

MDR-1 POLYMORPHISMS (G2677T & C3435T) IN B-CHRONIC LYMPHOCYTIC LEUKEMIA

Thesis

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List of Abbreviations

α	Alpha
μg	Microgram
μL	Microlitre
μ	Mu
ABCB1	ATP-binding cassette sub-family B member 1
ADP	Adenosine diphosphate
AEI	Allelic expression imbalance
ALC	Absolute lymphocyte count
ALL	Acute lymphoblastic leukaemia
AML	Acute myeloblastic leukaemia
ATP	Adenosine triphosphate
Bad	Bcl-2/BCL-XL-associated death promoter
Bak	Bcl-2 homologous antagonist/killer
Bax	Bcl-2-associated factor X
Bcl	B cell leukemia/lymphoma
B-CLL	B cell chronic lymphocytic leukemia
Bik	Bcl-2 interacting killer
$\beta 2\text{MG}$	Beta 2 microglobulin
CBC	Complete blood count
CCL21	Chemokine ligand 21
CCR7	Chemokine receptor type 7
CD	Cluster of differentiation
CHOP	Cyclophosphamide, doxorubicin, vincristin and prednisolone
CLL	Chronic lymphocytic leukemia
CML	Chronic myloid leukemia
DNA	Deoxyribonucleic acid
DW	Distilled water
dL	Decilitre

dsDNA	Double stranded DNA
EBV	Epstein Barr virus
EDTA	Ethylene diamine tetraacetic acid
FAB	French American British classification
FC	Fludarabin with cyclophosphamide
FCL	Follicular cell lymphoma
FCR	Fludarabin, cyclophosphamide and rituximab
FISH	Fluorescence in situ hybridization
FMC7	Mouse Anti-Human B-Cells
FR	Fludarabin with rituximab
g	Gram
Hb	Hemoglobin
HCL	Hairy cell leukemia
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
Hrk	Harakini, Bcl-2 interacting protein
HSCT	Haematopoietic stem cell transplantation
HSM	Hepatosplenomegaly
Ig	Immunoglobulin
IgVH	Immunoglobulin variable region heavy chain gene
IU	International unit
Kb	Kilo bite
kD	Kilo Dalton
L	Litre
LDH	Lactate dehydrogenase
LDT	Lymphocyte doubling time
M	Molar
Mcl-1	Myeloid cell leukemia sequence 1
MCL	Mantle cell lymphoma
MDR	Multiple drug resistance

MHC	Major histocompatibility complex
mAb	Monoclonal antibody
mL	Millilitre
mRNA	Messenger RNA
N	Normal
NCI	National Cancer Institute
NK	Natural killer
ng	Nanogram
OD	Optical density
P53	(P) means protein and the number after it represents the molecular weight of this protein
PB	Peripheral blood
PCR	Polymerase chain reaction
PCR-RFLP	Polymerase chain reaction restriction fragment length polymorphism
Pgp	P glycoprotein
PLL	Prolymphocytic leukemia
q	Long arm of the chromosome
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase PCR
REs	Restriction enzymes
rpm	Revolution per minute
sCD23	Soluble CD23
slg	Surface immunoglobulin
SLL	Small lymphocytic lymphoma
SLVL	Splenic lymphoma with villous lymphocytes
SNPs	Single nucleotide polymorphisms
sTK	serum thymidine kinase
TCR	T cell receptor
TLC	Total leukocytic count

t..... Translocation
UV.....Ultraviolet
VAD.....Vincristin,adriamycin and dexamethazone
VH..... Variable region heavy chain gene
WBC.....White blood cell
WHO World Health Organization
ZAP70..... Zeta-chain associated protein kinase

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Abstract

The human multidrug resistance (MDR-1) gene encodes P-glycoprotein (Pgp), which affects the pharmacokinetics of many drugs. Here, we investigated whether common MDR1 single nucleotide polymorphisms (SNPs) (C3435T and G2677T) affect predisposition to B-chronic lymphocytic leukemia (B-CLL). Genotyping was performed in 65 patients with CLL and 70 controls using polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP). We observed a higher frequency of carriers of 3435CT gene among B-CLL patients as compared to normal individuals (58.5% vs. 22.9%, $p < 0.001$). The genotypes 3435CT was associated with B-CLL, [odds ratio=4.8, 95% confidence interval=2.3-10.0]. Moreover, patient and control groups did not differ significantly regarding MDR1 genotype (G2677T). Furthermore, no correlation was shown between the MDR1 (3435 or 2677) genotypes and clinical and laboratory data of patient group. In conclusion, these data indicate that the MDR1 C3435T SNP may carry an increased risk of developing B-CLL.

Key Words:

PgP – MDR-1 – B-CLL .

INTRODUCTION

B-Chronic lymphocytic leukemia is one of the most common hematological malignancies that results in significant morbidity and mortality. It is caused by the clonal expansion of B cells with a distinctive morphological appearance and surface immunophenotype. One of the most striking features of CLL is the extent of its clinical variability. Some studies have been developed to understand this variability in terms of biological heterogeneity (*Pettitt, 2007*).

The incidence of CLL is higher among whites than blacks. The incidence of CLL is higher in males than in females, CLL is a disease that primarily affects the elderly (*Gribben, 2010*).

Most people are diagnosed without symptoms as the result of a routine blood test that returns a high white blood cell count, but as it advances CLL results in swollen lymph nodes, spleen, and liver, and eventually anemia and infections. Early CLL is not treated, and late CLL is treated with chemotherapy and monoclonal antibodies. It is now possible to diagnose patients with short and long survival more precisely by examining the DNA mutations, and patients with slowly-progressing disease can be reassured and may not need any treatment in their lifetimes (*Chiorazzi et al., 2005*).

The prognosis of patients with CLL varies widely at diagnosis. Some patients die rapidly, within 2-3 years of diagnosis, because of complications from CLL. Most patients live 5-10 years,

with an initial course that is relatively benign but followed by a terminal, progressive, and resistant phase lasting 1-2 years. During the later phase, morbidity is considerable, both from the disease and from complications of therapy (*Rai & Keating, 1997*).

Prognosis depends on the disease stage at diagnosis as well as the presence or absence of high-risk markers (*Kristinsson et al., 2009*).

The multidrug resistance 1 gene (MDR1) product P-glycoprotein (Pgp) is an important member of ATP-binding cassette transporter family that functions as an energy dependent xenobiotic efflux pump (*Gervasini et al., 2006*). Therefore, the most important physiological role of Pgp is the protection of the organism against toxic xenobiotic agents and environmental carcinogens (*Jamroziak et al., 2004 and Gervasini et al., 2006*). Moreover, as a wide range of important anticancer agents such as drugs used in chemotherapy can be actively extruded by this protein, Pgp expression in tumor cells is associated with multidrug resistance phenotype in some malignancies, including leukemia (*Jamroziak et al., 2004, 2005*).

Pgp is expressed at the highest level in adrenal gland; at an intermediate level in breast, stomach, colon, jejunum, rectum, liver, kidney, and placenta; and at a low level in testis, brain, bone marrow, and heart in humans. Its wide tissue distribution suggests that Pgp has a fundamental role in normal cellular metabolism (*Hitchins et al., 1988*).

MDR1 is a polymorphic gene with more than 40 single-nucleotide polymorphisms (SNPs). The most important MDR1 gene polymorphism is C3435T SNP, which is located on exon 26 and considered to be associated with decreased tissue protein expression and activity (*Hoffmeyer et al., 2000*).

A number of studies indicated that the presence of the 3435T allelic variant and particularly the homozygous mutant 3435TT genotype seems to be associated with a greater susceptibility to renal epithelial tumors (*Siegsmond et al., 2002*), colon cancer (*Kurzwski et al., 2005*), ulcerative colitis (*Schwab et al., 2003; Glas et al., 2004 and Farnood et al., 2007*), and acute lymphoblastic leukemia (*Jamroziak et al., 2004*).

Aim of the Work

The aim of the present study was to evaluate the role of two MDR1 gene polymorphisms (G2677T polymorphism) in exon 21 and (C3435T polymorphism) in exon 26 as risk factors for development of B-CLL and their relation to the clinical presentation of the patients.