gov/pubmed/20141430](http://www.ncbi.nlm.nih.gov/pubmed/20141430) |
| RKIP     | phosphatidyethanolamine binding protein 1                                   | [http://www.ncbi.nlm.nih.gov/pubmed/25015191](http://www.ncbi.nlm.nih.gov/pubmed/25015191) |
| CDC42    | cell division cycle 42                                                     | [http://www.ncbi.nlm.nih.gov/pubmed/19718053](http://www.ncbi.nlm.nih.gov/pubmed/19718053) |
| NOX4     | NADPH oxidase 4                                                             | [http://www.ncbi.nlm.nih.gov/pubmed/25928540](http://www.ncbi.nlm.nih.gov/pubmed/25928540) |
| PHLPP1   | PH domain and leucine rich repeat protein phosphatase 1                     | [http://www.ncbi.nlm.nih.gov/pubmed/19261608](http://www.ncbi.nlm.nih.gov/pubmed/19261608) |
| PHLPP2   | PH domain and leucine rich repeat protein phosphatase 2                     | [http://www.ncbi.nlm.nih.gov/pubmed/19261608](http://www.ncbi.nlm.nih.gov/pubmed/19261608) |
| STAT5    | signal transducer and activator of transcription 5                         | [http://www.ncbi.nlm.nih.gov/pubmed/25170013](http://www.ncbi.nlm.nih.gov/pubmed/25170013) |
| PIK3R2   | phosphoinositide-3-kinase, regulatory subunit 2 (beta)                     | [http://www.ncbi.nlm.nih.gov/pubmed/18704194](http://www.ncbi.nlm.nih.gov/pubmed/18704194) |
| PIK3CA   | phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha    | [http://www.ncbi.nlm.nih.gov/pubmed/18644865](http://www.ncbi.nlm.nih.gov/pubmed/18644865) |
| MTOR     | mechanistic target of rapamycin (serine/threonine kinase)                  | [http://www.ncbi.nlm.nih.gov/pubmed/21715304](http://www.ncbi.nlm.nih.gov/pubmed/21715304) |
Table 3: Cond...

| Gene      | Description & Functions                                                                 | Reference URL                                      |
|-----------|----------------------------------------------------------------------------------------|---------------------------------------------------|
| MYB       | v-myb avian myeloblastosis viral oncogene homolog                                        | http://www.ncbi.nlm.nih.gov/pubmed/2741649         |
| USP18     | ubiquitin specific peptidase 18                                                        | http://www.ncbi.nlm.nih.gov/pubmed/17374743        |
| BACH2     | BTB and CNC homology 1, basic leucine zipper transcription factor 2                     | http://www.ncbi.nlm.nih.gov/pubmed/11746976        |
| SELE      | selectin E                                                                             | http://www.ncbi.nlm.nih.gov/pubmed/15674360        |
| NOV       | nephroblastoma overexpressed                                                           | http://www.ncbi.nlm.nih.gov/pubmed/19623482        |
| NU1CD1    | NuC domain containing 1                                                                 | http://www.ncbi.nlm.nih.gov/pubmed/11416219        |
| FOLR3     | folate receptor 3 (gamma)                                                              | http://www.ncbi.nlm.nih.gov/pubmed/8110752         |
| MSI2      | musashi RNA-binding protein 2                                                          | http://www.ncbi.nlm.nih.gov/pubmed/12649177        |
| RARA      | retinoic acid receptor, alpha                                                          | http://www.ncbi.nlm.nih.gov/pubmed/8180390         |
| NUP98     | nucleoporin 98kDa                                                                      | http://www.ncbi.nlm.nih.gov/pubmed/24971156        |
| VPREB1    | pre-B lymphocyte 1                                                                     | http://www.ncbi.nlm.nih.gov/pubmed/23881307        |
| SOCS6     | suppressor of cytokine signaling 6                                                     | http://www.ncbi.nlm.nih.gov/pubmed/25172101        |
| CSF3R     | colony stimulating factor 3 receptor (granulocyte)                                     | http://www.ncbi.nlm.nih.gov/pubmed/23656643        |
| LHX2      | LIM homeobox 2                                                                         | http://www.ncbi.nlm.nih.gov/pubmed/14687986        |
| NPM1      | nucleophosmin (nucleolar phosphoprotein B23, nucleomembran)                            | http://www.ncbi.nlm.nih.gov/pubmed/25961029        |
| ABCG2     | ATP-binding cassette, sub-family G (WHITE), member 2                                    | http://www.ncbi.nlm.nih.gov/pubmed/24123600        |
| SMO       | smoothened, frizzled class receptor                                                    | http://www.ncbi.nlm.nih.gov/pubmed/18772113        |
| NUMB      | numb homolog (Drosophila)                                                               | http://www.ncbi.nlm.nih.gov/pubmed/21084860        |
| mir-31    | microRNA 31                                                                            | http://www.ncbi.nlm.nih.gov/pubmed/22511990        |
| TEC       | tec protein tyrosine kinase                                                            | http://www.ncbi.nlm.nih.gov/pubmed/22739199        |
| miR-155   | microRNA 155                                                                           | http://www.ncbi.nlm.nih.gov/pubmed/22511990        |
| RGS2      | regulator of G-protein signaling 2                                                     | http://www.ncbi.nlm.nih.gov/pubmed/7643615         |
| BLK       | BLK proto-oncogene, Src family tyrosine kinase                                         | http://www.ncbi.nlm.nih.gov/pubmed/22797726        |
| NAT8      | N-acetyltransferase 8 (GCN5-related, putative)                                         | http://www.ncbi.nlm.nih.gov/pubmed/24556617        |
| miR-564   | microRNA 564                                                                           | http://www.ncbi.nlm.nih.gov/pubmed/22511990        |
| ALOX5     | arachidonate 5-lipoxygenase                                                            | http://www.ncbi.nlm.nih.gov/pubmed/19503090        |
| CD44      | CD44 molecule                                                                          | http://www.ncbi.nlm.nih.gov/pubmed/16998483        |
| AXI       | AXI receptor tyrosine kinase                                                           | http://www.ncbi.nlm.nih.gov/pubmed/7521695         |
| FOXO3     | forkhead box O3                                                                        | http://www.ncbi.nlm.nih.gov/pubmed/18644865        |
| AKAP13    | A kinase (PRKA) anchor protein 13                                                      | http://www.ncbi.nlm.nih.gov/pubmed/8290273         |
| AHI1      | Abelson helper integration site 1                                                      | http://www.ncbi.nlm.nih.gov/pubmed/22183070        |
| SETBP1    | SET binding protein 1                                                                   | http://www.ncbi.nlm.nih.gov/pubmed/22566606        |
| IRF8      | interferon regulatory factor 8                                                          | http://www.ncbi.nlm.nih.gov/pubmed/24242069        |
| ETV6      | ets variant 6                                                                          | http://www.ncbi.nlm.nih.gov/pubmed/19480935        |
| PDGFB      | platelet-derived growth factor beta polypeptide                                        | http://www.ncbi.nlm.nih.gov/pubmed/2660925         |
| PDGFRA     | platelet-derived growth factor receptor, alpha polypeptide                             | http://www.ncbi.nlm.nih.gov/pubmed/19175693        |
| GATA2     | GATA binding protein 2                                                                 | http://www.ncbi.nlm.nih.gov/pubmed/19304323        |
| VEGFC     | vascular endothelial growth factor C                                                   | http://www.ncbi.nlm.nih.gov/pubmed/22169285        |
| AKAP12    | A kinase (PRKA) anchor protein 13                                                      | http://www.ncbi.nlm.nih.gov/pubmed/15287943        |
| SLC22A1   | solute carrier family 22 (organic cation transporter), member 1                        | http://www.ncbi.nlm.nih.gov/pubmed/23272163        |
| PRKDC     | protein kinase, DNA-activated, catalytic polypeptide                                   | http://www.ncbi.nlm.nih.gov/pubmed/11264175        |
| WNT       | wingless-type MMTV integration site family                                             | http://www.ncbi.nlm.nih.gov/pubmed/22823957        |
Then we checked all filtered variants in each individual for these known genes to find cancer-related genes but we did not identify any pathogenic mutation. Nevertheless, we identified polymorphisms in related genes, some of listed in Table 4.

**Discussion**

In this study we performed exome sequencing as a high throughput technology to identify genetic alterations other than BCR-Abl translocation or those that lead to this cytogenetic translocation at molecular level which finally cause CML. We used public databases to prepare a list of cancer genes for further analysis; however, no pathogenic mutation was identified. Moreover, we analyzed functional variants (coding region and splice site variants) bioinformatically, but no pathogenic mutation was found. Logically, there are two main reasons for such results in our survey; disease nature and the technique characteristics. A chromosomal translocation includes a DNA double strand break and repair more specifically, mis-repair.

**Table 4: Identified polymorphism in this study**

| Gene  | NM_ID            | Variant   | Function           |
|-------|------------------|-----------|--------------------|
| AKAP12| NM_144497        | rs3842128 | Inframe insertion  |
|       |                   | rs10872670| Missense           |
|       |                   | rs3734799 | Missense           |
| SETBP1| NM_001130110     | rs3085861 | Frameshift insertion|
|       |                   | rs663651  | Missense           |
|       |                   | rs3744825 | Missense           |
| FOLR3 | NM_000804        | rs71891516| Frameshift insertion|
| PIK3R2| NM_005027        | rs1011320 | Missense           |
| CD44  | NM_001001389     | rs9666607 | Missense           |
|       |                   | rs1467558 | Missense           |
| AXL   | NM_001699        | rs7249222 | Missense           |
| AKAP13| NM_006738        | rs2061822 | Missense           |
|       |                   | rs2061822 | Missense           |
|       |                   | rs2061824 | Missense           |
| SLC22A1| NM_003057      | rs683369  | Missense           |
|       |                   | rs628031  | Missense           |
| PDGFRA| NM_006206        | rs35597368| Missense           |
| SON   | NM_032195        | rs13433428| Missense           |
|       |                   | rs13047599| Missense           |

Accordingly, all genes implicated in homologous recombination and non-homologous end joining, as the two main DSB repair pathways, are putative candidate genes mutated before BCR-Abl translocation (6). Moreover, Alu elements have been involved in the pathogenesis of some complex translocations including BCR and ABL, but these are extremely rare (7). Leukemogenic chromosomal translocations, including fusions between BCR and ABL are present in the peripheral blood of healthy individuals (8). It was controversial because for decades it had been proposed that these translocations unavoidably led to leukemia. There are important hints in these results. First, it forcefully implies that this oncogenic translocation is not adequate to produce malignancy, but it instead produces a “pre-malignant” clone that requires additional, complementary, events to transform fully the cell. On the other hand, this result shows that detection of an oncogenic translocation is not equivalent to detection of a malignancy (9, 10). Second, this result makes a possible explanation for the observation that mice manipulated to overexpress...
an oncogenic fusion protein often do not grow leukemia. In these mice, one oncogenic mutation is integrated in the mouse germline, but leukemic transformation is not triggered until additional mutation(s) occur spontaneously as the mouse ages. However, most of these putative mutations have not been characterized (6) and we did not identify any pathogenic mutation in related genes as well.

In this study; however some polymorphic variants were identified among them; SNPs rs683369 and rs628031 in SLC22A1, found in all subjects, have previously been studied in relation to imatinib response. “SNP rs683369 and advanced disease stage are correlated with a high rate of loss of cytogenetic response or treatment failure to imatinib in CML patients” (11). We cannot determine the effect of this variant due to the chronic phase of the disease in our patients. Moreover, Chowbay et al. revealed a sub-haplotype region encompassing one exonic SNP (rs628031) surrounded by two intronic SNPs [IVS6-878C.A (rs3798168) and IVS7+850C.T] that is significantly associated with imatinib clearance (12). Except rs628031, two other polymorphisms of this sub-haplotype region were not detected in our subjects.

Exome sequencing has been a fast and cost-effective tool to identify recurrent, specific mutations in solid tumors and leukemias (13-15). Nevertheless, this recent technique has some limitations too. Two main technical limitations in NGS, which impair exome-sequencing results, are homologous sequences and guanine cytosine (GC) bias (16) which lead to alignment errors. Another technical consideration with exome sequencing is that variants located in UTRs, intronic, promoter, and intergenic regulatory regions are mostly missed. Although it is often difficult to interpret novel variants in such regions, there are known pathogenic variants in many genes that lie outside the exons.

**Conclusion**

It is the first report of exome sequencing in Philadelphia chromosome positive CML patients. We did not identify any pathogenic mutation in known cancer genes in our patients who can be due to CML pathogenesis or technical limitations.

**Ethical considerations**

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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**References**

1. Daley GQ (2004). Chronic myeloid leukemia: proving ground for cancer stem cells. Cell, 29, 119(3): 314-6.
2. Jabbour E1, Fava C, Kantarjian H (2009). Advances in the biology and therapy of patients with chronic myeloid leukemia. Best Pract Res Clin Haematol, 22(3): 395-407.
3. J. Zhao and S. Grant (2011). Advances in whole genome sequencing technology. Curr Pharm Biotechnol, 12(2):293-305.
4. M. Meyerson, S. Gabriel, and G. Getz (2010). Advances in understanding cancer genomes through second-generation sequencing. Nature Rev Genet, 10, 685–696.
5. Piazza R1, Valletta S, Winkelmann N, Redaelli S, Spinelli R, Pirola A, Antolini L, Mologni L, Donadoni C, Papaemmanuil E et al. (2013). Recurrent SETBP1 mutations in atypical chronic myeloid leukemia. Nat Genet, 45(1):18-24.
6. Peter D. Aplan (2006). Causes of oncogenic chromosomal translocation. Trends Genet, 22(1): 46–55.
7. D M Ross, M O’Hely, P A Bartley, P Dang, J Score, J M Goyne, M Sobrinho-Simoes, N C P Cross, J V Melo, T P Speed, T P Hughes and A A Morley (2013). Distribution of
genomic breakpoints in chronic myeloid leukemia: analysis of 308 patients. *Leukemia*, 27(10):2105-7.

8. Janz S, Potter M, Rabkin CS (2003). Lymphoma- and leukemia-associated chromosomal translocations in healthy individuals. *Genes Chromosomes Cancer*, 36 (3):211–223.

9. Mori H, Colman SM, Xiao Z, Ford AM, Healy LE, Donaldson C, Hows JM, Navarrete C, Greaves M (2002). Chromosome translocations and covert leukemic clones are generated during normal fetal development. *Proc Natl Acad Sci*, 99(12):8242–8247.

10. Izraeli S, Waldman D (2004). Minimal residual disease in childhood acute lymphoblastic leukemia: current status and challenges. *Acta Haematol*, 112 (1–2):34–39.

11. Kim DHD, Sriharsha L, Xu W, Kamel-Reid S, Liu X, Siminovitch K, Messner HA, Lipton JH (2009). Clinical relevance of a pharmacogenetic approach using multiple candidate genes to predict response and resistance to imatinib therapy in chronic myeloid leukemia. *Clin Cancer Res*, 15(14):4750–4758.

12. Singh O, Chan JY, Lin K, Heng CCT, Chowbay B (2012) SLC22A1-ABCB1 Haplotype Profiles Predict Imatinib Pharmacokinetics in Asian Patients with Chronic Myeloid Leukemia. *PLoS ONE*, 7(12): e51771.

13. Shah SP, Köbel M, Senz J, Morin RD, Clarke BA, Wieand KC, Leung G, Zayed A, Mehl E, Kalloger SE et al. (2009). Mutation of FOXL2 in granulosa-cell tumors of the ovary. *N Engl J Med*, 360:2719–2729.

14. Tiacci E, Trifonov V, Schiavoni G, Holmes A, Kern W, Martelli MP, Puccioni A, Bigema B, Pacini R, Wells VA et al (2011). *BRAF* mutations in hairy-cell leukemia. *N Engl J Med*, 364:2305–2315.

15. Mardis ER, Ding L, Dooling DJ, Larson DE, McLellan MD, Chen K, Koboldt DC, Fulton RS, Delehanty KD, McGrath SD et al. (2009). Recurring mutations found by sequencing an acute myeloid leukemia genome. *N Engl J Med*, 361:1058–1066.

16. Coonrod EM, Durtschi Fau - Margraf RL, MargrafRL Fau, Voelkerding KV (2013). Developing genome and exome sequencing for candidate gene identification in inherited disorders: an integrated technical and bioinformatics approach. *Arch Pathol Lab Med*, 137(3):415-33.