様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

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研究課題名（和文）Understanding how genetic variants in the oxytocin receptor gene (OXTR) confer the risk of autism spectrum disorder - a genetic and molecular biology analysis

研究課題名（英文）Understanding how genetic variants in the oxytocin receptor gene (OXTR) confer the risk of autism spectrum disorder - a genetic and molecular biology analysis

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研究成果の概要（和文）：OXTR遺伝子のmissese variantの機能解析を行ったところ、自閉症スペクトラム障害（ASD）患者において特異的に同定された1つの変異が機能獲得型変異のようであることが確認された。さらに、大規模全ゲノムシークエンスデータから機能変異の可能性が高いものをスクリーニングした，その結果をもとに、ディープラーニングのフレームワークを適用し、OXTRのNon-coding希少バリアントに対する転写およびエピジェネティック効果を予測しました。

研究成果の学術的意義や社会的意義
本研究では、オキシトシンの希少バリアントを網羅的にスクリーニングし、OXTRの転写活性と関連する可能性のある機能バリアントを同定しました。

研究成果の概要（英文）：We performed functional assay for missese variants in the OXTR gene and observed one variant that specifically identified in autism spectrum disorder (ASD) patients is like a gain-of-function variants. We further screened likely functional variants in large-scale whole-genome sequencing datasets.

研究分野：遺伝学関連

キーワード：Autism spectrum disorder　rare variant　Oxytocin receptor (OXTR)　Functional study

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Recent studies demonstrate that oxytocin plays a critical role in regulating a wide range of social behaviors including pair bonding, maternal parenting and formation of social memory. Despite tremendous progress in linking genetic variants in *OXTR* to a wide range of phenotypes and promising results from clinical trial studies, it remains unknown through which genetic and molecular mechanisms these common SNPs exert their influences; and how molecular level changes subsequently modulate complex social behaviors

２．研究の目的

To understand the functional consequences of OXTR variants and to screen rare variants in OXTR gene.

３．研究の方法

We generated expression vectors of missense mutation identified and performed in vitro calcium imaging analysis to measure the oxytocin receptor activation activity. In addition,

we analyzed whole-genome sequencing (WGS) data of autism spectrum disorder family samples and non-psychiatric samples, by comparing the allele frequency with that obtained from the gnomAD dataset which includes 126,216 exomes and 15,136 whole-genome sequences. We further applied deep learning frameworks to infer the likely transcriptional and epigenetic effects for non-coding rare variants in *OXTR*.

４．研究成果

By calcium imaging analysis, we demonstrated a gain-of-function variant which was identified from ASD patients. This variant was predicated to be located in a so-called “polar pocket” site which is indispensable for the activation of OXTR. Naturally we hypothesized that this variant is likely to be a loss-of-function mutation and will abolish the function. To test this idea, we made the expression

vector for WT and R150S and made stable expressed cell line. When applied oxytocin, the ligand to OXTR and measure the activation activity by Calcium imaging. However, we found that, rather than being a loss-of-function effect, this variant seems have higher response than WT (Figure1).

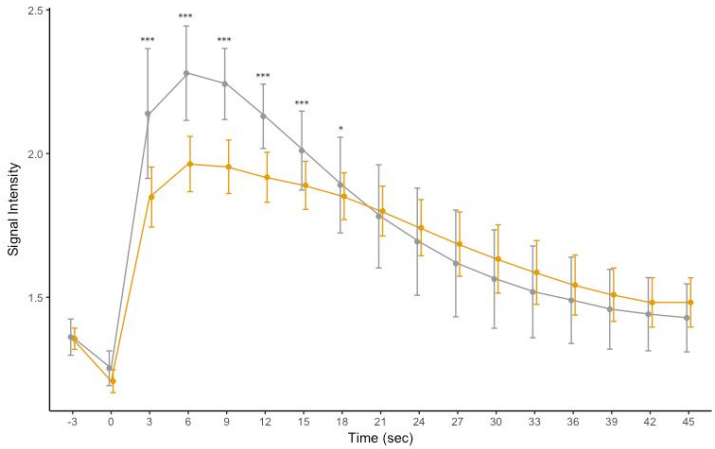

Figure 1: the calcium response curve to oxytocin for wild-type (yellow) and variant identified in ASD (grey).

The modeling suggested that the variant might strengthen the R73-D136 hydrogen bond, and also stabilize the R73-D153 interaction. In addition, we identified 24 rare missense mutations in ASD patients which were absent from the large scale public database. Furthermore we screened rare non-coding variants in OXTR and we applied several deep learning frameworks to infer the likely transcriptional and epigenetic effects. The rs2254298 has been implicated to be associated with autism and a wide range of psychiatric traits, however, the genetic mechanism remains yet unknown. We identified rs2268494, which is in linkage disequilibrium (D' =1) is likely to be functional critical and influence CTCF binding, and affect the regulate the gene expression of OXTR. We further identified two rare variants in the Japanese population that were predicted to exert significant effects in the promoter region of *OXTR*. Finally, we utilized the AlphaFold2 program to analyze the possible structural changes caused by coding variants in *OXTR* which were located in the predicated “polar pocket” sites.

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関