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Loss-of-function variation in the *DPP6* gene is associated with autosomal dominant microcephaly and mental retardation

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ABSTRACT

The molecular basis of autosomal dominant microcephaly, a disorder associated with small head circumferences that results in variable mental retardation, is largely unknown. In the present study, we conducted a variation analysis of the *DPP6* gene in patients with autosomal dominant microcephaly and variable mental retardation. The copy number variation analysis of *DPP6* was performed on DNA samples from 22 patients with microcephaly using high-resolution, array-based genomic hybridization, and sequence analysis was performed to screen mutations in another 50 microcephalic patients. Two de novo deletions and one missense mutation in familial microcephalic patients were identified. The transfection of plasmids encoding green fluorescent protein-pLLU2G-shDPP6 fusion proteins in mouse brains revealed that the decreased expression of the *DPP6* gene slightly reduced the weight of the mouse brains and resulted in mouse learning disabilities compared with their wild-type littermates. Our data indicate that the loss-of-function variations in DPP6 are associated with autosomal dominant microcephaly and mental retardation. DPP6 appears to play a major role in the regulation of proliferation and migration of neurons in neurogenesis, most likely by participating in neuronal electrical excitability, synaptic integration, and plasticity.

1. Introduction

Microcephaly is a rare condition characterized by a small occipitofrontal head circumference (OFC) that is 2 or more standard deviations below the mean (−2 SD) according to age and gender [1]. It has an approximate incidence rate of 0.1–2% in the general population [2]. Microcephaly can present in syndromic and non-syndromic forms, often accompanied by variable degrees of mental retardation. The severity of mental retardation has been related to the degree of microcephaly and the abnormalities in the brain anatomy [3]. Isolated microcephaly has been defined given the following clinical features: uncomplicated by other abnormalities; present at birth; associated with normal pregnancies, deliveries, and postnatal periods; associated with or without early psychomotor retardation; and non-progressive. The brain has a normal architecture except for being small [4]. The genetic factors that can cause isolated microcephaly are mostly Mendelian autosomal dominant, recessive, or X-linked genes, and some cases are caused by other rare chromosomal aberrations [5]. Individuals with autosomal dominant microcephaly exhibit some differences from those with autosomal recessive inheritance: Firstly, the stature of the patients with autosomal dominant microcephaly can be in the normal range; Secondly, patients with autosomal dominant microcephaly are generally categorized as being moderately to mildly or borderline retarded, and many of them are capable of leading relatively normal lives [6]. Moreover, investigators have identified families that have autosomal dominant microcephaly and short stature with normal intelligence [7,8]. The molecular basis of autosomal dominant microcephaly is largely unknown. Dipeptidyl-peptidase-like protein 6 (*DPP6*), a critical component

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protein in voltage-gated potassium (Kv) transmembrane channels in neurons, is located on chromosome 7q36.2 and is thought to play distinct roles in brain development. The *DPP6* protein is an integral membrane glycoprotein with a short cytoplasmic N-terminal domain, one transmembrane domain, and a long extracellular C-terminal domain. The function of *DPP6* is associated with Kv4 channels, facilitating the surface expression and modifying the kinetic and steady-state properties of the channel by regulating subthreshold-activating A-type K+ currents [9,10]. In mice, the loss of *DPP6* has been proven to result in abnormal electrophysiological neuron characteristics [11,12]. To date, abnormal variations in *DPP6* have been associated with amyotrophic lateral sclerosis (ALS) [13] and idiopathic ventricular fibrillation [14]. Collectively, *DPP6* plays fundamental roles in neuronal and excitable cell function, and *DPP6* dysfunction has been implicated in neurological disorders and diseases.

In the present study, we performed whole genome copy number variation analysis of 22 patients with either sporadic or familial microcephaly and mental retardation, and two de novo genomic deletions harbouring the *DPP6* gene were found in 2 patients. These findings prompted us to search for *DPP6* mutations in other microcephaly patients and construct a *DPP6*-knockdown mouse model using siRNA technology to further evaluate the microcephalic phenotype.

2. Materials and methods

2.1. High-resolution array-based comparative genomic hybridization

Peripheral blood samples from all participants were collected after informed consent and study approval by the Guangzhou Women and Children's Medical Center Institutional Review Board. Genomic DNA was extracted from blood using the Qiagen DNA blood Mini Kit (Qiagen, Germany). To identify candidate loci for microcephaly we first carried out a genome-wide copy number scan in 22 patients associated with microcephalic phenotypes using Affymetrix GeneChip Cytoscan HD array containing 2,696,550 markers for copy number analysis (Affymetrix, the USA). Array experiments were carried out according to the standard protocol of the manufacturer. The Affymetrix GeneChip Scanner 3000 7G was used in image processing. Copy number variations (CNVs) were called with the Affymetrix GeneChip Chromosome Analysis Software (CHAS 1.2.2). Array analysis was also performed on the blood sample of all parents of the patients and other 43 ethnically matched control subjects.

2.2. DPP6 gene mutational analysis

Genomic DNA was extracted from peripheral blood leukocytes from the parents of the above two individuals (Case BY0712 and Case BY2018), 50 other random microcephalic patients and 50 normal control subjects. All 26 exons of the DPP6 gene were amplified by means of the polymerase chain reaction (PCR) with the use of DPP6-exon primer, respectively. Sequences of the primers used to amplify the exons of DPP6 are available on request. Direct cycle sequencing of the PCR products was performed with the use of the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and analyzed with the use of the ABI Prism 310 Genetic Analyzer (Applied Biosystems).

2.3. Construction of plasmids

Full-length human shRNA oligo (shDPP6) were synthesized and annealed into double-strand. Vector pLLU2G (515 ng/μl) was digested by restriction enzymes HpaI (D1064A, TAKARA) and XhoI (D1094A, TAKARA). shDPP6 and Vector pLLU2G were ligated to generate pLLU2G-shDPP6(D2011A, TAKARA). The resulting plasmid encodes a fusion protein eGFP-pLLU2G-shDPP6 (eGFP fused to the upstream of hUbC promoter). DPP6-knockdown constructs pLLU2G-shDPP6-1, pLLU2G-shDPP6-2, pLLU2G-shDPP6-3 and pLLU2G-shDPP6-4 were generated from the wild-type construct by PCR reaction. After all the clones were sequence-verified (Invitrogen), the plasmid DNAs were prepared for microjection (Cyagen).

2.4. Immunohistochemistry analysis on the DPP6-knockdown mice brain tissue

After the hemi brain frozen sections were prepared, block endogenous peroxide blocking 3% H_2O_2 RT 10 min, washed with 0.01 M PBS (pH 7.2–7.4) for 2 min ×3 gentle shaking and block section with 5% BSA at room temperature for 15 min with gentle shaking, then incubate sections in primary antibody (DPP6, 1:50, abcam) at 4 °C for overnight, and then washed with 0.01 M PBS (pH 7.2–7.4) for 2 min ×3 gentle shaking. Incubate sections in PV-6001

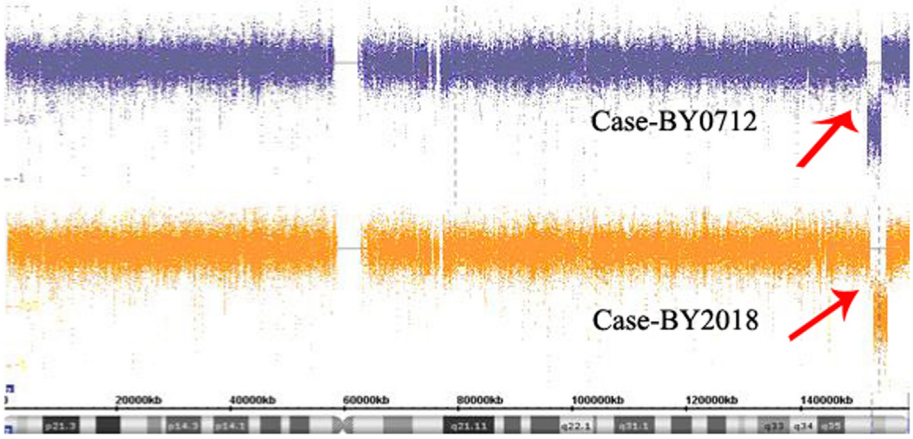

Fig. 1. The diagrammatic presentation of array-CGH detection in microcephalic patients of Case BY0712 and Case BY2018. The array result displays the scatterplot of genomic DNA copy number. The log 2 ratio of normal two copies is corresponding to base line "0" on the scatterplot, the log 2 ratio less than 0 indicates deletion (both cases). The deletion with 336 kb size in Case BY0712 maps to chromosome position 153,649,777–153,985,995 and deletion with 362 kb size in Case BY2018 maps to position: 153,829,386–154,191,684 on chromosome 7q36.2 region (red arrow), within which harbouring DPP6 gene.