

REVIEW ARTICLE

Copy Number Variations in Adult-onset Neuropsychiatric Diseases

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Abstract: Adult-onset neuropsychiatric diseases are one of the most challenging areas of medicine. While symptomatic treatments are available, for most of these diseases the exact pathomechanism is not known, thus, disease-modifying therapies are difficult to conceptualize and find. The two most common and best studied neuropsychiatric diseases affecting higher cortical functions in humans are schizophrenia and Alzheimer's disease; both diseases have high heritability, however, the genetic architecture is not fully elucidated. Robust Single Nucleotide Variant (SNV) studies have identified several loci with modest effect sizes. While Copy Number Variants (CNV) make an important contribution to genetic variation, CNV GWAS suffer from dependence on mainly SNP arrays with underperforming genotyping accuracy. We evaluated dynamic range of the assays for three types of CNV loci, including biallelic deletion, high copy gain, and fusion gene, to assess the depth of exploration of the contribution of CNVs to disease susceptibility. Despite the suboptimal genotyping, novel mechanisms are emerging and further large-scale studies with genotyping assays optimized for CNV detection are needed. Furthermore, the *CHRFAM7A* human-specific fusion gene association warrants large scale locus specific association studies in AD, schizophrenia, bipolar disorder and ADHD.

ARTICLE HISTORY

Received: June 14, 2016

Revised: March 01, 2017

Accepted: March 13, 2018

DOI:

10.2174/1389202919666180330153842

Keywords: Copy number variation, CNV, GWAS, Neuropsychiatric diseases, Adult onset, Structural variants.

1. INTRODUCTION

In the era of genome-wide interrogation of susceptibility loci using microarray approaches for a number of diseases in a systematic way, a vast amount data is available for analysis. As the initial GWAS phase was built on SNVs as a genetic marker map, the majority of data was generated on assays that were optimized to decipher biallelic single nucleotides. Through whole-genome scanning methods that enable us to interrogate the human genome at a resolution between that of cytogenetic analysis and DNA sequencing, a new perspective of human genetic variation was observed - the wide variation in the copy number of submicroscopic DNA segments. Copy Number Variant (CNV) is defined as a DNA segment that is 1 kb or larger and is present at variable copy number in comparison with the reference genome. CNV can be classified as deletions, duplications, deletions and duplications at the same locus, multi-allelic loci, and complex rearrangements [1].

CNVs are major contributors to genetic variance, thus, it is conceivable that they may confer susceptibility to or cause disease [2]. CNVs influence gene expression, phenotypic variation and adaptation by altering gene dosage. Gene expression variation as a model of complex phenotype found that 18% of the gene expression traits were associated with

CNVs [3]. CNVs are estimated to be enriched 50-fold for gene expression quantitative trait loci compared to SNVs [4]. CNVs were found to play a role in an increasing number of human diseases, including schizophrenia [5], major depressive disorder [6] and autism [7, 8].

The recombination events resulting in CNVs may be frequent. It has been shown at the whole genome level that about 0.2% of biallelic CNV genotypes exhibit mendelian discordance in parent-offspring trios [2]. Multiallelic CNVs are regions of recurrent mutations contributing to the evolution of human phenotypes and to disease susceptibility [9, 10].

Pathogenic CNVs may be more amenable to therapy than other types of genetic variation. CNVs alter gene dosage, thus, modification by small molecules may be possible in contrast to mutation events resulting in loss of function or toxic gain of function. The *CMT1A* duplication rat model treated with a progesterone antagonist had correction of gene dosage and clinical and pathological improvement [11].

As systematic reporting became feasible with the Database of Genomic Variants, an alternative genetic marker map using CNVs emerged. While for biallelic CNVs, SNPs can serve as markers, for the multiallelic CNVs SNPs are unable to decipher the CNV alleles. Robust bioinformatics development efforts to call CNVs from the SNP arrays characterized the following years. With optimization of the methods, genotyping error rates were found to be acceptable for deletions and borderline for duplications.

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This study summarizes CNV GWAS in adult-onset neuropsychiatric diseases and discusses emerging mechanisms from these association studies. Using publicly available datasets and orthogonal assays, genotyping accuracy is estimated to understand the robustness of the SNP based GWAS in the quest of the missing heritability.

2. METHODS

2.1. Literature Search

A literature search was performed to identify published CNV GWAS studies in adult-onset neuropsychiatric disease. Utilizing PubMed, the following search criteria were entered and searched from 2000 to July, 2016: 1) “Copy Number GWAS Alzheimer’s”, 2) “Copy Number GWAS Schizophrenia”, 3) “Copy Number GWAS Bipolar Disorder”, 4) “Copy Number GWAS ADHD”, 5) “Copy Number GWAS Suicide”, 6) “Copy Number GWAS OCD”, 7) “Copy Number GWAS Lewy Body Dementia”, and 8) “Copy Number GWAS Frontotemporal Dementia”.

The resulting papers were manually reviewed and searching the reference lists of the selected papers identified additional papers. Papers were included/excluded based on the presence or absence of a clear phenotype and disease with a preference for true GWAS studies. Additional enrichment studies were included in the presence of a disease association and phenotype. GWAS were selected for further extraction of the following information: disease, phenotype, sample size, assay type, design, CNV association chromosomal location, nearest gene, and significance level of the association (Table 1).

2.2. Experimental Procedure

Publicly available GWAS datasets including the Alzheimer's Disease Neuroimaging Initiative (ADNI), the National Institute on Aging - Late Onset Alzheimer's Disease Family Study (NIA-LOAD), and the Translational Genomics Research Institute series 2 (*TGEN2*) Study were obtained from NCRAD or through collaboration. Samples for orthogonal genotyping were obtained from the Texas Alzheimer Research and Care Consortium (TARCC). The Institutional Review Board exempted the study based on deidentified sample and data sharing.

ADNI: This data set consisted of 360 AD cases, 200 Mild Cognitive Impairment (MCI) cases and 193 controls (mean age: 78.6±5.3 years; 46.3% women) that were previously genotyped using the Illumina 610-Quad BeadChip. AD cases were between the ages of 55 and 90 (mean AAO: 71.9±8.1 years; 44.6% women) and met NINCDS/ADRDA criteria for AD [12]. The ADNI data used in this report were obtained from the ADNI database (adni.loni.ucla.edu). Genotyping was performed on the Illumina Human610-Quad.

NIA-LOAD: The NIA LOAD Family Study recruited families with two or more affected siblings with LOAD and unrelated, normal controls similar in age and ethnic background. A total of 1,819 cases and 1,969 controls from 1,802 families were recruited through the NIA-LOAD study, NCRAD, and the University of Kentucky and included for analysis, of which a subset of 882 cases and 906 unrelated controls were used in this study. For cases, the proband was

selected from each family. The controls included only those samples that were neurologically evaluated to be normal and were not related to a study participant. Genotyping was performed on the Illumina Human610-Quad.

TGEN2: Among the TGEN2 data analyzed were 1014 clinically- and neuropathologically-characterized brain donors, and 584 controls without dementia or significant AD pathology. Inclusion criteria were: self-defined ethnicity of European descent, neuropathologically confirmed AD or neuropathology present at levels consistent with status as a control, and age of death greater than 65. Autopsy diagnosis was performed by board certified neuropathologists and was based on the presence or absence of the characterization of probable or possible AD. Where it was possible, Braak and Braak staging and/or CERAD classification were employed. Samples derived from subjects with a clinical history of stroke, cerebrovascular disease, comorbidity with any other known neurological disease, or with the neuropathological finding of Lewy bodies were excluded. Genotyping was performed on the Affymetrix 6.0 microarray.

TARCC samples: The TARCC cohort with comparable sample size and cohort characteristics was used for genotyping as gold standard. The TARCC study included 781 subjects (AD and normal controls). Probable AD was diagnosed based on NINCDS-ADRDA criteria [12]. The methodology of the Texas Alzheimer Research and Care Consortium project has been described [13]. Exclusion criteria included a Hachinski score >4 and clinical or imaging evidence of a stroke. The control group consisted of non-demented subjects; inclusion criteria were the following: unrelated to cases, age over 55 years, normal performance on activities of daily living, and CDR global score 0 (by surrogate historian), and controls underwent neuropsychological testing to confirm status.

ADNI, NIA-LOAD and TGEN AD GWAS datasets were normalized using GoldenHelix and the Affymetrix data was corrected for 16 PCs. Segmentation of the data was performed with the circular binary segmentation algorithm implemented in Golden Helix. The best performing segment within the region was selected. CNV states were kept numeric and the kernel distributions were plotted. Dynamic range of the assays as a surrogate for genotyping accuracy was evaluated for three CNV structures with allele frequencies between 5-20%: 1) biallelic deletion upstream from *CREB1*; 2) multiallelic gain in an olfactory receptor cluster on chr14; and 3) a biallelic fusion gene *CHRFAM7A* on chr15. Locus specific orthogonal assays on the TARCC samples were used for genotyping as gold standard.

For the *CREB1* deletion, LR-PCR defined CNV state qualitatively [14]. For the olfactory receptor cluster, MLPA was used to quantitatively decipher CNV states [15]. For the *CHRFAM7A* fusion gene, a breakpoint specific TaqMan assay was used [14].

3. RESULTS

The initial PubMed literature search results were as follows: 1) “Copy Number GWAS Alzheimer’s” - 29 results 2) “Copy Number GWAS Schizophrenia” - 152 results, 3) “Copy Number GWAS Bipolar Disorder” - 56 results, 4)

Table 1. CNV GWAS studies in adult onset neuropsychiatric diseases.

Reference	Disease	Phenotype	Sample Size	Assay	Design	Chromosomal Location	Closest Gene	P-value
[13]	AD + P	+/- P	440/593/136	Illumina HumanOmni1-Quad	Enrichment	19p13.3	APC2	0.059*
[16]	AD	case/control	331/368+531	Illumina Human Hap550K	Tag SNP assoc.	2q32.1 / 11q22.3 / 15q13.3	CHRNA7	0.053 #
[18]	AD	case/control	146/313/181	Illumina 610-quad chip	Enrichment	3, 5, 6, 14, 22	4 gene clusters	0.005*
[17]	AD	case/control	794/196	Illumina 610-quad chip	Enrichment	2p16.3 / 7q31.1 / 15q13.3 / 16p13.11 / 17p12	CHRFAM7A / IMMP2L - CSMD1, HNRNPCL1, SLC35F2 / NRXN1, ERBB4	0.059*
[15]	AD	case/control	3260/1290	Illumina 610-quad chip	Enrichment	15q11.2 / 15q13.3 / 16p13.1	CHRNA7 / CHRFAM7A	0.22*
[10]	AD	AAO	40	aCGH	GWAS	14q11.2	CREB1	0.032#, 0.035#
[19]	AD	Familial	1536	NA	Enrichment	NA	NA	NA
[14]	AD	AAO	781	Affymetrix 6.0	GWAS	2q33.3 / 4p16.3 / 7p22.3 / 13p13 / 14p14 / 15q13.3	LINC00550 / CHRFAM7A / GALNTL6 / BX248273 / EIF3IP1	0.000523# / 1.37E-05# / 0.000726# / 4.22E-08# / 1.97E-06# / .0000679#
[9]	AD	AAO	781	Affymetrix 6.0	GWAS	1q32.2 / 2q14 / 3q22.1 / 6p12	CPNE4 / CR1, BIN1, CD2AP	0.033#
[20]	AD + P	+/- P	496/639/156/958	Illumina HumanOmni1-Quad	Enrichment	16p11.2	SPN, CORO1A, QPRT, MAZ, MAPK3	1*
[21]	AD	case/control	559/554	Illumina HumanHap 650Y	Enrichment	15q11.2	TUBGCP5, CYFIP1, NIPA2, NIPA1, WHAMML1	0.037*
[24]	SCZ	case/control	1073/1148	Illumina HumanHap	Enrichment	8p22, 16p13.11-p12.4, 22q11.2	many genes / NDE1	0.003*
[25]	SCZ + P	+/- P	1433/33250	HumanHap300 chip/550 chip, Affymetrix 6.0	GW <i>de novo</i> CNV	1q21.1, 15q11.2, 15q13.3	GJAS8 / CYFIP1 / CHRNA7	2.31×10 ⁻⁵ # / 9.57×10 ⁻⁴ # / 1.0# / 2×10 ⁻³ #
[26]	SCZ	case/control	91/92	aCGH	Enrichment	15q11.2 / 15q13 / 21q22 (c-s)	CYFIP1 / CHRNA7 / KCNE1-2	NA
[23]	SCZ	case/control	3945/3611	Affymetrix 6.0	Enrichment	1q21.1 / 3q29 / 15q13.3 / 16p11.2/22q11.21	TFRC to BDH1/FAM108A3 to NBPFL1 / CHRNA7 / SPN to CORO1A / 43 genes	.05* / 0.02* / 0.11* / <0.05* / .002*
[27]	SCZ	case/control	4719/5917	Affymetric 5.0, and 6.0	Enrichment	3q29 / 16p11.2 / 17q12 / 22q11.2,	NA	0.018* / 0.0037* / 0.0031* / 0.009*
[28]	SCZ/BD + P	+/- P	1564/1748	-	Enrichment	20p12.2	PAK7	NA

(Table 1) contd....

Reference	Disease	Phenotype	Sample Size	Assay	Design	Chromosomal Location	Closest Gene	P-value
[22]	SCZ	case/control	166/52	Affymetrix 6.0	Enrichment	1p13.3-31.1 / 15q11.2	PDE4B / CYFIP1, NIPA1, NIPA2 & GCP5	NA
[29]	SCZ	case/control	6682/11255	Illumina Arrays	Enrichment	1p36.33 / 15p57.67 / 16p11.2	SLC1A1 / GNB1, CALML6, TMEM52, KIAA1751, GABRD, CGNL1	0.023* / 0.0005* / 0.0019*
[30]	SCZ	case/control	790/1347, 662	Illumina HumanOmni1-Quad, Affymetrix 6.0	Enrichment	16p11.2	ATP2A2, CLN3	0.018*
[31]	SCZ	case/control	1699/824	aCGH	Enrichment	1q21.1, 3q29, 7q11.23, 15q11.2-q13.1, 15q13.3, 16p11.2 and 22q11.21	many genes	9.3x10 ⁻⁹ *
[36]	OCD/TS	case/control	1613/1086/1789	Illumina 610-quad chip	Enrichment	3p26.3 / 16p13.11 / 22q11	PARK2 / CNTN6	0.08*
[35]	Suicide	case/control	475/1133	Illumina HumanOmni1-Quad	GWAS	6p22.2 / 12q12	LRRK2 / large histone H1 gene cluster	< 0.05 #
[32]	BD	case/control	2691/8842	NA	Enrichment	1q21.1 / 3q29 / 16p11.2	NA	0.022* / 0.03* / 2.3x10 ⁻⁴ *
[33]	ADHD	case/control	896/2455	Illumina human660W and Affymetrix 6.0	Enrichment	15q13.3	CHRNA7	0.79*
[34]	ADHD	case/control	400/526	Illumina HumanOmni1-Quad	Enrichment	13q21.33	NA	0.3*

Key: AD - Alzheimer's Disease, ADHD - Attention Deficit Hyperactive Disorder, BD - Bipolar Disorder, NA - Not Applicable, OCD - Obsessive Compulsive Disorder, P - Psychosis, SCZ - Schizophrenia, TS - Tourette Syndrome

P-values designated with a (*) are reported from enrichment studies. P-values designated with a (#) are reported from GWAS studies.

“Copy Number GWAS ADHD” - 35 results, 5) “Copy Number GWAS Suicide” - 2 results, 6) “Copy Number GWAS OCD” - 3 results, 7) “Copy Number GWAS Lewy Body Dementia” - 0 results and 8) “Copy Number GWAS Frontotemporal Dementia” - 1 result.

After manual review of the resulting articles, the following were identified as GWAS or enrichment studies and included for further review: 1) 11 studies in AD [14-24] 2) 9 studies in schizophrenia [25-34] 3) 1 study in Bipolar Disorder [35] 4) 2 studies in ADHD [36, 37] 5) 1 study in Suicidal Behavior [38] and 6) 1 study in OCD [39]. 19 studies were case-control design, 3 used a quantitative phenotype (AAO) and 3 investigated qualitative endophenotypes (psychosis, suicide).

The assays used in these studies include the Affymetrix Genome-Wide Human SNP Array 5.0, 6.0 and 500K Array set and the following Illumina assays: Human Hap550K, Human-610 Quad BeadChip, Human Omni1-Quad BeadChip and Human660W-Quad BeadChip. A majority of studies were designed to assess enrichment of rare variants while few were correlation trend studies. Sample sizes were re-

ported from 40 - 33,250. Allele frequencies of the detected CNVs were reported between 0.17 - 29%. Statistical significance was modest.

The studies in this review reported a total of 22 CNV loci and the following loci were reported multiple times: 1q21.1, 3q29, 15q11.2, 15q13.3, 16p11.2, 16p13.11 and 22q11.2.

3.1. Disease-Specific CNVs

3.1.1. Alzheimer's Disease

We reviewed 11 papers investigating CNVs and AD through GWAS. CNVs were identified in AD patients in these studies at the following chromosomal locations: 2q32.1, 11q22.3, 14q11.2, 15q11.2, 15q13.3, 16p11.2, 16p13.11, 17p12 and 19p13.3. 36% of these studies reported an association with the 15q13.3 locus and 18% reported an association with the 16p13.11 locus.

15q13.3 harbors the human $\alpha 7$ neuronal nicotinic acetylcholine receptor gene, *CHRNA7*, along with its chimeric fusion gene *CHRFAM7A*. The study of Heinzen *et al.* [19] identified a duplication in *CHRNA7* at 15q13.3 ($p=0.053$).

The study of Swaminathan *et al.* [20] reproduced this association in *CHRFAM7A* on chromosome 15 as well as identifying CNV calls overlapping a novel gene, *IMMP2L*, on chromosome 7. Chapman *et al.* [18] studied CNVs previously associated with increased risk of AD and detected CNVs in the 15q13.3 region at lower rates. The ordered subset approach detected the *CHRFAM7A* association [14].

16p13.11 duplications were reported in the studies of Swaminathan *et al.* [20] and Chapman *et al.* [18]. 16p13.11 encompasses the *NDE1* gene associated with abnormalities in brain development [27].

Additional CNV associations in AD were reported on 2p16.3 involving the *NRXN1* gene [24], on 2q33.3-34 affecting *ERB4* [20], a duplication on 15q11.2 affecting up to 5 genes (*CYFIPI1* and *NIPAI1*) [24] and on 19p13.3 involving *APC2* [16].

3.1.2. Schizophrenia

We reviewed 9 GWAS of CNVs and schizophrenia that identified a total of 14 CNVs at the following chromosomal locations: 1p13.3, 1q21.1, 1q36.33, 3q29, 8p22, 15p11.2, 15q13.3, 15p57.67, 16p11.2, 16p13.11, 17q12, 20p12.2, 21q22 and 22q11.2. CNVs at 16p11.2 were identified in 50% of the studies and those at 15q13.3 and 22q11.2 were each identified in 40% of the reviewed schizophrenia CNV GWAS.

The 16p11.2 loci was reported in Schizophrenic patients in the studies of Levinson *et al.* [26], Szatkiewicz *et al.* [30], Rees *et al.* [32], Guha *et al.* [33] and Kushima *et al.* [34] encompassing multiple genes through both duplications and deletions.

The *CHRNA7* gene and its chimeric fusion gene *CHRFAM7A* have been implicated in schizophrenia and other psychosis. GWAS CNV studies by Kushima *et al.* [34], Stefansson *et al.* [28], Tam *et al.* [29] and Levinson *et al.* [26] all identified associations at 15q13.3 for which *CHRNA7* is an affected gene. A deletion in the 15q11.2 region encompassing the *CYFIPI1* gene was found in the studies of Kushima *et al.* [34], Stefansson *et al.* [28], Tam *et al.* [29] and Rudd *et al.* [25]. The studies of Kushima *et al.* [34], Need *et al.* [27], Levinson *et al.* [26] and Szatkiewicz *et al.* [30] also reported an enrichment of the deletion of the VCFS locus on 22q11.2.

Stefansson *et al.* [28], Kushima *et al.* [34], and Levinson *et al.* [26] also identified a deletion at 1q21.1, a gene-rich region affecting the *GJAS8* gene previously associated with Schizophrenia. Levinson *et al.* [26] further identified duplications of *VIPR2* on 3q29. Additional CNVs have been identified including the following loci: a single duplication at 21q22 affecting *KCNE1-2* gene [29], 20p12.2 implicating *PAK7* [31], 1p31.3-p31.1 encompassing *PDE4B* [25] and 1p36.33 involving duplications of five genes [32].

3.1.3. Other Neuropsychiatric Diseases

Five CNV GWAS were performed to identify CNVs associated with Bipolar Disorder, suicidal behavior, ADHD and OCD. In BD, enrichment of CNVs on 1q21.1, 3q29 and 16p11.2 were reported [35]. CNV GWAS with OCD found association signals at 3p26.3, 16p13.11 and 22q11 [39]. Sui-

cidal behavior was associated with CNVs at 6p22.2 and 12q12 loci [38]. ADHD was associated with the 15q13.3 locus in 2 independent studies [36, 37].

3.2. Dynamic Range of Three Representative CNV Loci

Kernel distribution of log2ratios are depicted in Fig. (1) for the three loci and contrasted to the gold standard assay for each locus. Dynamic range for the biallelic deletion locus was acceptable for 2/3 datasets. The multiallelic high copy number locus on chr14 harboring *OR4K2* was detectable with MLPA and Agilent aCGH. The SNP arrays failed to detect the high copy number states. The *CHRFAM7A* fusion gene harbors the exact sequence of *CHRNA7* and *FAM7A*, thus dynamic range in the microarrays is diminished. The unique breakpoint sequence allows detection by TaqMan assay with superior dynamic range.

4. DISCUSSION

4.1. Emerging Pathways and Shared Mechanisms

The most common CNV association with neuropsychiatric diseases is at the 15q13.3 locus harboring *CHRNA7* and the chimeric *CHRFAM7A* gene. It is associated with AD, schizophrenia, BD and ADHD.

CHRFAM7A is a human-specific fusion gene, a result of multiple chromosomal rearrangements including duplication, deletion and inversion events on chromosome 15 during evolution [40]. The region has ongoing instability and various neurological phenotypes have been reported in the context of deletions and duplications [41-43]. The *CHRFAM7A* fusion gene harbors a part of the alpha 7 nicotinic acetylcholine receptor ($\alpha 7$ nAChR), *CHRNA7*, and 4 new exons of the *FAM7* sequence corresponding to a part-functional *CHRNA7* due to *FAM7A* replacing a portion of the extracellular domain of *CHRNA7* and a part-kinase (*FAM/ULK4*) sequence [40]. It can be present in 0 (ancestral, rare), 1 (10-15% of the population) and 2 copies in the human genome; furthermore, the orientation can be direct or inverted, and the predicted proteins differ [40]. This gene product acts as a dominant negative regulator of $\alpha 7$ nAChR, allowing it to regulate *CHRNA7* gene function and highlighting its significance in neuropsychiatric diseases such as AD, BD, ADHD and schizophrenia.

The evolution of *CHRFAM7A* lends some insight into the rarity of *CHRFAM7A* identification by GWAS. As *CHRFAM7A* is partially identical to *CHRNA7*, it remains a challenge to separate the two genes by microarray probes. The unique sequence is limited to the breakpoint sequence, thus, PCR primers need to span the breakpoint sequence for genotyping assays. Furthermore, the fusion gene has both direct and inverted orientation with distinctive protein predictions. While the *CHRFAM7A* direct orientation protein is predicted at 42-45 kD, the inverted protein is smaller and the distance between the Kozak consensus sequence from initiation makes native translation unlikely.

Despite these limitations, *CHRFAM7A* was associated with AD and schizophrenia in each four independent studies. *CHRFAM7A* is a human specific fusion gene, thus, functional studies are sparse. *CHRNA7/CHRFAM7A* are implicated in $\alpha 7$ nAChR receptor function and inflammation.

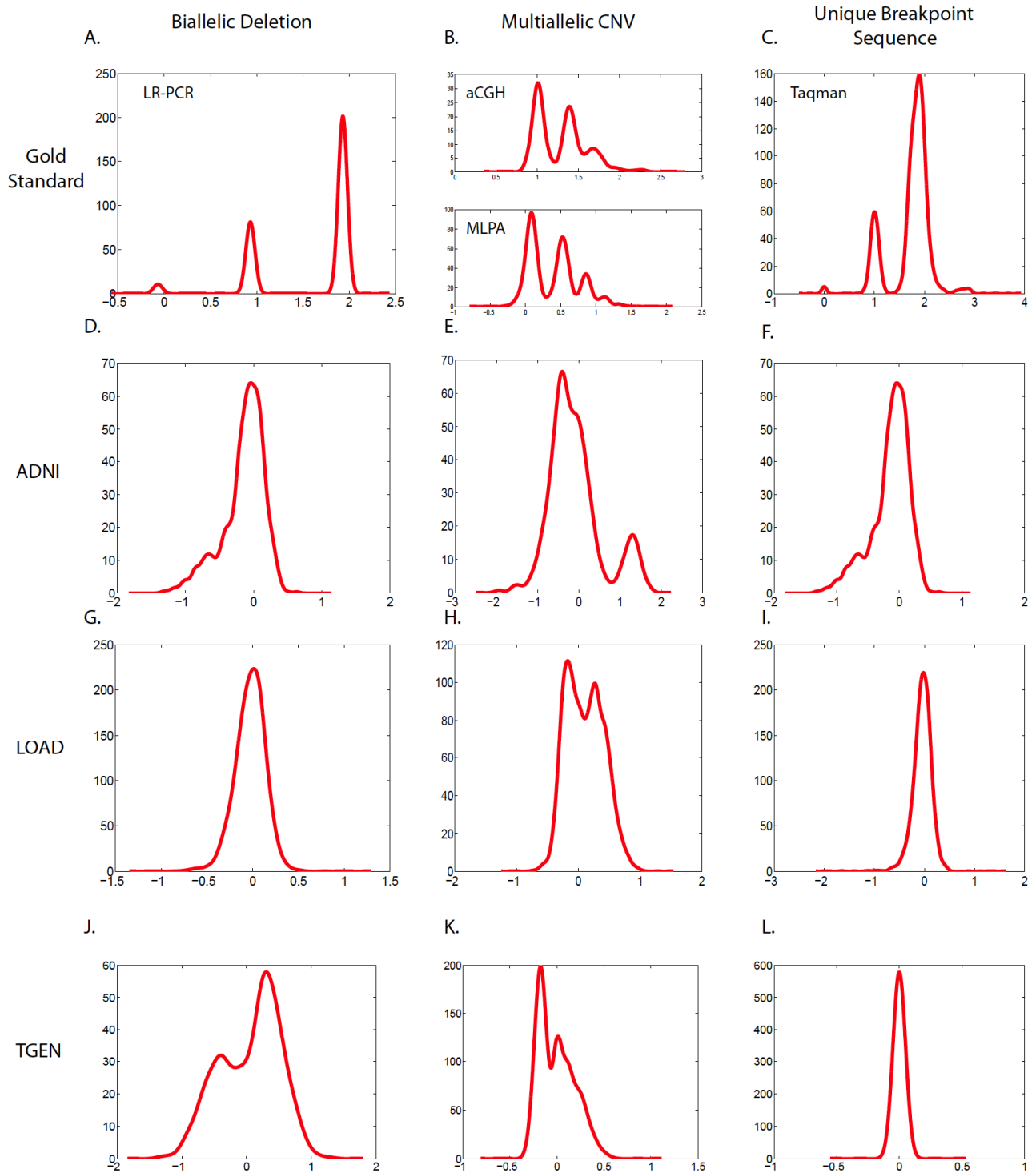


Fig. (1). Kernel distribution of log₂ratios is depicted for three characteristic CNV structures: 1) biallelic deletion upstream from *CREB1*; 2) multiallelic gain in an olfactory receptor cluster on chr14; and 3) a biallelic fusion gene *CHRFAM7A* on chr15. Locus specific orthogonal assays were used for genotyping as gold standard. For the *CREB1* deletion LR-PCR defined CNV state qualitatively (A). For the olfactory receptor cluster aCGH and MLPA was used to quantitatively decipher CNV states (B). For the *CHRFAM7A* fusion gene a breakpoint specific TaqMan assay was used (C). Dynamic range in the GWAS datasets: ADNI (D-F), LOAD (G-I) and TGEN (J-L) suggest high genotyping error rates. The deletion allele was adequately detected in 2 out of 3 datasets (D, G and J), while the high copy number state (E, H and K) and the fusion gene (F, I and L) were not detected.

Amyloid Beta 1-42 and $\alpha 7$ nAChR are co-expressed in Alzheimer's disease brains [44]. Amyloid Beta 1-42 binds with high affinity to the $\alpha 7$ nAChR and the receptor facilitates

internalization of Amyloid Beta 1-42 through endocytosis in cell culture [44], supporting the observation that neurons expressing the $\alpha 7$ nAChR are selectively vulnerable in AD.

$\alpha 7$ nAChR expressed in vascular smooth muscle cells is implicated in cerebral amyloid angiopathy, one of the pathological hallmarks of AD [45]. In light of the difficulties in differentiating between *CHRNA7* and *CHRFAM7A* mRNA and protein products, caution should be exercised when interpreting literature; the functions attributed to *CHRNA7* may overlap the functions attributed to *CHRFAM7A* in humans.

Recent reports elucidated the strong presence of *CHRFAM7A* on the periphery in addition to neuronal tissue, including Peripheral Blood Mononuclear Cells (PBMC), lymphocytes, synoviocytes and HL-60 cells. It is expressed in the hippocampus, cortex, corpus callosum, thalamus, putamen, caudate nucleus, and cerebellum. Due to the detection overlap, it may also be present where $\alpha 7$ nAChRs has been detected, such as vascular and brain endothelial cells, bronchial epithelial cells, keratinocytes, astrocytes, synoviocytes, thymocytes, lymphocytes, bone marrow cells, monocytes, macrophages, microglia, and astrocytes.

Both *CHRFAM7A* and *CHRNA7* are expressed in macrophages. While traditionally $\alpha 7$ nAChR was felt to be essential for vagus nerve regulation of acute pro-inflammatory cytokine release during systemic inflammatory response in animal models, in humans, *CHRFAM7A* is predominant (200-1000X compared to *CHRNA7*) in the macrophages, suggesting a human-specific immune response. LPS, nicotine and IL-1Beta inhibited *CHRFAM7A* expression.

Inflammation in the CNS is thought to contribute to AD, schizophrenia and even depression and bipolar disorder. Emerging bodies of evidence suggest a central role of microglia in the inflammation and neurodegeneration link. As microglia are the resident macrophages in the CNS originating from mesenchymal progenitor cells in the yolk sack, it is plausible that *CHRFAM7A* gene dosage may affect microglia function and, thus, susceptibility to neurodegenerative and neuropsychiatric diseases.

While *CHRFAM7A* has been associated with adult neuropsychiatric diseases, the association signals have not been robust. This can be attributed to the high genotyping error rate based on the logR distributions from the four SNP microarray datasets. Well-powered studies with breakpoint specific assays are needed to conclude the relevance of these associations. NGS datasets may offer an alternative to the breakpoint-specific TaqMan assay in the future.

The CNV association with deletions and duplications at the 16p11.2 locus was found in 26% of studies. Duplications at the 16p11.2 locus have been implicated in schizophrenia, AD with psychosis [23], ADHD and other neurocognitive diseases [20].

CNVs in this chromosomal region can affect approximately 25 genes, many of which have been linked to multiple neuropsychiatric phenotypes including: *SPN*, *QPRT*, *JCLN*, *KIF22*, *MAZ*, *PRRT2*, *MVP*, *CDIPT*, *PIS*, *SEZ6L2*, *ASPHD1*, *KCTD13*, *FKSG86*, *TMEM219*, *TAOK2*, *HIRIP3*, *INO80E*, *DOC2A*, *ALDOA*, *PPP4C*, *TBX6*, *YPEL3*, *GDPD3*, *MAPK3*, and *CORO1A* [23]. Of these genes, *SPN* (*CD43*), *CORO1A*, *QPRT*, *MAZ*, and *MAPK3* have been implicated in AD [23]. In particular, expression of *MAPK3* and altered *SPN* expression in human microglia has been reported in AD

along with higher levels of *QUIN* [23]. 16p13.11 encompasses the *NDE1* gene known to bind to *DISC1* during brain development [27]. Mutations of *DISC1* were found in patients with Schizophrenia and other neuropsychiatric disorders [27]. Furthermore, *MAZ* was identified as a biomarker for schizophrenia [23].

Association of the 22q11.2 locus was reported in 18% of the studies. Velocardiofacial Syndrome (VCFS) is linked to increased susceptibility to schizophrenia and 25-30% of VCFS patients will develop schizophrenia or psychosis [46, 47]. However, deletions in 22q11.2 in patients without VCFS have also been linked to an increased risk of schizophrenia [32]. The 22q11.2 loci is associated with the *CDC45* gene involved in the cell cycle and DNA replication and *PRODH* which has been implicated in neuropsychiatric diseases. In addition to *CDC45* and *PRODH*, the genes that are deleted in the 22q11.2 region include *TBX1*, *GNB11*, and *COMT* and are implicated in cortical development and early neuronal migration [46]. Haploinsufficiency of *Tbx1* and *Gnb11* in a mouse model was shown to cause impairments in Pre-Pulse Inhibition (PPI), an endophenotype of schizophrenia [46]. *CDC45* has been associated with schizophrenia in SNV GWAS [46]. The catechol-O-methyltransferase (*COMT*) gene has also been investigated for its role in the disease phenotype of 22q11.2 deletions. *COMT* is involved in the dopaminergic dysfunction seen in psychosis and abnormal executive functioning [46].

Three additional CNVs were reported multiple times at the following loci: 1q21.1, 3q29, and 16p13.11. The 1q21.1 locus has been implicated in Autism Spectrum Disorder (ASD), Schizophrenia, Bipolar Disorder and various neurodevelopmental disorders [34, 35] and is reported in 18% of the CNV GWAS [25, 26, 28, 35]. Patients with the 1q21.1 microdeletion may present with various phenotypes including facial dysmorphism and developmental delay [48]. The genes *PRKAB2*, *CHD1L*, *BCL9*, *GJA5*, and *GJA8* are all located in the 1q21.1 region, but none are sufficient to cause the associated 1q21.1 phenotypes [48].

3q29, harboring two schizophrenia candidate genes *PAK2* and *DLG1* [49], was reported in 15% of the studies [26, 30, 35]. The *PAK2* gene is highly expressed in the brain and is involved in the inhibition of *Rac1*, a regulator of spine morphology [49]. *DLG1* protein binds *neuroigin*, whose function has been implicated in ASD; *DLG1* also binds a Glutamatergic receptor subunit, *GluR1*, thought to be involved in schizophrenia [49]. 3q29 microdeletions are often associated with ASD features, microcephaly and dysmorphism [49].

The 16p13.11 locus has been implicated in ADHD, developmental delay, and aggression [50], and was reported in 18% of the studies. 16p13.11 deletions and duplications affect two notable genes, *NDE1* and *NTANI*, involved in numerous neuropsychiatric diseases [50]. *NDE1* is involved in centrosome localization and it is hypothesized that *NDE1* haploinsufficiency may cause microcephaly in patients with 16p13.11 deletion [50]. The *NTANI* gene encodes for asparagine-specific N-terminal amidase and has been tied to abnormalities in spatial memory and spontaneous activity in animal models [50].

4.2. Limited Dynamic Range Suggests High Genotyping Error Rates on SNP Arrays

While disease associations suggesting novel mechanisms emerged from the SNP array based CNV GWAS, the power is limited. The Kernel distributions for the three studied loci on SNP arrays contrasted to the corresponding locus-specific orthogonal genotyping assay suggest poor dynamic range to decipher CNVs from GWAS data, except for simple biallelic deletions. Loss of high copy number states and inability to distinguish between fusion gene (*CHRFAM7A*) and parent gene (*CHRNA7*) result in high genotyping error rates, and potentially loss of detection of the pathogenic allele. These limitations undermine traditional GWAS analyses by decreasing power. In the reported GWAS studies high resolution superior dynamic range assays (aCGH) have seldom been used; the few studies had small sample sizes and thus were not powered to detect relevant associations. In the reported CNV GWAS, common variants have been mostly omitted due to difficulty with binning of CNV states. Numeric logR data and correlation trend statistics have been used in a limited number of studies. Optimization attempts excluded common CNVs, CNVs in regions with low copy repeats and, for most studies, there was a conservative size limitation. It was further revealed that SNP arrays are underperforming for CNV analysis [51]. SNP arrays detected 4-16% of CNVs between 30-100kbp in size, 18-36% for sizes 100-150kbp and 24-62% in the 150-1000kbp range [52]. Different analytic tools applied to the same raw data typically yield CNV calls with <50% concordance. Moreover, reproducibility in replicate experiments is <70% for most platforms [53]. In addition, there is a detection bias toward deletion *versus* duplication and multicopy loci [53]. Segmental duplications further undermine CNV detection [53].

As Next Generation Sequencing (NGS) is becoming feasible for large sample sizes, these sequencing efforts led to the emergence of new challenges in CNV detection and the bioinformatics approaches are evolving [54-56]. The four main methods for detecting CNVs from NGS data are Read-Pair (RP), Split-Read (SR), Read Depth (RD), and Assembly (AS), with their own limitations [54]. While RP methods are excellent in identifying precise breakpoints, they are limited in detecting small events or events within segmental duplications. The SR method is inferior in detecting large scale CNVs and underperform in segmental duplications. Both of these methods identify the exact size, however they cannot measure dosage. RD can determine dosage, and its dynamic range can be improved by using a reference sample, a similar calculation that is applied in microarray analysis. The AS method is reference genome independent, but requires excessive computational power, and has limitations in repetitive sequences and segmental duplications. Combined approaches building on strengths of multiple analyses are being developed to optimize CNV detection [54-56].

However, even whole-genome sequencing projects (The 1000 Genomes Project) have limitations of sparse data (6-7 fold coverage) and CNV detection bias. The depth results in limited power to detect rare variants and CNV. In addition, linkage disequilibrium precludes inference. The sensitivity depends on CNV type, reaching 88% for deletions, 65% for duplications and 32% for inversions [57]. On the ends of the

size spectrum detection rates drop. Short reads miss >88% of insertions and deletions between 50bp-1kbp length [58].

CONCLUSION

NGS strategies may offer an alternative as larger sample sizes are captured, and will increase sensitivity especially for fusion genes and inversions. Detection of the high CNV states by NGS needs to be validated experimentally to determine sensitivity and specificity. Between the powerful SNP-GWAS studies and the ongoing Whole Genome Sequencing projects, the field has not adequately assessed the contribution of copy number variation to the genetic architecture of the adult onset neuropsychiatric diseases. Large scale studies with deep sequencing, orthogonal long-read sequencing technologies and a combination of experimental and computational methods are needed to elucidate haplotype structure and fully explore the role of CNVs in disease. *CHRFAM7A* is an interesting association signal detected in multiple studies and the genotyping assays mostly detected the homozygous deletion alleles. The recurrent observation and the human specificity of this gene imply a role in human specific brain function and, thus, warrants further functional studies in appropriate model systems.

LIST OF ABBREVIATIONS

AD	=	Alzheimer's Disease
ADHD	=	Attention Deficit Hyperactivity Disorder
ASD	=	Autism Spectrum Disorder
BD	=	Bipolar Disorder
CNV	=	Copy Number Variation
GWAS	=	Genome Wide Association Study
OCD	=	Obsessive Compulsive Disorder
SCZ	=	Schizophrenia
SNP	=	Single Nucleotide Polymorphism
SNV	=	Single Nucleotide Variant
SV	=	Structural Variants
VCFS	=	Velocardiofacial Syndrome

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

None declared.

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