Genetic factors influencing stroke susceptibility

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Abstract

Stroke is an inflammatory disorder and homeostasis among inflammatory mediators play critical role to develop clot that choke to cerebral artery. Polymorphism in genes that involve directly in inflammatory process and homocysteine metabolism may be responsible for mild to moderate risk of Stroke. The purpose of the present study was to estimate the influence of polymorphism in genes involved in regulation of inflammatory process and homocysteine metabolism on stroke susceptibility. Some of the homocysteine metabolizing genes and some of the inflammatory genes have independently significant effect on stroke susceptibility. Polymorphic gene or genetic factor as a whole is risk factor for stroke and influence the disease, diagnosis, effect of medicine on patients as well as the other risk factors.

Keywords: Inflammation, homocysteine, polymorphism, oxidative stress

Introduction

A sudden loss of brain function caused by a blockage or rupture of a blood vessel of the brain, resulting in necrosis of brain tissue (called a cerebral infarct) and characterized by loss of muscular control, weakening or loss of sensation or consciousness, dizziness, slurred speech, or other symptoms that vary with the extent and severity of brain damage. Also called cerebrovascular accident. According to WHO, stroke was second common cause of world wide mortality in 1993 and responsible about 4.4 million deaths mostly from developed countries. In 1999 deaths due to stroke reached 5.54 million but two third of these deaths from developing countries. In 2005, this data is increased up to 7.5 million and two-thirds of these individuals live in low income and middle-income countries. Now stroke is a leading cause of death and disability worldwide (Lopez et al., 2006). Demographic changes, urbanization, and increased exposure to major stroke risk factors will fuel the stroke burden in the future. By 2025, four out of five stroke events will occur in people living in these regions.

The most basic incident of blockage of blood vessel is endothelial injury after which complex mechanisms of inflammation start inside to endothelial membrane and forms a hard clot within endothelium (blood Vessels). The inflammation is life saving processes in their regulated manner which retard pathogen’s entry into tissue but when regulatory proteins and enzymes involved in inflammatory process are synthesized by polymorphic genes the process could be life threaten.

Homocystenine is also an inflammatory vasotoxin, at the damaged sites, homocysteine (Hey) mediated enhanced lipid peroxidation and generation of free radicals results into inflammation (Libby et al., 2002). An increased homocysteine in the blood is thus related with acute endothelial dysfunction, and oxidative stress is implicated in inflammatory process. Under normal metabolic circumstances, there is a strict balance between homocysteine formation and elimination. However, under the conditions of reduced or total loss of activity of homocysteine metabolizing enzyme due to mutation in corresponding gene, this metabolic balance is disturbed resulting into hyperhomocystenimia (Ueland et al., 1993).

The relationship between stroke and polymorphism in inflammation regulatory genes as well as homocysteine metabolizing genes could be stabilized because even a tiny crake (rupture) in blood vessel due to high blood pressure will initiate an uncontrolled inflammatory process in that individual who carry any one or more of these genes and made more susceptible for stroke then those have a regulated inflammatory process and homocysteine metabolism.
Homocysteine metabolism, and inflammation regulating system, suggests a strong and significant effect for several at-risk alleles.

In the near future, understanding the contribution of stroke genetic factors will lead to improvements in prevention and treatments for neurovascular diseases. Progress in molecular genetics has allowed the identification, through genome-wide linkage analysis, of various candidate genes.

**The vasotoxic effect of homocysteine**

Homocysteine is a sulfur-containing amino acid, COOHCH(CH₃CH₂SH)NH₂, that is formed during methionine metabolism. Oxidation of two homocysteine molecules yields the disulfide, two protons (H⁺), and two electrons (e⁻).

\[ 2 \text{RSH} \rightarrow \text{RSSR} + 2 \text{H}^+ + 2 \text{e}^- \]

In the presence of metal ions and oxygen, it can autooxidize, generating highly reactive partially reduced oxygen species such as superoxide and hydrogen peroxide (Ian et al., 2000). The superoxide produced from the reaction can react readily with nitric oxide (NO) to form the oxidant peroxynitrite (ONOO⁻). The endothelium-derived NO is able to mediate most of the anti-atherothrombotic functions of the endothelium. Therefore, a reduction in the bioavailability of NO constitutes an important step in the pathobiology of atherosclerotic vascular disease.

The increased concentration of homocysteine autoxidized with trace metal ions, generating reactive oxygen species such as superoxide anion, hydrogen peroxide, hydroxyl and thiol free radicals (Munday, 1989; Schöneich et al., 1989), which is able to reduce Cu²⁺ into Cu⁺ of ceruloplasmin. Cu⁺-Ceruloplasmin has a protective effect on LDL oxidase. HCY up to 100 µmol/l stimulated LDL oxidation in presence of CP inside the arteries (Heinecke et al., 1987; Hirano et al., 1994). Oxidized LDL has many characteristics that potentially promote atherogenesis. LDL is a chemo-attractant for circulating monocytes (Quinn et al., 1987) (Fig.1).

**Genetic regulation of homocysteine**

The three enzymes contributed to homocysteine metabolism, when there is an excess of methionine ingested, homocysteine follows the transulfuration pathway, through which homocysteine is converted automatically to cysteine. The first reaction in this pathway is catalyzed by vitamin B6 dependent enzyme Cystathionine β synthase (CBS) (Finkelstein et al., 1990). Under conditions with a negative methionine balance homocysteine follows another pathway in which, the homocysteine is remethylated into methionine and this step is catalyzed by Methionine synthase (MS), which uses B₁₂ as coenzyme and methylene-tetrahydrofolate (MTHF) as substrate. The formation of MTHF from tetrahydrofolate is catalyzed by Methylene-tetrahydrofolate reductase (MTHFR) (Engbersen et al., 1995).

**The polymorphisms in regulatory genes and their effect on enzyme activity**

**MTHFR**

The C677T mutation of the MTHFR gene, which leads to the synthesis of a thermo labile form of MTHFR that is responsible for 50% of the MTHFR activity (D’Angelo and Selhub, 1997; Frostell et al., 1995; Kang et al., 1993; Kluijtmans et al., 1996). This autosomal recessive mutation provokes a moderate hyperhomocysteinemia because mutated enzyme has reduced binding with their substrate. It is hypothesized that the serum levels of homocysteine and the MTHFR polymorphism could influence the risk Stroke (Frostell et al., 1995).

**CBS**

Substitution of thymine in the place of cytosine at position 833 (833T→C) is the most frequent polymorphism of Cystationine β Synthase (CBS) gene. Due to this substitution, the enzyme show irresponsiveness for their coenzyme PLP (pyridoxal phosphate) and reduce their activity to catalyze homocysteine, hence, pyridoxine treatment can decrease the hyperhomocysteinemia (Ian, 1991).

**MS**

Methionine Synthase gene shows a common polymorphic form (2756A→G), base transition results into conversion of aspartic acid to glycine, changing the crucial binding site of coenzyme (Vit. B₁₂) and therefore might influence in the secondary structure with possible reduced functional activity, and the function can restore with vit. B12 treatment. (Zang and Die, 2001).

**Leukotrienes an inflammatory activator**

Leukotrienes are short-lived but potent pro inflammatory molecules secreted from macrophages, neutrophils, eosinophils and mast cells (Fig.1). Arachidonic acid is precursor of leukotrienes. First it converted into leukotriene A₄, which is then converted to leukotriene B₄ or to leukotriene C₄ (LTC₄). LTC₄ is subsequently converted to leukotriene D₄ (LTD₄) and leukotriene E₄ (LTE₄). LTE₄ binds to receptors of endothelial cells, and acts as a potent attractor of neutrophils, induces recruitment of CD8⁺ T lymphocytes, and promotes leukocyte adhesion to blood vessel. LTC₄, LTD₄, and LTE₄, can cause altered endothelial cell permeability and vascular smooth muscle cell migration. The effects resulting from leukotriene activity are involved in the early stages of an inflammatory response (Steve et al. 2008).
Fig. 1: Showing the disturbed biochemical events responsible to initiate and elevate the inflammation elevate endothelial Inflammation.

(IL=Interlukine1, Cox=cyclooxygenase, LDL= Low density lipoprotein, RA= receptor antagonist , ROS=Reactive Oxygen Species, NO= Nitric oxide, AMP= Adinosine monophosphate, ACE= Angiotensine converting enzyme, MTHFR= Methyline tetrahydrofolate reductase, CBS= Cystathinine Beta Synthase, NFkB= Nuclear factoe Kappa- light-chain-enhencer of activated B cel l)

**Genetic regulation of leukotrienes**

Arachidonate5-lipoxygenase-activating protein (ALOX5AP) encodes the 5-lipoxygenase-activating protein (FLAP), which is an essential regulator of the biosynthesis of leukotriene A4 (LTA4) this catalyze the conversion of Arachidonic acid into LTA4 (Jerrard et al., 2003; Helgadottir et al., 2005).

**The polymorphisms in regulatory genes and their effect on enzyme activity**

The polymorphism of SG13S114 A/T in ALOX5AP gene at promoter region increases the binding affinity of RNA polymerase to its promoter (Sayers et al., 2003) and increase the production of FLAP hens increases the production of chemokines, proinflammatory cyto-kines in the macrophages and vascular cell wall and the secretion of these inflammatory factors could deteriorate the inflammation, formation of atherosclerosis, stenosis, damage and rupture of the blood vessels (Libby et al., 2002, Elin Lõhmussaar et al., 2005 and Helgadottir et al., 2006).

**Cyclic Adenosine Monophosphate (cAMP) passes inflammatory signals within cells**

cAMP is a second messenger, used for intracellular signal transduction, such as transferring the effects of inflammatory signals which cannot pass through the cell membrane. It is involved in the activation of protein kinases and regulates the effects of inflammatory induction signals (Fig.1).

**The genetic regulation of cAMP within the cells**

Phosphodiesterase (PDE) is phosphodiester cleaving enzyme. The subfamily of PDE enzymes are classified on the basis of substrate diesters into 11 families, namely PDE1-PDE11. PDE4D is the family of enzyme which breaks phosphodiester bond of cAMP (Fig-1), degraded them and maintain the appropriate level and duration of action of cAMP within the cell (Solveig et al., 2003). cAMP is secondary signaling molecule which involve to provoke genes to produce inflammatory mediators by several types of inflammatory cells. Hence extent of signal depends on concentration of cAMP and concentration of cAMP is regulated by PDE4D.

**The polymorphisms in regulatory genes and their effect on enzyme activity**

Because PDE4D degrade cAMP, therefore responsible subside to inflammatory process by stop massaging through cAMP to genes that produce inflammatory proteins.
this gene is polymorphic that effect to catalytic efficiency of PDE4D it will be impact on inflammation process hence on Stroke susceptibility. The SNP42, SNP45 and SNP56 are associated with Stroke (Robert et al., 2006). The 83 T/C is associated with ischemic stroke in a Han Chinese population (NLi et al., 2010). AA an AG genotype of SNP 41 associated with high risk of acute Stroke (Alex et al., 2010 and Bondarenco et al., 2010).

**IL-1RA -Interleukin 1 receptor antagonist an inflammation regulatory protein**

Interleukin 1(IL-1) is synthesized and produced by macrophages, monocytes, fibroblasts and dendritic cells of immune system on response to infection. IL-1 play central role in unregulated inflammation and Stroke susceptibility (Jasmina et al., 2005). Allele2 produces functionally deferent IL-1RA which produced by IL-1RA gene and competitively binds with IL-1 receptors and prevents the cell signaling in IL-1 activated cells.

**The regulation of IL-1 by IL-1RA**

IL-1 exhibit their effects on several types of cells by binding with receptors present on these cells. The biological regulation of IL-1 provoked inflammation is carried out by the IL-1 Receptor Antagonist (IL-1RA) which produced by IL-1RA gene and competitively binds with IL-1 receptors and prevents the cell signaling in IL-1 activated cells.

**The polymorphic effect of IL-1RA**

There is one allele carrying variable number of tandem repeats (VNTR) called allele2 showing positive association with unregulated inflammation and Stroke susceptibility (Jasmina et al., 2005). Allele2 produces functionally deferent IL-1RA which show low affinity for IL-1 receptor on deferent cells and displace less efficiently to IL-1 from these cells and inflammation can not be subsided (Lee et al., 2004 and Bradford et al., 2007).

**Cyclooxygenase 2**

Cyclooxygenase (COX) is an enzyme which catalyses the oxygenation of Arachidonic acid and convert it into potent inflammatory mediators Prostaglandin, Prostacycline and Thromboxans. The two isoform of this gene associated with inflammation Cox 1 and COX2. The function of this isoenzyme is similar but site specific. Cox1 is present in most of the tissue of the body in sufficient amount to catalyze reaction and initiation of inflammation whereas COX2 is the enzyme of endothelial membrane (blood vessels) and under normal circumstances its undetectable amount is present. It is inducible enzyme and up regulated after initial induction of inflammation by COX1 or other pro-inflammatory molecules and initiate acute inflammation within blood vessels (Justice and Carruthers, 2005).

**The polymorphic effect of COX2**

Cipollone et al., 2004, identified a new variant in the COX-2 prometer, a guanine to cytosine substitution at position –765 (–765GC), and showed that this variant is located within a putative binding site for the transcription factor Sp1. Hence this polymorphic variant is producing less amount of COX-2 to initiate acute endothelial inflammation and has protective effect on stroke.

**The concentration of Oxidized LDL and inflammation**

Oxidized low density lipoprotein (LDL) has been shown to modulate the expression of multiple gene products associated with inflammation in several deferent cell types including Macrophages and Neutrophills. The effect is depending on concentration of oxidized LDL (Hamilton and Major, 1996). The LDL transported in to arterial wall and trapped in the three dimensional cage work of fibril secreted by the artery wall cells (Nawab et al., 1991). The trapped LDL is oxidized in the sub endothelial space by secreting oxidative products from arterial wall cells. The oxidized LDL is recognized by Monocytes and oxidized LDL receptors of arterial wall which are notregulated by cholesterol content of cell. The result is a massive accumulation of cholesterol. Such cholesterol loaded cell has a foamy cytoplasm and have been called foam cells (Fig-1). The oxidized LDL induces macrophages to bind arterial wall and this binding of macrophages induces the arterial wall to produce monocytes activating factor. This reciprocal activation induces massive inflammation in the arterial wall (Judith et al., 1995).

**The regulation of concentration of oxidized LDL by HDL and Paroxonase.**

High density Lipoprotein (HDL) was found to protect against LDL oxidation and prevent the production of mildly oxidized LDL by arterial wall cells. The proteineous part of HDL (ApoA-1) associated with an enzyme called Paroxonase which has antioxidant activity, hence protect LDL against oxidation. The concentration of oxidized LDL is determined by the concentration of HDL associated Paroxonase (Watson et al., 1994). Paraoxonase, a 45 kDa glycoprotein, is well known for its ability to detoxify organophosphate (Primo-Parmo et al., 1996).

**The polymorphic effect of paroxonase**

A polymorphism of the paroxonase gene affects position 54 is associated with stroke and cardiovascular diseases. This mutation involves a methionine to leucine replacement in translated paroxonase. The mutation occurs in the NH2-terminal region of the peptide where a highly hydrophobic sequence may facilitate binding of paroxonase to HDL. The consequence of this polymorphism is lower concentration of paroxonase, because it has lower affinity with apoA-I. A positive correlation is present between apo A-I, the structural peptide of HDL, and paroxonase concentrations (Marie-
Claude et al., 1997). Another polymorphism is also shown associated with Stroke susceptibility in young population which leads to a glutamine to arginine substitution at position 192 (Voetsch et al. 2004).

Conclusions

Stroke is multi-factorial disease in which several environment, life style and prolonged hypertension are responsible to arise and progress of disease. In spite of this the two individual with same above factors may respond in deferent manner, because deferent individual may have slightly deferent (Polymorphic) gene variant which form a qualitatively and/or quantitatively changed protein and/or enzyme which may regulate the important pathway. Changed protein and enzymes will disturb the biochemical pathway and would produce toxic level of normal biochemical for endothelial or would produce reduced amount of protecting biochemical for endothelial. The two individual will be affected by there environment and life style factors according to their biochemical levels and respond in deferent manner to their environment and other factors. So, the genetic factor as a whole is risk factor for Stroke and influence the disease diagnosis, effect of medicine on patients as well as the other risk factors. It can change the approach to access disease and handle to cure. This type studies can help to us for early detection of risk population for stroke and the risk can be minimize by improvement of other factors. The findings of meta-analysis studies are clear in which some of the homocysteine metabolizing genes and some of the inflammatory genes have independently significant effect on Stroke susceptibility. Single nucleotide polymorphism and variable number of tandem repeats in described genes is found significantly risk for stroke.

References


phospholipids in MM-LDL are transferred to HDL and are hydrolyzed by HDL-associated esterases. *Circulation.* 90: 1-353.
