# Pre-micro RNA polymorphism detection in small versus large vessel disease in stroke patients

**Thesis**

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# List of Abbreviations

| Abb. | Full term |
|---|---|
| ACA | Anterior cerebral artery |
| AGEs | Advanced glycation end products |
| ASCO | Atherosclerosis; small vessel disease; cardiac sources; other causes classification |
| CAS | Carotid artery stenosis |
| CBC | Complete blood count |
| CT | Computed tomography |
| DM | Diabetes mellitus |
| DNA | Deoxyribonucleic acid |
| ECG | Electrocardiogram |
| EF | Ejection fraction |
| ELAMs | Endothelial leukocyte adhesion molecule |
| FLAIR | Fluid-attenuated inversion recovery |
| GWAS | Genome wide association |
| HbA1c | Glycated hemoglobin |
| HDL | High density lipoprotein |
| HTN | Hypertension |
| HWE | Hardy-Weinberg Equilibrium |
| ICA | Internal carotid artery |
| ICAM-1 | Intercellular adhesion molecule-1 |
| IHD | Ischemic heart disease |
| INR | International normalized ratio |
| LAD | Left atrial diameter |
| LDL | Low density lipoprotein |
| LDL-C | Low-density lipoprotein cholesterol |
| LVD | Large vessel disease |
| MCA | Middle cerebral artery |
| miRNAs | MicroRNAs |
| MRA | Magnetic resonance angiography |
| MRI | Magnetic resonance imaging |
| mRNA | Messenger RNA |

# List of Abbreviations Cont...

| Abb. | Full term |
|---|---|
| MRS | Modified Rankin scale |
| MSLL | Multifocal signal loss lesions |
| NIHSS | National institute of health stroke scale |
| OX-LDL | Oxidized LDL |
| PCR RFLP | Polymerase chain reaction based restriction fragment length polymorphism |
| PCR | Polymerase chain reaction |
| PT | Prothrombin time |
| PTT | Partial thromboplastin time |
| RNA | Ribonucleic acid |
| ROS | Reactive oxygen species |
| SNP | Single nucleotide polymorphism |
| SPARKLE | Subtypes of Ischemic Stroke Classification System |
| SSS-TOAST | Stop Stroke Study |
| SVD | Small vessel disease |
| TG | Triglycerides |
| TIA | Transient ischemic attack |
| TOAST | Trial of ORG 10172 in Acute Ischemic Stroke |
| VCAM-1 | Vascular cell adhesion molecule-1 |
| WHO | World Health Organization |

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# INTRODUCTION

Ischemic stroke is thought to have a polygenic basis, but identification of stroke susceptibility gene and quantification of associated risks has been hindered by conflicting results from different studies ***(Casas et al, 2004).***

While considerable progress has been made over the past years in identifying important genetic risk factors underlying predisposition to venous thrombosis, similar insight into the genetic component of arterial thrombosis has not established ***(Crowther and Kelton, 2003)***.

MicroRNAs (miRNAs) are a class of small, non-coding RNA molecules with 21–23 nucleotides in length that negatively regulate gene expression at the post-transcriptional level through translational repression or mRNA degradation ***(Ji and Wang, 2009).***

There are more than a thousand miRNAs reported in a human genome. Each miRNA is estimated to target hundreds of mRNAs or multiple miRNAs cooperatively regulate the same mRNA suggesting their complex and broad range of regulatory potential. There is an increasing body of evidence that miRNAs play a critical role in the control of key biological processes including development, differentiation, growth, and metabolism as well as pathophysiology of neurodegenerative disease, cancer and cardiovascular disease ***(Lewis et al, 2005).***

The crucial role of miRNA in cardiovascular system has become more evident with the identification of a number of miRNAs that are involved in cardiac/endothelial function as well as angiogenesis, and that show altered expression associated with cardiovascular disease risk. One of the possibilities for the altered amount of miRNAs associated with a disease is genetic variations that can affect the expression or processing to become mature miRNAs ***(Zampetaki et al, 2012).***

Indeed, it is well demonstrated that SNPs (single nucleotide polymorphism) or mutations in miRNAs sequence may alter miRNAs expression and/or maturation in cancer ***(Amos et al, 2008).***

Recent studies have shown that three potentially functional polymorphisms (rs2910164, rs11614913, and rs3746444) in pre-miRNAs (hsamiR-146a, hsa-miR-196a2, and hsa-miR-499, respectively) were associated with an increased risk for cancers, congenital heart disease and dilated cardiomyopathy ***(Hu et al, 2008).***

However, data from investigation on whether genetic variants of miRNA have an influence on ischemic stroke risk is still scarce, highlighting the need for evaluation of genetic variations in miRNAs by association analyses with the disease ***(Horikawa et al, 2008).***

# Aim of the Work

The aim of this study was to investigate the association between three potentially functional polymorphisms (rs2910164, rs11614913, and rs3746444) in pre-miRNAs (hsamiR-146a, hsa-miR-196a2, and hsa-miR-499, respectively) and stroke subtypes (small vessel disease and large vessel disease) in a sample of Egyptian stroke patients.

Chapter 1

# EPIDEMIOLOGY OF STROKE

Epidemiology is the study of the distribution and causes of health-related conditions in specific populations, and the use of this study to control health problems ***(Samet et al, 2009).***

According to the World Health Organization (WHO), 15 million people develop stroke worldwide each year. Of these, 5 million die, and another 5 million are left permanently disabled ***(MacKay et al, 2016).***

The Framingham Heart Study was one of the most notable studies. The incidence of stroke has been determined from this prospective study over 55 years of follow-up of 5184 men and women. The average annual incidence of stroke events increased with age, almost doubling in successive decades. This pattern was seen for all cerebrovascular events, including isolated TIAs and hemorrhage. Overall, the total initial completed stroke event rates were 5.89 per 1000 in men and 4.91 per 1000 in women, yielding a 20% excess in men ***(Sacco et al, 1989).***

Egypt is the most populated nation in the Middle East with an estimated population of 94.8 million people (inside the Republic) according to the final results of 2017. Official national statistics indicate that diseases of the circulatory

system, including stroke, are the leading cause of death in Egypt where they account for about one third of all deaths. Stroke accounts for 6·4% of all deaths and is ranked 3rd after heart disease and gastrointestinal (especially liver) diseases ***(Annual Bulletin of Mortality Statistics, 2017).***

In Egypt, a multi-center study was conducted by ***Abdallah and Mostafa*** in 2014 that revealed that the prevalence rate of stroke is around 963/ 100,000.

There is paucity in the number of studies representing Cairo and Alexandria citizens who have a higher socioeconomic status and better access to healthcare facilities than those living in rural areas, and represent more than 25% of Egypt's population ***(Abdullah and Mostafa, 2014).***

Epidemiological studies suggest racial and ethnic differences in the risk of stroke. Higher incidence of all stroke types and higher mortality rates were observed in Blacks and some Hispanic/Latino Americans compared to whites ***(Cruz-Flores et al, 2011).***

In 2009, ***Roger*** and his colleagues conducted a study about stroke death rates among black versus white Americans. They found increased number of deaths from stroke among blacks compared with whites in the population aged <65 years, in which, for example, there is a black/ white mortality ratio of 3.7 among men aged 45 to 54 years.

Furthermore, genetic factors play a role in incidence of stroke. A meta-analysis of cohort studies showed that a positive family history of stroke increases the risk of stroke by ≈30% (OR, 1.3; 95% CI, 1.2–1.5; P<0.00001) ***(Flossmann et al, 2004).***

# Chapter 2

# THE HERITABILITY OF STROKE

Heritability is defined as phenotypic variation among individuals either in traits or in risk of disease that is attributable to genetic variability. Family history and verified familial occurrence studies, particularly twin studies ***(Brass et al, 1992; Bak et al, 2002)***, were substantial in proving that part of the variability recorded in the occurrence of stroke was attributable to genetic variation ***(Falcone et al, 2014).*** Family history of stroke persists in these studies is an independent risk factor ***(Flossmann et al, 2004).***

New methods have used data from genome-wide association studies (GWAS) to confirm the heritability of stoke. Genome-wide complex trait analysis estimates the heritability of ischemic stroke to about 37.9%. Heritability varies with different stroke subtypes being 40.3% for large-vessel disease and 32.6% for cardioembolic but lower for small-vessel disease (16.1%) ***(Bevan et al, 2012)***.

Given the wide range of phenotypes that characterize a clinical stroke, it is evident that many genes are involved in contributing to stroke risk. Genes may influence other genes which can impact true heritability. The majority of family and genetic studies have been studying populations of European descent. The distribution and heritability of stroke may differ

according to ethnicity ***(Lisabeth et al, 2005).***

Underlying disorders for cerebral small vessel disease may be more heterogeneous than for large-artery atherosclerosis. This partly explains why heritability of large vessel disease is noticed to be higher ***(Jerrard-Dunne et al, 2003).***

***Jerrard-Dunne*** and his coworkers conducted a study in 2003 which revealed that a family history of stroke at < 65 years was a significant risk factor for both large-vessel disease and small-vessel disease. Cardioembolic stroke or stroke of undetermined etiology showed no significant associations.

Data from different studies indicated that increased risk of stroke in offspring with a parental history of stroke was most associated with atherosclerotic brain infarction. This included small-vessel occlusion, 'lacunar' infarctions, and large-artery atherosclerosis ***(Seshadri et al, 2010).***

Heritability of phenotypes, such as MRI markers of subclinical disease has also been investigated. Brain magnetic resonance scans were performed on 2012 individuals in the cohort and offspring of the Framingham study. The degree of white matter hyperintensities measured on brain MRI is estimated to have heritability as high as 55–80% ***(Atwood et al, 2004).***

Neuroimaging biomarkers of cerebral small vessel