Fanconi anemia: A paradigm of the Molecular detection of common mutations in Egyptian Fanconi anemia patients

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I declare that this thesis has been composed by my self and the work therein has not been submitted for a degree at this or any other university.

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Contents

Page

Abstract I

Acknowledgement II

List of Abbreviations III

List of Figures V

List of Tables VII

Introduction and Aim of the Work 1

1. Review of Literature .....

1.1. Fanconi anemia 2

1.1.1 Definition 2

1.1.2 Incidence and prevalence 2

1.1.3. Clinical features 3

1.1.3.1. Hematological aspects 3

1.1.3.2. Non hematological clinical aspects 5

1.1.3.3. Cancer predisposition 10

1.1.4. Cellular phenotype 11

1.1.4.1. Chromosome breakage and chromosome Aberrations 13

1.1.4.2. Crosslinker sensitivity 15

1.1.4.3. Cell cycle disturbance 16

1.1.4.4. Oxygen Sensitivity 17

1.1.4.5. Deregulated apoptosis 19

1.1.4. 6. RAD51 foci formation 19

1.1.5. Cytogenetic abnormalities 20

1.1.6. Cancer development 23

1.1.7. Differential diagnosis 24

1.1.8. Molecular phenotype 24
1.1.8.1. Background 24
1.1.8.2. The FA/BRCA biological pathway 27
1.1.8.3. The Fanconi Anemia pathway and Homologous Recompination proteins (HR)..... 39
1.1.8.4. The Fanconi Anemia pathway and Translesion synthesis protein (TLS) 40
1.1.8.5.The Fanconi Anemia pathway, Oxidative Stress, Cytokine Sensitivity 40
1.1.8.6. Fanconi anemia genes 43
1.1.9. Genotype - phenotype correlation in Fanconi anemia 58
1.1.10. Treatment and Manifestation 61
1.1.10.1. Androgen and corticosteroides 62
1.1.10.2. Hematopoietic growth factor 63
1.1.10.3.Hematopoietic Stem cell Transplantation.(HSCT) 63
1.1.10.4. Cancer treatment 64
1.1.10.5. Therapies Under Investigation 64
2. Materials and Methods..... 66
2.1. Subjects 66
2.2. Methods 66
2.2.1. Clinical evaluation 66
2.2.2. Cytogenetic studies 67
2.2.3. Molecular studies 67
2.2.3.1. DNA extraction 67
2.2.3.2. Detection of missense mutation 2574C→ G (S858R) in exon 27 of FANCA gene 70

2.2.3.3. Detection of mutation 3788 – 3790del in exon 38 of FANCA gene 74
2.2.3.4. Detection of mutations in exon 34 and exon 43 of FANCA gene 79
2.2.3.5. Single Stranded Conformational Polymorphism (SSCP)…… …..... 72
2.2.3.6. DNA Sequencing …….. 81
2.2.3.7. Detection of intragenic deletion of exon 27 in FANCA gene …… 81
3. Results …… 85
3.1. Clinical data …… 85
3.2. Molecular Data …….. 87
3.2.1 Patients had homozygous deletion mutation of exon 27 in the FANCA gene …….. 87
3.2.2 Result of 2574C → G (S858R) mutation in exon 27 of the FANCA gene …… 88
3.2.3 Results of 3788-3790del mutation in exon 38 of the FANCA gene …….. 89
3.2.4 Results of mutations screening in exon 34 and exon 43 of the FANCA gene …… 90
3.3 Genotype-phenotype correlations…… 91
3.3.1 The anthrometical, clinical, hematological and cytogenetic data of patients with the homozygous deletion in exon 27 of the FANCA gene…….. 91
4. Discussion …….. 117
Summary …….. 138
References …….. 140
Arabic Summary ……

Abstract

Fanconi anemia: A paradigm of the Molecular detection of common mutations in Egyptian Fanconi anemia patients.

Rehab Mostafa Mohamed Mosaad

Introduction: FA-A is the most frequent complementation group representing approximately two-thirds of the FA patients in the majority of countries. Aim of the work: screening the most common mutations in exons 27, 34, 38 and 43 of FANCA gene in the Egyptian Fanconi anemia patients and Phenotype /genotype correlation to evaluate the severity of the disease with the gene mutations. **Subjects and Methods**: Thirty Egyptian FA cases, their age range from 5 to 15 years ,descending from unrelated consanguineous pedigrees were recruited from the outpatient clinic of the Clinical Genetics department, NRC. Control group was 10 unrelated matching age and sex with no family history of Fanconi anemia. All patients had positive chromosomal breakage studies with diepoxybutane (DEB) confirming the diagnosis of FA. Amplification of FANCA gene exons, Restriction Analysis, DNA sequencing for patients and controls was done. **Results**: 20 % (6/30) of our patients had homozygous deletion of exon 27 of the FANCA gene , this detected deletion needs more studies to define the boundaries ; these patients presented with severe phenotype, 67% were born with low birth weight. All patients had cafe´ au lait spots, hyper pigmentation, and 83% had skeletal abnormalities. Moreover, chromosomal breakage (DEB test) in this group was with an average of 11.5 break/ cell . No 2574C→G (S858R) mutation in exon 27 or c.3788_3790 del TCT mutation in exon 38 in the FANCA gene wsa detected in our studied patients. Moreover, no new mutations were detected in exons 34 and 43 of the FANCA gene. This is the first step in defining the mutation spectrum of FA in Egyptian patients.

Keywords: Fanconi anemia, FANCA gene, DEB, 3788-3790del mutation**.**

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List of Abbreviations

AA	:	Aplastice Anemia
AIDs	:	Antibody Immunodeficiency Syndrome
AMD	:	Amplification Mismatch Detection
AML	:	Acutemyeloid Leukemia
Arg	:	Arginine
bp	:	Base pair
BMT	:	Bone Marrow Transplantation
BMF	:	Bone Marrow Failure
BSA	:	Bovine serum albumin
CAB	:	Congenital Abnormality
CDDP	:	Cisplatin
cM	:	Cent Morgan
CNS	:	Central Nervous Systen
Cod	:	Codon
CTD	:	C-terminal Domain
CU+	:	Reduced state of copper ions
CVS	:	Chorionic Villi Sampling
dATP	:	Deoxyadenosine 5'-triphosphate
dCTP	:	Deoxycytidine 5'-triphosphate
DEB	:	Diepoxybutan
Df	:	Dilution factor
DGGE	:	Denaturing gradient gel electrophoresis
dGTP	:	Deoxyguanosine 5'-triphosphate
DMSO	:	Dimethylsulfoxide
dNTP's	:	Deoxynucleoside 5'-triphosphate
DTT	:	Dithiothreitol
dTTP	:	Deoxythimidine 5'-triphosphate
dUTP	:	Deoxyuridine 5'-triphosphate
EDC	:	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
EDTA	:	Ethylene diamine tetra acetic acid
ER	:	Endoplasmic Reticulum

ERGIC		Endoplasmic Reticulum Golgi Intermediate Compartment.
EVI1	:	Ecotropic Virus Integration Site 1 gene
FA	:	Fanconi Anemia
FA-A		Fanconi Anemia complementation group A
G-CSF	:	Granulocyte-Colony Stimulating Factor
Hb	:	Hemoglobin
HD	:	Helical Domain
HDR	:	Homology-directed DNA Repair
HNSCC	:	Head and Neck Squamous Cell Carcinoma
HR	:	Homologous Recombinase
HSCT	:	Hematopoitic Stem Cell Transplatation
ICL	:	Interstrand DNA crosslinking
IFAR	:	International Fanconi Anemia Registry.
IgG	:	Immunoglobulin G
IR	:	Ionizing Radiation
LC3	:	Light Chain 3
kb	:	Kilo base
KDa	:	Kilo Dalton aminacid
MAP1	:	Microtubule-associated Protien
Mb	:	Mega bite
MCA	:	Multiple Congenital Anomalies
MDS	:	Myelodysplastic Syndrome
MMC	:	Mitomycin C
mRNA	:	messenger Ribonucleic Acid
NHEJ	:	Nonhomologous End Joining
N-Linked	:	Linked to Nitrogen terminal
NLS	:	Nuclear Localization Signals
NM	:	Nitrogen Mustard
OD	:	Optical density
O- linked	:	Linked to terminal oxygen
PCR	:	Polymerase chain reaction
pg	:	Picogram

PHA	:	Phytohemagglutan A
PHD	:	Plant Homeodomain
PL	:	Phospholipids.
PT	:	Prothrombin Time
RFLP	:	Restriction fragment length polymorphism
ROS	:	Reactive Oxygen Sensitive.
SDS	:	Sodium Dodecyle Sulphate
SSA	:	Single Strand Annealing
SCC	:	Squamous Cell Carcinomas
SCE	:	Sister Chromatide Exchange
SSCP	:	Single-strand confirmation polymorphism
TAE	:	Tris acetate-EDTA
TAR	:	Thrombocytopenia-absent radii
TBE	:	Tris borate-EDTA
TCT	:	Thrombin clotting time
TD	:	Tower Domain
TE	:	Tris-EDTA
TLS	:	Translesion Synthesis Protien
TNF-a	:	Tumor Necrosis Factor -a
Tris	:	Tris (hydroxymethyl) aminomethane
UBE2T	:	Ubiquitin Conjugating Enzyme E2.
UV	:	Ultraviolet

List of Figures

		Page
Fig. (1) :	The FA/BRCA biological pathway	26
Fig. (2) :	Ubiquitylation in response to interstrand crosslinks, highlighting Fanconi anemia proteins and the associated factors necessary for repair.	34
Fig. (3) :	Schematic of the Fanconi anemia DNA repair pathway	38
Fig. (4) :	Schematic representation of the thirteen human Fanconi anemia proteins	48
Fig. (5) :	Marked increase in the chromosomal breakage induced by DEB in FA patients with the characteristic isochromatid exchange.	106
Fig. (6) :	Mild increase in the chromosomal breakage induced by DEB in FA patients with the characteristic isochromatid exchange.	107
Fig. (7) :	Analysis of the amplified DNA products of homozygous intrgenic deletion of Exon 27 mutation in FANCA gene of Egyptian FA patients**.**	108
Fig. (8) :	Analysis of the amplified DNA products of 2574C⟶ G (S858R) mutation in exon 27 in FANCA gene.	109
Fig. (9) :	Analysis of the amplified DNA products of 3788-3790del mutation in exon 38 in FANCA gene.	110

Page

Fig.(10) :	Analysis of the amplified DNA products of exon 34 in FANCA gene	111
Fig.(11) :	Analysis of the amplified DNA products of exon 43 in FANCA gene	112
Fig.(12) :	SSCP analysis of the amplified productsof exon 34 of FANCA gene	113
Fig.(13) :	SSCP analysis of the amplified products of exon 43 of FANCA gene	114
Fig (14) :	Partial sequence of FANCA gene exon 34 PCR fragment, showing normal sequencesing forward primer.	115
Fig (15) :	Partial sequence of FANCA gene exon 43 PCR fragment, showing normal sequencesing forward primer.	116

List of Tables

		Page
Table (1) :	Frequency of abnormalities in Fanconi anaemia.	5
Table (2) :	Congenital malformations in patients in the International Fanconi Anemia Registry (IFAR).	8- 9
Table (3) :	Thirteen complementation groups of Fanconi Anemia	41- 42
Table (4) :	The sequence of PCR primers	84
Table (5) :	Anthropometric data of patients.	93-95
Table (6) :	Clincal data of patients.	96-99
Table (7) :	Hematological and cytogenetic data of patients.	100-101
Table (8) :	Anthropometric data of patients with homozygous intragenic exon 27 deletion mutation in the FANCA gene.	102
Table (9) :	Clincal data of patients with homozygous intragenic exon 27 deletion mutation in the FANCA gene	103
Table (10) :	Hematological and cytogenetic data of patients with homozygous intragenic exon 27 deletion mutation in the FANCA gene	104
Table (11) :	Molecular data of the patients (1-6).	105

Introduction

Fanconi anemia (FA; MIM 227650) is a genetically heterogeneous chromosomal instability syndrome associated with multiple congenital abnormalities, progressive pancytopenia, and predisposition to both hematologic malignancies and solid tumors ***(Morgan et al., 2005)***.

It is a very rare (1–5 per million) autosomal recessive or X-linked (FA-B group) disease ***(Callen et al., 2005***; ***Kutler and Auerbach 2004***).FAis caused by a genetic defect in a cluster of proteins responsible for DNA repair ***(D'Andrea, 2010)***. Fanconi anemia cells are hypersensitive to DNA cross-linking agents, such as diepoxybutane (DEB) and this provides a valuable laboratory test to support the clinical diagnosis ***(Auerbach, 2009)***.

Fanconi anemia is caused by biallelic mutations in one of at least 15 FA genes that are responsible for the known FA complementation groups (A, B, C, D1 [*BRCA2*], D2, E, F, G, I, J [*BRIP1*], L, M, N [*PALB2*], O [*RAD51C*], and P [*SLX4*]) ***(Hucl and Gallmeier, 2011).***FA-A is the most frequent complementation group representing approximately two-thirds of the patients in the majority of countries accounting to 60-66 %. ***(Kennedy and D'Andrea, 2005).***

To our knowledge, Fanconi anemia mutations have not yet been investigated in the Egyptian population, characterized by a heterogeneous ethnic background and a high rate of consanguinity.

Aim of this work:

The aim of the present work was screening of the most common mutations of FANCA gene in the Egyptian Fanconi anemia patients and Phenotype /genotype correlation to evaluate the severity of the disease with the gene mutations.

1.1. Fanconi anemia

1.1.1. Definition

FA (MIM 227650) is a rare genetic disease characterized by childhood-onset bone marrow failure (aplastic anemia), cancer/leukemia susceptibility, multiple congenital abnormalities and cellular hypersensitivity to interstrand DNA crosslinking (ICL) agents, such as MMC, diepoxybutane (DEB), melphalan, cisplatin and cyclophosphamide ***(Wang ,2007)*** . It is an autosomal recessive disease (all complementation groups except FA-B group) or X-linked (FA-B group) ***(Taniguchi , 2009).***

FA is a genetically heterogeneous disease caused by biallelic mutations in one of at least 15 FA genes which are responsible for the known FA complementation groups (A, B, C, D1 [*BRCA2*], D2, E, F, G, I, J [*BRIP1*], L, M, N [*PALB2*], O [*RAD51C*], and P [*SLX4*]). The latter two genes are still thought of as tentative as they do not fall within a very easily characterized compartment biologically and have very few representative individuals ***(Hucl and Gallmeier, 2011).***

1.1.2. Incidence and Prevalence

FA is a very rare disease (1–5 per million) and its heterozygous carrier frequency is estimated to be 1/300 ***(Auerbach et al., 2001).*** In some populations (Ashkenazi Jewish, Spanish Gypsy, and black South African) the carrier frequency of FA is estimated at around 1:100 ***(****Kutler and Auerbach 2004* ***&*** *Callen et al., 2005****&*** *Morgan et al .,2005*).The median age at diagnosis is 6.5 years for boys and 8 years for girls, but the age at diagnosis ranges from 0 to 48 years. From 1981 to 1990, the median age at death was only 19 years; by 2000 the median age had reached 30 years, probably because of improved medical care ***(Alter, 2003b).***