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Case Study

Genes Profiling of a Patient with Chronic Myeloid Leukemia on Illumina MiSeq Platform: A Cases Report

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Abstract

CML is a malignant disease of the pluripotent hematopoietic stem cell characterized by the Philadelphia chromosome (Ph) and a rearrangement between the BCR gene (break-point cluster region) and the ABL gene. Albeit multiple advances of research in this field, the molecular mechanisms leading to establishment and development of this disease are not completely understood. The incidence of CML is influenced by different parameters like: demography, gender, ethnicity and socio-economic difference.

We undertook this study to establish a repertoire of genes involved in the symptomatology of CML in Hail region (Saudi Arabia). We ran exome sequencing on blood genomic DNA (gDNA) from a 55-year-old Saudi patient on the Illumina Platform (San Diego, CA USA) as part of a pilot study preluding the establishment of genomic database in relation with this disease. The sequencing results were analysed using the GATK Bioinformatics suit system.

Indel realignment followed by base recalibration and HaplotypeCaller program generated a VCF file which outputs all the variants including SNPs and INDELS. After filtration and application of diseases association program and removal of genes of low quality and synonymous mutations, we obtained 244 unique genes that have been found to be associated with Leukemia. After intronic filtration variants, we obtained exonic variants: BCL6, CASP7, ILIA, RAGI, MMP8 and 14 are genes with possible association with CML. These genes are of great of interest in that, they are linked in the development and progression of CML. Lastly, using R-language data manipulation software and successive filtration of our raw data before exonic filtration, we obtained reduction variants. JAK1 and MLP resulted to be the variants of interest resulting from this merge and these two variants are associated with Myeloproliferative Disorders Philadelphia Chromosome.

Case Report

A 55 years old tribal ma n came to King Khalid Hospital, Ha'il, KSA, suffering from left abdominal pain, weight loss and extreme fatigue. CBC showed increased WBC and differential count revealed the presence of elevated neutrophils with the presence of precursors. He was then suspected of suffering from chronic myelocytic leukemia (CML). The patient was transferred to King Faisal Specialist Hospital (KFSH) in Riyadh, where cytogenetic study reveals the presence of the Philadelphia chromosome and underwent subsequent treatment. Whole blood sample was sent to our lab for genetic profiling using the whole genome sequencing (WGS) technology.

Exome Library Preparation and DNA Sequencing

High quality gDNA was purified from whole blood using a Genomic DNA Purification Kit (QIA amp DNA Blood Mini Kit from Qiagen, Hilden, Germany as reported initially [1]. Brief, the library construction was conducted using the Illumina Nextera Kit (V3 Chemistry).50 ng of gDNA was utilised using transposase based chemistry to create exome libraries. 2x 75 paired end cycles were performed (+1 cycle to each forward and reverse read to allow for phasing/pre-phasing).

Bioinformatics Analysis

fastq files were concatenated from multiple runs

Adapter trimming and base quality scores (less than Q30) were removed using Cutadapt

FastQC was used to check primary and post trimmed sequences

Alignments to the reference human genome (hg19) were conducted using BWA (version 0.7.15)

The Genome Analysis Tool Kit (version 3.0.0) was used for base quality score recalibration, variant calling following by hard filtering to identify high quality variants for downstream analyses

SnpEffv4.1 was exploited to determine in silico impacts upon protein function of candidate genes

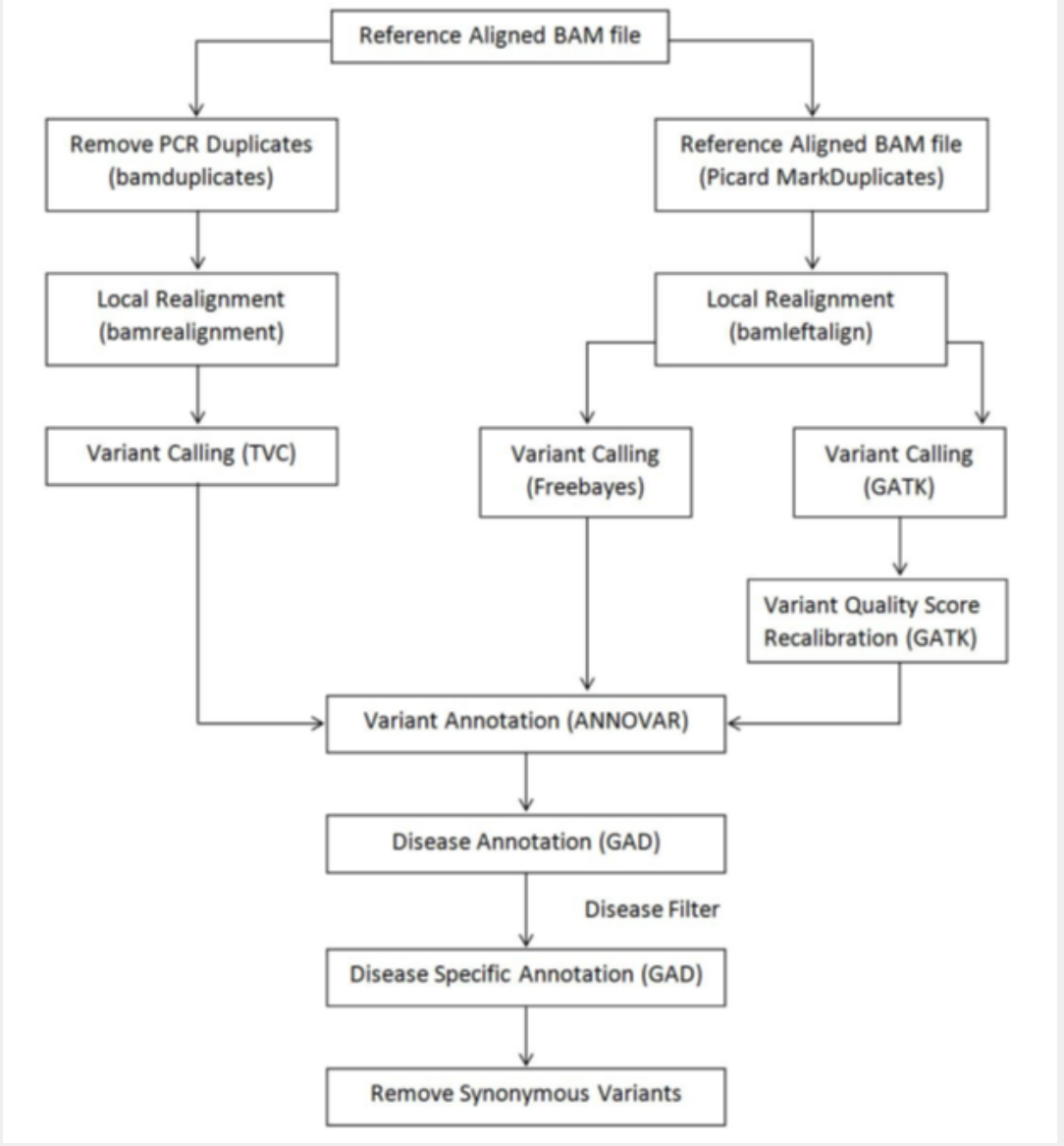

Figure 1: *Exome sequencing analysis pipeline listing various steps and tools from GATK.*

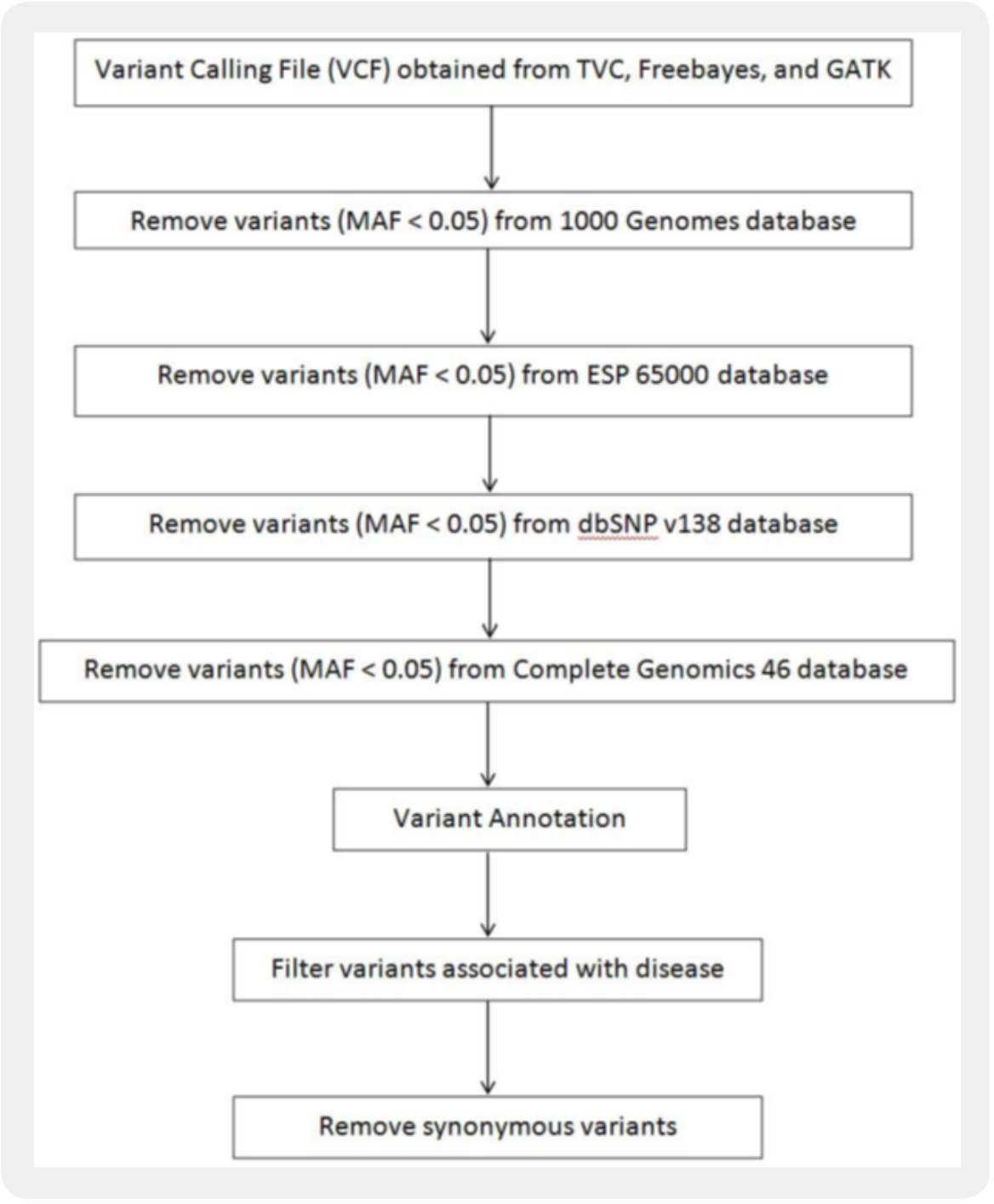

Figure 2: *Variant reduction pipeline performed using ANNOVAR tool.*

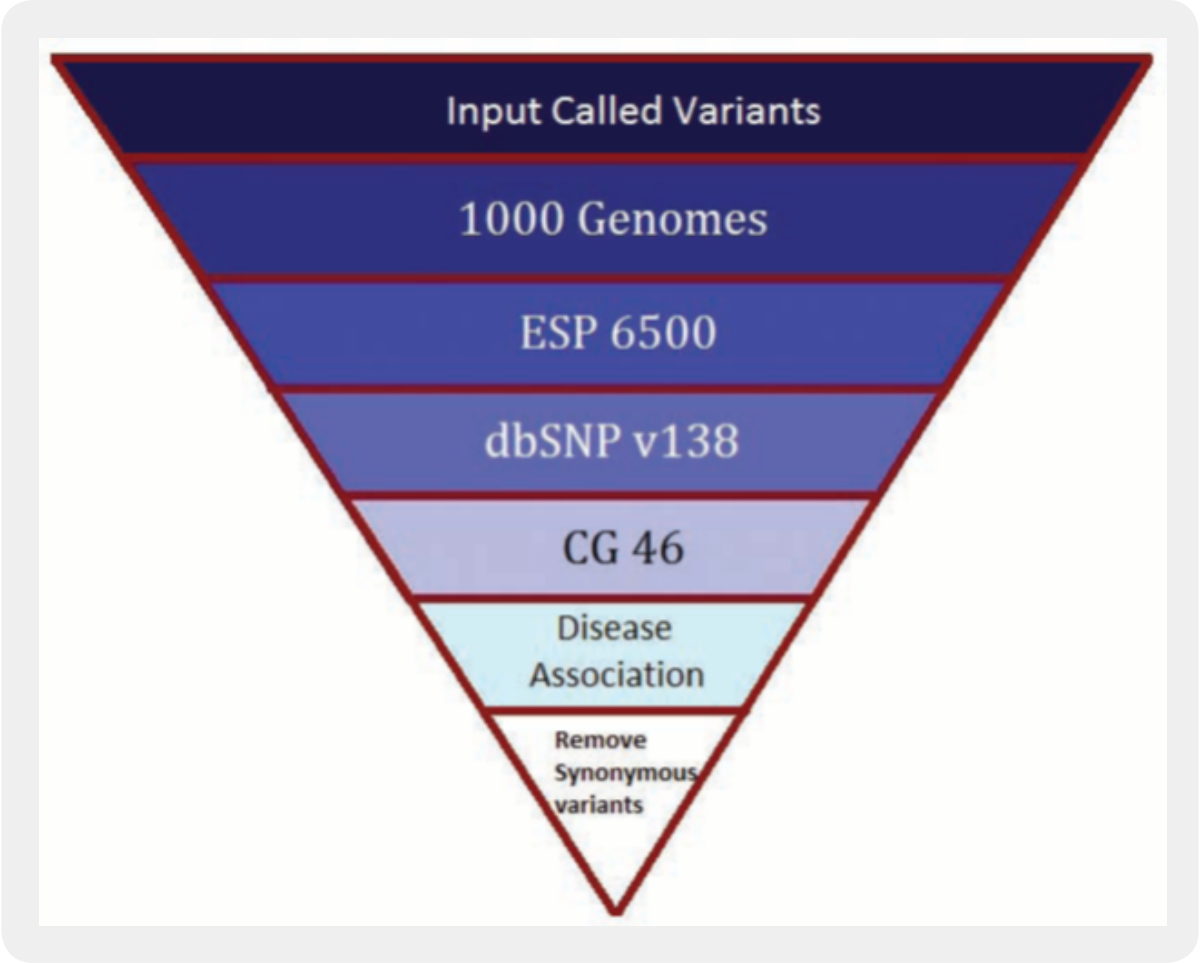

Figure 3: *Schematic of the steps illustrating variant reduction protocol performed for Sample-1 and Sample-30. (Please note that variants were filtered having MAF lower than 0.05.).*

Discussion

The pathophysiology and the mechanisms leading to CML development still remained completely un-understood. CML is a malignant disease of the pluripotent hematopoietic stem cell characterized by the Philadelphia chromosome (Ph) [2] (25) and a rearrangement between the BCR gene (break-point cluster region) and the ABL gene [3] (26). Clinically, CML is characterized by a massive expansion of immature progenitors and precursors that leave the marrow microenvironment prematurely [4] (27). Although normal progenitors coexist with the malignant clone in CML, their growth seems to be inhibited, possibly as consequence of some anomalies in CML microenvironment itself [5] (28). The mechanisms that underlie the massive expansion of Ph + hematopoietic progenitors, precursors, and mature cells are also not understood and why CML progenitors circulate prematurely in the blood is not completely understood neither [6].

We undertook WES to access the genetic profile of this patient suffering from leukemia. The variants profile in table 1 corresponding to exonic reduction shows multiple genes of interest like: BCL6, CARD8, CASP7, FBXW7, IL1A, IL4R and RAG1. Indeed, CASP7 is strongly involved in cancer development. Park *et al* [7] have demonstrated an association between CASP7 and CASP14 in the genetic polymorphisms with the risk of childhood leukemia development by running the minimum *P*-value (min*P*) and the false discovery rate (FDR) test. This approach confirms our findings characterized by exonic frameshift deletion of two TT in CASP 7 located on chromosome 10.

Table 1: *List of critical variants after filtration of non-synonymous and frame-shift deletion*

Gene.ref-Gene	Chr	Start	End	Ref	Alt	Func.refGene	ExonicFunc.refGene
APEX1	14	20925154	20925154	T	G	exonic	nonsynonymous SNV
BCL6	3	187446211	187446211	C	T	exonic	nonsynonymous SNV
CAPN2	1	223900408	223900408	C	G	exonic	nonsynonymous SNV
CAPN9	1	230895340	230895340	C	A	exonic	nonsynonymous SNV
CARD8	19	48737706	48737706	A	T	exonic	nonsynonymous SNV
CASP7	10	115439641	115439642	TT	-	exonic	frameshift deletion
CCNH	5	86695274	86695274	A	G	exonic	nonsynonymous SNV
CD5	11	60892606	60892606	A	G	exonic	nonsynonymous SNV
CDC25C	5	137665323	137665323	G	A	exonic	nonsynonymous SNV
CDC6	17	38457151	38457151	G	A	exonic	nonsynonymous SNV
CENPF	1	214811244	214811244	C	G	exonic	nonsynonymous SNV
CTLA4	2	204732714	204732714	A	G	exonic	nonsynonymous SNV
CYP1A1	15	75014027	75014027	A	G	exonic	nonsynonymous SNV
CYP1B1	2	38298203	38298203	C	G	exonic	nonsynonymous SNV
CYP4B1	1	47282772	47282772	C	T	exonic	nonsynonymous SNV
DCLRE1C	10	14968855	14968855	G	C	exonic	nonsynonymous SNV
							SNV
EGF	4	110914427	110914427	A	T	exonic	nonsynonymous SNV
EZH2	7	148525904	148525904	C	G	exonic	nonsynonymous SNV
FBXW7	4	153247170	153247170	G	-	exonic	frameshift deletion
FMO2	1	171168545	171168545	T	C	exonic	nonsynonymous SNV
FMO3	1	171076966	171076966	G	A	exonic	nonsynonymous SNV
GNAS	20	57415876	57415876	C	A	exonic	nonsynonymous SNV
GNB3	12	6954864	6954864	G	A	exonic	nonsynonymous SNV
GSTA2	6	52617731	52617731	C	G	exonic	nonsynonymous SNV
GSTP1	11	67352689	67352689	A	G	exonic	nonsynonymous SNV
GSTZ1	14	77794283	77794283	T	C	exonic	nonsynonymous SNV
GZMB	14	25100282	25100282	A	G	exonic	nonsynonymous SNV

HIST1H1T	6	26108168	26108168	G	A	exonic	nonsynonymous SNV
HLA-C	6	31239827	31239827	C	T	exonic	nonsynonymous SNV
HLA-DRB5	6	32485524	32485524	C	A	exonic	nonsynonymous SNV
HSPA1L	6	31777946	31777946	C	T	exonic	nonsynonymous SNV
IGF2R	6	160494409	160494409	A	G	exonic	nonsynonymous SNV
IL13	5	131995964	131995964	A	G	exonic	nonsynonymous SNV
IL1A	2	113537223	113537223	C	A	exonic	nonsynonymous SNV
IL4R	16	27357927	27357927	C	T	exonic	nonsynonymous SNV
ITGAE	17	3657175	3657175	T	C	exonic	nonsynonymous SNV
KCNE4	2	223917983	223917983	T	G	exonic	nonsynonymous SNV
KIR2DS4	19	55350979	55350979	A	G	exonic	nonsynonymous SNV
KIR3DL1	19	55340906	55340906	G	A	exonic	nonsynonymous SNV
LIG4	13	108863591	108863591	G	A	exonic	nonsynonymous SNV
LTA	6	31540556	31540556	T	C	exonic	nonsynonymous SNV
MCM4	8	48885436	48885436	T	A	exonic	nonsynonymous SNV
MDC1	6	30680721	30680721	G	A	exonic	nonsynonymous SNV
MMP14	14	23306048	23306048	C	T	exonic	nonsynonymous SNV
MMP8	11	102593248	102593248	T	C	exonic	nonsynonymous SNV
MTHFD1	14	64908845	64908845	G	A	exonic	nonsynonymous SNV
MTRR	5	7870973	7870973	A	G	exonic	nonsynonymous SNV
MYH11	16	15820863	15820863	C	T	exonic	nonsynonymous SNV
NAT2	8	18258316	18258316	G	A	exonic	nonsynonymous SNV
NLRP11	19	56321414	56321414	C	A	exonic	nonsynonymous SNV
NLRP4	19	56369189	56369189	G	A	exonic	nonsynonymous SNV
NLRP8	19	56487603	56487603	A	G	exonic	nonsynonymous SNV
P2RX7	12	121592689	121592689	T	C	exonic	nonsynonymous SNV
PARD3	10	34649103	34649103	A	C	exonic	nonsynonymous SNV
RAG1	11	36595600	36595600	A	G	exonic	nonsynonymous SNV
RCAN3	1	24861704	24861704	G	A	exonic	nonsynonymous SNV
SELP	1	169566313	169566313	C	T	exonic	nonsynonymous SNV
SLC22A1	6	160551204	160551204	G	C	exonic	nonsynonymous SNV
SP110	2	231072709	231072709	C	T	exonic	nonsynonymous SNV
TGFB1	19	41858921	41858921	G	A	exonic	nonsynonymous SNV
TYK2	19	10488926	10488926	C	T	exonic	nonsynonymous SNV
ULK3	15	75130093	75130093	T	C	exonic	nonsynonymous SNV
XRCC2	7	152346007	152346007	C	T	exonic	nonsynonymous SNV
XRCC3	14	104165753	104165753	G	A	exonic	nonsynonymous SNV
ZNF230	19	44515514	44515514	C	A	exonic	nonsynonymous SNV

Table 2: *Disease associated with Myeloproliferative Disorders Philadelphia Chromosome*

Gene.ref Gene	Chr	Start	End	Ref	Alt	Func.ref Gene
JAK1	chr1	65419919	65419919	T	C	intronic
JAK1	chr1	65392026	65392026	T	C	intronic
JAK1	chr1	65397542	65397542	A	G	intronic
MPL	chr1	43809533	43809533	A	-	intronic
MPL	chr1	43812075	43812075	G	A	intronic
MPL	chr1	43814864	43814864	T	C	intronic

The development and growth of any types of cancer is under the control of cancer-initiating cells (CICs), a cell population with the same attribute like stem cells [8]. In addition to their self-renewal and multipotency properties, stem cells are endowed with their own cell cycle features. Uncovering key mechanisms underlying their cell cycle control will shed the light in the regulation of the self-renewal and differentiation processes [9]. The (Fbxw7) is a key regulator gene of the cell cycle involved in the maintenance of normal stem cells and CICs Takeishi *et al.* [10,11] The Fbxw7 gene products are characterized by three isoforms (Fbxw7α, β, and -γ) that differ only at their amino termini, with each isoform possessing the dimerization domain. Fbxw7 plays pivotal roles in cell division, growth, and differentiation by targeting several proteins - including: c-Myc, Notch1, Notch4, c-Jun, and cyclin E - for degradation [12,13]. Fbxw7 binds each of these substrates through a conserved phosphorylated domain known as the Cdc4 phosphodegron. Given that most of these proteins targeted by Fbxw7 for degradation are proto-oncoproteins, Fbxw7 has been thought to function as a tumor suppressor. Indeed, in heterozygous mutations Fbxw7 have been detected in several types of human cancer, including T-cell acute lymphoblastic leukaemia (T-ALL), T-cell lymphoma, and cholangiocarcinoma [13,14]. Nearly three-quarters of these mutations are point mutations that result in amino-acid substitutions at key positions in the WD40 repeats and consequent disruption of substrate binding. These clinical observations thus indicate that Fbxw7 is crucial for preventing carcinogenesis as a result of its role in cell cycle regulation. Our reductional variants list after filtration of intronic variants indicates frameshift deletion of G.

Conclusion

In summary, we have run the whole exome sequencing on Saudi male adult blood genomic DNA using Illumina platform. Analysis and subsequent filtration identified 244 unique genes have been found to be associated with Leukemia. Exonic filtration reveals 14 gene of interest that are associated to the development and progression of CML. Of importance is the identification of two variants: JAK1 and MLP which are associated with Myeloproliferative Disorders Philadelphia Chromosome. More studies remained to be conducted via SNPs genotyping specifically associate these variants to CML and ultimately conduct clinical studies.

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