Screening of GATA-1 Mutation in Patients with Down Syndrome

Thesis

Submitted for partial fulfillment of the requirements of Doctorate Degree of Medical Genetics

By

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Nada Hammad Abd El Fattah

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List of Abbreviations

Abb.	Full term
AFP	Alpha fetoprotein
AMKL	Acute megakaryocytic leukemia
APP	β-amyloid precursor protein
ASD	Atrial septal defect
AVSD	Atrio-ventricular septal defect
Aβ	β-amyloid peptide
CBC	complete blood count
CEP	Congenital erythropoietic porphyria
CH	Congenital hypothyroidism
CHD	Congenital heart disease
CRP	C-reactive protein
DNA	Deoxyribo-nucleic acid
DS	Down syndrome
DSCR	Down syndrome critical region
FISH	Flourescent in situ hybridization
GI	Gastrointestinal
GIT	Gastrointestinal tract
HPLC	high performance liquid chromatography
HSCs	Hematopoietic stem cells

List of Abbreviations

Abb.	Full term
Mb	Megabase
MEP	Megakaryocyte-erythrocyte progenitor
ML-DS	Myeloid leukemia in Down syndrome
MPNs	Myeloproliferative neoplasms
MI	meiosis I
MΠ	Meiosis Π
NT	Nuchal translucency
PAD	Patent ATRIAL duct
PCR	POLYMERASE chain reaction
PT21	Partial trisomy 21
RNA	Ribonucleic acid
Ss	Sanger sequencing
TAM	Transient abnormal myelopoiesis
TMD	Transient myeloproliferative disease
uE3	Unconjugated estriol
VSD	$ventricular septal defect
WHO	World health organization
XLT	X-linked thrombocytopenia
XLTT	X-linked thrombocytopenia with thalassemiA
β-hCG	Human chorionic gonadotrophins

INTRODUCTION

In children with Down syndrome (DS), the risk of developing acute megakaryocytic leukemia (AMKl) is estimated to be 500 times higher than in children without DS ***(kanezaki et al., 2010).***

Interestingly, neonates with DS are at a high risk 5-10% of developing a hematologic disorder referred to as transient myeloproliferative disease (TMD) ***(Hitzler, 2007)***. The World Health Organization (WHO) defines TMD as increased peripheral blood blast cells in neonates with DS ***(Vardiman et al., 2009)***.

In about 60% of cases, TMD resolves spontaneously within the first 3 months of life ***(Massey et al., 2006).*** A small proportion of babies with TAM will die from their disease, usually due to liver failure caused by hepatic fibrosis and blast cell infiltration ***(Klusmann et al, 2008; Gamis et al, 2011).***

An estimated 20% to 30% of babies with TMD subsequently develop Ml-DS (Myeloid leukemia in DS). Thus, TMD is an important clinical problem ***(Klusmann et al., 2008).***

Acquired mutations in exon 2 of the hematopoietic transcription factor *GATA-1* mapped at Xp11.23 are consistently present in the affected cells of children with TMD and Ml-DS, leading to expression of N-terminally truncated GATA-1 protein ***(Cabelof et al., 2009).***

GATA-
1 ıs a transcrıptıon factor that comprehensıvely regulates the gen es that are ımportant for the development of erythroıd and megak aryocytıc cells. Accumulatıng evıdence supports the notıon that d efects ın GATA-
1 functıon are ıntımately lınked to hematopoıetıc dısorders ***(Shım ızu and Yamamoto, 2012)***.

GATA-
1 mutatıon analysıs showed that 8.5% of DS neonates had a *GA TA-*
1 mutatıon detected by Sanger sequencıng/ denaturıng hıgh perfo rmance lıquıd chromatography (Ss/DHPlC) ***(Roberts et al., 2013)*** . Sımılar to estımates from retrospectıve studıes (5%-
10%) ***(Malınge et al., 2009).***

Xu et al. (2003) found the presence of GATA-
1 mutatıons ın 21 patıents out of 22 patıents Down syndrome wıth TMD and ın 12 patıents out of 18 patıents Down syndr ome wıth AMKl.

Roberts et al. (2013) suggest that a practıcal and sensıtı ve defınıtıon of TMD ıs the presence of blasts >10% on bloo d smears and a GATA-1 mutatıon detected by Ss/DHPlC.

Aim of the Work

The aım of thıs study ıs to screen DS neonates wıth perıpheral blasts for GATA-1 mutatıons. Thıs facılıtates regular clınıcal and laboratory follow up and ensures approprıate management of cytopenıas that may precede AMl, ıncludıng the tımıng of antıleukemıc therapy, any possıbılity of use of GATA-1 as a marker for cure.

Chapter 1

DOWN SYNDROME

Introduction

Trisomy for human chromosome 21 is the most frequent live-born aneuploidy and results in Down syndrome (DS). This is a well recognized syndrome with variable phenotypic expression *(Gardiner et al., 2010).*

The incidence of trisomy 21 is influenced by maternal age *(Wiesman et al., 2009)*, It occurs in one in approximately 691 and 1000 newborns in the USA and Europe, respectively *(Jiang et al., 2015)*.

Genotype of DS

Chromosome 21 is the smallest human autosome. It consists of ~50 Mb of DNA. The short arm is very small and all of the unique genes that have been located to this chromosome have been mapped to the long arm of the chromosome as shown in figure 1 *(Kola and Hertzog, 1997).*

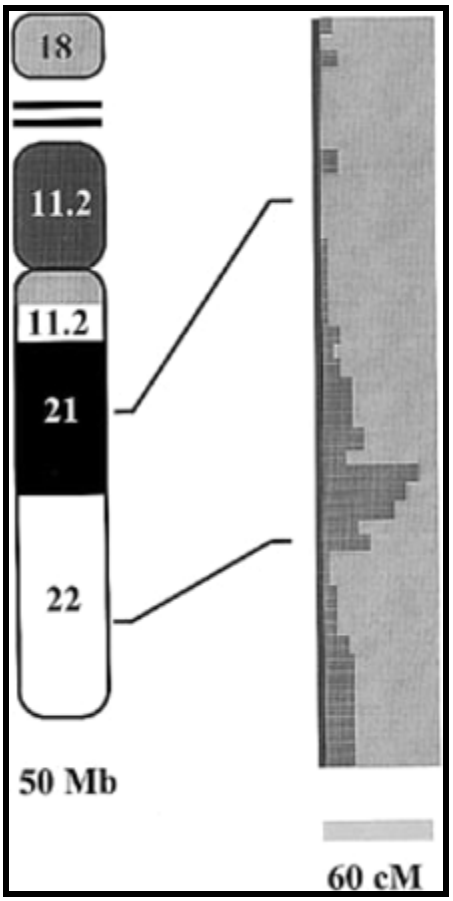

figure (1): Map of the frequency of expressed sequences encoded on different parts of human chromosome 21 ***(Kola and Hertzog, 1997).***

The smallest chromosomal region in common among individuals who share a given feature is referred to as a 'Down syndrome critical region' (DSCR). The best-defined DSCR extends ~5 Mb from *D21S17* to *MX1* in band **21**q22.3. This segment contains about 33 conserved genes ***(Olson et al., 2007).***

Mechanısms of DS

1. Nondısjunctıon:

Nondısjunctıon occurs ın about 95% of people wıth Down syndrome ***(Shın et al., 2010).***

Nondısjunctıon ıs the faılure of homologous chromoso mes or sıster chromatıds to segregate to separate daughter cell s durıng cellular dıvısıon. When thıs type of error occurs durı ng meıosıs, some of the resultıng gametes wıll have too many or too few chromatıds compared wıth the expected haploıd n umber (aneuploıdy**)** ***(Mıddlebrooks et al., 2014).***

In~90% **of** trısomy 21 ındıvıduals, the addıtıonal chro mosome ıs maternal ın orıgın, ~70% **of** the maternal errors h ave been found to occur durıng meıosıs I (MI), whıle the oth er 30% occur durıng meıosıs II (MII) ***(lamb et al., 1997)***.

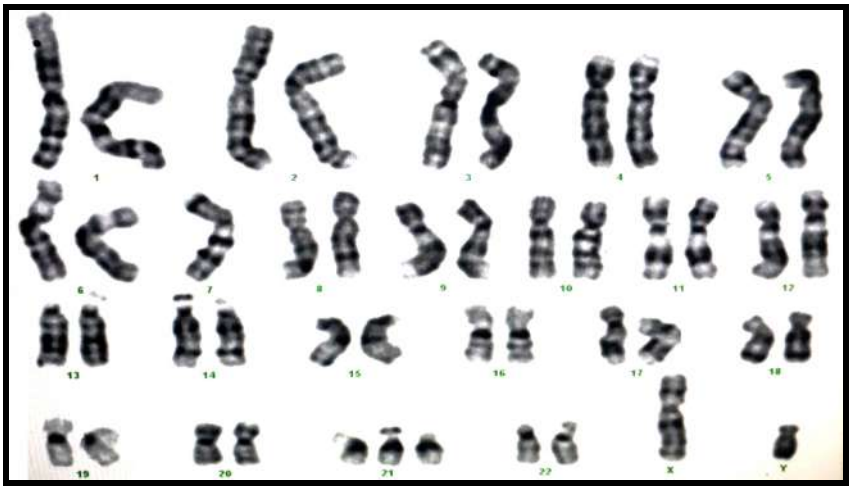

figure (2): Karyotype of a patıent wıth nondısjunctıon Down syndrome: 47, XY, +21. ***(Genetıcs Unıt, Aın Shams Unıversıty)***

2. Translocatıon:

This type accounts for a small percentage of people with Down syndrome (about 3%) ***(Shin et al., 2010)***.

There is extra chromosome 21 material attached (translocated) onto another chromosome. For parents of a child with Down syndrome due to a translocation, there may be an increased chance of Down syndrome in future pregnancies. This is because one of the two parents may be a carrier of a balanced translocation ***(GHR, 2012).***

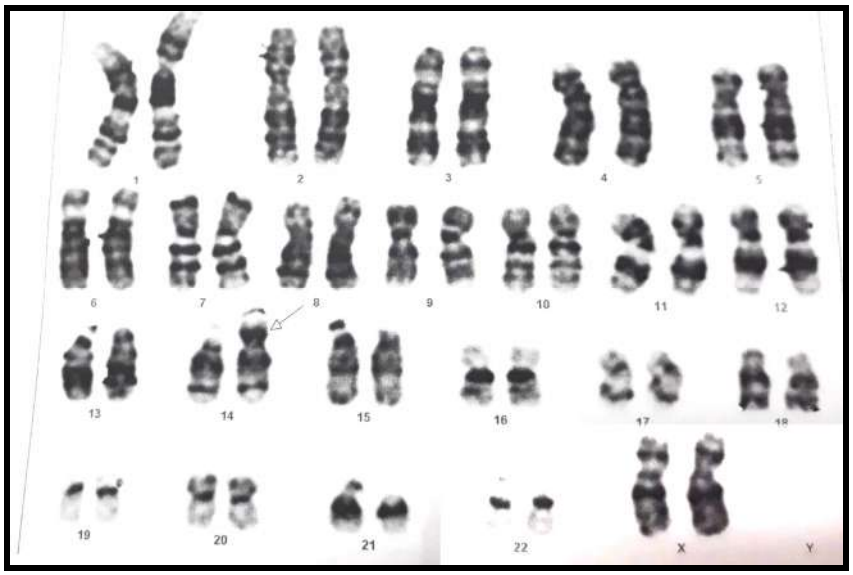

figure (3): Karyotype of a patient with translocation Down syndrome: 46, XX, der (14;21), +21 ***(Genetics Unit, Ain Shams University).***