Segmental Copy Number Loss of SFMBT1 Gene in Elderly Individuals with Ventriculomegaly: A Community-Based Study

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Abstract

Objective  Idiopathic normal pressure hydrocephalus (iNPH) is clinically important as a treatable gait disturbance or preventable dementia by shunt operation. We have recently reported that approximately 1.5% of the elderly living in a Japanese community showed ventriculomegaly with features of iNPH on MRI (VIM), which may represent a preclinical stage of iNPH. The purpose of the present study was to identify a possible genetic change in VIM subjects.

Methods  Eight subjects with VIM and 10 healthy individuals were examined for copy number variations (CNV) with a CNV-targeted whole-genome oligonucleotide microarray (Agilent 400 K CNV array). Another panel of 100 healthy Japanese individuals was screened for CNV by whole-genome using the deCODE-Illumina CNV 370 K chip. Immunohistochemical examination of the human brain was performed using an avidin-biotin-peroxidase complex method.

Results  Among several genetic changes observed, a copy number loss within the SFMBT1 gene was seen in half of the VIM cases (4 of 8 cases), that was rare among the Japanese control subjects (0/10 by Agilent 400 K CNV array or 1/100 by deCODE/Illumina CNV 370 K chip). Immunohistochemical examination of the human brain revealed that the SFMBT1 protein was localized mainly in the arterial walls, the ependymal cells, and the epithelium of the choroid plexus, all of which play a crucial role in the CSF circulation.

Conclusion  A segmental copy number loss of the SFMBT1 gene may be involved in the pathological process in some individuals with VIM/iNPH.

Keywords: AVIM (asymptomatic ventriculomegaly with features of iNPH on MRI), CNV (copy number variation), NPH (normal pressure hydrocephalus), SFMBT1 (Scm-like with four MBT domains protein 1), ventriculomegaly


Introduction

Idiopathic normal pressure hydrocephalus (iNPH) occurs in the elderly and is characterized by a clinical triad of gait disturbance, cognitive impairment, and urinary incontinence (1-3). The diagnosis of iNPH has recently progressed by use of brain magnetic resonance imaging (MRI). Kitagaki et al described that, in addition to ventriculomegaly of the brain, a disproportional narrowing of the subarachnoid space and cortical sulci at the high convexity of the cerebrum is a hallmark for iNPH on brain MRI (4). These MRI features have been proven to be useful in the diagnosis of iNPH and are now included in the “Guidelines for Management of iNPH” by the Japanese Society of NPH (5).

Since 2000, we have been conducting a community-based prospective study of 790 Japanese elderly individuals using brain MRI (6-10). Among the subjects examined (n=790),...
Table 1. Clinical Characteristics of the VIM* Cases

<table>
<thead>
<tr>
<th>case#</th>
<th>age</th>
<th>sex</th>
<th>Sx (1)</th>
<th>Sx (2)</th>
<th>SFMBT1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70</td>
<td>M</td>
<td>no</td>
<td>no</td>
<td>CN loss</td>
</tr>
<tr>
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<td>72</td>
<td>M</td>
<td>no</td>
<td>D</td>
<td>CN loss</td>
</tr>
<tr>
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<td>70</td>
<td>M</td>
<td>D</td>
<td>D</td>
<td>CN loss</td>
</tr>
<tr>
<td>4</td>
<td>61</td>
<td>M</td>
<td>D+G</td>
<td>D+G</td>
<td>CN loss</td>
</tr>
<tr>
<td>5</td>
<td>72</td>
<td>F</td>
<td>D</td>
<td>D</td>
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<tr>
<td>8</td>
<td>61</td>
<td>M</td>
<td>no</td>
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<td>N</td>
</tr>
</tbody>
</table>

*VIM: ventriculomegaly with features of iNPH on MRI
M: male, F: female, CN: copy number, N: normal
Sx (1): symptoms at first exam.
Sx (2): symptoms 4-8 years later
no: no symptoms
D: demential/cognitive impairment
G: gait disturbance

we found 12 individuals who showed ventriculomegaly with features of iNPH on MRI (VIM) (1.5%); 8 subjects (1%) were asymptomatic (asymptomatic VIM: AVIM), and the remaining 4 (0.5%) had neurological symptoms consistent with iNPH (possible iNPH). Two of the 8 subjects with AVIM later developed gait disturbance and/or cognitive decline during a follow-up period of 4-8 years (10). Therefore, AVIM may represent a preclinical, asymptomatic stage of iNPH (10).

In the clinical settings, iNPH is important because the neurological symptoms are treatable by ventriculo-peritoneal shunt operations; thus, iNPH is called “treatable gait disturbance” and/or “preventable dementia”. However, in a considerable number of patients with iNPH, the once-improved symptoms by shunting worsen again several years after the surgical operation (11, 12), indicating that the shunt operation is just a symptomatic procedure, but not a causal therapy for iNPH. In order to establish a causal therapy for iNPH, it is crucial to clarify a molecular basis underlying the disturbance of cerebrospinal fluid (CSF) circulation in iNPH. However, little is known about a molecular basis of the disease. Since familial occurrence of iNPH has been reported (13), a genetic component may play some role in the pathogenesis of iNPH. Recently, the importance of copy number variations (CNVs), such as loss, duplication, and triplication of a gene, has been recognized as an inter-individual genetic variation across the human genome (14). CNVs were also found to play a significant role in the susceptibility to “sporadic” diseases, such as psoriasis (15), Crohn’s disease (16), glomerulonephritis in systemic lupus erythematosus (17), and HIV (18).

In the present study, we tested the hypothesis that CNV may be related to the pathogenesis of VIM as a genetic predisposing factor, and found that half of the VIM cases had a segmental copy number loss of the SFMBT1 (Scm-like with four MBT domains protein 1) gene, although rare in normal individuals.

Methods

Subjects

Twelve individuals at the ages of 61-72 years with VIM were followed up in our community-based cohort study of Japanese elderly (10); 8 of whom gave informed consent to genetic analysis (Table 1). VIM was defined as having “the features of iNPH on MRI”, which included ventriculomegaly as assessed by the Evans index > 0.3 and a disproportional narrowing of the subarachnoid space and cortical sulci at the high convexity of the cerebrum, as described previously (4, 10). Cognitive impairment was determined to be positive if the score of mini-mental state examination (MMSE) was lower than 24 or the score of Hasegawa dementia scale-revised version (HDS-R) was lower than 20. Qualified neurologists examined all of the subjects, and gait disturbance was determined to be positive if the subject showed walking difficulty that was not explained by orthopedic diseases or any other neurological diseases including Parkinson’s disease, progressive supranuclear palsy, cortico-basal degeneration, motor neuron disease, cerebrovascular diseases, etc. The 8 individuals with VIM were examined for CNV with the Agilent 400 K Human whole-genome CNV microarray together with 10 healthy 70-year-old individuals with no ventriculomegaly on MRI nor any neurological symptoms, who were recruited as controls from the participants in our cohort study. Another panel of 100 healthy Japanese control individuals (all 70 years of age), who were negative for ventriculomegaly on MRI and neurological symptoms, was also examined for CNV with the deCODE-Illumina CNV370K chip. Written informed consent was obtained from all the subjects. The study was approved by the Medical Ethics Committee of the Yamagata University Faculty of Medicine.

CNV typing

Genomic DNA was extracted and purified from peripheral leukocytes in whole-blood samples using a QIAamp DNA blood kit (QIAGEN, Tokyo, Japan). We performed whole-genome screening for CNVs using CNV-targeted oligonucleotide array based on array-based comparative genomic hybridization (array CGH) to detect CNV changes recurrent among the VIM cases. For this purpose, we used the Agilent 400 K Human whole-genome CNV microarray (Agilent Technologies, Santa Clara, CA), which consists of 487,008 probes, including 392,824 CNV probes. CNV array experiments were performed according to the manufacturer’s instructions (19, 20). CNV call and filtering were carried out as described previously (21-24).

deCODE-Illumina CNV370K chip analysis

Another panel of 100 healthy Japanese individuals, as control, was screened for CNV by whole-genome using the deCODE-Illumina CNV370K chip (Illumina Infinium sys-
as likely candidates for hematological diseases.

**Results**

**Clinical characteristics of VIM cases**

The clinical characteristics of the eight cases with VIM are summarized in Table 1. Five cases (cases 1, 2, 6-8) were asymptomatic at the first examination, but one of them (case 2) later developed cognitive decline during a follow-up period of 8 years. The remaining 4 cases were asymptomatic during the period. Three cases (cases 3-5) had cognitive decline and/or gait disturbance at the first examination, and the symptoms remained unchanged at the second examination 4-8 years later.

**Frequent copy number loss of SFMBT1 gene in VIM cases**

To explore possible genomic copy number changes, we conducted whole-genome oligonucleotide CNV microarray analysis (Agilent 400 K CNV array) targeted to CNV-rich regions in the human genome. The results showed that some particular CNVs displayed loss in multiple cases of VIM but not in the 10 controls. Among them, the most frequent copy number loss was found at CNV in the SFMBT1 gene on the chromosome region 3p21.1 in cases 1-4 (Table 1 and Fig. 1). The copy number loss was heterozygous, and occurred at the 12 kb region within intron 2 of the SFMBT1 gene (Fig. 1). Such a copy number loss was absent in all of the non-VIM, normal Japanese individuals (n=10) that we examined in the same Agilent 400 K CNV array analysis. We then examined another panel of 100 non-VIM, normal
Japanese individuals for CNV by deCODE-Illumina CNV 370 K chip. Among the 100 individuals examined, only one displayed this copy number loss, indicating that this CNV is rare in the Japanese population.

To examine whether the SFMBT1 protein was really expressed in the human brain, we performed an immunohistochemical examination and found that intense immunostaining of SFMBT1 was observed in the smooth muscle cells of the medial layer of the arteries in the subarachnoid space and brain parenchyma (Fig. 2A, B). The endothelial cells of the blood vessels, the epithelial cells of the choroid plexus, and the ependymal cells lining the cerebral ventricles were also SFMBT1-positive (Fig. 2A-E). Although weak in intensity, SFMBT1 immunostaining was also detectable in the neuropil (region between neuronal cell bodies in the gray matter) and occasionally in neurons. The immunostaining was localized mainly in the cytoplasm; no apparent nuclear immunostaining of SFMBT1 was found in the brain. In the sections of bone marrow, however, nuclear staining of SFMBT1 was encountered in some hematological cells (Fig. 2F).

To check the specificity of SFMBT1 immunostaining, we performed an absorption test. The result showed that the addition of the antigen peptide to the primary antibody solution completely abolished the SFMBT1 immunostaining (Fig. 3).

**Discussion**

In the present study, CNV analysis among the VIM cases detected a frequent copy number loss (4 of 8 cases of VIM) of the 12 kb region within intron 2 of the SFMBT1 gene on the chromosome region 3p21.1 but not in any of the 10 Japanese control subjects by examination with the Agilent 400 K CNV array. Only one of the 100 Japanese individuals displayed a copy number loss when examined with the deCODE-Illumina CNV370K chip, suggesting that this copy number loss in the SFMBT1 gene is rare in the Japanese
population. Redon et al (14), Wang et al (27) and Cooper et al (28) described its frequencies as being 0.37% (1/270), 9.8% (11/112), and 11.9% (15/126), respectively, by using the human HapMap panel of EB virus-transformed lymphoblast cell line. The frequencies in mixed population of Han Chinese (CHB) and Japanese (JPT) were described as 6.7% (6/90) and 7.8% (7/90), respectively (29, 30). The difference in frequency of the copy number loss in the region of the SFMBT1 gene may be due to the differences in the definitions of the CNV structure, the experimental platforms employed, the ethnicities examined, and/or the source of DNA (peripheral blood or transformed cell line).

The mechanism by which the deletion variant of SFMBT1 intron 2 contributes to the gene expression remains undetermined. One may speculate that a large deletion within intron 2 may modulate transcriptional regulation through disturbance of splicing or chromatin structuring. In this context, it is worth noting the presence of the binding site of BAF155 (Brg-1-Associated Factor, 155 kD) within intron 2 of the SFMBT1 gene. BAF155 is a ubiquitous component of the SWI/SNF chromatin-remodeling complex (31). The deletion in intron 2 may influence chromatin structuring of the gene or the genome region. Alternatively, the CNV variant might be in linkage disequilibrium with other genetic variations, which influence the function and/or structure of the SFMBT1 gene or nearby gene(s).

The present study has some limitations derived from the small number of VIM cases examined. Since VIM is a relatively rare condition (only 12 cases were identified among the 760 elderly residents in a Japanese community) (10), in the present study it was only possible to examine genetically 8 subjects with VIM. Therefore, it is not proper, at present, to draw a conclusion that a copy number loss in the SFMBT1 gene confers susceptibility to VIM. However, the present study seems to provide a novel molecular target for VIM/iNPH research, and will pave the way to search for a molecular pathogenesis of the disease. It is necessary to conduct a nationwide survey to collect sufficient cases of VIM/iNPH for genetic, case-control, and functional/biological studies, leading to the development of a new therapeutic strategy for iNPH.

The human SFMBT1 gene shares a high similarity with the Drosophila Scm (sex comb on midleg) gene. The SFMBT1 gene encodes a protein with 866 amino acid residues, which contains four malignant brain tumor (MBT) repeat domains (32, 33). In the adult mouse, SFMBT1 mRNA has been reported to be expressed most abundantly in the testis, but many other tissues, including the brain, also express SFMBT1 mRNA (34). At present, however, no information is available about the expression and localization of SFMBT1 protein in human tissues, including the brain. In the cell-lines of human origin, expression of the SFMBT1 protein has been reported to be detectable only in specific cell types, mainly those of hematological origin, such as the erythroblastic K562, myeloblastic HL-60, and B-cell lymphoblastic Daudi cells (32). In contrast, the protein was barely detected in epithelial cell lines derived from the uterus (HeLa), breast (MCF7), and kidney (HEK293) (32). In erythroblastic K562 cells, the SFMBT1 protein was shown to be localized in the nucleus (32). In the present study, in agreement with a previous report (32), some hematological cells had SFMBT1 protein in the nucleus. In the human brain, however, SFMBT1 immunostaining was observed in the cytoplasm of the cells, including the smooth muscle cells in the medial layer of the arteries, the endothelial cells of the blood vessels, the ependymal cells lining the ventricles, and the epithelial cells of the choroid plexus. SFMBT1 has been shown to bind to the N-terminal tail of histone H3 and to repress transcriptional activity in cultured cells (32). The present observation, in which the protein was localized in the cytoplasm, suggests that SFMBT1 might have another, yet undetermined, function in the cytoplasm. Alternatively, the SFMBT1 protein may be shuttled between the nucleus and the cytoplasm under certain conditions.

![Figure 3. SFMBT1 immunostaining (A) is completely abolished by the addition of the antigen peptide to the primary antibody solution (B). A and B are serial sections.](image-url)
ther biochemical study is warranted to elucidate the precise function and localization of the SFMBT1 protein in the human tissue.

CSF is secreted from the choroid plexus into the cerebral ventricles. After moving through the ventricles into the subarachnoid space, CSF is finally absorbed by the pachionian granulations into the superior sagittal sinus. It has recently been reported that a significant amount of CSF is also absorbed by the blood vessels of the subarachnoid space, leptomeninges, and their underlying brain parenchyma (35). With respect to the pathogenesis of iNPH, decreased compliance of the intracranial arteries is considered to be crucial for ventricular dilatation (35). In the present study, we observed SFMBT1 immunostaining in the choroid plexus, ependyma, and blood vessels, all of which are important structures for secretion, flow, and absorption of CSF, respectively. Therefore, it is conceivable that the dysfunction of SFMBT1 may predispose individuals to develop VIM with advancing age. As the next step of the study, it would be intriguing to examine whether clinically definite, shunt-responsive patients with iNPH also have a copy number loss in the SFMBT1 gene. The clinical application of this analysis for a large number of iNPH patients would need the establishment of a simple, target-specific method for measuring the copy number loss, such as target-specific genomic amplification (36). Although autopsy of an iNPH patient is extremely rare in the world, it is also important to perform an immunohistochemical examination of the iNPH brain for SFMBT1 protein.

The authors state that they have no Conflict of Interest (COI).

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