Molecular Approach to Turkish Pediatric Stroke Patients

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Stroke can be defined as focal cerebral damage due to either vascular occlusion or rupture of vessels with subsequent bleeding and neurologic deficits. Stroke secondary to vascular occlusion may occur in the venous drainage of the brain, sinovenous thrombosis (SVT) or in the arterial supply to the brain, arterial ischemic stroke (AIS). Parenchymal damage or infarction of the brain occurs commonly as a squeal of AIS and less commonly of SVT[1]. Stroke is a rare event in childhood. Several figures estimated the incidence at 0.6-1.2 per 100.000 children per year[1]. Figures from Turkey, are hospital based studies indicating that one out of 170 hospitalized pediatric patient experience thrombosis; of which stroke compromises almost one third of the total thrombotic patients[2,3].

Stroke has certain differences between pediatric patients and adults. First it is common in adults, which results in rapid recognition and the potential for early treatment. Second, vascular occlusive strokes in adults are mostly secondary to arteriosclerosis which is not the case in pediatric patients. Third there are special properties of haemostatic system during infancy and childhood. Fourth, there are several developmental differences in the cerebrovascular and neurologic systems[1].

It is important to note that the incidence of thrombotic stroke in children appears to increase. This is probably due to the enhanced sensitive diagnostic tests and surviving of the children with previously lethal diseases such as congenital heart disease, prematurity, acute lymphoblastic leukemia. These two factors led the pediatricians to become aware of the problem.

Although several causes or potential risk factors exist for the occurrence of stroke in children, in about one third of these patients, no obvious cause or underlying disorder can be diagnosed[4-6].

The majority of children (65%) have at least two risk factors with 40% having more than three risk factors[7]. Numerous clinical and environmental conditions leading to a prothrombotic state such as the use of central catheters, trauma, surgery, vessel abnormalities, malignancies, sickle cell disease, autoimmune diseases, cardiac malformations, renal diseases, endocrine disorders , infections etc. were reported[8]. Various prothrombotic disorders particularly affecting the physiological anticoagulant systems have attributable risk for stroke. Prothrombotic disorders can be classified as either congenital or acquired (anticardiolipin antibodies, or Lupus anticoagulants). Congenital disorders include activated protein C resistance (APCR) and gene defects. Most common gene defects include G-A transition at nucleotide 1691 in exon 10 of the factor V

gene causing APC resistance and a G-A transition in the 3' untranslated region of the prothrombin gene at nucleotide position 20210 (G-A) which is associated with increased levels of prothrombin activity[9-11].

Clinical significant relationships have been confirmed between thromboembolism and deficiencies of antithrombin (AT), protein C (PC) and protein S (PS). These are aberrations in the natural anticoagulant systems that occur in plasma and commonly first occur in the presence of acquired risk factors such as surgery, dehydration, and catheters. The prevalence of congenital deficiencies of AT, PC and PS is low, even among patients with familial thrombosis[1].

Two recent prospective studies reported prothrombotic abnormalities in 34% (25 of 73) and 30% of children with acute ischemic stroke[12,13]. Vielhaber et al reported genetic and acquired risk factors of thrombophilia in 31 out of 32 pediatric patients with cerebral venous sinus thrombosis[14].

Further, there exist other mutations possibly effecting to the pathogenesis of stroke in pediatric patients. So, in this mini review, we aimed to compile mutation data in Turkish pediatric stroke patients.

FACTOR V MUTATIONS

Factor V 1691 G-A

The most common cause of thrombophilia is the G-A substitution at the nucleotide 1691 of factor V gene leading a single amino acid alteration in one of the three cleavage sites, ie Arg instead of Gln at position 506. This common mutation causes activated protein C resistance[15]. The prevalence of FV1691A varies among populations. It was reported between 7-10% in healthy Turkish population[16-18]. Our latest data on healthy Turkish subjects revealed 29 FV1691A carriers among 324 (9.0%) ; it was 17% in Antalya and 12.2 % in Turkish Cypriots[19].

Case reports and case series suggest that FV1691A may be a risk factor childhood stroke[20-27]. And further the presence of this mutation plays a role in early onset[20-22,28]. Even it is a genetic risk factor in ischemic stroke of cardiac origin[29].

We reported that FV1691A mutation is also important for the pathogenesis of cerebral infarct[25]. Our latest figures showed that 17 (22%; one being homozygote) of the 77 pediatric stroke patients carried FV1691A mutation[19]. It is interesting that there is a reported hemophilia A patient having cerebral infarct associated with FV1691A mutation[30].

Factor V 4070 A-G (His 1299 Arg)

A4070G (FV1299 His-Arg) polymorphism in exon 13 of the factor V gene (this allele is part of a haplotype named R2) was shown to influence circulating FV levels and contribute to the activated protein C (APC) resistance phenotype[31,32]. Plasma samples from the carriers contain an increased ratio of the more highly glycosylated and more thrombogenic isoform of factor V (V1) which is a more potent cofactor for thrombin generation, and a less potent cofactor for APC-mediated inactivation of factor VIIIa in vitro[33,34]. Most of the previous reports accepted A4070G as a thrombogenic risk factor on the occurrence of deep vein thrombosis, however some of the existing data failed to show the possible role of HR2 haplotype as an independent risk factor for VTE[35-40]. Double heterozygosity for FV1691A and FV 4070G conferred a 3-to 6-fold increase in the relative risk of venous thromboembolism compared with FV R506 Q alone[41,42].

Frequency of FV 1299 G mutation in Turkish population is high as 8.5%[40,43]. We previously reported 26 FV1691A carriers among 129 thromboembolic patients of which six had FV4070G mutation (23.0%) with a 6.7 fold risk compared to controls. It is interesting that of these 6 patients, two patients carried prothrombin 20210A mutation and one patient had protein C deficiency at the same time. Two of the PT 20210A carriers had mesenteric artery thrombosis. The other three had clinical presentation of cerebral infarct, vascular graft thrombosis and Budd Chiari Syndrome[40,42]. Protein C deficient patient was a four year old female child with the diagnosis of cerebral thrombosis[44]. Further, we reported a homozygous ß-thalassemia patient with multiple cerebral emboli due to FV 1299 (His-Arg) mutation[45]. This data lead us to study the distribution of FV 1299 His-Arg mutation in forty six Turkish Pediatric cerebral infarct patients below the age of 18[46]. Ten of the patients were found to carry FV 1299 His-Arg mutation (21.7%), one being homozygous. The cerebral infarct risk for FV 1299 was found to be 2.5 (CI 95% 0.9-7.2) for all group. When all underlying possible conditions were excluded, the incidence of FV4070 mutation increased to 33.3%. The risk also increased to 3.9 (CI 95% 1.2-12.3) indicating

a possible role of R2 haplotype in the pathogenesis of the stroke in pediatric patients[46].

Prothrombin 20210 G-A

Prothrombin 20210 G-A alteration causes a "gain of function" in the coagulation system with an increase of prothrombin levels associated with an increased potential to form thrombin[11]. Prevalence of the mutation varies among different populations. It is about 2.6% in Turkish population[47,48]. Several studies revealed that PT20210A does not represent a risk factor for pediatric stroke[23,28,29]. On the contrary, our study in Turkish pediatric stroke patients and Nowak-Göttl et al's study found that PT20210A is a risk factor[24,25]. Our latest data showed that 11 (14.2%) of 77 patients carry PT20210 mutation[19]. It is important to emphasize here that three of our patients had combination of FV 1691A mutation; one had FV 4070A-G[25,46].

The other two patients had an underlying diagnosis of cystic fibrosis and isolated glucocorticoid deficiency[49,50]. Further meta-analysis of this mutation in these particular patients is needed to enlighten the role in the pathogenesis of pediatric stroke.

HOMOCYSTEIN METABOLISM

Hyperhomocysteinaemia is a known risk factor for cerebrovascular, peripheral vascular, coronary heart disease and thrombosis[51,52]. Heterozygosity and/or homozygosity for mutations in the enzymes involved in homocysteine metabolism may confer an increased risk for thrombosis by causing hyperhomocyteinemia[53]. Metabolic traffic of homocysteine occurs along two major interchanges that lead either, via remethylation, to methionine or, through irreversible transsulfuration, first to cystathionine and then to cysteine. Methylenetetrahydrofolate reductase (MTHFR), Methylenetetrahydrofolate dehydrogenase, methionine synthase (MS), methionine synthase reductase, Cobalamin coenzyme synthesis and cystathionine -Synthase (CBS) are the enzymes that play role in homocyteine metabolism. Deficiency of the enzyme activities result in hyperhomocysteinemia and homocystinuria[51-53].

A common mutation MTHFR (677 C-T; Ala-Val) in homocysteine metabolism have been shown to cause increased plasma homocysteine levels thus causing a predisposition to thrombosis[53]. Recently, another mutation in the same gene, the 1298 (A-C) mutation, which changes a glutamate into an alanine residue decreasing MTHFR activity has been reported to be a risk factor for neural tube defects. However this mutation was not associated with increased plasma homocysteine levels[54].

Although the mutations related to homocysteine metabolism possibly increase the risk of stroke, the data are conflicting[23-25,55]. Other genotypes related to homocysteine metabolism i.e. MTHFR 677 C-T, MTHFR 1298 A-C; MTHFD 1958 G-A; MTRR 66 A-G; and risk assessment of double gene alterations after FV 1691A mutation excluded revealed that neither of the risk alleles of the homocysteine related genes alone increased the risk for the occurrence of stroke[55].

OTHER POSSIBLE MUTATIONS

Plasminogen Activator Inhibitor-1 4G/5G Polymorphism

A decreased fibrinolytic activity due to increased levels of plasminogen activator inhibitor-1 (PAI-1) has been shown in patients suffering deep vein thrombosis[56]. Elevated plasma PAI-1 levels are associated with the 4G allele of a 4G/5G insertion/deletion polymorphism located in the promoter region 675 bp upstream from the transcription start sequence of the PAI-1 gene[57]. The thrombotic risk of carrying PAI-1 4G allele was found to be controversial in previous studies[56-59]. Further, Junker et al concluded that concurrence of FVL and homozygosity for the 4G allele of the PAI-1 4G/5G polymorphism leading to an increased risk for cerebral sinus thrombosis[58]. However a recent study in older women suggested of an important contribution of PAI-1 in cerebrovascular pathology. PAI-1 4G4G homozygotes had a markedly reduced risk of cerebrovascular mortality compared with PAI-1 5G5G homozygotes[60].

Our data and Nowak-Göttl et al's data indicated that PAI-I 4G/5G alteration does not have any thrombotic effect in pediatric stroke patients with and without FV 1691A[61,62].

Endothelial Nitric Oxide Synthase Gene 298 Glu/Asp Variant

Nitric oxide (NO) has an important physiological role in regulating vascular tone and is also relevant to

many pathological processes including hypertension and atherosclerosis. Endothelial constitutive nitric oxide synthase (ecNOS) is the key enzyme in determining basal vascular wall NO production[63]. The gene encoding ecNOS is located on chromosome 7q35-36 and comprises 26 exons spanning 21 kb[64]. It was reported that ecNOS locus has a substantial effect on the variance of plasma NO[65]. A number of polymorphisms have been identified in the ecNOS gene. Among them eNOS 298 Glu-Asp was studied in Turkish pediatric stroke patients[66]. Distribution of 298 Asp allele was found to be 0.279 in pediatric cerebral infarct patients. When compared to controls (0.237), the difference was not significant (p= 0.59). Five (11.6%) of the pediatric cerebral infarct patients carried 298 Asp allele in homozygous state. On the contrary it was (3.6%) in the control group. When underlying factors were excluded, of the 19 patients four (21.05%) had homozygous 298 Asp allele and the frequency of the 298 Asp allele was 0.447. When compared to controls the difference was significant (p= 0.058) with an odds ratio 5.7 (CI, 95% 1.2-28)[66]. Further study is needed to determine the role of eNOS polymorphisms for the occurrence of CVA

Platelet Integrin a2ß1 Haplotypes

Platelet-dependent thromboembolism is an underlying mechanism in the pathogenesis of stroke. The platelet integrin a2ß1, also known as glycoprotein Ia-IIa, is one of the major collagen receptors present on platelets[67]. Platelets adhere to collagen exposed in sub endothelial structures and become activated with the help of glycoprotein Ia-Iia[68-70]. Two silent point mutations on GPI (a2) were found to be associated with the expression density of GPIa-IIa on the platelet surface[71,72]. GPIa 807T/873A is associated with a higher expression of the receptor. Moreover the expression density of GPIa-IIa could be correlated to the rate of platelet attachment to collagen type I[73]. Further, nucleotide polymorphisms in the a2 gene was found to define three alleles of which these polymorphisms was found to be a risk factor for the development of stroke[74].

Although A recent report by Carlsson et al indicated that T807/A873 sequence might be a genetic risk factor for the development of stroke in adults with the age of < 50 years, in our data, there was no significant difference for the haplotypes when controls compared to pediatric stroke group[74,75].

Endothelial Cell Protein C/APC Receptor Exon III 23 bp Insertion

An endothelial cell-specific transmembrane that binds protein C and activated protein C (APC) on the cell surface was named as endothelial cell protein C/APC receptor (EPCR). The human EPCR gene was cloned and a 23 bp insertion of exon III of the gene was suggested that might contribute to thrombosis in myocardial infarction and deep vein thrombosis[76-78]. Of the 127 pediatric thrombosis patients two of the cases carried 23 bp ins mutations. The clinical diagnoses of these two patients were retinal vein thrombosis and cerebral infarct respectively. Cerebral infarct patient (18 months old/female) also carried the prothrombin 20210 a mutation in heterozygous state. These two patients did not have factor V 1691A and 4070G mutations[79]. Although, the frequency of EPCR gene 23 bp insertion is low in our population, it may have effect on the pathogenesis of pediatric thrombosis which needs further investigation with other possible mutations at the gene.

Plasma Platelet-Activating Factor (Val279Phe)

Deficiency of plasma platelet-activating factor (PAF) acetylhydrolase resulting from a missense mutation (Val279Phe) in exon 9 of the gene has been described exclusively in the Japanese population and rarely in Turkey. A pediatric stroke patient was also described[80].

Tumor Necrosis Factor-Alpha Gene Polymorphism (-308 G-A)

Tumor necrosis factor-alpha (TNF-a) is an immunomodulatory cytokine, playing an important role in clot formation by activating platelets, monocytes and endothelial cells and inducing procoagulant substances such as negatively charged phospolipids or tissue factor. There exist inter-individual variation of TNF-a production, which is genetically controlled. A polymorphism due to a G to A transition at nucleotide -308 in the TNF-a gene promoter is a much stronger transcriptional activator than the common allele that is designated as TNF 2 allele[81-83].

Although previous studies revealed no link betwe-

en the genetic regulation of TNF production and venous thromboembolic disease, and antiphospholipid syndrome in adults; Carroll et al concluded that carrying TNF 2 allele could have a slight protective effect against the occurrence of stroke in sickle cell disease patients of the 50 pediatric stroke patients we were not able to detect this mutation[2-3,84,85].

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