The Receptor for Advanced Glycation End Products (RAGE) Gene Polymorphisms in Pediatrics with Sickle Cell Disease and Sickle Thalassemia

Thesis

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By

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List of Abbreviations

Abb.	Full term
ACS	*Acute chest syndrome*
AD	*Alzheimer's disease*
AGEs	*Advanced glycation end products*
CBC	*Complete blood count*
cRAGE	*Cleaved RAGE*
DN-RAGE	*Dominant negative RAGE*
ERK	*Extracellular signal-regulated kinase*
esRAGE	*Endogenous secreting RAGE*
HMGB-1	*High-mobility group box-1*
HPFH	*Hereditary persistent fetal haemoglobin*
HPLC	*High performance liquid chromatography*
ICAM	*Intercellular adhesion molecule*
IEF	*Isoelectric focusing*
IQR	*Interquartile range*
ISCs	*Irreversible sickle cells*
JAK	*Janus kinase*
JNK	*c-Jun N-terminal kinase*
K2 – EDTA	*Potassium Ethylene Diamine Tetra Acetic acid*
LDH	*Lactate dehydrogenase*
MAPK	*Mitogen activated protein kinases*
MCP-1	*Monocyte chemoattractant protein-1*
MCV	*Mean corpuscular volume*
mDia-1	*Mammalian diaphanous-1*
MGB	*Minor groove binder*
MS	*Multiple sclerosis*
NF-kβ	*Nuclear factor*
NFQ	*Nonfluorescent quencher dye*
NO	*Nitric oxide*
NS	*Non significant*
PBX2	*Pre-B-Cell Leukemia Homeobox2 gene*
PCR	*Polymerase chain reaction*

List of Abbreviations cont...

Abb.	Full term
PCV	*Packed cell volume*
PI3K	*Phosphoinositol 3-kinase*
PKC	*Protein kinase C*
PRR	*Pattern-recognition receptor*
RA	*Rheumatoid arthritis*
RAGE	*Receptor for advanced glycation end product*
ROS	*Reactive oxygen species*
S	*Significant*
SCA	*Sickle cell anemia*
SCD	*Sickle cell disease*
SD	*Standard deviation*
SLE	*Systemic lupus erythromatosis*
SNPs	*Single nucleotide polymorphism*
SPSS	*Statistical Package for the Social Sciences).*
SR	*Scavenger receptor*
sRAGE	*Soluble form of RAGE*
STAT	*Signal transducer and activator of transcription*
Tm	*Melting temperature*
TNF	*Tumor necrosis factor*
TRCA	*Transient red cell aplasia*
VCAM	*Vascular cell adhesion molecule*
VOC	*Vasoocclusive crisis*

INTRODUCTION

Sickle cell disease (SCD) is a hemoglobinopathy characterized by unstable auto-oxidative sickle haemoglobin (HbS), chronic intravascular hemolysis, recurrent ischemia reperfusion injury and low grade inflammation, all contributing to an increased generation of reactive oxygen species (ROS) potentially contributing to the characteristic widespread organ damage ***(Van Beers et al., 2008).*** ROS and the end products of their oxidative reactions are potential markers of disease severity and could be targets for antioxidant therapies ***(Nur et al., 2011).***

Oxidative stress is an important feature of SCD that result in increased production and accumulation of advanced glycation end products (AGEs), which are not only well-established markers of oxidative stress but are themselves oxidatively active and have been demonstrated to serve as biomarkers of disease severity in SCD ***(Gerrits et al., 2008).***

The AGEs are generated by non-enzymatic glycation and glycoxidation of proteins and lipids. The interaction of AGEs with their cellular receptor, the receptor for advanced glycation end product (RAGE), which is expressed on different cells including endothelial cells and macrophages, triggers cell-specific signaling, resulting in enhanced generation of ROS, and in the activation of the transcription factor NF-kβ. This leads to sustained upregulation of pro-inflammatory mediators,

adhesion molecules, oxidants and to a dysfunctional cell phenotype ***(Nur et al., 2010).***

The ligand-RAGE axis has been shown to be intimately involved in the pathobiology of a wide range of diseases, including diabetes mellitus, atherothrombosis, immune/ inflammatory conditions, aging, cancer, and neurodegeneration ***(Santilli et al., 2009).***

The gene encoding RAGE is located on chromosome 6p21.3 in the major histocompatibility locus, a region of the genome containing a number of genes involved in immune and inflammatory responses and comprises 11 exons. Polymorphisms of RAGE gene in the promoter and the exons affected the expression and function of RAGE and may influence development of complications by altering AGE-RAGE interaction ***(Jang et al., 2007).***

AIM OF THE WORK

Investigate the association of the polymorphisms of RAGE gene with SCD patients and assess the influences of these polymorphisms on the disease progression & complications together with studying their correlation with different clinical and laboratory parameters.

Chapter 1

OVERVIEW OF SICKLE CELL DISEASE

Sickle cell disease (SCD) is an inclusive term for a group of related β-hemoglobinopathies characterized by the predominance of sickle haemoglobin (HbS) within erythrocytes. This arises from a single nucleotide substitution in the β-globin gene (HBB) that yields a mature β-globin protein with a hydrophobic valine instead of a hydrophilic glutamic acid at the sixth amino acid position ***(Quinn, 2016).***

Genetic background of SCD:

Although the molecular lesion is a single-point mutation, the sickle gene is pleiotropic in nature causing multiple phenotypic expressions associated with complex genetic interactions and modifiers that constitute the various complications of sickle cell disease in general and sickle cell anemia in particular ***(Ballas et al., 2012).***

The genetic Forms of SCD:

SCD is composed of diverse genotypes ***(Table 1):***

- Homozygous state or sickle cell anemia (HbSS)
- Heterozygous carrier state or sickle cell trait (HbAS)
- Sickle cell/haemoglobin C disease (HbSC)

- Sickle cell/ β thalassemia
- Sickle cell anemia with coexistent α thalassemia
- Sickle cell/hereditary persistent fetal haemoglobin (HPFH)
- Sickle cell/ Hb Lepore disease
- Sickle cell/ HbD disease
- Sickle cell/ HbO arab disease
- Sickle cell/ HbE disease
- Other sickling haemoglobins:

In addition to HbS, there are at least 13 haemoglobins (e.g haemoglobins $C^{Ziquinchor}$, C^{Harlem}, $C^{Ndjamena}$) that have both the β6 glutamic acid to valine substitution and an additional substitution, resulting from second point mutation in the same β globin chain. These haemoglobins also have a positive solubility test since they are prone to polymerise but generally exhibit different electrophoretic and chromatographic properties from haemoglobin S. They have clinical significance similar but not necessarily identical to that of HbS ***(Bain et al., 2017)***.

Table 1: Genetic forms of SCD

Genotype	Mean haemoglobin (g/L)	MCV	Haemoglobin electrophoresis (%)				
			S	A	F	A_2	Other
SS	81	N	80–95	–	2–20	N	–
SS –α/αα, SS –α/–α	86, 92	↓, ↓	80–90, 80–90	–, –	2–20, 2–20	3.3–3.8, 3.3–3.8	–, –
SC	110	↓	40–50	–	1–4		C: 40–50
S/β^0-thalassaemia	88	↓	75–90	–	2–20	4–6	–
S/β^+-thalassaemia	115	↓	50–85	5–30	2–20	4–6	–
SD Punjab	82	N	40	–	2.5–5	2–3	D Punjab: 50
SO Arab	81	N	45	–	4–7		O Arab: 45
S Lepore	110	↓	75	–	3.5–40	2	Lepore: 10
SE	130	↓	60	–	4		E: 30–35
S/HPFH	137	N or ↓	60–70	–	25–35	1.5–2.5	–
AS*	N	N	30–45	50–65	2–5	N	–

*Sickle cell trait is asymptomatic.
MCV, mean corpuscular volume.

(Hoffbrand et al., 2016)

Genetic modifiers of sickle cell disease:

Sickle cell anemia is associated with unusual clinical heterogeneity for a Mendelian disorder. Fetal Hb concentration and coincident α thalassemia are the major modulators of the phenotype of the disease **(*Tewari et al., 2015).***

HbF inhibits the polymerization of deoxy-HbS; thus depending on its level and pattern of distribution across erythrocytes, it can ameliorate or prevent nearly all complications of SCD ***(Quinn, 2016).***

Coinherited α-thalassemia can also modify the phenotype of HbSS by reducing the intracellular concentration of HbS that in turn decreases HbS polymer-induced cellular damage, which ameliorates hemolysis. Paradoxically, there is also evidence