# A Single Base-Pair Deletion in Exon 7 in *qgaa*1 Gene Responsible for Acid Maltase Deficiency in Japanese Quail (*Coturnix Coturnix Japonica*)

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**Abstract**

By sequence analysis in ten quails with acid maltase deficiency (AMD), we have identified the mutation in *qgaa*1 gene, but not in *qgaa*2 gene. No mutations were detected in either *gaa* gene in ten normal quails. AMD quails had a novel G deletion at nucleotide position 1639 in exon 7 in *qgaa*1 gene (designated 1639delG). This deletion causes a frameshift altering the amino acid sequence from position 366 on and introduces a premature stopcodon at amino acid position 390. A second *qgaa*2 gene having frequent polymorphisms in the sequence of cDNAs and extremely shorter introns than *qgaa*1 gene, may account for the low level of serum acid alpha glucosidase (GAA) activity and the reduction of clinical severity in AMD quails.

**Key words**: acid alpha-glucosidase (GAA), acid maltase deficiency (AMD), Japanese quail, *qgaa* genes.

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Acid maltase deficiency (AMD), or Pompe's disease, is caused by a deficiency of lysosomal enzyme acid alpha-glucosidase (GAA; synonym, acid maltase) [9, 12, 14], and is a heterogeneous autosomal recessive disease with three major phenotypes - infantile, juvenile, and adult [9, 12]. The infantile form is characterized by cardiomegaly and skeletal myopathy, with death within the first two years [14]. The juvenile and adult-onset forms are characterized by a slow progression of skeletal myopathy [9].

A Japanese quail with the AMD was found 21 years ago in Japan [13], and an inbred strain was established recently [6]. The skeletal muscle, liver and heart are specially affected by an abnormal accumulation of glycogen [11, 13]. The clinical traits are similar to those of patients with juvenile type AMD [6]. Preclinical trials for the gene therapy [8, 10, 16] and the enzyme replacement therapy [6, 20] were made for the first time in this animal model.

In order to determine the molecular basis for the AMD in quails, we cloned a full length of the *qgaa*1 cDNA and found that the reduction of GAA activity in AMD quails is due to a lack of its mRNA [7]. The present paper describes the genomic and mutation analysis of *qgaa*1 and *qgaa*2 genes in AMD quails.

## Methods

### *Quails*

AMD (RW line) and corresponding normal (PNN line) quails were provided from the bird colony in the Nippon Institute for Biological Science in Kobuchizawa, Yamanashi, Japan. The care of quails and the experimental protocol were approved by the Ethics Review Committee for Animal Experimentation of National Institute of Neuroscience, NCNP.

### *Isolation and characterization of quail gaa1 and gaa2 genomic clones*

A quail genomic Lambda GEM12 library was constructed from quail blood cells and screened with a full-length of quail gaa1 cDNA or gaa2 cDNA. Eleven clones and 6 clones were screened and mapped to check their constructions for *qgaa*1 and *qgaa*2, respectively. The inserts were sequenced using a thermo-sequence kit (Amersham). Genomic DNA isolated from normal and AMD quail blood cells was used as a template in a polymerase chain reaction (PCR). PCR reaction mixtures contain primers specific for introns near every exon in *qgaa*1 and *qgaa*2 gene. Amplified DNA fragments were sequenced using the thermo-sequence kit and compared

to the sequence structure of every exon between genomic DNAs from normal and AMD quails. The nucleotide sequences of *qgaa*1 and *qgaa*2 genes were registered in AB081289 and AB081290 of the DDBJ, respectively.

*5'-Rapid amplification of cDNA ends (RACE) of gaa1 and gaa2 mRNA*

Total RNA from quail liver was prepared by the guanidine thiocyanate / CsCl method. A total of 1 micro gram RNA was subjected to the reverse transcriptase reaction using the oligonucleotide AAC TGA TCG GCG AAG ATC AG for *qgaa*1 and AAC TGG TCC GCA AAG AAC AG for *qgaa*2 to prime the synthesis. 5'-cDNA sequence was amplified using the 5'-RACE kit (Life Technologies, Inc.) according to the manufacture's conditions. The first round of PCR amplification was performed with the anchor primer included in the kit as well as primer ACG GTG GTG TTG AGC AGC AC for *qgaa*1 and GAA CGG ATC CTG GAG GAG CT for *qgaa*2, respectively. The second round of amplification used the anchor primer and primer CAT GCC CAG CAC TGT CTG GT for *qgaa*1 and GTC ACG TTG TCC GCC CTG TA for *qgaa*2, respectively. DNA flagment were isolated, cloned into the GEM-T Vector (Promega), and sequenced.

## Results and Discussion

*Gene structure of qgaa1 and qgaa2*

The *qgaa*1 gene was approximately 9 kb long, and contained 20 exons, while *qgaa*2 gene was approximately 6 kb long, and contained 19 exons (Fig. 1a). The exon number of *qgaa*1 was identical to that of human *gaa* gene, but the full length was shorter than human *gaa* gene having 20kb long [1, 3]. We identified an intron that separate the 5' untranslated leader sequence from exon 2 containing the initiation of translation. This region contained two exons, exon1a and exon1b either of which function as a promoter for the translation of *qgaa*1 starting at the initiation codon in exon 2. Exon1a and exon1b are 537bp and 84 bp in length, respectively. There is an intron with 751 bp length between exon1a and exon1b, and another intron with approximately 1kb long between exon1b and the initiation codon in exon 2 (ATG). The *qgaa*1 and *qgaa*2 promoter region contains GC rich sequences and has features characteristic of a housekeeping gene [5]. The distinct TATA motifs were lacking. These regions have no significant homology to the human *gaa* promoter regions.

The GAA1 is essential enzyme for the degradation of glycogen in glycogenosomes. This is likely supported by following aspects. First, introns of the *qgaa*2 gene in normal and AMD quails were extremely short; 107 bp in average length. Since the transcripts from the genes with introns shorter than 69 bp were spliced correctly at low efficiency [19], the *qgaa*2 gene is functionally impaired. Second, the frequent polymorphisms detected all over the genomic *qgaa*2, except at a catalytic site in exon 9

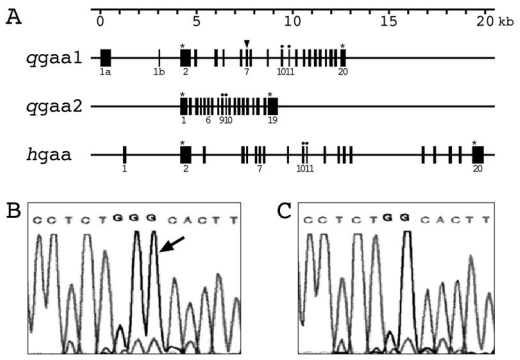


*Figure 1. Comparison of the structure between the human acid alpha-glucosodase (hgaa) gene and two quail acid alpha-glucosodase genes, qgaa1 and qgaa2 (a). Position of the start and stop codons are indicated by an asterisk. An arrow head indicates the location of mutation on exon 7 responsible for the quail acid maltase deficiency (AMD). The sequence in ten normal quails was identical to the previously reported qgaa1 sequence [7]. However, ten AMD quails had a novel G deletion at nucleotide position 1639 in exon 7 of gaa1 gene (designated 1639delG) (b and c). A nucleotide of G pointed by an arrow in qgaa1 gene of normal quail (b), was deleted in AMD quail (c).*

and exon 10 [1], may cause insufficient expression of GAA2 by altering the three-dimensional structure of the enzyme. In contrast, there were no polymorphisms in any regions in *qgaa*1 gene. Third, in transient enzyme assay by cultured COS cells, the GAA2 activity was far weaker than the GAA1 activity (data not shown). Taken together, these results suggest that the low residual level of GAA2 is account for the reduction of a clinically severe dysfunction in AMD quails lacking the expression of principal *qgaa*1 gene.

*AMD quail has one-point mutation in qgaa1 gene*

Mutations that occur most frequently in patients with the AMD are missense, nonsense, and frameshift mutations. These are a deletion of exon 18 (del exon 18) [15, 18], a single base-pair deletion (525delT) in exon 2 [18], and the IVS1 (-13T-G) transversion in the consensus sequence of the acceptor splice site of intron 1 [4]. The del exon 18 and the deletion of a single base (525delT) lead to premature termination of GAA synthesis. Homozygotes for del exon 18 or 525delT have the infantile form of AMD and no GAA activity. The truncated 525delT protein affects the stability of the mRNA [2, 18]. The *qgaa*1 mRNA transcripts of AMD quail are not stable leading to the degeneration soon after the initiation of mRNA transcription [7] .

The sequence in ten normal quails was identical to the previously reported *qgaa*1 and *qgaa*2 sequence. However, ten AMD quails had a novel G deletion at nucleotide position 1639 in exon7 of the *qgaa*1 gene (desig-

nated 1639delG) (Fig. 1b and c). The 1639delG causes a frame-shift, altering the amino acid sequence from position 366 on and introduces a premature stop-codon at amino acid position 390. Since AMD quails are homozygous for this mutation on both alleles, the truncated protein caused by the instability of mRNA, leads to the severely affected infantile form. However, the clinical traits of AMD quails more closely resemble the juvenile form of human AMD. The residual GAA activity (approximately 16% of normal levels) was always detected in the blood serum of AMD quails [17]. The answer to this discrepancy between genotype and phenotype came from the fact that AMD quails had a second gene, *qgaa*2, which synthesizes the residual GAA. The AMD quails exhibited clinically milder symptoms than AMD patients with infantile form.

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**Abbreviations**

Acid alpha-glucosidase gene (*gaa*), acid alpha-glucosidase (GAA), acid maltase deficiency (AMD).

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