Dioxin Sensitivity-Related Two Critical Amino Acids of Arylhydrocarbon Receptor May Not Correlate with the Taxonomy or Phylogeny in Avian Species

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ABSTRACT: There are two arylhydrocarbon receptor (AhR) isoforms in birds, AhR1 and AhR2. The varying sensitivity of AhR is reported to be related to two critical amino acids at positions 325 and 381 in the AhR1 ligand-binding domain. In this study, seven avian species whose in vivo dioxin sensitivity was known, and 13 species with no data regarding their in vivo dioxin sensitivity were examined. The two critical amino acids in the ligand-binding domain were investigated in avian species, and the results were compared with the taxonomy or phylogenetic trees for the bird AhR proteins. We found that the two critical amino acids did not correlate with the taxonomy or phylogeny of these proteins, suggesting that dioxin sensitivity was independent of taxonomy.

KEYWORDS: aryl hydrocarbon receptor, avian species, dioxin, ligand-binding domain.

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Environmental pollutants, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), halogenated aromatic hydrocarbons (HAHs) and polycyclic aromatic hydrocarbons (PAHs), can induce serious toxicity in avian species. The types of toxicity induced include teratogenic, immunotoxic and reproductive toxicity [1, 2, 19, 23, 31]. The dioxin concentration required to induce these types of toxicity varies significantly between birds [3]. The large difference in dioxin sensitivity among avian species is reported to be dependent on the arylhydrocarbon receptor (AhR) protein [11, 17], which has a role in the induction of toxicity [10, 21, 27].

AhR is a basic-helix-loop-helix/Per Arnt Sim (PAS) family protein and a transcription factor activated by ligand binding [6]. When not bound to a ligand, AhR remains in the cytosol, forming a complex with heat shock protein 90 (HSP90), AhR-associated protein (XAP2 or ARA9) and p23 [7, 24]. Once bound to a ligand, AhR is translocated to the nucleus [32] where it forms a heterodimer with an AhR nuclear translocator (Arnt), which then binds to the xenobiotic responsive element (XRE) [20, 28]. After binding to XRE, transcription of the CYP1A1, CYP1A2 and AhR repressor (AhRR) genes is activated [13].

Avian species have two AhR isoforms, AhR1 and AhR2 [33, 34], whereas most mammals possess only one. The dominant isoform of AhR differs among bird species [18], and there are large differences in function, even within the same AhR isoform. For example, although avian AhR1s are highly conserved (>90%) among species, there are large interspecies differences in their sensitivity to dioxins, which can be explained by differences in their ligand-binding affinities and transactivation abilities.

It is reported that AhR sensitivity can be predicted from the two amino acids at positions 325 and 381 of AhR1 [17]. Chicken is well known to be the only avian species which has a sensitive type of AhR, however, the sensitivity of AhR isoforms in broad avian species is still unclear. In this study, several kinds of avian species, which were chosen from phylogenetic trees of birds, were investigated to determine their dioxin sensitivity. The amino acid sequences of AhR1 and AhR2 were determined for each species, and compared to their taxonomic and phylogenetic classifications.

MATERIALS AND METHODS

Animals: Bird species analyzed in this study were selected considering clade of phylogenetic tree based on DNA sequences [12]. A one-year-old female blue-eared pheasant (Crossoptilon auritum), a male ruddy shelduck (Tadorna ferruginea), a one-year-old male mallard (Anas platyrhynchos), two male great horned owls (Bubo virginianus), two male and one female bar-headed goose (Anser indicus), one male Indian peafowl (Pavo cristatus), one 12-year-old male goose (Anser anser), one 19-year-old female black-headed ibis (Threskiornis melanocephalus), one male swan goose (Anser cygnoides), two male snowy owls (Bubo scandiacus), one female Chilean flamingo (Phoenicopterus chilensis), one eight-year-old male Humboldt penguin (Spheniscus humboldti), one female cape barren goose (Cereopsis novaehollandiae) and one gender-undetermined black-crowned night heron (Nycticorax...
Table 1. Primers for avian AhR

| Avian AhR       | Forward 5'-sequence-3' | Reverse 5'-sequence-3' |
|-----------------|------------------------|------------------------|
| Avian AhR Full  | CAGGATGAAACCCCAATGTCAC | GTGACATAAAATCCTAGATGCAAA |
| Avian AhR1-1    | GAGTGAACCCCCAATGTCACCTA | ATCCTGTTCGAAAAATTCATA   |
| Avian AhR1-2    | TCATCGCAGTTACGATGCCCT  | AACACAGACTCATGTCGCCTTA  |
| Avian AhR1-3    | TGCCCTTCATGTGTTCACCTGTTA | TCAAATGTGAAATCCTCCAT    |
| Avian AhR1-4    | CAGCTCTGTCAAAAGATGAAA  | TTACATAATCCACTAGA       |

RESULTS

Two critical amino acids in the AhR1 and AhR2 proteins:

Based on the two critical amino acids in the ligand-binding domain of AhR1 [17], the avian species we examined could be divided into three groups. The first group, with amino acids 325-Ile and 381-Ser, consisted of the ostrich and chicken. The chicken is reported to be a highly sensitive species to TCDD [14, 17, 18, 34]. The blue-eared pheasant, Indian peafowl, black-footed albatross and swan goose composed the second group, possessing amino acids 325-Ile and 381-Ala. Other avian species, including the bar-headed goose, mallard, ruddy shelduck, cape barren goose, snowy owl, great horned owl, peregrine falcon, black-headed ibis, Humboldt penguin, Chilean flamingo, common cormorant and the black-crowned night heron, belonged to the last group, harboring amino acids 325-Val and 381-Ala (Figs. 1 and 2; Table 2).

The species could also be divided into three groups according to the amino acids in the AhR2 ligand-binding domain. The group possessing the amino acids 325-Leu and 381-Ala comprised the ostrich, blue-eared pheasant and Indian peafowl. The chicken is the only one species to constitute the group of species to possess 325-Val and 381-Ser AhR2. Other avian species belonged to the group harboring the amino acids 325-Val and 381-Ala (Figs. 1 and 2; Table 2).

AhR1 and AhR2 phylogenetic analyses: The phylogenetic tree constructed from the avian AhR1 sequences indicated that the ostrich was distinct from the other avian species. Also, Galliformes, including chicken, pheasant and peafowl, Ciconiiformes, including albatross, tern, cormorant, penguin, flamingo, falcon and heron and Strigiformes, including the snowy owl and great horned owl, grouped close together.
**Fig. 1.** Amino acid sequence alignment of AhR1. The amino acid sequences of the ligand-binding domain from AhR1s were aligned using the ClustalX2 software, and the accession numbers are listed in the Materials and Methods. Boxes indicate the two critical amino acids at positions 325 and 381. * indicates the species whose AhRs are cloned and sequenced in this study.

**Fig. 2.** Amino acid sequence alignment of AhR2. The amino acid sequences of part of the ligand-binding domain from AhR2s were aligned using the ClustalX2 software, and the accession numbers are listed in the Materials and Methods. Boxes indicate the two critical amino acids at positions 325 and 381. * indicates the species whose AhRs are cloned and sequenced in this study. Snowy owl, common tern and black-headed ibis are not shown in this figure.
Fig. 3. Phylogenetic analysis of avian AhR1. DNA sequences of AhR1s were aligned by CLUSTAL W using the MEGA5 program. Human AhR (L19872) was added as an outgroup. Alignment was performed with a length of about 2,000 bases, including the functional domains, PAS-A, PAS-B and the Q-rich domains and excluded the regions containing gaps. The phylogenetic tree was constructed by ML method using MEGA5. The number of bootstrap replications was set to 500. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. Tamura-Nei model was applied in nucleotide sequences. Swan goose, bar-headed goose, goose, ruddy shelduck, cape barren goose and black-headed ibis are not shown in this figure.

Table 2. The critical amino acids in ligand-binding domains of AhR1 and AhR2 with in vivo sensitivity

|                | AhR1 325 | AhR1 381 | AhR2 325 | AhR2 381 | Order | In vivo Sensitivity |
|----------------|----------|----------|----------|----------|-------|----------------------|
| Ostrich        | I        | S        | L        | A        | St    |                      |
| Chicken        | I        | S        | V        | S        | G     | High [16]            |
| *Blue-eared Pheasant | I      | A        | L        | A        | G     | Middle [3, 22]a)     |
| *Indian Peafowl| I        | A        | L        | A        |       |                      |
| *Swan Goose    | I        | A        | V        | A        |       |                      |
| *Bar-headed Goose | V    | A        | V        | A        |       |                      |
| *Goose         | V        | A        | V        | A        |       | low [5]              |
| *Mallard       | V        | A        | V        | A        |       | low [3, 5, 16]       |
| *Ruddy Shelduck| V        | A        | V        | A        |       |                      |
| *Cape Barren Goose | V   | A        | V        | A        |       |                      |
| Black-footed Albatross | V | A        | V        | A        | C     |                      |
| Common Cormorant | V       | A        | V        | A        | C     | low [26]b)           |
| Peregrine Falcon | V       | A        | V        | A        | C     | low [15]c)           |
| Common Tern    | V        | A        | –        | –        | C     | low [4, 15]          |
| *Black-headed Ibis | V      | A        | V        | A        | C     |                      |
| *Humboldt Penguin | V       | A        | V        | A        | C     |                      |
| *Chilean Flamingo | V      | A        | V        | A        | C     |                      |
| *Black-crowned Night Heron | V | A        | V        | A        | C     |                      |
| *Snowy Owl     | V        | A        | V        | A        | Sg    |                      |
| *Great Horned Owl | V    | A        | V        | A        | Sg    |                      |

The two amino acids in AhR1 and AhR2 at positions 325 and 381 of each avian species were indicated. I: isoleucine, S: serine, A: alanine, V: valine, L: leucine. * indicates the species whose AhRs are cloned and sequenced in this study. Abbreviations for each order are; St: Struthioniformes, G: Galliformes, A: Anseriformes, C: Ciconiiformes and Sg: Strigiformes. a) In vivo sensitivity of common pheasant or ring-necked pheasant (Phasianus colchicus). b) In vivo sensitivity of double-crested cormorant (Phalacrocorax auritus). c) In vivo sensitivity of American kestrel (Falco sparverius).
on the tree. These groupings were consistent with the taxonomic groupings of the avian species (Fig. 3).

In the case of AhR2, the phylogenetic tree was also linked to the taxonomy of the species with the exception of the ostrich and great horned owl. The orders Galliformes, Ciconiiformes and Anseriformes in phylogenetic tree were all assembled the same as that of AhR1 (Fig. 4).

DISCUSSION

In mammals, several factors have been reported to determine the ligand-binding affinity or dioxin sensitivity to AhR-induced toxicity. In C57BL/6 and DBA/2 mice, large differences in AhR ligand-binding affinity result from AhR point mutations at codon 375 in the ligand-binding domain [8]. Similarly, Han/Wistar rats, which are insensitive to dioxin, harbor a point mutation at position 497 in AhR [25].

Avian species are distinct in that they possess two AhR isoforms, AhR1 and AhR2, and the type of AhR dominantly expressed varies among avian species [18]. Most species dominantly express AhR1, and AhR1 ligand-binding affinity is reported to directly correlate with CYP1A transactivation ability [18]. Critical mutations which decide dioxin sensitivity have been found in avian species at positions 325 and 381 of AhR1 [17]. Head et al. [14] showed these two amino acids are effective for predicting avian dioxin sensitivity. In the study, avian species are divided into three groups according to the key amino acids in AhR1. The most sensitive group is with AhR1 of 325-Ile and 381-Ser, middle for 325-Ile and 381-Ala, and the least sensitive for 325-Val and 381-Ala. In this study, the blue-eared pheasant, goose and mallard are newly classified according to the two amino acids, and we found that the grouping corresponds with in vivo sensitivity also in these cases.

In the current study, we investigated whether similar avian species would have similar levels of dioxin sensitivity. Indeed, the phylogenetic trees constructed from the amino acids sequences of AhRs gave results in-keeping with the evolutionary history of these proteins [12]. Unexpectedly, even though the amino acids sequences of AhRs highly reflected the taxonomy, the identity of the two critical amino acids, at positions 325 and 381, did not correspond to the phylogenetic trees of AhR or taxonomy. In fact, these two amino acids were not conserved in the orders, Galliformes and Ciconiiformes [14, 34]. Therefore, our new findings suggested that these key amino acids at positions 325 and 381 are independent from the other amino acids sequences of AhRs, so that they cannot be predicted from the phylogenetic tree or from taxonomy. That is, the ligand-binding affinity or the dioxin sensitivity of each avian AhR protein cannot be determined from the taxonomy.

The amino acid sequence of the AhR1 ligand-binding domain was highly conserved among different species. In the ostrich, multiple amino acid changes were found throughout the ligand-binding domain, compared with other avian species. This ostrich AhR1 was reported to possess high trans-
activation ability in our previous study, similar to chicken AhR1 [11].

Regarding the two critical amino acids, avian species harboring isoleucine at position 325 in AhR1 were chicken, peafowl, pheasant, albatross, swan goose and ostrich. All species in the order Galliformes examined in this study, including chicken, peafowl and pheasant, possessed isoleucine at position 325. However, albatross and swan goose were the only species to possess this amino acid in their respective orders, Ciconiiformes and Anseriformes. Only two of the avian species, chicken and ostrich from the orders Galliformes and Struthioniformes, respectively, possessed a serine at position 381. Species within the order Galliformes, such as peafowl and pheasant, did not harbor this amino acid. Taken together, these findings indicate that the two critical amino acids are independent of taxonomy or even phylogeny of the full-length amino acid sequence of AhR1. Therefore, we conclude that it is difficult to predict the dioxin sensitivity of avian species from taxonomy or evolutionary history.

In the case of avian AhR2, the amino acid sequence of the ligand-binding domain was not as highly conserved as that of AhR1. In terms of the two critical amino acids in the ligand-binding domain, the only species to possess 381-Ser in AhR2 was the chicken. This amino acid was not conserved in the AhR1 and AhR2 of ostrich. In addition, we identified the amino acid leucine at position 325 in pheasant, peafowl and ostrich. This amino acid has not previously been reported at this position, and its corresponding AhR function is therefore unknown. It will be of interest to investigate the function or ligand-binding affinity of this type of AhR protein. Future researches are also required to fully investigate the avian AhR2 protein and its role in avian dioxin sensitivity.

In conclusion, the two critical amino acids at positions 325 and 381 in the ligand-binding domain of AhR were investigated in several bird species, and the results were compared with the taxonomy or phylogenetic trees for the AhR proteins. The two critical amino acids did not correlate with the taxonomy or phylogeny of these proteins, and dioxin sensitivity was independent of taxonomy.

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REFERENCES

1. Bird, D. M., Tucker, P. H., Fox, G. A. and Lagüé, P. C. 1983. Synergistic effects of aroclor 1254 and mirex on the semen characteristics of American Kestrels. Arch. Environ. Contam. Toxicol. 12: 633–639. [Medline] [CrossRef]

2. Blankenship, A. L., Hilscherova, K., Nie, M., Coady, K. K., Villalobos, S. A., Kannan, K., Powell, D. C., Bursian, S. J. and Giesy, J. P. 2003. Mechanisms of TCDD-induced abnormalities and embryo lethality in white leghorn chickens. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 136: 47–62. [Medline] [CrossRef]

3. Brunström, B. and Reutergårdh, L. 1986. Differences in sensitivity of some avian species to the embryotoxicity of a PCB, 3,3',4,4'-tetrachlorobiphenyl, injected into the eggs. Environ. Pollut. 42: 37–45. [CrossRef]

4. Brunström, B. and Halldin, K. 1998. EROD induction by environmental contaminants in avian embryo livers. Comp. Biochem. Physiol. C, Pharmacol. Toxicol. Endocrinol. 121: 213–219. [Medline] [CrossRef]

5. Brunström, B. 1988. Sensitivity of embryos from duck, goose, herring gull, and various chicken breeds to 3,3',4,4'-tetrachlorobiphenyl. Poult. Sci. 67: 52–57. [Medline] [CrossRef]

6. Burbach, K. M., Poland, A. and Bradfield, C. A. 1992. Cloning of the Ah-receptor cDNA reveals a distinctive ligand-activated transcription factor. Proc. Natl. Acad. Sci. 89: 8185–8189. [Medline] [CrossRef]

7. Denis, M., Cuthill, S., Wikstrom, A. C., Poellinger, L. and Gustafsson, J. A. 1988. Association of the dioxin receptor with the M90,000 heat shock protein: a structural kinship with the glucocorticoid receptor. Biochem. Biophys. Res. Commun. 155: 801–807. [Medline] [CrossRef]

8. Ema, M., Ohe, N., Suzuki, M., Mimura, J., Sugawa, K., Ikawa, S. and Fujii-Kuriyama, Y. 1994. Dioxin binding activities of polymorphic forms of mouse and human arylhydrocarbon receptors. J. Biol. Chem. 269: 27337–27343. [Medline]

9. Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783–791. [CrossRef]

10. Fernandez-Salgueiro, P. M., Hilbert, D. M., Rudikoff, S., Ward, J. M. and Gonzalez, F. J. 1996. Aryl-hydrocarbon receptor-deficient mice are resistant to 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced toxicity. Toxicol. Appl. Pharmacol. 140: 173–179. [Medline] [CrossRef]

11. Fujisawa, N., Darwish, W. S., Ikenaka, Y., Kim, E., Lee, J., Iwata, H., Nakayama, S. M. M. and Ishizuka, M. 2013. Molecular evaluation of a new highly sensitive aryl hydrocarbon receptor in ostriches. Poult. Sci. 92: 1921–1929. [Medline] [CrossRef]

12. Hackett, S. J., Kimbatt, R. T., Reddy, S., Bowie, R. C. K., Braun, E. L., Braun, M. J., Chojnowski, J. L., Cox, W. A., Han, K. L., Harshman, J., Huddleston, C. J., Marks, B. D., Miglia, K. J., Moore, W. S., Sheldon, F. H., Steadman, D. W., Witt, C. C. and Yuri, T. 2008. A phylogenetic study of birds reveals their evolutionary history. Science 320: 1763–1768. [Medline] [CrossRef]

13. Hahn, M. E. 2002. Aryl hydrocarbon receptors: diversity and evolution. Chem. Biol. Interact. 141: 131–160. [Medline] [CrossRef]

14. Head, J. A., Hahn, M. E. and Kennedy, S. W. 2008. Key amino acids in the Aryl Hydrocarbon Receptor predict dioxin sensitivity in avian species. Environ. Sci. Technol. 42: 7535–7541. [Medline] [CrossRef]

15. Hoffman, D. J., Melancon, M. J., Klein, P. N., Eisemann, J. D. and Spann, J. W. 1998. Comparative developmental toxicity of planar polychlorinated biphenyl congeners in chickens, American kestrels, and common terns. Environ. Toxicol. Chem. 17: 747–757. [CrossRef]

16. Jin, X., Kennedy, S. W., Di Muccio, T. and Moon, T. W. 2001. Role of oxidative stress and antioxidant defense in 3,3',4,4',5-pentachlorobiphenyl-induced toxicity and species-differential sensitivity in chicken and duck embryos. Toxicol. Appl. Pharmacol. 172: 241–248. [Medline] [CrossRef]

17. Karchner, S. I., Franks, D. G., Kennedy, S. W. and Hahn, M. E. 2008. Key amino acids in the Aryl Hydrocarbon Receptor predict dioxin sensitivity in avian species. Environ. Sci. Technol. 42: 7535–7541. [Medline] [CrossRef]

18. Kim, E. Y., Iwata, H., Yasui, T., Inoue, N., Lee, J. S., Franks, D.
G., Karchner, S. I., Hahn, M. E. and Tanabe, S. 2008. Molecular basis for differential dioxin sensitivity in birds: characterization of avian AHR isoforms. pp. 81–86. In: Interdisciplinary Studies on Environmental Chemistry, Vol. 1, Biological Responses to Chemical Pollutants (Murakami, Y., Nakayama, K., Kitamura, S-I., Iwata, H. and Tanabe, S. eds.), TERRAPUB, Tokyo.

19. Larson, J. M., Karasov, W. H., Sileo, L., Stromborg, K. L., Hanbidge, B. A., Giesy, J. P., Jones, P. D., Tillitt, D. E. and Verbrugge, D. A. 1995. Reproductive success, developmental anomalies, and environmental contaminants in double-crested cormorants (Phalacrocorax auritus). Environ. Toxicol. Chem. 5: 553–559.

20. Matsushita, N., Sogawa, K., Emi, M., Yoshida, A. and Fujiy-Kuriyama, Y. 1993. A factor binding to the xenobiotic responsive element (XRE) of P-4501A1 gene consists of at least two helix-loop-helix proteins, Ah receptor and Arnt. J. Biol. Chem. 268: 21002–21006. [Medline]

21. Mimura, J., Yamashita, K., Morita, M., Takagi, T. N., Nakao, K., Emi, M., Sogawa, K., Yasuda, M., Katsuki, M. and Fujiy-Kuriyama, Y. 1997. Loss of teratogenic response to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in mice lacking the Ah (dioxin) Receptor. Genes Cells 2: 645–654. [Medline] [CrossRef]

22. Nosek, J. A., Sullivan, J. R., Craven, S. R., Gendron-Fitzpatrick, A. and Peterson, R. E. 1993. Embryotoxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the ring-necked pheasant. Environ. Toxicol. Chem. 12: 1215–1222.

23. Peden-Adams, M., Alonso, K., Godard, C., Skipper, S., Mamburn, W., Hoover, J., Charbonneau, C., Henshel, D. and Dickerson, R. 1998. Effects of environmentally relevant concentrations of 2,3,7,8-TCDD on domestic chicken immune function and CYP450 activity: F1 generation and egg injection studies. Chemosphere 37: 1923–1939. [Medline] [CrossRef]

24. Perdew, G. H. 1998. Association of the Ah receptor with the 90kDa heat shock protein. J. Biol. Chem. 263: 13802–13805.

25. Poljanjvrita, R., Wong, J. M., Li, W., Harper, P. A., Tuomisto, J. and Okey, A. B. 1998. Point mutation in intron sequence causes altered carboxyl-terminal structure in the aryl hydrocarbon receptor of the most 2,3,7,8-tetrachlorodibenzo-p-dioxin-resistant rat strain. Mol. Pharmacol. 54: 86–93. [Medline]

26. Powell, D. C., Auierich, R. J., Meadows, J. C., Tillitt, D., Kelly, M. E., Stromborg, K. L., Melancon, M. J., Fitzgerald, S. D. and Bursian, S. J. 1998. Effects of 3,30,4,40,5-pentachlorobiphenyl and 2,3,7,8-tetrachlorodibenzo-p-dioxin injected into the yolks of doublecrested cormorant (Phalacrocorax auritus) eggs prior to incubation. Environ. Toxicol. Chem. 17: 2035–2040.

27. Prasch, A. L., Teraoka, H., Carney, S. A., Dong, W., Hiraga, T., Stegeman, J. J., Heideman, W. and Peterson, R. E. 2003. Aryl hydrocarbon receptor 2 mediates 2,3,7,8-tetrachlorodibenzo-p-dioxin developmental toxicity in zebrafish. Toxicol. Sci. 76: 138–150. [Medline] [CrossRef]

28. Reyes, H., Reisz-Porszasz, S. and Hankinson, O. 1992. Identification of the Ah receptor nuclear translator protein (Arnt) as a component of the DNA binding form of the Ah receptor. Science 256: 1193–1195. [Medline] [CrossRef]

29. Tamura, K. and Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10: 512–526. [Medline]

30. Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28: 2731–2739. [Medline] [CrossRef]

31. Walker, M. K., Pollen, R. S. and Smith, S. M. 1996. Expression of the Aryl hydrocarbon receptor (AhR) and AhR nuclear translocator during chick cardiogenesis is consistent with 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced heart defects. Toxicol. Appl. Pharmacol. 143: 407–419. [CrossRef]

32. Whitelaw, M. L., Göttlicher, M., Gustafsson, J. A. and Poellinger, L. 1993. Definition of a novel ligand binding domain of nuclear bHLH receptor: co-localization of ligand and hsp90 binding activity within the regulable inactivation domain of the dioxin receptor. EMBO J. 12: 4169–4179. [Medline] [CrossRef]

33. Yasui, T., Kim, E. Y., Iwata, H. and Tanabe, S. 2004. Identification of aryl hydrocarbon receptor 2 in aquatic birds; cDNA cloning of AHR1 and AHR2 and characteristics of their amino acid sequences. Mar. Environ. Res. 58: 113–118. [Medline] [CrossRef]

34. Yasui, T., Kim, E. Y., Iwata, H., Franks, D. G., Karchner, S. I., Hann, M. E. and Tanabe, S. 2007. Functional characterization and evolutionary history of two Aryl hydrocarbon receptor isoforms (AhR1 and AhR2) from avian species. Toxicol. Sci. 99: 101–117. [Medline] [CrossRef]