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学位論文名	Six novel mutations detected in GALC gene in 17 Japanese patients with Krabbe disease and new genotype-phenotype correlation （日本人クラッベ病患者 17 人における遺伝子解析による６つの新規変異と遺伝子型―表現型相関について）
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論文内容の要旨

〔 Aim 〕

Krabbe disease (globoid cell leukodystrophy：GLD, MIM 245200) is an autosomal recessive neurodegenerative disorder caused by the deficiency of galactocerebrosidase (GALC) (EC 3.2.1.46). Approximately 90% of patients exhibit the early infantile form, first manifesting symptoms before six months old and experiencing rapid disease progression over the ensuing one or two years. The remaining 10% demonstrate late-onset Krabbe disease and are classified into one of three classes, late-infantile type, juvenile type or adult type, depending on the onset period and disease progression rate. Molecular cloning of the human GALC gene has led to molecular-level analyses of Krabbe disease. To date, the number of GALC mutations reported worldwide is more than 60, displaying molecular heterogeneity. Whereas several papers reported Krabbe disease mutations in Japanese patients, clear genotype-phenotype correlations remain obscure, due to the small number of subjects studied. We evaluated the GALC gene in 17 Japanese patients, classifying mutations as related to clinical phenotype. Here, we report the common mutations and the correlation between such mutations and their clinical severity.

〔 Methods and Results 〕

We studied 17 unrelated Japanese patients with Krabbe disease. Diagnoses were determined in our laboratory by reduced GALC activity in either fibroblasts or leukocytes. According to the age at onset, patients were classified into one of four clinical phenotype groups, including infantile onset：9 patients；late-infantile onset：2 patients; juvenile onset：4 patients and adult onset: 2 patients. After informed consent, genomic DNA was prepared from patients' peripheral blood leukocytes and/or cultured skin fibroblasts using standard methods, and entered into the subsequent studies. For the screening of 12Del3Ins mutation previously reported and relatively common mutation genomic DNA samples were amplified and the product was digested with *Hinf* I. I66M＋I289V, is a unique mutation identified in the Japanese population to date. Only when two single-nucleotide substitutions (I66M, I289V) resided on the same allele, was their combination (I66M＋I289V)

proved to be a pathogenic mutation (Furuya et al. 1997). To detect I66M and I289V, genomic DNA samples were amplified using two pairs of primers, and the products were digested with *Eco* RV and *Sal* I, respectively. All the digested fragments were subjected to electrophoresis. When both I66M and I289V were heterozygous in a patient, mutation analysis of the patient's parents with screening for I66M and I289V was necessary to clarify whether I66M and I289V resided on the same allele. For all observed digestion patterns different from the normal control, the corresponding fragments were re-amplified for direct sequencing analysis. For screening of the 30 kb deletion mutation, a previously reported and common mutation in Caucasians, genomic DNA samples were amplified using three primers according to the method described previously. For all patients, all of the 17 exons and exon-intron boundaries were amplified by polymerase chain reaction. DHPLC analysis was performed with the WAVE DNA Fragment Analysis System equipped with a DNASep Column. PCR-amplified products exhibiting a heteroduplex profile were re-amplified and used for direct sequencing analysis. For patients with no mutations or only one mutation by common mutation screening or DHPLC, GALC mutation analysis was performed by sequencing cDNA. Total RNA was extracted from cultured skin fibroblasts or lymphocytes, and first-strand cDNA synthesis was performed. The coding region was amplified, and PCR products were directly sequenced. For the screening of two novel missense mutations (S257F, L364R), PCR amplification of genomic DNA from 100 healthy individuals was performed and the product was digested with *Eco* 57I and *Aci* I, respectively.

The results of detected mutations are summarized with reference to reported mutations in Japanese patients.

The most frequent mutation (30kb large deletion) in Caucasians was not found in our Japanese patients. Fourteen different mutations identified in this study were found in 27 detected alleles of 17 patients, including nine missense mutations (I66M＋I289V, I234T, S257F, G270D, P302A, L364R, L618S, T652P), two nonsense mutations (W115X and R204X), one small deletion (393delT), one small insertion (1719-1720T) and one deletion/insertion (12Del3Ins). Of these mutations, six were novel (W115X, R204X, S257F, L364R, 393delT and 1719-1720insT). All GALC mutations in Japanese patients with their frequencies, including those previously reported, are summarized. The distribution of the clinical phenotype for each mutation is summarized. For rare mutations, those detected less than twice, the genotype-phenotype correlation remains indeterminate ; however, in rather common mutations, the tendency between genotype and phenotype was observed. The distribution of the phenotype for 12Del3Ins, T652P and R515H was observed mostly in infantile-type Krabbe disease, while I66M＋I289V, G270H and L618S were detected mostly in the adult type and never in the infantile form of the disease.

〔 Summary 〕

In this report we detected 27 mutant alleles in 17 patients. We found six novel mutations in the GALC gene in Japanese patients with Krabbe disease. As reported in the literature, mutations of the GALC gene in Krabbe disease exhibited great heterogeneity. It was considered hard to screen the GALC mutation because there is no common mutation in Japanese patients; however, as our results show, several common mutations exist. The most common mutation in Japanese patients is 12Del3Ins with a 0.22 allele frequency. The second most frequent mutation, I66M＋I289V, exhibits 0.15 prevalence. The most common mutation (12Del3Ins) along with the two other mutations (T652P and R515H) in the homozygous state resulted in the classic infantile phenotype. The second most common mutation, I66M＋I289V, contributed to late onset-type Krabbe disease, as the homozygous state of this mutation was found only in the adult type, the mildest form of the disease, while the heterozygous state was detected in the juvenile or adult form. This concordance strongly suggests that the existence of this mutation leads to the mild clinical phenotype. Since screening for this mutation might directly reveal a mild phenotype of Krabbe disease in Japanese patients, we propose a screening method using restriction enzyme digestion with PCR fragments for I66M and I289V as being viable toward that end. For the missense

mutations, G270D and L618S, similar concordance is shown, demonstrating that it will be effective to screen this mutation to estimate the mild phenotype.

論文審査の結果の要旨

クラッベ病（Krabbe disease；Globoid cell leukodystrophy）は常染色体性劣性遺伝病であり、リソソーム酵素である galactocerebrosidase（GALC）の欠損により、psychosine が蓄積し、中枢、末梢神経のミエリン形成細胞が障害を受け、脱髄を引き起こす疾患である。発症年齢によって４つの臨床表現型に分けられ：乳児型、晩期乳児型、若年型、成人型がある。発症率は日本では 20 万に１人くらいと考えられる。この GALC 遺伝子は 1993 年にクローニングされ、世界では、既に 60 くらいの変異が見付かり、欧米人において 30 kb large deletion 変異が一番多くて 40-50%であるが、日本人クラッベ患者においては、変異の頻度、遺伝子型―表現型相関がまだ不明である。

本研究では、日本人クラッベ病患者 17 人について遺伝解析を行い、６つの新規変異が見いだされた。以前報告された日本人クラッベ患者 11 人の遺伝子変異解析の結果を含めて、変異頻度、遺伝子型と表現型相関を検討した。20 種類の変異があり、６つの頻度の高い変異を合わせると 70%となることが明らかになった。その中、３つの変異が軽症タイプであり、少なくとも１つの変異があれば、患者さんは軽症タイプとなると考えられる。

以上の結果は、日本人クラッベ患者の頻度、分布、遺伝子型と表現型相関を解明した研究成果であり、学位の授与に値すると考えられる。