博 士 論 文 の 要 旨 及 び 審 査 結 果 の 要 旨

氏名	HOSSAIN Md Ibrahim
学位	博士（医学）
学位記番号	新大院博（医）第 821 号
学位授与の要件	学位規則第 4 条第 1 項該当
博士論文名	Motoneuron degeneration in the trigeminal motor nucleus innervating the masseter muscle in dystonia musculorum mice （ジストニア マスキュローラム マウスの咀嚼筋を支配する三叉神経運動核における運動ニューロン変性）
論文審査委員	主査　教授　日比野　浩 副査　教授　佐藤　昇 副査　教授　竹林　浩秀

博士論文の要旨

(Background and purpose)

Dystonia musculorum mice show movement disorder such as dystonia and cerebellar ataxia, which have mutation in the *dystonin* (*Dst*) gene. *Dst* gene encodes a cytoskeletal linker protein. *Dystonia musculorum* mice are used as animal models to investigate the human disease known as hereditary sensory and autonomic neuropathy type VI, since some of the patients are known to have mutation in the *DST* gene and to show similar phenotype including peripheral neuropathy with decreased longevity. Although *dt* mice had originally been reported to show sensory neuron degeneration in the peripheral nervous system, it was still unclear whether neuronal cell death also occur in the central nervous system (CNS). It is also unknown the reason why the *dystonia musculorum* mice show weight loss and decreased longevity. We tried to investigate the histopathological phenotypes in the CNS and systemic condition of the *dystonia musculorum* mice.

(Methods)

Dst genetrap (Dst^{Gt}) homozygous mice at postnatal stages were used for histological analyses including immunohistochemistry and *in situ* hybridization, as well as electrophysiological analysis, electromyogram.

(Results)

In order to investigate the histopathological phenotypes in the CNS of Dst^{Gt} homozygous mice, applicants performed immunohistochemistry using anti-neurofilament antibody and anti-cleaved caspase 3 antibody, and then, found abnormal neurofilament accumulation and cleaved caspase 3 positive motoneurons in the trigeminal motor nucleus of the Dst^{Gt} homozygous brainstem at postnatal day 21 and 28. Applicants also found reduced proliferation of oligodendrocyte progenitor cells at postnatal day 21. Other abnormalities in the trigeminal motoneurons including induction of *CHOP* (an endoplasmic reticulum stress marker), *ATF3* and *lipocalin 2* (stress markers) were observed in the Dst^{Gt} homozygous mice at P21 and P28. Reduced number of trigeminal motoneurons and reduced size of trigeminal nucleus were also observed in the Dst^{Gt} homozygous mice at P28. Applicants further showed that the abnormal and irregular myelin structure and macrophage infiltration in the mandibular nerve, which contains axons of trigeminal motoneurons. Atrophy

of the masseter skeletal muscles were observed with increased expression of *atrogin-1* (a skeletal muscle atrophy marker) in Dst^{Gt} homozygous mice at P28. Finally, electromyographic studies indicated Dst^{Gt} homozygous mice have uncontrolled and weaker masseter muscle activities than that of control mice.

(Discussion)

Applicants have shown that there was degeneration of trigeminal motoneurons and a reduction in size of trigeminal motor nuclei in the Dst^{Gt} homozygous mice. The motoneurons, which abnormally accumulated neurofilament, also expressed the stress markers *CHOP*, *ATF3* and *Lcn2*. Applicants also demonstrated macrophage infiltration into mandibular nerve and masseter muscle atrophy with weak muscle contraction. All of these data suggest that *dystonia musculorum* mice have difficulty in mastication of food, which may be one of reasons for growth retardation at post-weaning period. Applicant think that there is a possibility that oligodendrocyte progenitor cells proliferation is reduced due to malnutrition with a low food intake, because reduced proliferation of oligodendrocyte progenitor cells in the Dst^{Gt} homozygous brain were observed around P21, which is weaning stage.

審査結果の要旨

Dystonin（*Dst*）遺伝子の変異を有する *Dystonia musculorum* マウスは、ジストニアや小脳変性などの運動障害と末梢神経障害を示し、ヒト遺伝性感覚自律神経ニューロパチーのモデルとして使われている。このマウスでは、末梢神経系において感覚神経細胞死が認められるが、中枢神経系に細胞死が生じているかは不明である。さらに、体重減少や寿命短縮が起こる理由も謎である。そこで本研究では、生後 21 日と 28 日の *Dst* 遺伝子トラップ(Dst^{Gt})マウスの脳を組織学的に検討した。

免疫組織学的な解析により、neurofilament の異常な集積や cleaved caspase 3 の発現を示す運動ニューロンが、三叉神経運動核に見出された。ER ストレスマーカータンパク質などの増加も確認された。その運動ニューロンの数は減少しており、三叉神経運動核の大きさは低下していた。オリゴデンドロサイト前駆細胞の増殖も抑制されていた。また、三叉神経運動ニューロンの軸索を含む下顎神経の構造変化とマクロファージの浸潤も明らかとなった。さらに、咬筋の萎縮を伴った咀嚼力の低下を観察した。これらの事象による低栄養が体重減少などにつながることが強く示唆された。

本研究は、ヒト神経疾患モデルの症状が発生する仕組みの一端を明確に示した点で、学位論文としての価値があると判定した。