The Role of Aquaporin-4 Polymorphisms in the Development of Brain Edema After Middle Cerebral Artery Occlusion

Ilka Kleffner, MD; May Bungeroth; Hagen Schiffbauer, MD; Wolf-Ruediger Schäbitz, MD; E. Bernd Ringelstein, MD; Gregor Kuhlenbäumer, MD

Background and Purpose—Some patients develop severe brain edema after complete middle cerebral artery occlusion, whereas others do not. Aquaporin-4 (AQP4) is the main water channel in the brain and has been shown to be critical for the development of brain edema after ischemia. We asked whether genetic variation in the AQP4 gene is related to the severity of brain edema after middle cerebral artery occlusion.

Methods—We genotyped 10 single nucleotide polymorphisms distributed across the AQP4 gene in 41 patients with middle cerebral artery occlusion with and without severe brain edema and assessed single marker association as well as the linkage disequilibrium structure across AQP4.

Results—One single nucleotide polymorphism (rs9951307) at the 3’ end of AQP4 was associated with severe brain edema (dominant model, P<0.01; OR, 0.10; 95% CI, 0.02 to 0.49 for the protective G-allele). Linkage disequilibrium across AQP4 was low; no clear haplotype blocks could be identified for the assessment of haplotype association.

Conclusions—This explorative study shows that genetic variation in AQP4 might contribute to brain edema formation after middle cerebral artery occlusion and warrants further investigation.

Key Words: brain ▪ cerebral infarct ▪ edema ▪ genetics ▪ middle cerebral artery occlusion ▪ polymorphisms

Severe brain edema is a frequent complication of complete middle cerebral artery occlusion (MCAO), often causing severe morbidity or death. Therapeutic options, often applied as rescue therapies, are limited and refer to decompressive craniectomy, hypothermia, and osmoticuretic therapy.1 Although some patients develop little edema without midline shift, other patients with a comparable infarct size proceed to life-threatening brain swelling.2 The cause of these differences has hardly been investigated so far. To find the best therapeutic option for every patient, it would be helpful to know which patient will develop a malignant brain edema and to find a surrogate parameter reliably anticipating brain swelling.

Aquaporin-4 (AQP4) is a major water channel in the brain tissue.3 It is localized in astrocyte foot processes ensheathing cerebral microvessels and ependymal cells. AQP4-deficient mice are relatively resistant to ischemia-induced brain edema,4 and AQP4 expression is regulated in the cerebral cortex in response to cerebral ischemia.5 These findings suggest an important role of AQP4 in the development of brain edema after ischemia. The aim of our studies was to investigate the role of genetic variations in the AQP4 gene in the development of brain edema after MCAO and malignant stroke.

Patients and Methods

The study was approved by the local ethics committee. Forty-one patients with complete MCAO within the last 2 years treated in the Department of Neurology at the University of Münster were identified retrospectively. Complete MCAO was defined by CT and CT angiography on admission. Twenty-two patients fulfilled the criteria of severe brain edema (9 male; mean age, 73 years; SD, 13 years) defined by the following criteria on cranial CT: (1) midline shift; (2) compression of ventricles; (3) herniation; and (4) loss of gyration (Figure), whereas 19 patients showed no or little edema (8 male; mean age, 64 years; SD, 11 years). Clinical signs such as impaired consciousness, disturbance of pupillomotorics, use of external ventricular drainage, and craniotomy further supported but did not define the diagnosis of brain edema. Venous blood samples for genomic DNA isolation were taken from each patient. Ten single nucleotide polymorphisms (SNPs) distributed over the genomic representation of the AQP4 gene (one SNP per 2 kb) were chosen, including the only known missense SNP (rs3906956; Thr278Met). Because no clear linkage disequilibrium (LD) blocks are found in the AQP4 gene (www. hapmap.org), SNPs were distributed approximately evenly across the 2 isoforms of the gene6 (supplemental Figure I, available online at http://stroke.ahajournals.org). SNPs rs9951307, rs3906956, rs11661256, and rs48800773 were genotyped by polymerase chain reaction restriction fragment length polymorphism, whereas SNPs rs14393, rs1058427, rs1058424, rs335929, rs162005, and rs162004 were genotyped by direct DNA sequencing. Details are available on request. Accordance with Hardy-Weinberg equilibrium and the LD structure across the AQP4 gene were
assessed in the whole study population using the program Haploview (www.broad.mit.edu/mpg/haploview) and7. Association was evaluated for all markers on a genotype basis using Fisher exact test. For markers showing significant association, we calculated the association under the assumption of a dominant or recessive genetic model.

Results

This study assessed the genetic association between 10 SNPs in the AQP4 gene and severe brain edema caused by complete MCAO. The genotypes of all 10 SNPs were in agreement with Hardy-Weinberg equilibrium (Table 1). On a genotype basis, only the SNP rs9951307 showed evidence for an association with severe brain edema (P = 0.01, Table 1). Calculation under the assumption of a dominant genetic model demonstrated that the G allele is associated with protection from severe brain edema (Table 2). SNP rs9951307 is located in the genomic DNA downstream of the AQP4 gene. It is not in significant LD with any of the other SNPs analyzed in this study. However, we did not detect any larger blocks of high LD across the whole AQP4 gene. For this reason, no haplotype association analysis was performed.

Discussion

AQP4 is the predominant water channel in the glial processes of brain tissue. AQP4-deficient mice are partially protected against cytotoxic brain edema caused by focal cerebral ischemia.8 AQP4 expression is upregulated in response to cerebral ischemia, and downregulation in the infarct core might reduce edema formation.5 This exploratory investigation on the influence of genetic variations in the AQP4 gene on the development of brain edema after complete MCAO demonstrated a possible dominant protective role of the G allele of SNP rs9951307. The association would not hold after conservative correction for multiple testing, eg, Bonferroni correction. SNP rs9951307 is located approximately 1 kb downstream to the AQP4 gene in a poorly conserved region without known transcription factor-binding sites. Because rs9951307 does not alter the amino acid sequence, it is unlikely that it alters the function of AQP4. However, rs9951307, or another polymorphism in LD with it, might

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Table 1. SNP-SNP Designation According to dbSNP*: Genetic Association Results for Genotypes

<table>
<thead>
<tr>
<th>SNP</th>
<th>rs162004 (HWpval = 0.31)</th>
<th>rs162005 (HWpval = 0.55)</th>
<th>rs4800773 (HWpval = 1.00)</th>
<th>rs11661256 (HWpval = 0.93)</th>
<th>rs3906956 (T278 mol/L) (HWpval = 0.07)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genot</td>
<td>CC</td>
<td>CG</td>
<td>GG</td>
<td>P</td>
<td>AA</td>
</tr>
<tr>
<td>Edema</td>
<td>5</td>
<td>9</td>
<td>8</td>
<td>0.93</td>
<td>15</td>
</tr>
<tr>
<td>No edema</td>
<td>4</td>
<td>7</td>
<td>8</td>
<td>11</td>
<td>7</td>
</tr>
</tbody>
</table>

rs335929 (HWpval = 0.78)

<table>
<thead>
<tr>
<th>SNP</th>
<th>rs1058424 (HWpval = 0.77)</th>
<th>rs14393 (HWpval = 0.31)</th>
<th>rs1058427 (HWpval = 0.93)</th>
<th>rs9951307 (HWpval = 0.68)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genot</td>
<td>CC</td>
<td>CG</td>
<td>GG</td>
<td>P</td>
</tr>
<tr>
<td>Edema</td>
<td>14</td>
<td>8</td>
<td>0</td>
<td>0.28</td>
</tr>
<tr>
<td>No edema</td>
<td>15</td>
<td>4</td>
<td>0</td>
<td>12</td>
</tr>
</tbody>
</table>


HWpval indicates Hardy-Weinberg P value as calculated by Haploview (for details, see Barrett, Bioinformatics, 2005); Genot, genotypes; Edema, genotype distribution in the group of patients with severe edema; No edema, genotype distribution in the group of patients without severe edema; P, P value (Fisher exact test).

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Table 2. SNP-SNP Designation According to dbSNP*: Genetic Association Between rs9951307 Under the Assumption of a Dominant or Recessive Genetic Model

<table>
<thead>
<tr>
<th>SNP</th>
<th>rs9951307 Dominant</th>
<th>rs9951307 Recessive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genot</td>
<td>AG + GG</td>
<td>AA</td>
</tr>
<tr>
<td>Edema</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>No edema</td>
<td>17</td>
<td>2</td>
</tr>
</tbody>
</table>


Genot indicates genotypes; Edema, genotype distribution in the group of patients with severe edema; No edema, genotype distribution in the group of patients without severe edema; P, P value (Fisher exact test); Dominant, assumption of a dominant genetic model with reference to the rare allele; Recessive, assumption of a recessive genetic model with reference to the rare allele.

Figure. CT scan of a patient with MCAO and severe brain edema.
influence AQP4 expression if the polymorphism was located, for example, in an enhancer element. Although the core promoter elements located upstream of AQP4 have already been characterized in detail,9 nothing is known about the localization of other cis-acting regulatory elements like enhancers. LD in the AQP4 gene does not seem to extend over large distances because neither our investigation nor the HapMap project demonstrated any large blocks with high LD across the gene. We conclude that genetic variations around the AQP4 gene may be involved in the development of brain edema and that this knowledge could help to predict the development of malignant brain swelling after MCAO.

Clear limitations of this study are the small sample size and the age difference between patients with and without severe brain edema. It is well known that younger patients tend to develop more severe brain edema after brain infarction. The fact that our patients with severe edema were on average 9 years older than the ones without severe edema argues against the hypothesis that severe edema was due to the well-known effect of younger age. Further association studies in larger patient cohorts and functional investigations of the critical polymorphisms are needed to definitively elucidate the role and regulation of AQP4 in the development of ischemic brain edema.

Disclosures

None.

References

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