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GENETIC ANALYSIS OF RHABDOMYOLYSIS-ASSOCIATED GENES: AN AUTOPSY CASE OF METHAMPHETAMINE-RELATED HYPERTHERMIA AND ACIDOSIS.

Abstract: In an autopsy case of methamphetamine (MA)-related hyperthermia and acidosis, we investigated the genetic background of hyperthermia and muscular hyperactivity in relation to rhabdomyolysis. We examined mutations in the *ryanodine receptor1* (*RYR1*) gene, which is associated with malignant hyperthermia, the *very long-chain acyl-CoA dehydrogenase* (*VLCAD*) gene, which is associated with rhabdomyolysis, the *carnitine palmitoyltransferase II* (*CPT II*) gene, which is the most common cause of recurrent rhabdomyolysis in adults and the *cytochrome P450* (*CYP)2D6* gene which encodes MA-metabolizing enzyme. There were two homozygous and three heterozygous silent mutations in the three hot-spot regions in the *RYR1* gene. There was no mutation in the *VLCAD* gene. In the *CPT II* gene, the subject was found to be homozygous for two amino acid substitutions, ^{352}Phe>Cys and ^{368}Val>Ile in exon 4. In the *CYP2D6* gene, the subject was heterozygous for ^{100}C>T, ^{1661}G>C and ^{4180}G>C causing ^{34}Pro>Ser, a silent mutation and ^{486}Ser>Thr, respectively, in the *CYP2D6*10A* allele.

Introduction

We report an autopsy case of methamphetamine (MA)-related hyperthermia and acidosis. It was recently reported that MA causes rhabdomyolysis, myoglobinuria, and acute renal failure[1]. There is a possibility that rhabdomyolysis can be triggered by fragility of muscular cells or a reduction in the metabolism of the causative agent, which are caused by genetic background. The *ryanodine receptor1* (*RYR1*) is mainly expressed in skeletal muscle where it mediates Ca^{2+} release from the sarcoplasmic reticulum, following depolarization of the plasmalemma. Mutation in the *RYR1* gene have been found in association with malignant hyperthermia[2]. The *very long-chain acyl-CoA dehydrogenase* (*VLCAD*) gene is an enzyme catalysing the dehydrogenation of long-chain fatty acids in the first step of mitochondrial fatty acid oxidation. Recognized heritable causes of rhabdomyolysis are defects in fatty acid oxidation[3]. The *carnitine palmitoyltransferase* (*CPT*) enzyme system plays an important role in the transfer of long chain fatty acids from the cytosolic compartment to the mitochondrial matrix, where beta-oxidation occurs. CPT II deficiency is an important cause of recurrent rhabdomyolysis[4]. MA is metabolized in the liver by the *cytochrome P450* (*CYP)2D6*[5].

Materials and methods

Examined case

A man in his twenties was found dead in a rice field with his clothes scattered around.

Autopsy findings: He was 177 cm tall and weighed 74 kg. The rectal temperature was 40°C at the postmortem examination. There were many small abrasions and subcutaneous bleeding on the body. Rigor mortis was relatively advanced. The brain was edematous, and various organs were congested. There was a blood-like solution and bubbles in the trachea and bronchus.

His cause of death was diagnosed by histological, toxicological and other examination. Especially, to diagnose the acute renal disorder followed by rhabdomyolysis, immunostaining of kidney was performed with antibodies against myoglobin, 70 kDa heat shock protein (HSP70), 8-hydroxy-2'-deoxyguanosine (8-OH-dG), 4-hydroxy-2-nonenal (4-HNE), superoxide dismutase Cn/Zn enzyme (SOD), and 50kDa oxygen regulated protein (ORP-150). Immunostaining of skeletal muscle was also performed with antibodies against myoglobin.

Genetic analysis

For the genetic analysis, genomic DNA was isolated from blood samples using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The mutational analysis was performed using direct sequencing. The *RYR1* gene contains 106 exons. Intronic primers for amplification from genomic DNA were designed for each exon within the three mutational hot-spot regions. The *VLCAD* gene contains 20 exons and we designed primer pairs for all exons of the *VLCAD* gene. Mutation in the *CPT II* gene was analyzed according to the method of Kaneoka et al[4]. CYP2D6 was analyzed all exons using designed primer pairs specific to the *CYP2D6* gene. All PCR products were sequenced directly on an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) using a BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems).

Results and Discussion

Cause of death

Histologically, severe congestion was found in all organs, and was especially marked in the lung, liver, kidney, and spleen. The lungs were also edematous. In the heart, there were no abnormal findings. In the proximal tubules, the epithelia were swollen and their nuclei were enlarged. Immunohistochemical findings are shown in Table 1. In the kidney, myoglobin and 8-OHdG were negative. HSP70, 4-HNE, SOD and ORP-150 were positive. Myoglobin immunoreactivity was decreased in the skeletal muscle.

In blood from the heart, 0.75 μg/ml MA was detected by GC/MS analysis, and was 16.8 μg/ml in the urine, and 6.2 μg/ml in the stomach contents. Amphetamine was also detected in the blood by GC/MS analysis.

Autopsy findings, histological findings, immunohistochemical findings and toxicological analysis revealed his cause of death was MA-related death, such as hyperthermia and metabolic acidosis caused by muscular hyperactivity.

Genetic analysis

So, we performed a mutational analysis of 4 rhabdomyolysis-associated genes (Table 2). In the *RYR1* gene, there were two homozygous and three heterozygous silent mutations in the three hot-spot regions, but there was no mutation causing an amino acid substitution. Mutation of the *VLCAD* gene was not found. In the *CPT II* gene, the subject was found to be homozygous for two amino acid substitutions, ^{352}Phe>Cys and ^{368}Val>Ile in exon 4 (Fig. 1). However, it has been reported that the ^{352}Phe>Cys and ^{368}Val>Ile substitution alone did not affect enzyme activity in vitro[6 7]. The *CYP2D6* gene is highly polymorphic, causing no, decreased, normal or increased enzyme activity. A relationship between increased drug concentrations and rhabdomyolysis has been reported. In the *CYP2D6* gene, the subject was heterozygous for ^{100}C>T, ^{1661}G>C and ^{4180}G>C causing ^{34}Pro>Ser, a silent mutation and ^{486}Ser>Thr, respectively, in the *CYP2D6*10A* allele (Fig. 2). The subject was heterozygous for the *CYP2D6*1* allele and *CYP2D6*10A* allele. The *CYP2D6*10* allele, which includes both the *CYP2D6*10A* and *CYP2D6*10B* variants, is widely observed in Japanese. CYP2D6*10 encodes an unstable enzyme with reduced catalytic activity[8]. It is possible that an alteration of CYP2D6 activity changes the metabolism of MA.

Conclusions

His cause of death was considered to be hyperthermia and acidosis caused by muscular hyperactivity. There was no mutation which causes the amino acid substitution in the *RYR1* and *VLCAD* genes. In the *CPT II* gene, the subject was found to be homozygous for two amino acid substitutions, ^{352}Phe>Cys and ^{368}Val>Ile in exon 4. It has been reported that these substitutions did not affect enzyme activity in vitro. In the *CYP2D6* gene, the subject was heterozygous for ^{100}C>T, ^{1661}G>C and ^{4180}G>C causing ^{34}Pro>Ser, a silent mutation and ^{486}Ser>Thr, respectively, in the *CYP2D6*10A* allele. CYP2D6*10A is associated with a decreased metabolic clearance of CYP2D6 substrates.

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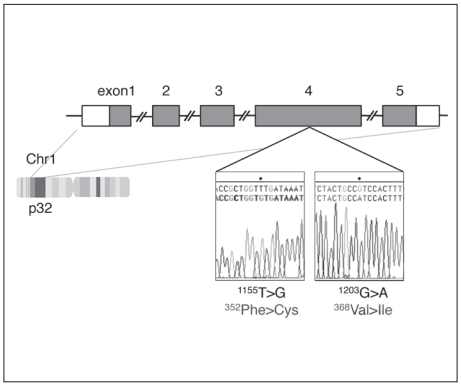

Figure 1 – Partial nucleotide sequences of the *CPT II* gene.

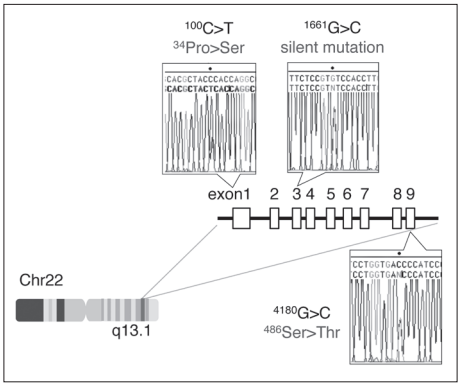

Figure 2 – Partial nucleotide sequences of the *CYP2D6* gene.

Immuno-reaction	Glomerulus	Proximal tuble	Distal tuble
Myoglobin	–	–	–
HSP70	+	–	–
8-OHdG	–	–	–
4-HNE	–	–	+
SOD	–	+	–
ORP-150	–	+	+

Table 1 – The results of immunohistochemical staining (+; positive, –; negative)

Gene	Nucleotide change	Exon (s)	Amino acid change	Zygosity
RYR1	^{1668}G>A	15	Silent mutation	Homo
	^{7281}C>T	45	Silent mutation	Hetero
	^{7584}C>T	47	Silent mutation	Homo
	^{11754}T>A	85	Silent mutation	Hetero
	^{14256}A>C	98	Silent mutation	Hetero
VLCAD	none	–	–	–
CPT II	^{1155}G>A	4	^{352}Phe>Cys	Homo
	^{1203}G>A	4	^{368}Val>Ile	Homo
CYP2D6	^{100}C>T	1	^{34}Pro>Ser	Hetero
	^{1661}G>C	3	Silent mutation	Hetero
	^{4180}G>C	9	^{486}Ser>Thr	Hetero

Table 2 – Identified mutations