Comparative Nucleotide Sequence of Encoding for Quaking Protein of Birds and Mammals

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Abstract: Partial nucleotide sequences of eight kinds of bird (common crane, gray pelican, goose, black swan, bewisk's swan, whooper swan, ostrich, and chicken) gene encoding quaking protein were analyzed. Variously sized bands were observed in all eight birds compared with the same sized band in mammals based on nested PCR results, and all sequence data showed a very high level of homology. Three bases (CD223(Ala)) in exon 6 of all eight bird species were defective compared with sequence data from the mouse, and both the existence and defect of 24 bases (CD211 ~ 219) in exon 6 of the eight birds were confirmed on complementary DNA sequence analysis. These findings suggest that birds generate specific alternative sequencing data, there are many alternative splicing variants, and the avian quaking gene may be a specific marker differentiating between birds and mammals.

The mouse *quaking I* (*qkI*) gene, encoding a KH domain containing RNA-binding protein [1], was first isolated as a candidate gene for neurological mutation, *quaking viable,* and was known as a model for human leukodystrophy [2]. Genes highly homologous to *qkI* have been found in various animal species from nematodes to humans [3-7], and mutations in these genes result in various developmental defects other than demyelination. It is therefore believed that members of the *qkI* gene family are essential for multiple biological processes. Herein, we report the isolation and sequence analysis of eight kinds of avian *qk*.

Genomic DNA was isolated from whole blood cells of eight kinds of bird (common crane, gray pelican, goose, black swan, bewisk's swan, whooper swan, ostrich, and chicken) by the standard method. RNA was isolated from whole blood cells of these birds with Trizol LS reagent (Invitrogen, USA). The ostrich was one-week-old and the others were adults, and none showed abnormality according to neurological examinations. Complementary DNA synthesis was performed using the SuperScript Preamplification System (Invitrogen, USA). Primers used for the reverse transcription reaction were qk-5c (5'-CAAAGGCGATTACCAG TTAAC-3') and qk-5D (5'-GGTTAGATGGTAAGACGAA C-3'). The PCR primers were designed from highly conserved nucleotide sequences between mice and humans based on the results obtained in our previous study [3, 4]. Using these cDNA and gDNA as a template, PCR and nested PCR were performed for amplifications using the following primer sets: e2-4(5'-AGCTGCGGAGCCTGCAATAT-3'), EH5-3(5'-CTCCATCAGCTGCATCTTC-3'), qk-5c, qk-5d, EH5-5(5'-GAAGATGCAGCTGATGGAG-3'), and e6-u (5'-GCATGACAGCGGTCTGTATTT-3'). The PCR reactions were performed with the following cycling parameters: at 94°C for 4 min, then 38 cycles comprising denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 60 s. Nested PCR reactions were performed for 30 cycles. Amplified cDNA and gDNA products were directly sequenced using the Dye Terminator cycle sequencing kit with an ABI 373A DNA sequencer (Applied Biosystems). Primers used for sequence determination were EH5-5 and e6-u.

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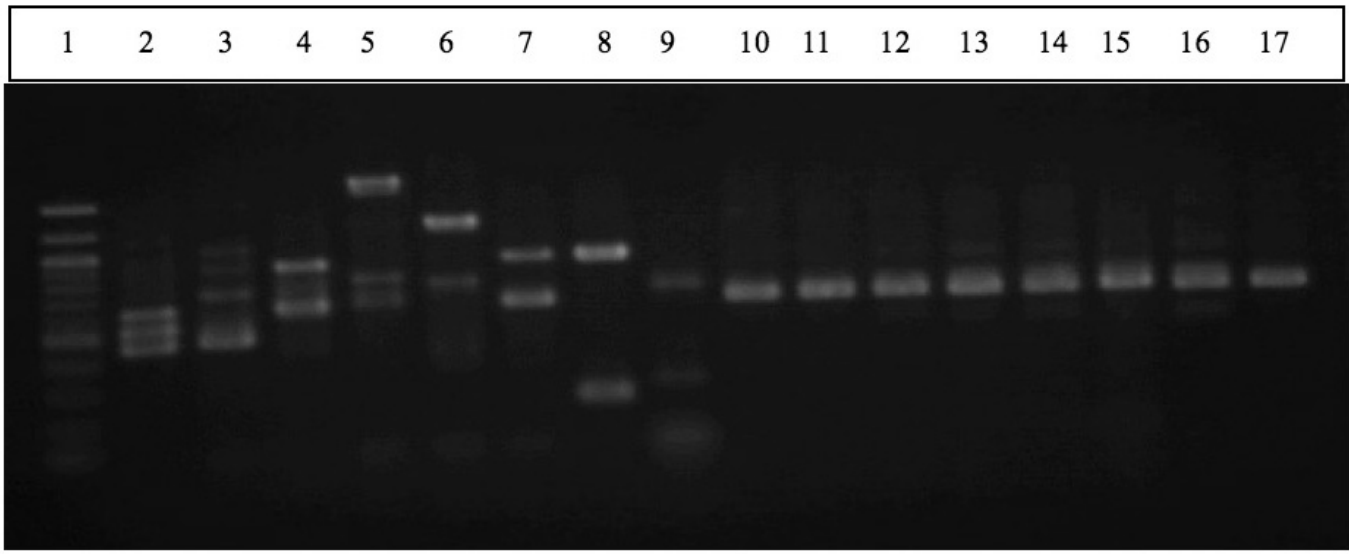

Fig. (1) Electrophoresis pattern for intron 5 – exon 6 of eight kinds of bird and eight kinds of mammal. primers: e2-4 & EH5-3, 1.5% TBE gel, 100V, 40min, lane 1: 100bp ladder marker; lane 2: common crane; lane 3: grey pelican; lane 4: goose; lane 5: black swan; lane 6: bewisk's swan; lane 7: whooper swan; lane 8: ostrich; lane 9: chicken; lane 10: mouse; lane 11: rat; lane 12: rabbit; lane 13: dog; lane 14: cat; lane 15: cow; lane 16: pig; lane 17: horse.

Electrophoresis patterns were detected (Fig. **1**). Many variously sized bands were clearly confirmed in all eight kinds of bird, compared with one same-sized band and a very thin differently-sized band in eight kinds of mammal. Nucleotide sequences of these cDNA and gDNA *quaking* genes around intron 5 - exon 6 are shown in parallel with the *qkI* sequence in Fig. (**2**). Nucleotide sequences of all avian *qk* showed an absence of three bases (CD223 (Ala)) compared to *qkI* and just one amino acid (Fig. **2**). The alternative splicing (-24 bp (CD211~219 **:** CA/GCC/CTT/GCC/TTT/TCT/CTT/GCA/G)) in exon 6 of all avian *qk* was also confirmed.

The sequence of our chicken *qkI* and that of Mezquita's chicken *qkI* [2] are the same, but the source organs are different. This shows that the three bases defect and the twenty four base alternative splicing in exon 6 of *qk* are normal and

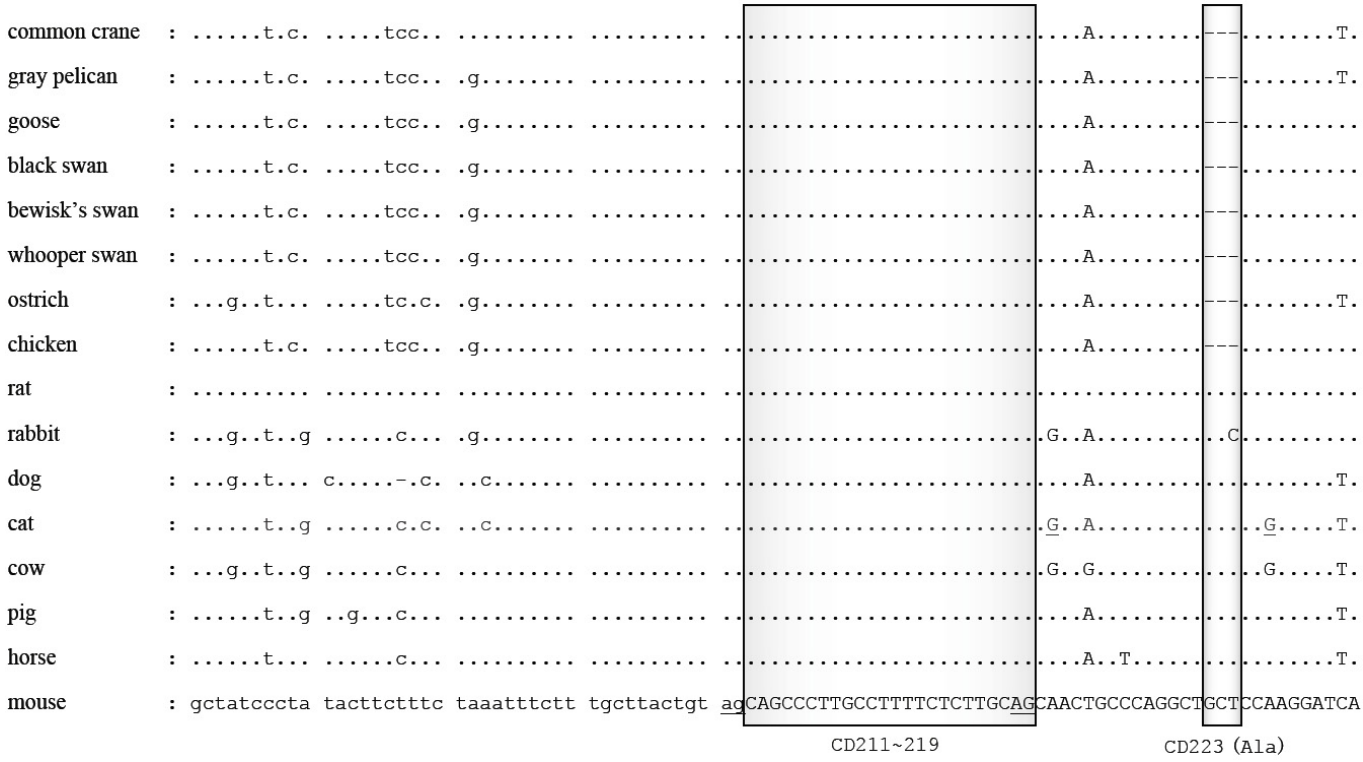

Fig. (2). Intron 5 - exon 6 of genomic DNA of whole blood cells of eight kinds of bird and eight kinds of mammal, (dot): same base with base of mouse, small letter: base in intron 5, large letter: base in exon 6, _(underline): splicing base, -: defected base.

ubiquitous in the chicken. In addition, the structures of exon 6 of *qk* were very highly conserved in all birds, but were not so highly conserved between birds and mammals. Fujita reported the alternative splicing patterns for *qkI* in the rat brain [8]. This article suggests that splicing occurs in a very small portion, is usual in birds.

The evolutionary dichotomy between birds and mammals began more than three hundred million years ago. The high-level homology of the *quaking* gene was confirmed in the same class of animals from our data, but there was a lower homology between different classes, Aves and Mammalia. This suggests that this *qk* is a specific marker differentiating between birds and mammals.

The sequence of avian *qk* showed that it easily underwent alternative splicing (Murata *et al*. personal data, preparation). In the mammals, alternative splicing occurred, but effective repair systems such as nuclease might be active to maintain protein conformity. In these birds, its activity may be weak, or the activity of spliceosome or legated enzyme may be strong. The spliceosome may be good analytical tool to investigate the development and differences between birds and mammals.

The sequence data reported in this paper were deposited in the DNA Data Bank of Japan (DDBJ) as follows: common crane: *grgrqkI-01* AB284384, gray pelican: *pecrqkI-01* AB284385, goose: *ananqkI-01* AB284386, black swan: *cyatqkI-01* AB284387, bewisk's swan: *cycoqkI-01* AB284388, whooper swan: *cycyqkI-01* AB284389, ostrich: *stcaqkI-01* AB284391, and chicken: *gagaqkI-01* AB284390.

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