WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

A Review: DNA extraction from saliva of stroke patients by magnetic nanoparticles-based method

Abhishek Uniyal

Department of Biotechnology, School of Basic and Applied Science, Shri Guru Ram Rai University, Dehradun-248001, Uttarakhand, India
Email.id: Uniyalabhishek180@gmail.com

ABSTRACT

In Methylenetetrahydrofolate reductase (MTHFR) gene, C677 polymorphism is a risk factor for stroke. DNA from 70 healthy controls and 70 stroke patients was isolated from saliva using by magnetic nanoparticles-based method and from conventional methods by using blood.Bythe result of Real-time PCR exposed that the C677T polymorphism was genotyped through PCR using DNA extracted from both blood and saliva samples.Results of genotype were confirmed by gene sequencing, and for blood and saliva samples result were consistent.Frequency of TT genotype was remarkably higher in stroke patients than in controls.

Homocysteine levels were remarkably higher than controls in both of TT genotype groups. Hence for saliva samples this magnetic nanoparticles-based method used to screen for MTHFR C877T polymorphism in target populations.

Keywords:Magnetic nanoparticles,neural regeneration,stroke,braininjury,homocysteine,gene polymorphism,methylenetetrahydrofolatereductase,gene screening,gene polymorphism,neuro-regeneration, grants-supported paper.

INTRODUCTION

Stroke is a main causing factor of disability and mortality in China.Annually more than 2 million incidents of stroke in China,with 6-7 million survivors of stroke annually. Besides thrombolytic therapy,there are no effective treatments for stroke patients,which applies only 1 to 2% of stroke patients.Therefore, through the modification of risk factors.

For the occurrence of stroke,there are many traditional factors such as age,diabetes, hyperlipidemia,hypertension and smoking,but these features do not account for all cases. In one third of stroke patients no obvious disorder can be diagnosed.Gene defects which related to stroke may act as a risk factor. Frosst etal first represented a candidate genetic risk factor for stroke:-a C-T polymorphism at nucleotide 677 in MTHFR gene (methylenetetrahydrofolate reductase gene),due to its role in catalyzing the production of 5-methylenetetrahydrofolate,a co-substrate for the transformation of homocysteine to methionine.The MTHFR gene is situated at 1p36.3 and consists of 11 exons in which polymorphism appearing at exon 4. for a 677 C-T polymorphism of the MTHFR gene about 10% of the population is homozygous(TT).The TT polymorphism starts reduction in enzyme and subsequent 20% elevation of plasma homocysteine,as a result inhyperhomocysteinemia,which is thrombogenic and atherogenic,contributing to the physiologic mechanisms of stroke.

MTHFR C677T polymorphism role has been widely thoughtful across the world in distinct populations,but the results remain disputable.Variation in methodological and ethnicity differences may account for this discrepancy.The C677T mutation may develop the risk of stroke and analysis of genetic sequences are more critical for the primary prevention of stroke. People with defective genes diagnosed earlier and treated for stroke in advance.

Generally,the genetic diseases detection is rather complicated on a large scale due to the invasive sampling and a DNA extraction process. Blood sample is the most common source of DNA for genetic testing,but it is painful,invasive,and risk infection, making subjects frequently resistant to submit and limiting our ability to play screening of target populations widely.Moreover,the classic methods for DNA purification from blood are time consuming and laborious. Many steps are involved in the DNA purification from blood, including proteinase treatment,detergent mediated lysis,extractions with ethanol precipitation and organic solvents,which also expand the epidemic risk of the test technicians.In such study, we addressed the development of a simple, reliable,rapid,and industrially scale protocol for extraction of DNA from saliva samples. This method is based on the use of magnetic nanoparticles.Magnetic bead-based applications were first developed during the 1980.The major advantages of bead related methods for the small DNA purification include expanded surface area for DNA immobilization, reduced incubation time as well as increased sensitivity.This method has been used in detection of gene for forensic casework and in research areas with plants,animals and microorganisms.Different studies of magnetic nanoparticles to diagnosis disease related on small sample volumes from hair, serum and blood.In someresearches have concentrated on the application of magnetic bead related methods to extract DNA from saliva for use in clinical medicine. Saliva as samples is best alternative source of genomic DNA, it is a painless and noninvasive collection.Saliva samples are much more efficient,convenient,and accessible comparedto blood,if DNA can be purified and extracted.There-fore DNA extraction from saliva samples by magnetic nanoparticles-based method are promising.

The motive of this study was to build a high sensitivity, high accuracy screening manner for fast genetic study of DNA extracted from stroke patient's

saliva. A magnetic bead-based method was built for detection of defective genes. According the experimental results exhibit that this nanoparticles-based method for saliva extraction was more noninvasive and effective than traditional methods using blood as a sample.

REFERENCES

[1] Wu J. Beijing: People's Medical Publishing House; 2005. Neurology. [Google Scholar]
[2] Xu X, Li J, Sheng W, et al. Meta-analysis of genetic studies from journals published in China of ischemic stroke in the Han Chinese population. *Cerebrovasc Dis.* 2008;26(1):48–62. [PubMed] [Google Scholar]
[3] Akar N, Akar E, Ozel D, et al. Common mutations at the homocysteine metabolism pathway and pediatric stroke. *Thromb Res.* 2001;102(2):115–120. [PubMed] [Google Scholar]
[4] Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet.* 1995;10(1):111–113. [PubMed] [Google Scholar]
[5] Goyette P, Pai A, Milos R, et al. Gene structure of human and mouse methylenetetrahydrofolate reductase (MTHFR) *Mamm Genome.* 1998;9(8):652–656. [PubMed] [Google Scholar]
[6] Hankey GJ, Eikelboom JW. Homocysteine and stroke. *Lancet.* 2005;365(9455):194–196. [PubMed] [Google Scholar]
[7] Gaughan DJ, Barbaux S, Kluijtmans LA, et al. The human and mouse methylenetetrahydrofolate reductase (MTHFR) genes: genomic organization, mRNA structure and linkage to the CLCN6 gene. *Gene.* 2000;257(2):279–289. [PubMed] [Google Scholar]
[8] Morita H, Kurihara H, Tsubaki S, et al. Methylenetetrahydrofolate reductase gene polymorphism and ischemic stroke in Japanese. *Arterioscler Thromb Vasc Biol.* 1998;18(9):1465–1469. [PubMed] [Google Scholar]
[9] Soriente L, Coppola A, Madonna P, et al. Homozygous C677T mutation of the 5,10 methylenetetrahydrofolate reductase gene and hyperhomocysteinemia in Italian patients with a history of early-onset ischemic stroke. *Stroke.* 1998;29(4):869–871. [PubMed] [Google Scholar]
[10] Mejia Mohamed EH, Tan KS, Ali JM, et al. TT genotype of the methylenetetrahydrofolate reductase C677T polymorphism is an important determinant for homocysteine levels in multi-ethnic Malaysian ischemic stroke patients. *Ann Acad Med Singapore.* 2011;40(4):186–191. [PubMed] [Google Scholar]
[11] Oh SH, Kim NK, Kim HS, et al. Plasma total homocysteine and the methylenetetrahydrofolate reductase 677C>T polymorphism do not contribute to the distribution of cervico-cerebral atherosclerosis in ischemic stroke patients. *Eur J Neurol.* 2011;18(3):491–496. [PubMed] [Google Scholar]
[12] Li Z, Sun L, Zhang H, et al. Elevated plasma homocysteine was associated with hemorrhagic and ischemic stroke, but methylenetetrahydrofolate reductase gene C677T polymorphism was a risk factor for thrombotic stroke: a multicenter case-control study in China. *Stroke.* 2003;34(9):2085–2090. [PubMed] [Google Scholar]
[13] Angeline T, Jeyaraj N, Granito S, et al. Prevalence of MTHFR gene polymorphisms (C677T and A1298C) among Tamilians. *Exp Mol Pathol.* 2004;77(2):85–88. [PubMed] [Google Scholar]
[14] Banerjee I, Gupta V, Ganesh S. Association of gene polymorphism with genetic susceptibility to stroke in Asian populations: a meta-analysis. *J Hum Genet.* 2007;52(3):205–219. [PubMed] [Google Scholar]
[15] Giles WH, Croft JB, Greenlund KJ, et al. Total homocyst(e)ine concentration and the likelihood of nonfatal stroke: results from the Third National Health and Nutrition Examination Survey, 1988-1994. *Stroke.* 1998;29(12):2473–2477. [PubMed] [Google Scholar]
[16] Zhang G, Dai C. Gene polymorphisms of homocysteine metabolism-related enzymes in Chinese patients with occlusive coronary artery or cerebral vascular diseases. *Thromb Res.* 2001;104(3):187–195. [PubMed] [Google Scholar]
[17] Wald DS, Bishop L, Wald NJ, et al. Randomized trial of folic acid supplementation and serum homocysteine levels. *Arch Intern Med.* 2001;161(5):695–700. [PubMed] [Google Scholar]
[18] Lee M, Hong KS, Chang SC, et al. Efficacy of homocysteine-lowering therapy with folic Acid in stroke prevention: a meta-analysis. *Stroke.* 2010;41(6):1205–1212. [PMC free article] [PubMed] [Google Scholar]
[19] Almeida OP, Marsh K, Alfonso H, et al. B-vitamins reduce the long-term risk of depression after stroke: The VITATOPS-DEP trial. *Ann Neurol.* 2010;68(4):503–510. [PubMed] [Google Scholar]
[20] Toole JF, Malinow MR, Chambless LE, et al. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: the Vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial. *JAMA.* 2004;291(5):565–575. [PubMed] [Google Scholar]