Genomic Profiles of Stroke in Blood

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Abstract—These studies show that gene expression changes in most patients by 2 to 3 hours after ischemic stroke, and in all patients studied by 24 hours. (Stroke. 2007;38[part 2]:691-693.)

Key Words: diagnostic methods ■ gene regulation ■ genomics ■ ischemia ■ immunology ■ inflammation ■ pathobiology ■ pathogenesis ■ stroke

Our group set out to develop a “stroke” blood test in 1999 after a move from University of California, San Francisco to the University of Cincinnati. The data presented at this Princeton Conference were the culmination of animal and human studies that took over 7 years to complete. The same session included a presentation by Dr Alison Baird demonstrating very similar findings from humans with ischemic stroke even though they used a different method of cell collection in blood (peripheral blood monocytes) and used a different Affymetrix microarray chip. Nonetheless, these studies will pave the way for developing blood tests for stroke that will not only aid in diagnosis but will point to the causes of the stroke (eg, cardioembolic versus atherosclerotic), as well as possibly guiding acute treatment with tissue-type plasminogen activator (tPA) and other drugs, and perhaps pointing to prognosis and the risk of future strokes.

Proof of Principle Animal Studies of Stroke
To determine whether gene expression would change in blood and brain after different types of brain injury, the following study was performed.1–3 Adult rats were subjected to ischemic strokes, intracerebral hemorrhages, kainate-induced status epilepticus, hypoxia, insulin-induced hypoglycemia, or sham surgery and compared with untouched control animals. Animals were allowed to survive for 24 hours after each type of injury at which time the brain and blood of these animals was assayed for gene expression (RNA levels) on microarrays.

The findings in the brain showed that there were genes shared by all of the types of injury including such genes as GFAP that likely represented acute responses to all of the injuries. In addition, there were unique signatures of gene expression that were unique to each type of injury including ischemic stroke, intracerebral hemorrhage, hypoglycemia and status epilepticus. That is, there were specific changes of gene expression in the brain that were different depending on the type of brain injury.2

Blood produced similar findings. After stroke, hemorrhage, hypoglycemia and status epilepticus, there were large changes of gene expression in the peripheral blood that were common to all of the injuries.1 The common changes of gene expression in the blood were postulated to be caused by “stress.” This was supported by the fact that the “sham-surgery” animals had similar changes of gene expression in the blood.1 Most importantly, however, there were changes of gene expression in the peripheral blood of rats 24 hours after ischemic stroke that were different from hemorrhage, hypoglycemia and status epilepticus. Though no single gene correlated with a given type of injury, a group of genes provided a disease signature, fingerprint or profile for each type of injury including ischemic stroke.1

Rationale for Changes of Gene Expression in Peripheral Blood
Though it might not be apparent why these changes should occur, they were predicted from the very start. The changes of gene expression in peripheral blood include all of the cells in blood including white blood cells, red blood cells and platelets. In addition, neutrophils, monocytes, macrophages, and lymphocytes (CD4, CD8, Natural Killer cells) and B cells each have unique gene expression/RNA profiles.4 Once injury to an organ including brain occurs, there is a robust immune response to that injury, and the cells that respond change their gene expression. This change of gene expression can then be assayed by isolating RNA from blood and examining gene expression on microarrays that can detect all of the RNAs in the genome. Finally, the gene expression in any organ including the blood reflects the current environment of the organ as well as the underlying genetic program of a given individual. Hence, gene expression in peripheral...
blood can give a read out of acute injury, recent local environment, and the genetics of a given person.

**Blood Genomic Profiles in Genetic and Chromosomal Diseases**

To demonstrate various factors that can modify gene expression in peripheral blood, we showed that gender has a profound effect, with Y chromosome genes being highly expressed in males. Age has a profound effect on genomic profiles, with genes related to immunoglobulins being highly expressed in children. We also postulated that genetic diseases would be associated with characteristic changes of gene expression in peripheral blood, if the genes were expressed in blood cells. Hence, we showed that there were characteristic gene profiles in the blood of patients with Neurofibromatosis Type 1 and Tuberous Sclerosis type II, and also a characteristic profile in the blood of children with Down syndrome. The latter findings were of particular note because the Trisomy 21 chromosomal abnormality in Down syndrome produced a reliable profile in peripheral blood. In addition, the blood profile differed depending on whether the Down patients had congenital heart disease. In addition, the profile in the blood of patients with Tuberous Sclerosis also differed depending on whether they had autism.

We have also shown that patients with epilepsy treated with valproic acid have different blood genomic profiles compared with those treated with carbamazepine. These studies are important for emphasizing that any given drug or group of drugs can profoundly affect gene expression in the peripheral blood. We have also shown that children with migraine have different blood genomic profiles from children without migraine. Acute and chronic migraine appeared to be associated with platelet genes, whereas chronic migraine was associated with mitochondrial genes. The data point to the possibility that genomic profiling will make it possible to study complex genetic diseases like migraine, autism, Tourette’s and many others based on complex genomic profiles in the blood and other organs.

**Genomic Profiles of Stroke in Blood**

We then embarked on a study of gene expression in the blood of patients with ischemic stroke. This was done as a part of the CLEAR trial at the University of Cincinnati, in collaboration with Dr Joseph Broderick, Dr Arthur Pancioli, Dr Kenneth Wagner and Dr Edward Jauch who were the principal investigators on this study. Blood was drawn from patients into PAXgene tubes that stabilize RNA from whole blood. The RNA was examined using oligonucleotide microarrays in 15 patients at 2.4±0.5 hours, 5 hours and 24 hours after onset of ischemic stroke and compared with control blood samples. The 2.4±0.5-hour blood samples were drawn before patients were treated either with tPA alone or with tPA plus eptifibatide (the Combination approach to Lysis utilizing Eptifibatide And Recombinant tPA trial).

Nearly 100 genes were regulated in peripheral blood of these patients between 2 and 3 hours after the stroke. Most genes induced in whole blood at 2 to 3 hours were also induced at 5 and 24 hours. These genes included: matrix metalloproteinase-9; S100 calcium-binding proteins P, A12 and A9; coagulation factor V; arginase 1; carbonic anhydrase IV; lymphocyte antigen 96 (cluster of differentiation [CD]96); monocarboxylic acid transporter (6); ets-2 (erythroblastosis virus E26 oncogene homolog 2); elongation initiation factor-2α; cytoskeleton-associated protein 4; N-formylpeptide receptor; ribonuclease-2; N-acetylneuraminate pyruvate lyase; BCL6; and glycogen phosphorylase. The fold change of these genes varied from 1.6 to 6.8, and these 18 genes correctly classified 11/15 patients at 2.4 hours, 14/15 patients at 5 hours and 15/15 patients at 24 hours after stroke. It is notable that well over 1000 genes were significantly regulated in peripheral blood at 5 hours and 24 hours after stroke, representing a significant percentage of the genes expressed in these cells.

Though the numbers of patients were small, we also showed that patients on aspirin before their strokes had different genes expressed in their peripheral blood compared with the patients who had not been on aspirin. However, these aspirin-regulated genes were different from the stroke-regulated genes. There were also genes regulated as a function of race, gender, National Institutes of Health Stroke Scale (NIHSS) and reperfusion. However, most all of these genes were also different from the stroke-regulated genes.

**Genes Induced in Blood Acutely After Stroke Are Expressed in Polymorphonuclear Leukocytes and Monocytes**

In a separate study we had defined the genes that are characteristic of different cell types in peripheral blood of normal individuals. Using this gene library, we then determined which cell types the genes induced in the patients with ischemic stroke were expressed in. The large majority of the genes induced at 2 to 3 hours after stroke were expressed mainly in polymorphonuclear cells (neutrophils, polymorphonuclear leukocytes). By 24 hours there were significant numbers of genes also expressed in monocytes. This finding is important because it points to some of the explanations for the differences in gene lists produced by the studies of Baird and colleagues and those of this study. Moore et al used peripheral blood monocytes, and the Tang et al study used whole blood, thus accounting in part for differences in the studies. In addition, the study of Tang et al examined blood at 2 to 24 hours after stroke and the Moore study examined blood from less than a day out to 4 days or longer. Moreover, the 2 studies used different generation microarray chips that could have also affected the results.

However, to determine how well the genes discovered in the Moore et al study would predict the results of our study, we took the 156 genes shared between the study of Moore et al and those regulated in our study and inserted them into the PAM software. PAM generated a small list of genes from the study of Moore et al that correctly predicted 13 of 15 stroke samples at 24 hours in our study.

Thus, even though differing gene lists were produced in the 2 studies, they produced complementary results, and the essential findings were extremely similar. Most importantly, the genes regulated in the Moore et al study that were regulated in common with our study were nearly as predictive of stroke in the Tang et al study as the genes derived from the Tang et al study itself. This confirmation of results is...
certainly a first for this embryonic field and bodes well for future studies. Future studies will require detailed analyses to determine which genes or sets of genes are accounted for by risk factors such as hypertension, atherosclerosis, smoking, medications and other factors not controlled for in these studies using healthy controls. However, the fact that the majority of genes identified changed expression over the period of 2 to 24 hours suggests that these genes were regulated by brain infarction rather than associated risk factors that would have been constant over this short period of time.

**Future Blood Genomic Profiles of Stroke**

Since the publication of our study, we have also analyzed the data with respect to the causes of stroke in those first 15 patients. Though the numbers are small, we were able to discern a blood genomic profile that correlated with whether patients were believed to have had cardioembolic strokes or whether they had atherosclerotic strokes. The blood genomic profiles of cardioembolic stroke were evident in the 3, 5 and 24-hour blood samples, possibly suggesting an underlying difference of gene expression in the blood of these patients compared with others with different causes of stroke. These data need to be confirmed but point to an important use of this type of data in the future: determining stroke cause, pathogenesis, risk factors, and prognosis.

These studies likely represent just the beginning and likely will be associated with some missteps and mistakes. However, the basic premise will be upheld, and technological and analytical methods will continue to improve in order to bring this approach into the clinical arena. Many basic studies are needed before this, however, including those that will show that ischemic stroke has a different profile in blood compared with intracerebral hemorrhage and subarachnoid hemorrhage, and others that distinguish various types of ischemic stroke based on genomic profiles in peripheral blood and distinguish ischemic stroke from myocardial infarction. In many cases these studies cannot be performed in animal models because the animals have very different immune systems, have very different peripheral blood profiles compared with humans, do not have the same genetics as humans, and are not subject to the same diseases including hypertension and atherosclerosis as humans. Thus, the patient will become the laboratory, and studies will have to be carefully designed to protect individuals from premature use of this data, to maximize the information gained from each set of studies, and to ensure data reliability and reproducibility.

The clinical applicability of microarrays to stroke may be limited because it currently takes days to process blood on microarrays. However, RT-PCR can be used to yield the same information, and this can be performed in a matter of hours. Time may not be a factor in some future applications where patients might obtain baseline genomic profiles that are subsequently used to identify the cause of transient ischemic attacks or stroke, or identify an impending stroke or other vascular event, or are used to confirm the efficacy of a given drug for preventing or treating transient ischemic attacks or stroke.

**Disclosures**

None.

**References**

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