Comment to Dr. Zoeller's Speech

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Thank you for introduction. The title of my talk is "*In vivo* Model to Study the Thyroid Hormone Action in Developing Brain." I will focus mainly on the rodent cerebellum as a model system to assess the thyroid hormone action and, probably, endocrine disrupter action. Now, the first slide, please.

As Dr. Zoeller pointed out, some endocrine disrupters may act the brain through thyroid hormone system, particularly during the developmental period. Some action may be exerted through the thyroid gland in which thyroid hormone production could be altered by endocrine disrupters as presented by Dr. Zoeller.

In addition, since thyroid hormone acts many organs, which, in turn, regulate brain function, the change in thyroid hormone level induced by endocrine disrupters may alter the peripheral metabolism, which may alter the brain function indirectly.

Furthermore, thyroid hormone receptors are expressed widely in the central nervous system. Since the structure of thyroid hormone and some endocrine disrupters are similar, endocrine disrupters may directly act on the brain through thyroid hormone receptors. Thus, to study the effect of endocrine disrupters on the thyroid hormone system, it is necessary to clarify the site of its action. For this purpose, I would like to propose using rodent cerebellum as the model system. Next slide, please.

Dr. Zoeller's group also did a series of elegant works to identify thyroid hormone sensitive genes in the fetal cerebrum. His work is very important, of course, to understand the role of thyroid hormone on fetal brain development.

However, as a model system, I would suggest that rodent cerebellum could be useful. In this system, as shown in this slide, the decrease in thyroid hormone level during perinatal period results in abnormal development of the cerebellum, which includes relatively small cerebellum and depressed arborization of Purkinje cell dendrites. There are several evidences that thyroid hormone directly acts to develop the cerebellum, one of which will be presented by Dr. Kuroda's group in the following presentation. Next slide, please.

There are several advantages to use the rodent cerebellum as a model system. First, the development of rodent cerebellum occurs postnatally while that of other brain regions occurs prenatally. Actually, in the case of humans, cerebellum development occurs prenatally. Thus, this is very specific for rodent cerebellum.

Thus, perinatal hypothyroidism induces abnormal cerebellar development. In other words, we can precisely manipulate thyroid function during cerebellar development to study the site and mechanism of action.

Second, the structure of the cerebellar cortex is relatively simple compared to other brain regions. You can easily identify the cell type by microscope. The role of thyroid hormone on the development of each cell type has been clarified. Finally, most importantly, there is a critical period of thyroid hormone action to regulate cerebellar development.

During brain development, there is a narrow time window only during which a particular substance acts to a specific stage of brain development. This is called the critical period. In case of the rodent cerebellum, such a critical period of thyroid hormone action is within 14 days after birth. I will discuss later, but many substances that may be involved in thyroid hormone-mediated cerebellar development are also known. Next slide, please.

Morphologically, we and other investigators have already identified several abnormal cerebellar developments in hypothyroid mice. Again, as shown in this slide, arborization of Purkinje cell dendrite is depressed by hypothyroidism, and cellular migration of granule cell from external granule cell layer to internal granule cell layer is retarded. Next slide, please.

This is a little bit busy slide, but I do not want to talk too much in detail. This slide shows possible interaction of substances that may be involved in thyroid hormone-mediated cerebellar development. Again, I do not want to talk too much in detail, but you should identify that there are several important steps, which may be critical to induce thyroid hormone action.

First, T_4 preferentially crosses the blood brain barrier, and then T_4 is taken up by astrocytes, in which T_4 is de-iodinated to T_3, which is the active form. Then, T_3 is transported to neurons to bind nuclear thyroid hormone receptors that are ligand regulated transcription factor. Then, this T_3-TR complex may directly regulate genes essential for cerebellar development, or regulate other transcription factors, which then regulate critical genes.

In addition, thyroid hormone receptor action may be modulated by other transcription factors, which are developmentally regulated, or by phosphorylation such as PKC or CaM kinase. Another non-generic pathway mediated by T_4 is also proposed.

In this system, it is most likely that endocrine disrupter may inhibit or may activate T_3-thyroid hormone receptor binding. However, it should be noted that there are several other possible sites of action on endocrine disrupters.

In conclusion, the developing cerebellum could be useful to study the effect of endocrine disrupters on thyroid hormone-mediated brain development because of its time of development, structure, defined critical period of thyroid hormone action, and well-clarified substances involved in this system. By using this system, we are currently working to identify the possible site of action of endocrine disrupting agent. Thank you very much for your attention.