INTRODUCTION

Variable response to drugs has been shown in many clinical settings to be due to polymorphisms in key genes involved in drug transport, uptake, metabolism, or targeting. A number of studies have shown that pharmacogenetic variants can influence response to therapy and toxicity of therapy in children with acute lymphoblastic leukemia (ALL). The best-studied example is the gene thiopurine methyltransferase (TPMT) that modifies metabolism of 6-mercaptopurine (6-MP) ***(Evans et al., 2001; Davies et al., 2008).***

Thiopurine methyltransferase (TPMT) is a cytoplasmic enzyme that preferentially catalyze the S-Methylation of aromatic and heterocyclic sulphydryl compound, such as the thiopurine drugs e.g. 6-Mercaptopurine and thiogunine. These drugs form the same terminal metabolites. one major influence of thiopurine therapy is inherited activity of (TMPT) ***(Lynne, 1998)***.

A series of studies over 20 years have shown that TPMT genotype can modify both disease control and toxicity in children with ALL. However, multiple drugs are used to treat children with ALL, and more recent studies have identified additional loci that may modify response to therapy, including genes participating in folate metabolism, steroid response and drug transport, metabolism, and detoxification. In addition, analysis of the numerous "nongenetic" characteristics, such as age, white cell count, and race that influence treatment response in addition to polymorphic

genotypes is necessary to adequately assess the importance of germ line variation on antileukemic response ***(Relling et al., 1999; Krajinovic et al., 2004; Rocha et al., 2005).***

The activity of thiopurine methyltransferase (TPMT) exhibits genetic polymorphism, with approximately 1 in 300 individuals inheriting TPMT deficiency as an autosomal recessive trait, and about 11% having intermediate activity (ie, heterozygotes). Patients with TPMT deficiency accumulate excessive concentrations of 6-thioguanine nucleotides (TGNs) and develop severe toxicity when treated with standard dosages of mercaptopurine. High TPMT activity has been associated with lower concentrations of TGNs, yielding a higher risk of treatment failure in children with acute lymphoblastic leukemia (ALL). As the biochemical basis of these pharmacodynamic relationships has not been fully elucidated ***(McLeod et al., 1995).***

Accordingly, this study is conducted in attempt to investigate whether a specific host pharmacogenetic polymorphism can affect drug dosage and toxicity.

AIM OF THE WORK

To determine the frequency of Thiopurine Methyl-transferase (TPMT) gene polymorphism in children with Acute Lymphoblastic Leukemia and its correlation with response to chemotherapy and toxicity in children with Acute Lymphoblastic Leukemia.

ACUTE LYMPHOBLASTIC LEUKEMIA

Definition

Acute lymphoblastic Leukemia is a malignant clonal disease of bone marrow in which early lymphoid precursors proliferate and replace the normal hematopoietic cells of bone marrow ***(Seiter, 2002).***

Incidence:

Acute lymphoblastic leukemia (ALL) is the most common malignant disorder in childhood, representing nearly one third of all pediatric cancers ***(Ek et al., 2004).***

Annual incidence of ALL is about 30 cases per million people, with a peak incidence in people aged 2-5 years ***(Pui et al., 2008).*** In the US, each year, 2000-2500 new cases of childhood ALL are diagnosed. Internationally , the incidence rate is thought to be similar throughout the world to that in the United States ***(Rubnitz and Pui, 2003).***

Age:

The peak incidence as mentioned above occurs between 2-5 years, while in developed countries is 1-4 years. This may reflect underlying social-economic factors including infant care, breast feeding, hygiene and pollution ***(Hrusak et al., 2002).*** It is rare in infant below one year of age ***(Somjee et al., 2002).***

Sex:

In childhood ALL, males are more often affected than females with notable exception of female predominance in infancy ***(Smita et al., 1999).***

In Egypt ALL more common in males with male to female ratio 1.6 :1 ***(Khalifa et al., 1999).***

Racial and ethnic variation:

ALL incidence is higher among white children in most age groups. Incidence rates for ALL are highest in the United States, Australia, Costa Rica and Germany. Rates are intermediate in most European countries, and lowest in India and among black children in the United States ***(Smita et al., 1999).***

Etiology:

Childhood leukemia is a biologically diverse disease, so several pathways to its development are possible. All probably combine genetic susceptibility and exposure to external risk factors at a time when the child is vulnerable (**Dickinson,2005).**

Environmental factors:

Draper et al. (2005) investigated whether there is an association between distance home address at birth from high voltage power liens and the incidence of leukemia in children in England and Wales. They concluded that there is an association

between childhood leukemia and proximity of home address at birth to high voltage power lines, and the apparent risk extends to a greater distance than would have been expected from previous studies.

Steffen and coworkers (2004) analyzed the association between potential environmental exposure to hydrocarbons and the risk of acute childhood leukemia. Results showed an association between acute childhood leukemia and dwellings neighboring auto repair garages and petrol stations, which are benzene emitting sources. These finding could be to chance, although the strength of the association and the duration trend are arguments for a causal association

Also irradiation is an important environmental factor as the leukemogenic potential of ionizing radiation has been documented in studies of survivors of atomic bombing of Japan ***(Tomonaga et al., 1994).***

Viral infection have been consistently associated with some human cancer. Epestien –Barr virus (EBV) has been implicated as a causative factor in B-cell lymphoma, while the human T-cell leukemia lymphoma virus (HTLV-1 and HTLV-2)have been isolated from adults with T-cell leukemia ***(Judith, 1996).*** Infection with HTLV1 is associated with T-cell leukemia that may be predisposed by genetic lesions associated with HTLV1 infection such as mutation of P53 and deletion of P16 ***(Pombo, 2002).***

Observations of a peak age of development of childhood acute lymphoblastic leukaemia of 2–5 years, an association of industrialization and modern societies with increased prevalence of the disease, and the occasional clustering of childhood leukaemia cases (especially in new towns) have fuelled two parallel infection-based hypotheses by British investigators: Kinlen's population-mixing hypothesis and Greaves' delayed-infection hypothesis **(Figure 1)** ***(Greaves, 2006).***

Kinlen's hypothesis predicts that clusters of childhood cases of acute lymphoblastic leukaemia result from exposure of susceptible (non-immune) individuals to common but fairly non-pathological infections after population-mixing with carriers. The delayed-infection hypothesis of Greaves is based on a minimal two-hits model and suggests that some susceptible individuals with a prenatally acquired preleukaemic clone had low or no exposure to common infections early in life because they lived in an affluent hygienic environment. Such infectious insulation predisposes the immune system of these individuals to aberrant or pathological responses after subsequent or delayed exposure to common infections at an age commensurate with increased lymphoid-cell proliferation ***(Pui et al., 2008).***

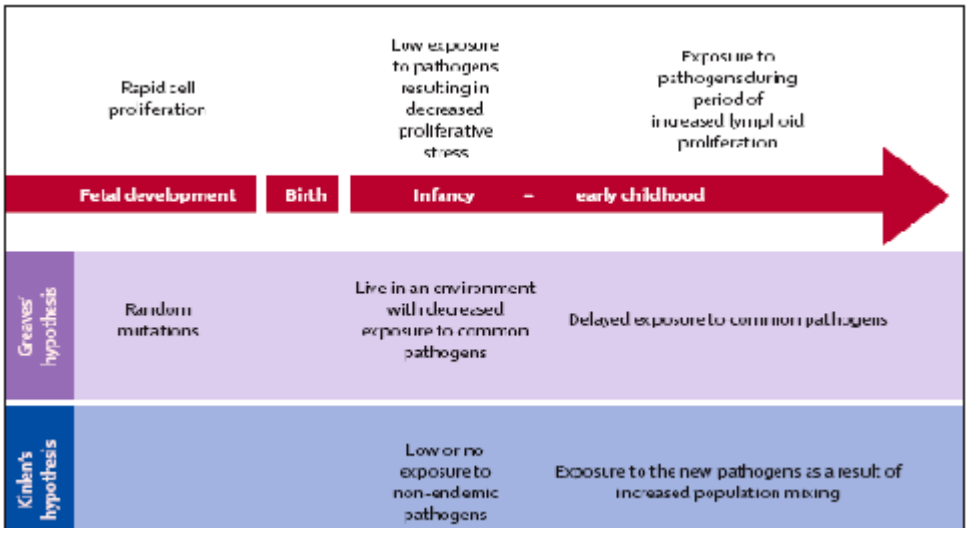

Figure (1): Infection-based models of leukaemia development ***(adapted from Pui et al., 2008).***

Genetic factor:

Studies suggest nearly a 20-fold increased risk of leukemia in individuals with down syndrome. Most of this increased risk appears in the first few decades of life. With the highest incidence in children less than 5 years of age. It is unknown why children with down syndrome are at such an increased risk of leukemia ***(Ross et al., 2005).***

The enzymes GSTM1, GSTT1, GSTP1, CYP1A1 and CYP2E1 are involved in the bioactivation and detoxification of a variety of xenobiotics present in food, organic solvents, tobacco smoke, drugs, alcoholic drinks, pesticides and environmental pollutants. Polymorphisms in the gene coding for these enzymes have been associated with increased susceptibility to different cancers, including hematologic malignancies. The data revealed

that carriers of the rare GSTP1 Val allele were at higher risk of ALL in children ***(Canelle et al., 2004).***

Retrospective identification of leukaemia-specific fusion genes, hyperdiploidy, or clonotypic rearrangements of immunoglobulin or T-cell-receptor loci in archived neonatal blood spots (Guthrie cards) and studies of leukaemia in monozygotic twins indicate clearly a prenatal origin for some childhood leukaemias ***(Greaves, 2006; Hong et al., 2008).***

Screening of neonatal cord-blood samples has revealed a putative leukaemic clone with the TEL-AML1 fusion gene (also known as ETV6-RUNX1) in 1% of newborn babies, a frequency 100 times higher than the prevalence of acute lymphoblastic leukaemia defined by this fusion gene later in childhood ***(Mori et al., 2002)***. The variable incubation period and clinical outcome of such cases, and the 10% concordance rate of leukaemia in identical twins with this genotype, support the notion that additional postnatal events are needed for full leukaemic transformation ***(Greaves and Wiemels, 2003).*** A recent study further established the presence of a preleukaemic clone with the TEL-AML1 fusion ***(Hong et al., 2008).***

Pathophysiology:

Normally, the bone marrow makes blood stem cells (immature cells) that develop into mature blood cells over time. A blood stem cell may become a myeloid stem cell or a lymphoid stem cell **(Figure 2)**

Acute lymphoblastic leukaemia is thought to originate from various important genetic lesions in blood-progenitor cells that are committed to differentiate in the T-cell or B-cell

pathway, including mutations that impart the capacity for unlimited self-renewal and those that lead to precise stage-specific developmental arrest ***(Armstrong, 2005 and Pui et al., 2008).***

In some cases, the first mutation along the multistep pathway to overt acute lymphoblastic leukaemia might arise in a haemopoietic stem cell possessing multilineage developmental capacity ***(Wang and Dick, 2005).*** The cells implicated in acute lymphoblastic leukaemia have clonal rearrangements in their immunoglobulin or T-cell receptor genes and express antigen-receptor molecules and other differentiation-linked cell-surface glycoproteins that largely recapitulate those of immature lymphoid progenitor cells within the early developmental stages of normal T and B lymphocytes ***(Pui et al., 2008).***

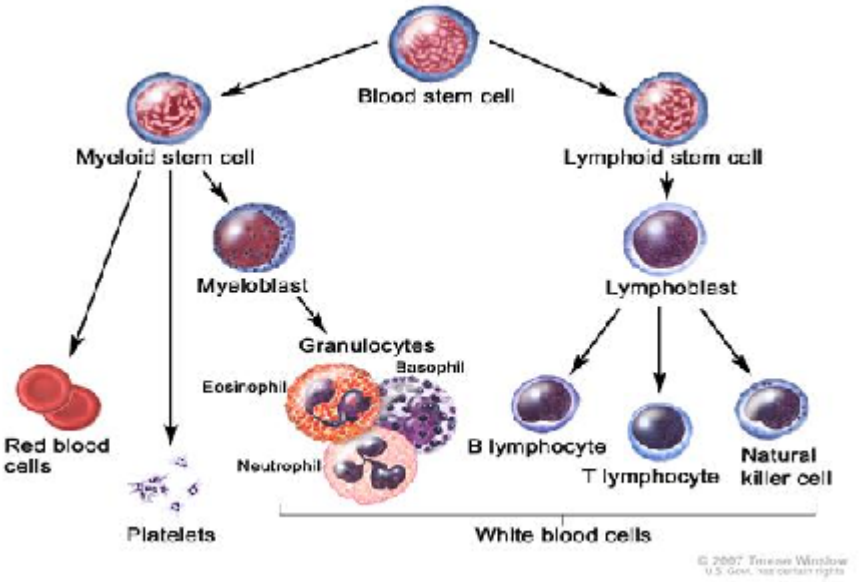

Figure (2): Normal Blood cell development. A blood stem cell goes through several steps to become a red blood cell, platelet, or white blood cell ***(adapted from Web site, NCI, 2009).***

Cytogenetics:

Karyotyping of leukemic cells is an important factor in the diagnosis of ALL and is also considered as an independent prognostic indicator, with an impact on the choise of treatment ***(Harrison and Foroni, 2002).***

Clonal cytogenetic abnormalities, both structural and numerical, are found in 80% of ALL cases ***(Mckenna, 2000).***

Chromosomal translocation*:*

Chromosomal translocations that activate specific genes are a defining characteristic of human leukaemias and of acute lymphoblastic leukaemia in particular ***(Armstrong, 2005)***. Gene-expression patterns studied in large series of newly diagnosed leukaemias have substantiated the idea that specific chromosomal translocations identify unique subtypes of the disease ***(Lu et al., 2005).***

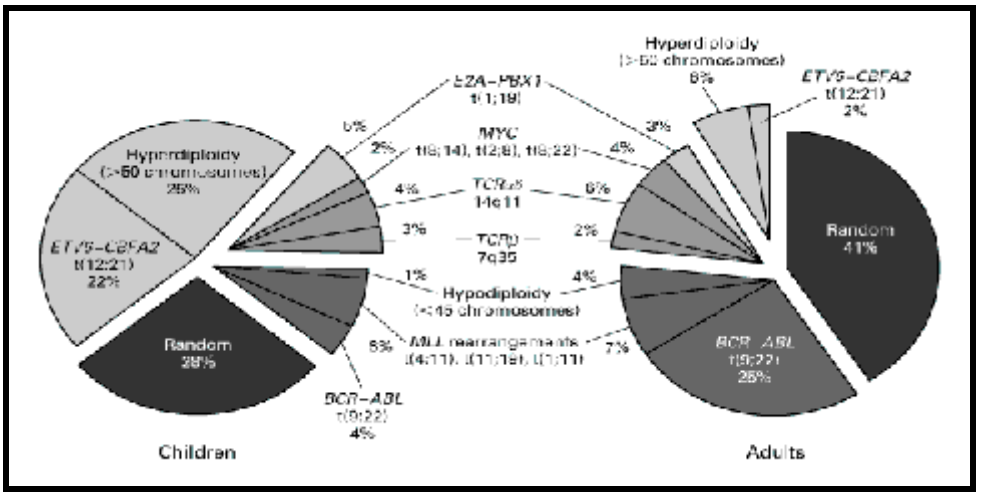

Figure (3): Cytogenic abnormalities in patient with ALL
(adapted from Pui and Evans, 1998).

Usually, translocations activate transcription-factor genes, which in many cases can control cell differentiation (rather than cell division perse), are developmentally regulated, and frequently encode proteins at the apex of important transcriptional cascades. These so-called master oncogenic transcription factors, which can exert either positive or negative control over downstream responder genes, are expressed aberrantly in leukaemic cells as one gene product or as a unique fusion protein combining elements from two different transcription factor ***(Pui et al., 2008).***

About 25% of cases of B-cell precursor acute lymphoblastic leukaemia, the most frequent form of acute leukaemia in children, harbour the TEL-AML1 fusion gene—generated by the t(12;21)(p13;q22) chromosomal translocation ***(Pui et al., 2008).***

Table (1): Common Cytogenetic translocations associated with specific molecular genetic abnormalities in ALL

Cytogenetic translocation	Molecular genetic abnormality	%
t (12;21)CRYPTIC	TEL-AML1 fusion	25.4%
t (1;19)(q23;p13)	E2A-PBX (PBX1) fusion	4.8%
t (9;22)(q34;q11)	BCR-ABL fusion(P185)	1.6%
t (4;11)(q21;q23)	MLL-AF4 fusion	1.6%
t (8;14)(q24;q32)	IGH-MYC fusion	
t(11;14)(p13;q11)	TCR-RBTN2 fusion	

(Pakakasama et al., 2008)

Although the molecular pathogenesis of TEL-AML1-positive leukaemia remains unclear, findings in mice establish the Tel gene as an important regulator of haemopoietic-cell development, essential for definitive haemopoiesis ***(Hock et al., 2004).*** Similarly, Aml1 gene is essential for definitive embryonic haemopoiesis ***(Pui et al., 2008).*** Thus, the presence of the TEL-AML1 fusion protein in B-cell progenitors seems to lead to disordered early B-lineage lymphocyte development, a hallmark of leukaemic lymphoblasts. Analysis of TEL-AML1-induced cord blood cells suggests that the fusion gene serves as a first-hit mutation by endowing the preleukemic cell with altered self-renewal and survival properties ***(Hong et al., 2008).***

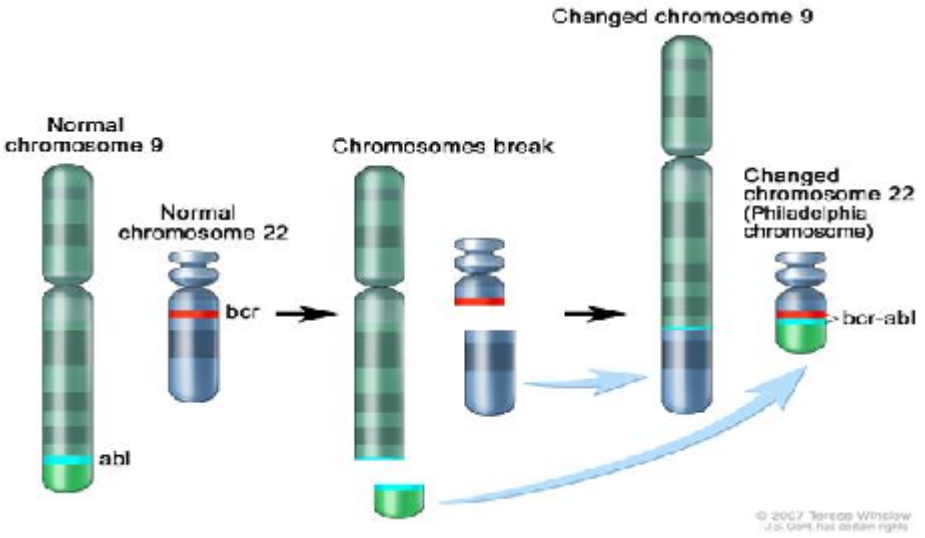

Figure (4): Philadelphia chromosome. A piece of chromosome 9 and a piece of chromosome 22 break off and trade places ***(adapted from web site; NCI, 2009).***

In adults, the most frequent chromosomal translocation is t(9;22), or the Philadelphia chromosome **(Figure 4),** which causes fusion of the BCR signalling protein to the ABL non-receptor tyrosine kinase, resulting in constitutive tyrosine kinase activity and complex interactions of this fusion protein with many other transforming elements, such as the signalling pathway for RAS (GTP-binding protein that activates target genes involved in cell differentiation, proliferation, and survival) ***(Ren, 2005)***.

As an activated kinase, BCR-ABL offers an attractive therapeutic target, and imatinib mesilate, a small molecule inhibitor of the ABL kinase, has proven to be effective against leukaemias that express BCR-ABL ***(Pui et al., 2008).***

Cooperating mutations:

Although chromosomal abnormalities are a hallmark of pathogenesis of acute lymphoblastic leukaemia, evidence suggests that they must act in concert with several other genetic lesions to induce overt leukaemia. A prime example is the biallelic deletion or epigenetic silencing of the cyclin-dependent kinase inhibitor 2A gene (CDKN2A), which encodes both the tumour suppressors p16INK4A and p14ARF and whose inactivation neutralises both the TP53 and retinoblastoma pathways in most cases of T-cell and many cases of B-cell precursor acute lymphoblastic leukaemia ***(Pui et al., 2008).***

In a genome-wide analysis of 242 cases of paediatric acute lymphoblastic leukaemia using high-resolution single

nucleotide polymorphism arrays, deletions, amplifications, point mutations, and other structural rearrangements were identified in genes encoding regulators of B-lymphocyte development in 40% of cases of B-cell precursor acute lymphoblastic leukaemia ***(Mullighan et al., 2007).***

The PAX5 gene was the most frequent target of somatic mutation, being altered in almost a third of cases. Deletions were also detected in other B-cell developmental genes, such as TCF3 (E2A), EBF1 (EBF), LEF1, IKZF1 (Ikaros), and IKZF3 (Aiolos). Finally, in T-cell acute lymphoblastic leukaemia, at least five multistep mutational pathways leading to frank leukaemia have been identified, and in some cases these pathways entail five or more documented genetic lesions ***(Grabher and Boehmer, 2006).***

Classification

The FAB classification

Subtyping of the various forms of ALL used to be done according to the French-American-British (FAB) classification .which was used for all acute leukemias (including acute myelogenous leukemia, AML).

- ALL-L1: small uniform cells
- ALL-L2: large varied cells
- ALL-L3: large varied cells with vacuoles (bubble-like features)