

Screening of GATA-1 Mutation in Patients with Down Syndrome

Thesis

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By

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قالوا

سببناك لا علم لنا
إلا ما علمتنا إنك أنت
العليم العظيم

صدق الله العظيم

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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
A β	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
MII.....	Meiosis II
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 is a transcription factor that comprehensively regulates the genes that are important for the development of erythroid and megakaryocytic cells. Accumulating evidence supports the notion that defects in GATA-

1 function are intimately linked to hematopoietic disorders (*Shimizu and Yamamoto, 2012*).

GATA-

1 mutation analysis showed that 8.5% of DS neonates had a GATA-

1 mutation detected by Sanger sequencing/ denaturing high performance liquid chromatography (Ss/DHPLC) (*Roberts et al., 2013*)

. Similar to estimates from retrospective studies (5%-10%) (*Mahng et al., 2009*).

Xu et al. (2003) found the presence of GATA-1 mutations in 21 patients out of 22 patients Down syndrome with TMD and in 12 patients out of 18 patients Down syndrome with AMKL.

Roberts et al. (2013) suggest that a practical and sensitive definition of TMD is the presence of blasts >10% on blood smears and a GATA-1 mutation detected by Ss/DHPLC.

AIM OF THE WORK

The aim of this study is to screen DS neonates with peripheral blasts for GATA-1 mutations. This facilitates regular clinical and laboratory follow up and ensures appropriate management of cytopenias that may precede AML, including the timing of antileukemic therapy, any possibility 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*).

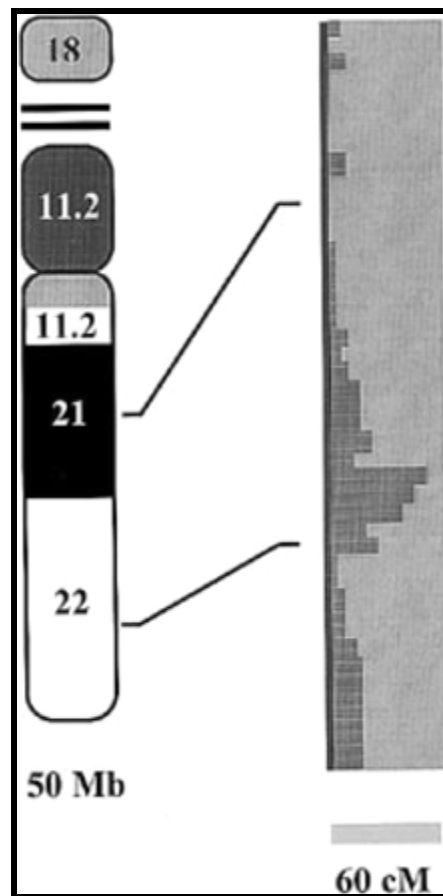


figure (1): Map of the frequency of expressed sequences encoded on different parts of human chromosome 21 (*Kola and Hertzog, 1997*).

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 *MXI* in band 21q22.3. This segment contains about 33 conserved genes (*Olsen et al., 2007*).

Mechanisms of DS

1. Nondisjunction:

Nondisjunction occurs in about 95% of people with Down syndrome (*Shin et al., 2010*).

Nondisjunction is the failure of homologous chromosomes or sister chromatids to segregate to separate daughter cells during cellular division. When this type of error occurs during meiosis, some of the resulting gametes will have too many or too few chromatids compared with the expected haploid number (aneuploidy) (*Middlebrooks et al., 2014*).

In ~90% of trisomy 21 individuals, the additional chromosome is maternal in origin, ~70% of the maternal errors have been found to occur during meiosis I (MI), while the other 30% occur during meiosis II (MII) (*lamb et al., 1997*).

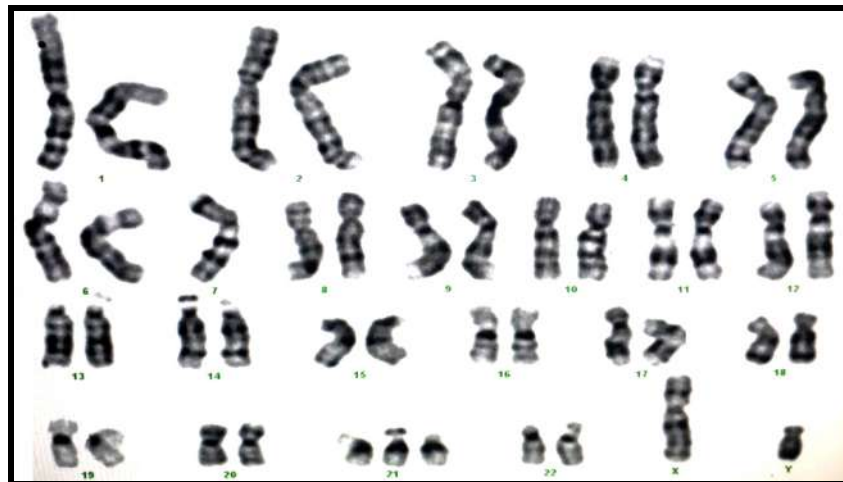


figure (2): Karyotype of a patient with nondisjunction Down syndrome: 47, XY, +21. (*Genetics Unit, Ain Shams University*)

2. Translocation:

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*).

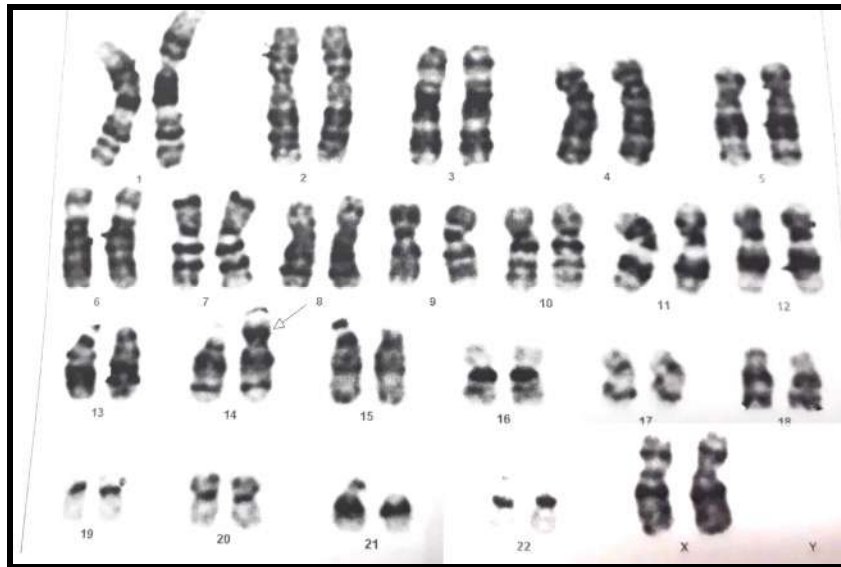


figure (3): Karyotype of a patient with translocation Down syndrome: 46, XX, der (14;21), +21 (*Genetics Unit, Ain Shams University*).