Gene Expression Profile of the Neonatal Female Mouse Brain After Administration of Testosterone Propionate

Yutaka Nakachi, PhD,* Mioko Iseki, BSc,* Tomotaka Yokoo, PhD,† Yosuke Mizuno, PhD,‡ and Yasushi Okazaki, MD, PhD*‡

*Division of Translational Research, Research Center for Genomic Medicine, Saitama Medical University, Hidaka, Saitama, Japan; †Experimental Animal Laboratory, Research Center for Genomic Medicine, Saitama Medical University, Hidaka, Saitama, Japan; ‡Division of Translational Research, Research Center for Genomic Medicine, Saitama Medical University, Hidaka, Saitama, Japan

DOI: 10.1111/jsm.12802

ABSTRACT

Introduction. Clinical care decisions for peripubertal adolescents with gender dysphoria (GD) should be made carefully. Furthermore, the identification of biomarkers is very important for rapid and accurate diagnosis of GD in young people.

Aim. The aim of this study was to investigate gene expression profiles during masculinization of the neonatal female mouse brain by testosterone and to identify biomarkers related to GD.

Methods. Microarray analysis was performed using RNAs extracted from the brains of neonatal mice treated by intraperitoneal injection of testosterone propionate during the sexual determination period. Sequence motif enrichment analysis for sex hormone receptor responsive elements was performed for the ”anking regions of genes that showed significant expression changes following administration of testosterone propionate.

Main Outcome Measures. We revealed a gene set with marked changes in expression during brain masculinization of neonatal female mice following administration of testosterone propionate.

Results. We identified 334 genes that showed differential expression in the masculinized neonatal female brain after testosterone propionate treatment. Interestingly, most of these genes are not reported to be expressed in a sexually dimorphic manner. Moreover, sequence motif enrichment analysis suggested that masculinization of the neonatal female brain by testosterone was controlled more by estrogen receptors than androgen receptors.

Conclusions. Differences in genes that are expressed differentially following administration of testosterone injection from known sexually dimorphic genes suggest that many GD-related genes are upregulated during female brain masculinization. The gene set identified in this study provides a basis to better understand the mechanisms of GD and delineate its associated biomarkers. **Nakachi Y, Iseki M, Yokoo T, Mizuno Y, and Okazaki Y. Gene expression profile of the neonatal female mouse brain after administration of testosterone propionate. J Sex Med 2015;12:887–896.**

Key Words. Brain; Sexual Differentiation; Gene Expression Profiling; Animal Model; Gender Dysphoria; Testosterone; Cross-Sex Hormone Treatment

Introduction

Gender Dysphoria (GD), previously known as gender identity disorder, is characterized by dissociation between psychological gender identity and physical sex [1–3]. This condition causes clinically significant distress or impairment in social, occupational, or other important areas of functioning. GD prevalence has been estimated as 1:11,900 for male-to-female GD and 1:30,400 for female-to-male GD [4]. Most affected individuals experience GD continuously from childhood [5,6]. The mental stress caused by the uneasiness with their assigned sex at birth is significant and often results

in self-injury and/or suicidal intent [7,8]. Although puberty suppression using a gonadotropin-releasing hormone agonist may be considered as a valuable contribution to the clinical management of GD in adolescents without the distress of irreversible development of secondary sex characteristics [9–12], clinical decisions for peripubertal adolescents with GD should be made cautiously [10]. Therefore, the identification of biomarkers is very important to provide rapid and accurate diagnosis of GD in young people.

Studies of the biological mechanisms in GD have been reported continually. For example, histological findings of sexually dimorphic areas in the brain, such as the bed nucleus of the stria terminalis and limbic nucleus, of male-to-female GD sufferers are more similar to those of the female than male [13,14]. Genetic studies of sexual differentiation of the human brain as well as sexual behavior indicate the contribution of genetic factors to GD [15–19]. However, in terms of steroidogenic enzymes and sex hormone receptors, there are no reports of genetic variants that are strongly associated with GD [20–23]. It has been considered that human GD is associated with sexual differentiation of the brain via sex hormones [10,19,24–26]. However, it is inadequate to consider that only steroidogenic enzymes and sex hormone receptors drive determination of gender identity because these genes are partly responsible for disorders of sexual development [27,28].

Thus, we present a novel hypothesis that genes directly related to GD are target genes of sex hormones and/or downstream genes of sex hormone signaling pathways as well as steroidogenic enzymes or sex hormone receptors. In particular, we believe that genes with a specific function in sexual differentiation of the brain might be related to GD and may be regarded as biomarkers. However, the specific gene regulation and molecular mechanisms during sexual differentiation of the mammalian brain are still unclear [29].

To identify GD-related genes as biomarkers for diagnosis, we examined gene sets regulated by sex hormones during sexual differentiation of the brain. To identify these gene sets, we performed comprehensive gene expression analyses of neonatal mice treated with an androgen reagent (testosterone propionate [TP]) during the period of sexual differentiation in the brain. Recent studies indicate that the mechanisms of brain sex differentiation are conserved among mammals, and TP administration to neonatal female mice induces masculinization of the GD-related sexual dimorphic area of the brain (the bed nucleus of the stria terminalis) and ensuing masculinized behavior [10,13,30]. Therefore, our expression analyses may lead to the identification of GD biomarkers.

Aim

The aim of this study was to investigate gene expression profiles during masculinization of the neonatal female mouse brain by testosterone and to identify biomarkers related to GD.

Materials and Methods

Neonatal Mice

C57BL/6J (CLEA Japan, Tokyo, Japan) wild-type neonatal mice were used in experiments. All animal studies were approved by the Institutional Animal Care and Use Committee of Saitama Medical University.

Sex Hormone Injection into Neonatal Mice

Six neonatal mice were subjected to each of the four treatment conditions: TP-injected female mice, vehicle control-injected female mice, TP-injected male mice, and vehicle control-injected male mice (Figure 1A). TP (1 mg in 20 μL of sesame oil per mouse [31]) or the vehicle control (20 μL of sesame oil only per mouse) was intraperitoneally injected into neonatal mice at postnatal day 2 (the early period of mouse brain sex differentiation). We isolated total RNA from whole brains at postnatal day 6, just prior to the start of cell apoptosis in the bed nucleus of the stria terminalis, which is related to sexual dimorphism in the mouse brain [30] (Figure 1A). Total RNA was extracted from homogenized whole brains using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Genomic DNA for sex identification of neonates was extracted from ear tissue using a REDExtract-N-Amp Tissue PCR Kit (Sigma, St. Louis, MO, USA). Sex determination of neonates was performed by PCR amplification of *Zfy1/Zfy2* genes using the following primer set: 5′-AAGATAAGCTTACATAATCACATGGA-3′/5′-CCTATGAAATCCTTTGCTGCACATGT-3′ (data not shown).

Quantitative Reverse Transcription Polymerase Chain Reaction for Confirmation of TP Effects

To confirm the in" uence on gene expression in the brain by intraperitoneal hormone injection, *Kiss1* expression was measured in each mouse before