



A proposed synergistic effect of *CSF1R* and *NMUR2* variants contributes to binge eating in hereditary diffuse leukoencephalopathy with spheroids

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Background: The genetic mechanisms of binge eating (BE) as a disease identity remain obscure. BE is usually viewed as a part of the behavioral variant of frontotemporal dementia (bvFTD) features. We encountered a family with hereditary diffuse leukoencephalopathy with spheroids (HDLS) that manifested uniformly with binge-eating-onset dementia. The genetic factors associated with the rare phenotype were investigated.

Methods: The detailed phenotypes of the patients were described. We performed whole-exome sequencing (WES) of family members and repeat-primed PCR to analyze the patients' expansion size of *C9orf72*, a well-established gene causing FTD. The WES results of additional HDLS patients without BE manifestations were also investigated.

Results: All affected individuals had a BE-dementia-epilepsy pattern of disease progression. A recurrent disease-causing mutation in *CSF1R* established the diagnosis of HDLS in the family. No abnormalities in the expansion size of *C9orf72* were detected. The concurrence of a recurrent *CSF1R* mutation and a rare variant in *NMUR2*, a gene functionally related to BE, was revealed in the affected family members. No potentially pathogenic variants in other known BE-associated genes were identified. Both the *NMUR2* variant and the *CSF1R* mutation cosegregated with the BE-dementia-epilepsy phenotype in the family. In three additional HDLS patients without BE, no pathogenic variants in *NMUR2* were detected.

Conclusions: We propose that synergistic genetic effects of *NMUR2* and *CSF1R* variants may exist and contribute to the development of the BE phenotype in HDLS. *NMUR2* is one of the potential susceptible genes in BE and may contribute in a background of a disrupted structural neuronetwork. Further studies in other BE-related disorders are required.

Keywords: Binge eating (BE); genetic modifier; hereditary diffuse leukoencephalopathy with spheroids (HDLS); *NMUR2*

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Introduction

Binge eating (BE) is the most common type of eating disorder and is characterized by a loss of control over eating, during which the individual consumes an unusually large amount of food within a short period of time in the absence of regular inappropriate compensatory behaviors (1). BE is seldomly mentioned among existing disease entities but is associated with substantial psychiatric symptoms (2,3). A strong genetic contribution has been suggested for BE (4,5). Family and twin studies have provided evidence that BE aggregates strongly in families independently of obesity (6). Variations in the melanocortin-4 receptor gene (*MC4R*) (7) and several other genes, including *TPH-1*, *HDAC4*, *ESRRA*, *DRD2*, and *BDNF*, and the serotonin-transporter-linked polymorphic region (5-HTTLPR) of *SLC6A4* (1,8), have been suggested to be associated with eating disorders, including BE (9). There are also genes that are functionally related to BE that were not screened in BE patients, such as neuromedin U receptor 2 (*NMUR2*) (10).

Hereditary diffuse leukoencephalopathy with spheroids (HDLS) is an autosomal dominant white matter disease caused by mutations in the protein tyrosine kinase domain of the colony-stimulating factor 1 receptor (*CSF1R*), encoded by the *CSF1R* gene on chromosome 5q32.4. HDLS is characterized by behavioral changes, memory decline, executive dysfunction, depression, motor impairments, and seizures (11,12). BE is not a common manifestation in HDLS. In a review study summarizing 51 HDLS patients (13), seven patients had documented hyperorality and dietary changes that are viewed as a part of the behavioral variant of frontotemporal dementia (bvFTD) features. HDLS is representative of primary microgliopathies, and patients' cranial MRI findings are consistent with frontal-predominant leukoencephalopathy and concomitant cerebral atrophy (14).

As BE disorder has always been complicated by psychiatric comorbidities, single-gene disease models with a BE phenotype would be realistic for studying its genetic mechanism with the benefit of a pure hereditary and clinical background. Recently, we encountered a family with *CSF1R*-related leukoencephalopathy that manifested uniformly with BE-onset dementia. In this study, we investigated the genetic factors associated with this rare phenotype.

Methods

We obtained informed and written consent from available family members, and the study was approved by the Ethical

Review Board of Peking Union Medical College Hospital. Detailed clinical manifestation of all the affected family members had been recorded. Genomic DNA was extracted from peripheral blood leucocytes using a QIAamp DNA Blood Midi Kit (Qiagen, Valencia, CA, USA) according to standard protocols. Exome enrichment of 3 µg genomic DNA (individual II-4, III-3, III-4 and III-5) was performed using the Agilent SureSelect Human Exon Kit capture library. The capture library was then loaded onto the HiSeq2000 platform. Raw image files were processed by Illumina base calling Software 1.7. Reads were aligned to a human reference sequence (UCSC assembly hg19, NCBI build 37), and genotypes were called using SAMtools or GATK. Variants were annotated using SeattleSeq (<http://sngs.washington.edu/SeattleSeqAnnotation131/>), SIFT (<http://sift.jcvi.org/>), and PolyPhen2 (<http://genetics.bwh.harvard.edu/pph2/>). Population frequencies for each variant were determined in dbSNP, the 1000 Genomes Project, and the NHLBI Exome Sequencing Project version ESP6500 exome variant server [<http://evs.gs.washington.edu/EVS/> (1 Dec 2013)]. For each sample, the coverage of the targeted region was more than 99.1%. Over 58% of at least 83 million reads were uniquely mapped to the targeted human exon regions to give an average depth of 70. Under such coverage, approximately 97% of targeted regions were covered by 4 reads or more, and 91% were covered by more than 20 reads.

Results

Clinical presentations

The family demonstrated an autosomal dominant inheritance pattern (*Figure 1*). The proband was a 43-year-old female who became apathetic and less social for two years. She often sat alone at family gatherings and showed diminished interrelatedness. With disease progression, cognitive impairment and urinary incontinence ensued. Her short-term memory declined quickly, and she was incapable of doing housework such as cooking meals. The patient gained weight, over 10 kg within 3 months, before her visit to our hospital because of compulsive eating at every mealtime. Her family also reported that she experienced episodes of unconsciousness with suspected convulsion. Neuropsychological testing revealed depression and comprehensive cognitive decline, including marked deficits in attention and executive dysfunction, short- and long-term memory decline, nonfluent aphasia, dyscalculia and a loss of visual space, with an MMSE score of 15/30 and an

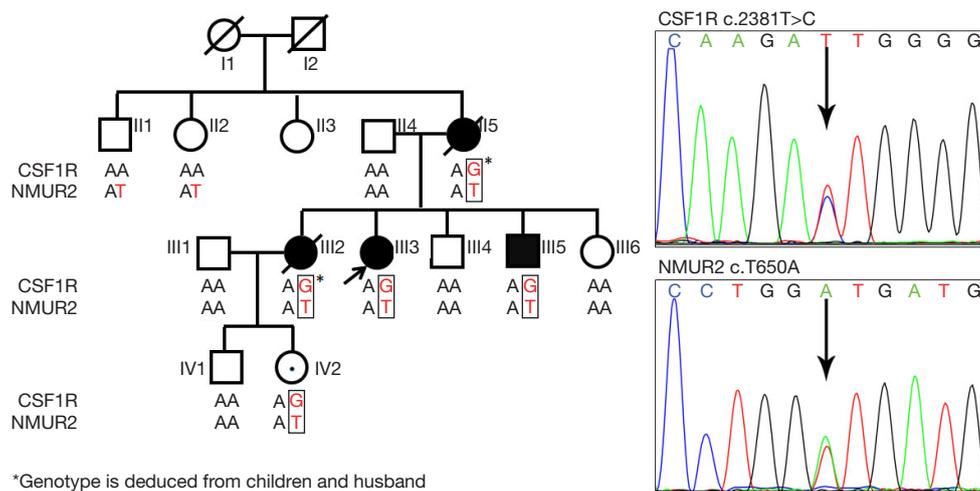


Figure 1 Haplotype of the family with hereditary diffuse leukoencephalopathy with spheroids (HDLS) complicated with a binge-eating phenotype. The proband is indicated by the arrow.

MoCA score of 13/30. Other neurological examinations were essentially unremarkable, with no paralysis, ataxia or sensory deficits. Routine blood and urine laboratory examinations were normal. Electroencephalography showed diffused slowing waves. Cranial MR imaging (*Figure 2A*) showed severe symmetric confluent bifrontal and biparietal white matter lesions, with marked cortical and subcortical atrophy. The corpus callosum was also involved. There were several foci showing hyperintensities on T2-weighted and fluid-attenuated inversion recovery (FLAIR) imaging as well as diffusion-weighted imaging with a decreased apparent diffusion coefficient. No contrast enhancement was seen. Subsequently, the activity of enzymes in the serum and leukocytes, including α - and β -galactosidase, α - and β -glucosidase, α -fucosidase, α -mannosidase, β -hexosaminidase, arylsulfatase A and galactocerebroside, as well as the serum level of very-long-chain fatty acids, were tested and were all within normal ranges. HDLS was diagnosed based on her clinical and genetic findings. The patient was given topiramate and antidepressant treatment. Her older sister (family member III-1) showed similar symptoms at the age of 37 and was admitted to our hospital in 2006. Personality changes and cognitive impairment were the initial features. She was described as being apathetic, having difficulties at work, and losing personal hygiene. She gained more than 10 kg of weight within 10 months because of compulsive eating. Brain MRI showed significant patchy and confluent bifrontal and biparietal periventricular white matter lesions, with moderate cortical and subcortical

atrophy. Lumbar puncture and stereotactic brain biopsy of the left frontal lobe were performed during hospitalization. CSF examination was unremarkable. Microscopic examination showed a reduction in and vacuolar degeneration of cortical neurons and reactive astrocytosis with axonal dilations (spheroids) in the subcortical white matter. The spheroids were GFAP positive but were negative for ubiquitin. No infarction, demyelination or lymphocytic infiltrates were observed. The cortical and medullary vessels were unremarkable. Pathological findings were in line with sclerosis leukoencephalopathy. Within five years after symptom onset, she became wheelchair-bound, had severe dementia with communication difficulties, and suffered from frequent seizure attacks before dying.

The proband's mother showed the exact similar pattern of disease progression. She began to show symptoms at the age of 58. After going through all the stages of binge eating, dementia and epilepsy, she finally became unresponsive and died five years after symptom onset.

The proband's 39-year-old younger brother reported no symptoms other than transient numbness in the right limbs. His brain MRI (*Figure 2B*) showed asymmetric and patchy lesions in the frontal periventricular white matter, which were identified as presymptomatic changes of HDLS (14). He was thus diagnosed with presymptomatic HDLS.

Genetic analysis

Whole exome sequencing (WES) data were estimated

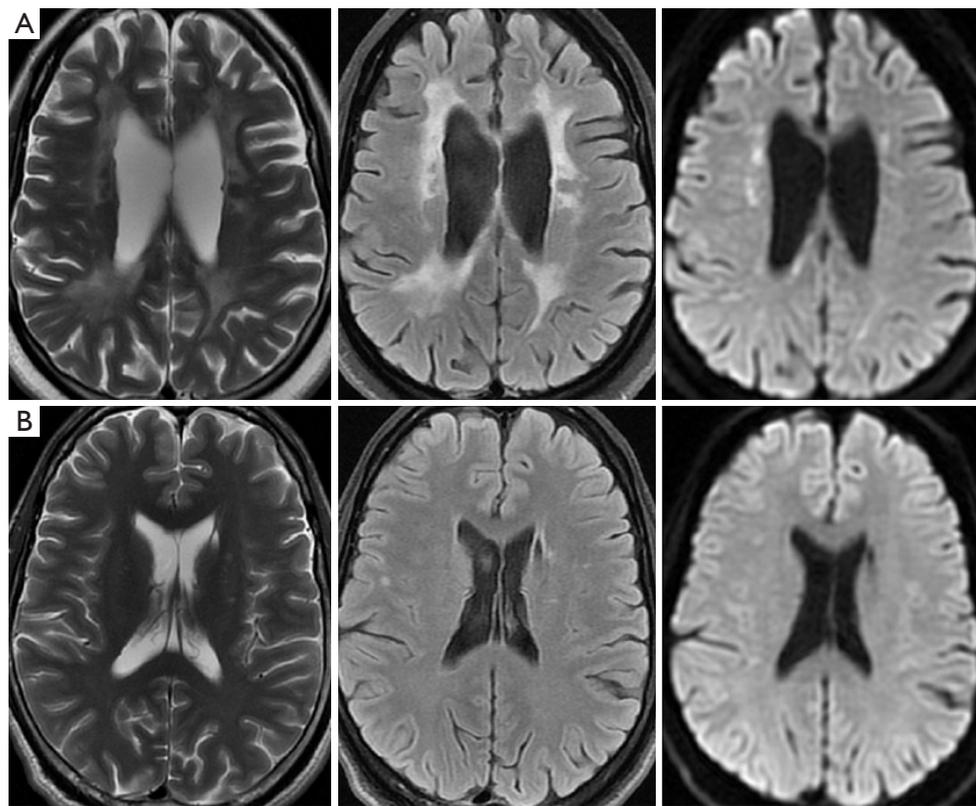


Figure 2 Brain imaging and pathology of the affected individuals with hereditary diffuse leukoencephalopathy with spheroids (HDLS). (A) Images of the proband. On T2-weighted images and fluid-attenuated inversion recovery magnetic resonance imaging, severe symmetric confluent white matter lesions with concurrent cortical and subcortical atrophy are shown. Some lesions are hyperintense in diffusion-weighted imaging with a decreased apparent diffusion coefficient. (B) Images of the proband's younger brother (individual III-5). Cerebral magnetic resonance imaging shows asymmetric and patchy lesions in the frontal periventricular white matter.

by strict screening in which only predicted pathogenic rare nonsynonymous, splicing and frameshift variants were selected. Potentially pathogenic variants in five genes (*CSF1R*, *EML1*, *NMUR2*, *PITPNM2* and *SPDYC*) were filtered out by exome sequencing in the four family members. Further analysis of linkage disequilibrium in the family by Sanger sequencing of the DNA of six family members (II-1, II-2, III-1, III-6, IV-1, IV-2) suggested a recurrent mutation in *CSF1R*, c.2381T>C(p. I794T) (15), cosegregated with the disease. The family was therefore genetically diagnosed with HDLS. Notably, individual IV-2, a 28-year-old female who is currently pregnant, is an obligate carrier of *CSF1R* mutation with no symptoms yet.

When inspecting rare variants that may be associated with BE, one single nucleotide variation (SNV), c.T650A(p. I217N), in *NMUR2*, was identified as being transmitted from the proband's maternal line. The reported

frequency is 0.046% (1/2,178) in dbSNP135 and 0.05% in the 1000 Genome Project database, which made it potentially pathogenic. We further examined the SNV in a noncommercial in-house database composed of Chinese Han individuals without neurological disorders and obtained a variant frequency of 0.3% (9/3,004). Single-nucleotide polymorphisms (SNPs) in the genes documented to be associated with eating disorders, including *MC4R*, *TPH-1*, *HDAC4*, *ESRRA*, *DRD2*, *BDNF* and *SLC6A4*, were also inspected for the proband. SNPs in those genes are listed in *Table S1*. However, these SNPs were either inherited from the proband's paternal line so that they were not involved with the disease or lost in the affected individuals (as in the genes of *MC4R* and *SLC6A4*). Since the disease phenotype mimics bvFTD, we also examined the hexanucleotide GGGGCC repeat expansion in *C9ORF72* by repeat-primed PCR with a previously reported method (16).

The result was negative with a 6-time repeat expansion size. The WES data of three genetically confirmed HDLS patients without BE manifestations were also investigated for *NMUR2* rare variants. They had 5, 2 and 1 SNPs, respectively. No rare variants with a minor allele frequency (MAF) less than 0.5% documented in the ExAC or dbSNP databases were identified.

Conclusions

Neuromedin U (NMU) is a gut-brain peptide that regulates food intake (17) and body weight (18) via the peripherally expressed NMU receptor 1 (NMUR1) and the central NMUR2. NMUR2 knockdown rats demonstrated significantly greater binge-type food consumption of the high-fat diet and showed a greater preference for higher-fat food than controls (10). However, despite its functional involvement with eating abnormalities, the *NMUR2* gene has never been screened in patients with associated disorders. This was mostly because BE in most scenarios is complicated with psychiatric comorbidities, which would infer a polygenic or additive genetic pattern of inheritance. Adults with BE usually experience anxiety disorders, mood disorders, impulse control disorders, or substance use disorders. These complex phenotypic backgrounds made genetic analysis impossible for BE studies.

In the family that we studied, HDLS patients had BE-onset dementia as a uniform presentation, and had a similar pattern of disease progression. A potentially pathogenic variation in *NMUR2* was carried by the first- and second-degree relatives of the proband's mother along with the *CSF1R* mutation. No other potentially pathogenic variants in the existing BE-associated genes were detected to cosegregate in the family. Additional analysis of three HDLS patients without BE symptoms revealed no rare variants in *NMUR2*. We therefore propose that these overlapping phenotypes of HDLS dementia and BE may be explained by shared genetic risk factors and that *NMUR2* is a genetic modifier of HDLS caused by *CSF1R* mutation. Carrying the very rare *NMUR2* variant would modify the disease phenotype, in the current case, contributing to the manifestation of BE in HDLS.

It should be noted that family members with the *NMUR2* variant but without the *CSF1R* mutation did not present with BE, suggesting that having *NMUR2* rare variant(s) would not be sufficient for developing the phenotype. In HDLS, diffused white matter lesions with frontal and parietal lobe predominance involving the periventricular

deep white matter and the dilation of the lateral ventricles detected by brain imaging were confirmed (19). Neuropathological findings include prominent lesions with myelin and axonal damage, neuroaxonal swelling (spheroid formation), and pigmented macrophages and glia (20). On cerebral MRI scans, the white matter lesions without gadolinium enhancement can be scattered and asymmetric during the initial stages of the disease but later become confluent, diffuse, and more symmetric. A thinning of the corpus callosum with signal abnormalities is observed even in the early stages of the disease (21). These are indications for diffuse disruption of the structure and function of the brain in HDLS. NMUR2 is a high-affinity receptor for NMU found in discrete regions of the central nervous system, particularly the paraventricular nucleus (PVN) of the hypothalamus, which may be responsible for mediating the anorectic effects of NMU (22). It is an intriguing hypothesis that *NMUR2* variation may contribute to the BE phenotype in a background of a disrupted neuronetwork and that *NMUR2* is possibly one of the candidate or susceptible genes of BE, as shown in rats (10).

Our results also highlight that genetic research will not only assist with the identification of risk alleles but also unlock additional risk factors dependent on genotypes. The advent of next-generation sequencing technologies and its rapid decline in sequencing costs have accelerated the identification of causative variants in most genetic disorders. The rare phenotype in certain diseases may be determined by combined or additive genetic deficits. It is appropriate to define a complete genetic profile of the affected individuals for a phenotype explanation.

In summary, we propose a synergistic genetic effect of *NMUR2* and *CSF1R* variants, which may result in the development of the BE phenotype in the current family with HDLS. Further analysis of *NMUR2* in additional BE-related diseases and models would be required to provide supporting evidence.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest

to declare.

Ethical Statement: The authors are accountable for all aspects of the work and for ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. We obtained informed and written consent from available family members, and the study was approved by the Ethical Review Board of Peking Union Medical College Hospital (No. ZS-1554).

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Supplementary

Table S1 Additional variants identified in the family in the genes documented to be associated with eating disorders

Gene	Variation	Exonic function	dbSNP135
<i>HDAC4</i>	c.C2565T(p.P855P)	Synonymous	rs1063639
<i>ESRRA</i>	c.G1055C(p.R352P)	Nonsynonymous	none
<i>DRD2</i>	c.T852C(p.H284H)	Synonymous	rs6275
<i>BDNF</i>	c.A450G(p.A150A); c.G196A(p.V66M)	Synonymous; nonsynonymous	rs2353512; rs6265