様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　３ 年　 ４ 月 ２３ 日現在

機関番号：１３９０１
研究種目：基盤研究(C)（一般）
研究期間：2018～2020
課題番号：１８Ｋ０７５５４

研究課題名（和文）次世代シーケンサーを用いた統合失調症多発家系の遺伝子解析と病因・病態解明

研究課題名（英文）NGS of multiple onset schizophrenia families

研究代表者
Ａｌｅｋｓｉｃ　Ｂｒａｎｋｏ（Aleksic, Branko）

名古屋大学・医学系研究科（国際）・特任准教授

研究者番号：６０５４７５１１

交付決定額（研究期間全体）：（直接経費）　　3,300,000円

研究成果の概要（和文）：統合失調症多発家系内では、de novo変異以外に、家系内患者間で共有されている稀な変異が、発症に強い影響を及ぼす変異と考えられる。従来、この稀な変異同定の主たる標的は、親から受け継がない孤発例の患者に新しく発生したde novo変異であった。以上を踏まえた本研究では、1日本人統合失調症多発家系のゲノムサンプリング(20家系、計約100名)、2家系内の患者に加え健常家族も含めた家系全体のエキソーム解析、3家系解析によりde novo 変異に加え患者間で共有する変異の抽出、4in silico解析による発症関連変異を基にした統合失調症発症に関わる遺伝子ネットワーク障害の特定、を実施した

研究成果の学術的意義や社会的意義
This research helped to alleviate misconceptions and reduce stigma through an improved understanding of the genetic cause of psychiatric disorders, and eventually offer support to patients and their families.

研究成果の概要（英文）：In this research we dissected genetic architecture of schizophrenia using families with multiple affected members. Until now most family based exome sequencing used trio based design and research was focused mainly on de novo variants (i.e. variants that are not present in parents but exist in affected children). In the current research besides de novo variants we focused on inherited variants. These are variants that are present in all affected member in one pedigree. In addition we investigated variants that are rare and of high impact (i.e. splice site mutation and/or nonsense variants), which may be family specific but exhibit incomplete penetrance (i.e. present in both affected and non affected members of the same family.

研究分野：biological psychiatry

キーワード：exome seqencing

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Schizophrenia (SZ) is a severe chronic psychiatric disease that is characterized by psychotic symptoms such as hallucinations and delusions. The lifetime risk of SZ is estimated to be 1%, with subsequent mortality of SZ patients is 2.5 times higher than in the general population. Autism spectrum disorder (ASD) is characterized by impaired social interactions and communications and by restricted, repetitive behavior. The prevalence of prototypical ASD is around 25 per 10000, and that of broad ASD is 116 per 100002. The heritability of SZ and ASD is estimated as 60–90% from population-based and twin studies. Both common and rare genetic variants are associated with the etiology of both disorders. Neurodevelopmental mechanisms and related molecules are strongly involved in both SCZ and ASD. Family, twin, and adoption studies have suggested strong evidence for the importance of genetic factors in the etiology of schizophrenia. However, although an estimated heritability is around 0.8, identifying the specific genetic risk factors of SZ is challenging. Genome-wide linkage scans have been performed in SZ, but the inconsistent replication results suggest that variants of major effect are unlikely to exist. On the other hand, genome-wide association studies of SZ have recently implicated a number of variants of small effect. Recent study (Nat. Genet. 42, 203–209 2010) suggested that expression of individual symptoms is determined by
the main chromosomal variation (e.g. 1q21.1 (deletion), 2p16.3 (deletion), 3q29 (deletion), 7q36.3 (duplication), 15q13.2 (deletion), 16p11.2 (duplication) and 16p13.11 (duplication)) that both predisposes to neuropsychiatric phenotypes as a single event and exacerbates neurodevelopmental phenotypes in association with other deletions or duplications. NGS is the necessary and sufficient analytical tool for the discovery of such genetic modifiers. We expect that, through NGS approach and large sample of SZ patients, it will be possible to discover and catalog, all potential genetic modifiers of the main chromosomal deletion. These modifiers could determine the large degree of variability between patients in the expression of symptoms. Having such variants catalogued in combination with deep clinical characterization of SZ patients in whom those variants are identified will enable us to understand molecular pathways relevant for development of specific symptoms observed in SZ patients. The deficiency in molecular pathways will give us opportunity to utilize more efficiently medication that are used in current clinical practice and establish therapeutic protocols for the SZ patients with deficiency in specific molecular-phenotypic pathway and concurrent symptomatic constellation

２．研究の目的

The results of previous studies suggested that the pattern of inheritance in SZ is combination of common variants and rare variants; with potentially many risk alleles of small effect size distributed across large numbers of loci. Given the challenges of studying rare variants with currently available sample sizes we used an approach to exome sequencing in SZ by sequencing multiplex families as an initial discovery strategy, followed by a case-control analysis.

３．研究の方法

We performed exome sequencing of affected members with SZ and some of the unaffected members from multiplex families and tested rare, segregating variants in an independent case-control sample. Identified variants were annotated with ANNOVAR. For annotation of potentially damaging variants, we followed the example of a recent schizophrenia exome sequencing study in defining 3 successively more inclusive annotation categories based on 5 bioinformatics algorithms (SIFT, PolyPhen-2 HVAR, PolyPhen-2 HDIV, LRT, and MutationTaster) provided in the Database for Nonsynonymous SNPs and Their Functional Predictions. The categories were characterized as nonsynonymous broad (evidence of damaging effect by any 1 of 5 different bioinformatics algorithms), nonsynonymous strict (evidence of damaging effect by all 5 different bioinformatics algorithms), and disruptive (canonical splice site, nonsense, or frameshift mutations).

４．研究成果

We found low frequency, segregating variants that were shown to be damaging using bioinformatics analysis. Our results are consistent with the allelic heterogeneity in SZ and suggest that large samples will be required to definitively identify candidate rare variants or genes associated with increased risk

for SZ.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　0件／うちオープンアクセス　1件）

１．著者名 Ishizuka K, Kimura H, Kushima I, Inada T, Okahisa Y, Ikeda M, Iwata N, Mori D, Aleksic B, Ozaki N.	４．巻 5
２．論文標題 Assessment of a glyoxalase I frameshift variant, p.P122fs, in Japanese patients with schizophrenia.	５．発行年 2018年
３．雑誌名 Psychiatr Genet.	６．最初と最後の頁 90-93
掲載論文のDOI（デジタルオブジェクト識別子） 10.1097/YPG.0000000000000204	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Yu Y, Lin Y, Takasaki Y, Wang C, Kimura H, Xing J, Ishizuka K, Toyama M, Kushima I, Mori D, Arioka Y, Uno Y, Shiino T, Nakamura Y, Okada T, Morikawa M, Ikeda M, Iwata N, Okahisa Y, Takaki M, Sakamoto S, Someya T, Egawa J, Usami M, Kodaira M, Yoshimi A, Oya-Ito T, Aleksic B, Ohno K, Ozaki N.	４．巻 8(1)
２．論文標題 Rare loss of function mutations in N-methyl-d-aspartate glutamate receptors and their contributions to schizophrenia susceptibility	５．発行年 2018年
３．雑誌名 Translational Psychiatry	６．最初と最後の頁 12
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41398-017-0061-y	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

〔その他〕

－

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