*Page 1: The Title Page*

Manuscript Type: ORIGINAL ARTICLE

Title: Basic characterization of heat shock protein 70 genes HSPA2 and HSPA8 from the Japanese quail, *Coturnix japonica*

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*Page 2: ABSTRACT & Key words*

**ABSTRACT**

We have identified two members of the heat shock protein (HSP) 70 family from the Japanese quail, *Coturnix japonica*. - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -. Taken together, the data suggest that *CjHSPA2* and *CjHSPA8* encode orthologs of *HSPA2* and *HSPA8* with the notable difference that *CjHSPA2* and *CjHSPA8* are heat shock-inducible genes, whereas *HSPA2* and *HSPA8* are constitutively expressed in a heat shock-independent manner.

**KEYWORDS**

gene structure, heat shock protein 70, HSPA2, HSPA8, Japanese quail

**ABSTRACT:** should be written within 200 words or less on page 2.

**U**p to five ***Key words*** and/or phrases (consisting of less than five words) can be written at the end of the abstract, and arranged in alphabetical order.

*Page 3 and following pages: text, acknowledgements, references, figure legends, figure(s) and table(s)*

**1 INTRODUCTION**

Proteins in the heat shock protein 70 family - - - - - - - - - - - - many proteins (Bradley-Johnson, 1994). - - - - - - - - - - - - - . The cytoplasm (CY) and endoplasmic reticula (ER) groups are - - - - - - (Beers & De Bellis, 2002). Seven CY genes have been identified in the human genome (Rutter et al., 2003). - - - - - - - - - - - --. Beers and De Bellis (2002) already reported in - - - - - - - chickens (*Gallus gallus*, or *Gg*).

 *CD1* molecules play an important role in the immune system, presenting lipid-contain - - - - - - (Beers & De Bellis, 2002; Rutter et al., 2003). *CD1* transcription is strong in chicken lymphoid tissues, particularly bursa and spleen (Miller M. et al., 2008, unpublished data). - - .

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**Unit.**

5 ml, 5 mol/L, 5%, 5ºC, 10,000×*g,* 5 hr, 5 min, 5 sec, 17:00 hours (the time),

p < .05, Means ± S*E, Means* ± *SEM*, Means ± *SD*

**2 MATERIALS AND METHODS**

**2.1 Animal care and nucleic acid isolation**

The management of Japanese quail and all procedures in the present study were performed according to the Animal Experimental Guidelines for Tokyo University of Agriculture. - - - - -

**2.2 Amplification of full-length cDNA sequences for three quail HSP70 genes**

Complete cDNA sequences for *CjHSPA2* and *CjHSPA8* were amplified using - - - - - - - - - - -

**3 RESULTS**

**3.1 Isolation and sequence of quail HSP70 family cDNA**

We identified cDNA sequences that - - - - - - - - - -. Therefore, mammals and avian belong to different clusters, with the exception that, at the amino acid level, *CjHSPA8* is more similar to *HsHSPA8* than it is to *GgHSC70* (Figure 1). - - - - - - - Figure 2 shows the results of -----.

 Accordingly, the conceptual translations of the quail HSP70 genes show a high level of sequence identity to chicken and human proteins.

**[Figure 1]**

A place to insert Figure and/or Table should appoint in text.

**4 DISCUSSION**

**4.1 *CjHSPA2* expression is elevated in response to heat shock treatment**

We next asked if any of the quail HSP70-related genes reported here respond to heat shock treatment. - - - - - -

**5 CONCLUSION**

Our knowledge regarding avian MHC has increased

**ACKNOWLEDGMENTS**

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If the work is supported by any grants, ACKNOWLEDMENTS should be written.

**REFERENCES**

**Example of reference with 2 to 7 authors**

Beers, S. R., & De Bellis, M. D. (2002). Neuropsychological function in children with maltreatment-related posttraumatic stress disorder. *The American Journal of Psychiatry*, *159*, 483–486. [https://doi:10.1176/appi.ajp.159.3.483](http://dx.doi.org/10.1176/appi.ajp.159.3.483)

Ramus, F., Rosen, S., Dakin, S. C., Day, B. L., Castellote, J. M., White, S., & Frith, U. (2003). Theories of developmental dyslexia: Insights from a multiple case study of dyslexic adults. *Brain*, *126*(4), 841–865. [https://doi:10.1093/brain/awg076](http://10.0.4.69/brain/awg076)

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Rutter, M., Caspi, A., Fergusson, D., Horwood, L. J., Goodman, R., Maughan, B., … Carroll, J. (2004). Sex differences in developmental reading disability: New findings from 4 epidemiological studies. *Journal of the American Medical Association*, *291*(16), 2007–2012. [https://doi:10.1001/jama.291.16.2007](http://10.0.3.233/jama.291.16.2007)

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**Book edition**

Bradley-Johnson, S. (1994). *Psychoeducational assessment of students who are visually impaired or blind: Infancy through high school* (2nd ed.). Austin, TX: Pro-ed.

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Shotton, M. A. (1989). *Computer addiction? A study of computer dependency* [DX Reader version]. Retrieved from http://ebookstore.tandf.co.uk/html/index.asp

Schiraldi, G. R. (2001). *The post-traumatic stress disorder sourcebook: A guide to healing, recovery, and growth* [Adobe Digital Editions version]. doi:10.1036/0071393722

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O'Keefe, E. (n.d). *Egoism & the crisis in Western values*. Retrieved from http://www.onlineoriginals.com/showitem.asp?itemID=135

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Freud, S. (1953). The method of interpreting dreams: An analysis of a specimen dream. In J. Strachey (Ed. & Trans.), *The standard edition of the completed psychological works of Sigmund Freud* (Vol. 4, pp. 96-121). Retrieved from http://books.google/books (Original work published 1900)

**Electronic material**

National Center for Biotechnology Information (NCBI). (1999). Nucleotide–nucleotide BLAST (blastn) [homepage on the Internet]. National Center for Biotechnology Information, Bethesda, MD; [cited 13 December 2002]. Available from URL:<http://www.ncbi.nlm.nih.gov/blast/>

**Ph.D. Thesis.**

Suzuki, Y. (2016). Study on the physiological functions of two endocrine factors derived from the liver, ANGPTL8 and Chemerin, in Ruminants. Ph.D. Thesis, Tohoku University, Sendai, Japan.

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*Reference list entry:* Smith, I. M. (1988). *U.S. Patent No. 123,445*. Washington, DC: U.S. Patent and Trademark Office.

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**SUPPORTING INFORMATION**

Additional Supporting Information may be found online in the supporting information tab for this article.

**Figure legends**

**FIGURE 1**  Morphological profiled on erythroid cells from equine peripheral blood during incubation in a two phase liquid culture system. Im Ebl: immature erythroblasts, M Ebl: mature erythroblasts, D Ery: denucleated erythroid cells. Values are expressed as mean ± *SE*

**FIGURE 2**  Changes in percentage of haemoglobin containing cells on erythroid cells from equine peripheral blood during incubation in a two phase liquid culture system. Im Ebl: immature erythroblasts, M Ebl: mature erythroblasts, D Ery: denucleated erythroid cells. Values are expressed as mean ± *SE*

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FIGURE 1

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**TABLE 1** Changes in percentage of blood group antigen expressed cells on erythroid cells from equine peripheral blood during incubation in a two phase liquid culture system

|  |  |  |  |
| --- | --- | --- | --- |
|  Blood System | A | C | D |
|  Factor | Aa | Ca | Dk | Dl |
|  Days of culture |  |  |  |  |
| 0 | 0 | 0 | 0 | 0 |
| 1 | 10.5 ± 3.3a | 7.1 ± 3.3a | 0 | 0 |
| 2 | 19.9 ± 3.3ab | 17.2 ± 5.1ab | 5.3 ± 5.1a | 3.2 ± 2.5a |
| 3 | 37.7 ± 2.4b | 31.8 ± 3.9b | 15.4 ± 4.2a | 12.5 ± 2.3a |
| 4 | 56.4 ± 4.1c | 54.5 ± 2.4c | 21.1 ± 1.4ab | 18.0 ± 3.5ab |
| 5 | 91.5 ± 1.5d | 78.3 ± 4.6d | 52.2 ± 2.1c | 51.0 ± 5.4c |

Values are expressed as mean ± *SE*. Different letters indicate significant differences (a < b < c < d, *p* < .05).

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