Genetic endothelial systems biology of sickle stroke risk

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Genetic differences in endothelial biology could underlie development of phenotypic heterogeneity among persons afflicted with vascular diseases. We obtained blood outgrowth endothelial cells from 20 subjects with sickle cell anemia (age, 4-19 years) shown to be either at-risk (n = 11) or not-at-risk (n = 9) for ischemic stroke because of, respectively, having or not having occlusive disease at the circle of Willis. Gene expression profiling identified no significant single gene differences between the 2 groups, as expected. However, analysis of Biological Systems Scores, using gene sets that were predetermined to survey each of 9 biologic systems, showed that only changes in inflammation signaling are characteristic of the at-risk subjects, as supported by multiple statistical approaches. Correspondingly, subsequent biologic testing showed significantly exaggerated RelA activation on the part of blood outgrowth endothelial cells from the at-risk subjects in response to stimulation with interleukin-1β/tumor necrosis factorα. We conclude that the pathobiology of circle of Willis disease in the child with sickle cell anemia predominantly involves inflammation biology, which could reflect differences in genetically determined endothelial biology that account for differing host responses to inflammation. (Blood. 2008;111:3872-3879)

Introduction

Many human diseases present in a clinically variable manner, yet the basis for the biologic phenomenon of phenotypic heterogeneity, the variation in presentation of any given disease, is generally unknown. We have used a specific example of this phenomenon to address our overarching hypothesis that genetic, inherited differences in endothelial biology can underlie the phenotypic heterogeneity of human vascular disease.

Sickle cell anemia, caused by inherited homozygosity for the mutant sickle hemoglobin, is a disease characterized by anemia, vascular occlusions, and chronic organ damage. It has an exceedingly complex pathophysiology and incredibly diverse clinical complications.[1] Among these, there are 3 stroke syndromes: clinically silent strokes occurring in children resulting from multifocal small vessel disease; hemorrhagic strokes occurring in adults; and clinical ischemic stroke, the classical stroke syndrome of sickle cell anemia.

Notably, approximately 10% of children with sickle cell anemia develop classic ischemic stroke, with peak age being approximately 5 years.[2,3] Risk factors include elevated white count, low blood hemoglobin, hypertension, and a prior neurologic event.[2-5] However, the primary risk factor is occlusive disease at the circle of Willis (CoW),[6,7] the encircling structure of medium to large vessels at the base of the brain. CoW disease is thought to be causal, as the strokes tend to be due to thrombosis occurring over the area of vessel wall abnormality, and the extent of stroke correlates with degree of CoW stenosis.[2,8] Stroke pathogenesis does not simply involve sickling in the vasa vasorum because vessels in the CoW do not have vasa vasorum.[9]

Our hypothesis predicts that those children with sickle cell anemia who develop CoW disease and are therefore at-risk for ischemic stroke have inherited different polymorphisms (affecting endothelial gene expression) from each other, but polymorphisms that exert similar downstream effects on the relevant biologic systems involved in CoW disease development. Indeed, HLA linkage[10] and sib-pair analysis[11] have suggested a familial predisposition to stroke in sickle cell anemia.

A limitation in understanding this problem is that the identity of the systems biology of CoW disease in sickle cell anemia is, indeed, not known. On the other hand, a great deal is known about the vascular pathobiology of the sickle cell anemia subject in general. Particularly notable is the fact that sickle disease is a systemic inflammatory state.[12] Thus, we expected that our study would probably implicate inflammation signaling as able to discriminate between the 2 study groups: sickle cell anemia children at-risk for ischemic stroke, by virtue of having CoW disease (n = 11); and sickle cell anemia children not-at-risk for stroke by virtue of not having CoW disease (n = 9).

Our approach uses endothelial sampling from carefully selected persons, with subsequent global microarray profiling for endothelial gene expression. Thus, the method is blind, for example, to

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