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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）Exome sequencing of schizophrenia using families with multiple affected members

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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 sequecing

１．研究開始当初の背景

Schizophrenia (SCZ) is a severe chronic psychiatric disease that is characterized by psychotic symptoms such as hallucinations and delusions. The lifetime risk of SCZ is estimated to be 1%, with subsequent mortality of SCZ patients is 2.5 times higher than in the general population1. 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 SCZ 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 (SZ). 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 (figure 1). 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

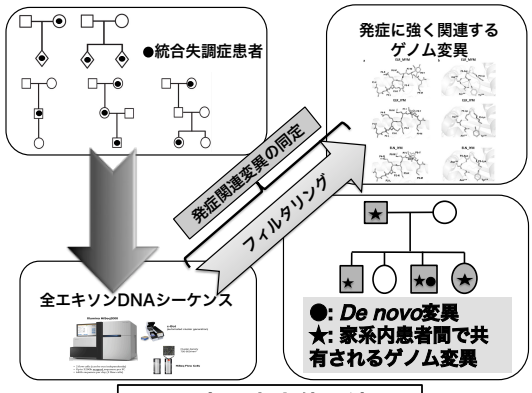

図１：本研究全体の流れ

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 (figure 2). Identified variants were annotated with ANNOVAR (using reference assembly (RefSeq, release 65;). 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).

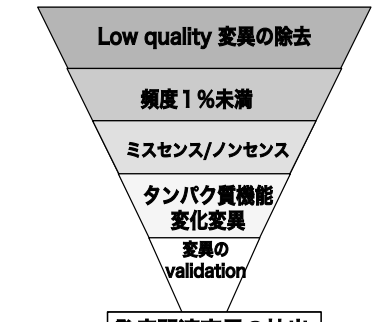

図2: フィルタリングによる変異の抽出過程

４．研究成果

We found rare (frequency <1%), segregating variants that were predicted to be damaging using bioinformatics analysis (figure 3). 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.

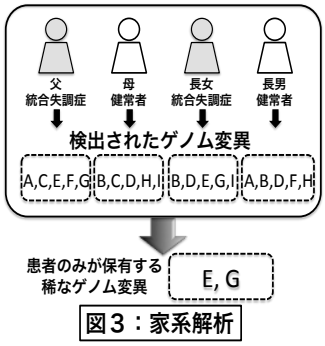

図３：家系解析

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

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〔図書〕（計 0 件）

〔産業財産権〕

○出願状況（計 0 件）

○取得状況（計 0 件）

〔その他〕
ホームページ等

なし

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なし

(4)研究協力者

なし