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A neurogenetic model for the study of schizophrenia spectrum disorders: the International 22q11.2 Deletion Syndrome Brain Behavior Consortium

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Abstract

Rare copy number variants contribute significantly to the risk for schizophrenia, with the 22q11.2 locus consistently implicated. Individuals with the 22q11.2 deletion syndrome (22q11DS) have an estimated 25-fold increased risk for schizophrenia spectrum disorders, compared to individuals in the general population. The International 22q11DS Brain Behavior Consortium is examining this highly informative neurogenetic syndrome phenotypically and genomically. Here we detail the procedures of the effort to characterize the neuropsychiatric and neurobehavioral phenotypes associated with 22q11DS, focusing on schizophrenia and subthreshold expression of psychosis. The genomic approach includes a combination of whole-genome sequencing and genome-wide microarray technologies, allowing the investigation of all possible DNA variation and gene pathways influencing the schizophrenia-relevant phenotypic expression. A phenotypically rich

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CONFLICT OF INTEREST

DMM-M has given lectures on 22q11DS for Natera; CA has been a consultant to or has received honoraria or grants from Abbot, AMGEN, AstraZeneca, CIBERSAM, Dainippon Sumitomo Pharma, Fundación Alicia Koplowitz, Forum, Instituto de Salud Carlos III, Gedeon Richter, Janssen Cilag, Lundbeck, Ministerio de Ciencia e Innovación, Ministerio de Sanidad, Ministerio de Economía y Competitividad, Mutua Madrileña, Otsuka, Pfizer, Roche, Servier, Shire, Schering Plough, Sunovio and Takeda. DF has been a consultant and/or advisor to or has received honoraria from AstraZeneca, Bristol-Myers Squibb, Eisai, Janssen, Lundbeck, Otsuka and Ministerio de Ciencia e Innovación, Ministerio de Sanidad, Ministerio de Economía. The other authors declare no conflict of interest.

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data set provides a psychiatrically well-characterized sample of unprecedented size (n = 1616) that informs the neurobehavioral developmental course of 22q11DS. This combined set of phenotypic and genomic data will enable hypothesis testing to elucidate the mechanisms underlying the pathogenesis of schizophrenia spectrum disorders.

INTRODUCTION

Advances in technology for the characterization of entire genomes, for example, next-generation whole-genome sequencing (WGS) and the availability of large samples with DNA, promise to propel our mechanistic understanding of neuropsychiatric disorders. Rare structural and other variants, including copy number variants (CNVs), have contributed to recent advances. Large rare CNVs throughout the genome have been identified as contributors to the etiology of schizophrenia, conferring significant risk with large effect sizes.[1–4] Arguably, none is more clinically relevant or feasible as a model for focused investigation than the recurrent 22q11.2 deletion underlying the 22q11.2 deletion syndrome (22q11DS).[4]

The link between 22q11DS and schizophrenia has long been recognized.[5–11] Multiple studies confirm that ~1 in 4 individuals with 22q11DS develop schizophrenia, and that ~1 in 100–200 individuals with schizophrenia in community samples have a 22q11.2 deletion.[12,13] These observations led a large international group of investigators to collaborate with the goal of identifying the underlying mechanisms of schizophrenia expression in 22q11DS that may be applicable to idiopathic schizophrenia in the general population. They agreed to share phenotypic information and existing DNA samples. Recognizing the potential of this collaboration to provide a wealth of data on the link between 22q11DS and schizophrenia spectrum disorders, the US National Institute of Mental Health (NIMH) has forged a collaborative effort to investigate this important neurogenetic syndrome through the newly established International 22q11DS Brain Behavior Consortium (IBBC). Here we provide the goals and underlying hypotheses of the IBBC, an overview of clinical aspects of 22q11DS and the rationale for focusing on this condition. We outline the phenotypic procedures and the genetics work flow, and summarize early findings.

GOAL OF THE CONSORTIUM

The IBBC is taking a multifaceted unbiased genome-wide approach to uncover genetic variation that contributes to the expression and high prevalence of schizophrenia in 22q11DS. The overarching hypothesis is that taking advantage of the magnifying effect of the 22q11.2 deletion and comprehensively studying genetic variation across the genome may elucidate genes and, more plausibly, gene networks and functional biological pathways that contribute to the etiology of schizophrenia and other neuropsychiatric phenotypes in 22q11DS and in the general population. A related hypothesis is that the study of youths with 22q11DS allows for the identification of early behavioral and/or cognitive markers associated with schizophrenia but preceding the onset of the first psychotic episode. It is expected that youths with 22q11DS and greater cognitive decline will share some of the genetic factors identified in individuals with schizophrenia. The expectations are that the

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biological pathways identified may be similar to those for individuals with schizophrenia without the 22q11.2 deletion, and that the elevated risk in 22q11DS will provide an enhanced effect size to identify the salient genetic factors and systems involved.[14–16]

THE 22Q11.2 DELETION SYNDROME

The 22q11.2 deletion is the most common chromosomal microdeletion (~1:4000 live births; ~1/1000 fetuses) associated with a highly penetrant genomic syndrome.[13] Over 90% of affected individuals have a ~3 million base pair (Mb) hemizygous deletion encompassing 46 protein-coding and 44 additional genes (Figure 1).[13,17,18] Some have smaller nested deletions within the interval.[13] The deletion typically occurs as a *de novo* event arising by non-allelic homologous recombination between chromosomes during meiosis, mediated by chromosome-specific low-copy repeats (LCRs) across the chromosome 22q11.2 region.[19–21]

The phenotypic presentation is heterogeneous, often involving multiple systems including cardiac, palatal, endocrine, immune, gastrointestinal, skeletal and, most commonly, neuropsychiatric abnormalities.[13] Brain dysfunction may be expressed as developmental delay and/or elevated prevalence of developmental neuropsychiatric disorders including attention deficit hyperactivity, autism spectrum, anxiety and psychotic disorders.[22–26] The ~ 25-fold increased risk for psychotic illness in 22q11DS is far greater than the estimated 3-fold increased risk of psychotic illness associated with general developmental delay.[6,27] Therefore, 22q11DS provides a unique opportunity to investigate mechanisms underlying the evolution of schizophrenia and schizophrenia spectrum features across the lifespan. The existence of mouse models of individual or multiple genes within the region of synteny to the human 22q11.2 region also lays the foundation for a broad array of translational research to understand molecular mechanisms.[17,18,28–32]

The fact that the genetic risk is identifiable prenatally and postnatally,[13] coupled with comparable age at onset, symptom pattern and early clinical signs, including neurocognitive deficits, all similar to idiopathic disease, support 22q11DS as a particularly promising genetic model for schizophrenia.[14]

SCHIZOPHRENIA SPECTRUM AND BRAIN BEHAVIOR PARAMETERS IN 22Q11DS

As in the general population, schizophrenia in 22q11DS commonly emerges in late adolescence to early adulthood and is characterized by positive symptoms (hallucinations, delusions), negative symptoms (amotivation, asociality) and disorganized behavior.[33–35] Within the context of the syndrome, response to antipsychotic medications appears similar to that in schizophrenia in the general population, considering the context of a multi-system disorder.[36,37]

Youths at risk for psychosis in the general population have been studied in help-seekers[38,39] and community samples.[40,41] Neurocognitive measures and neuroimaging parameters show abnormalities in at-risk individuals, suggesting the presence of neurobiological aberrations early in the psychosis process.[42–44] With a known high-risk for schizophrenia, 22q11DS

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provides the opportunity to systematically investigate early abnormalities in development as well as the emergence of psychotic illness in cross-sectional and prospective studies of individuals who share the same genetic abnormality.[45–47] Investigations applying standard procedures to assess subthreshold psychotic symptoms in 22q11DS have reported their presence across samples.[48–50] Integration of phenotypic parameters with genomics may generate mechanistic insights that lead to improved clinical diagnosis and offer new therapies.

Neurocognitive dysfunction is a central feature of schizophrenia[51] with intellectual decline evident years prior to the emergence of psychotic symptoms.[52,53] Similarly, in 22q11DS there is a steeper decline in verbal IQ associated with the emergence of psychotic disorders.[46,54] Divergence of intellectual trajectories between those who subsequently develop a psychotic disorder and those who do not is distinguishable from age 11 years onward.[54]

In schizophrenia, there are well-documented deficits in specific domains including verbal memory, executive functions and social cognition.[51,55] Comparable patterns of impairments evident in clinical high-risk populations,[56,57] and family members of patients,[58,59] implicate these as potential endophenotypes. Similar impairments in executive function, social cognition, non-verbal memory, working memory and visual-spatial function in 22q11DS,[24,45–47,60–62] suggest possible sharing of underlying neural networks.

Historically, studies of brain and behavior in 22q11DS have involved relatively small samples, with convergent findings emerging. Recognizing that multicenter samples are necessary to generate large data sets, enabling integration of genomic and phenotypic data, investigators with complementary expertise who had established an international collaborative effort to study 22q11DS came together to form the IBBC.

THE INTERNATIONAL 22Q11.2 BRAIN BEHAVIOR CONSORTIUM OBJECTIVES

The IBBC established several aims to harmonize existing cohorts of 22q11DS participants with both phenotypic data and DNA available to perform next-generation sequencing and other genome-wide genomic analyses: (1) to examine the neuropsychiatric and neurocognitive-behavioral phenotypes associated with schizophrenia spectrum disorders in 22q11DS; (2) to generate and analyze genome-wide data for >300 adults with 22q11DS, about half with schizophrenia and half, aged 25 years and older with no psychotic illness, and use the same strategy for at-risk youths with 22q11DS; (3) to develop and pilot commonly used measures to optimize assessment of neuropsychiatric and cognitive-behavioral phenotypes in 22q11DS as a platform for future prospective longitudinal studies; (4) to build coordinated resources in the public domain for the international scientific community. The genetic analyses involve genome-wide data from WGS and high-resolution microarrays, with the goal of identifying genetic pathways that may influence the expression of schizophrenia and related phenotypes.

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ORGANIZATIONAL STRUCTURE

The IBBC includes multiple international phenotyping sites, contributing DNA samples and phenotypic data, and genomic sites (Table 1; See author list and Supplementary Material for other IBBC members). The phenotyping working group provides neuropsychiatric and neurobehavioral expertise and the genomic working group the genetic analytic approach. An executive committee is comprised of 13 representatives from the working groups, including 9 members who lead the four phenotyping groups. The executive committee communicates regularly by conference calls and in-person meetings to implement research plans, communicate with NIMH, disseminate information to collaborative sites, arrange general meetings, oversee the development and quality of the database and the website and review proposals for ancillary studies and publications.

SAMPLE AND ELIGIBILITY CRITERIA

For inclusion in the study, the IBBC took advantage of available cohorts of well-characterized individuals with 22q11DS where DNA samples exist. Initial steps in the IBBC study involve extensive quality control. These include reviewing the phenotypic data, verifying the presence of a typical 22q11.2 deletion (Figure 1) and determining relatedness between and ancestry of participants with high-quality DNA samples. Table 2 details the sample characteristics.

PHENOTYPIC PROCEDURES

Psychiatric assessment

Harmonization of existing clinical psychiatric data for the IBBC has involved two main phases. To enable dichotomous ‘case-control’ categorization, the presence of schizophrenia (or related psychotic illness), or the absence of any psychotic illness in subjects assessed at age 25 years or older, is established for all subjects. Complementary methods enable the study of at-risk youths with 22q11DS.

Schizophrenia spectrum disorders—Across sites, a semi-structured diagnostic interview,[63,64] with collateral information and medical records, provided details on clinical presentation and longitudinal history for establishing a DSM-IV diagnosis of schizophrenia, schizoaffective disorder, or related psychotic disorders such as delusional disorder or psychotic disorder not otherwise specified. After extensive discussion, the few cases with a psychotic mood disorder were excluded from initial analyses. For phenotypic harmonization, two investigators with clinical expertise independently reviewed standardized clinical summaries (average 2–3 subjects/site). There was full consensus among the two reviewers and the diagnosis provided by the sites for case-control classification. Individuals of any age who met diagnostic criteria for a major psychotic disorder, predominantly schizophrenia, were included as ‘cases’.

Non-psychotic adults—Participants ≥25 years-old at the time of assessment, with no evidence for a psychotic disorder or psychotic mood disorder (that is, bipolar disorder or major depression with psychosis), were classified as ‘non-psychotic,’ as they were likely to

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be through the major risk period for developing psychosis. Consequently, individuals <25 years at the time of the last assessment without a psychotic disorder nor subthreshold psychotic symptoms are labeled as 'putative controls.' This categorization does not exclude the possibility of other neuropsychiatric conditions that are common in 22q11DS (for example, anxiety, attention deficit hyperactivity).[22]

Subthreshold psychosis youths—The majority of existing participants in the IBBC cohort are young, thus not yet through the risk period for developing schizophrenia when assessed (Table 2). Subthreshold psychosis is systematically assessed by applying established diagnostic interviews and scales.[65–67] Investigators with expertise in child and adolescent psychiatry reached a best estimate 'subthreshold psychosis' designation based on review of a standard clinical summary provided across sites (on average 3 subjects/site). Subthreshold psychosis is applied when positive psychotic symptoms are persistently reported, but severity and impact on function do not justify a formal diagnosis of a psychotic disorder. Most sites provided quantification of subthreshold symptom severity, based on structured interview data (Structured Interview for Prodromal Syndromes;[66] or Comprehensive Assessment of At-Risk Mental States[67]). Because many youths who have not yet passed through the risk period for the development of psychotic illness have been followed longitudinally at IBBC sites, data available on quantitative cognitive phenotypes relevant to schizophrenia risk, including decline in verbal abilities in individuals,[54] will also be used for genomic analyses.

Cognitive assessments

Standardized IQ scores, available for many subjects across sites, provide measures of general cognitive ability, verbal abilities, non-verbal abilities and selected executive functions. Using both cross-sectionally and longitudinally obtained standardized IQ scores allowed definition of an average cognitive trajectory for 22q11DS individuals, on which individual trajectories can be plotted to identify deviations from what is expected in this population. This approach has enabled generation of a quantitative measure of IQ decline as a phenotype of interest for genetic analyses.[46,54] To enhance commonality across other neurocognitive variables, several considerations were applied: representation across at least five IBBC sites, and comparability of neurocognitive test versions, administration procedures and scoring. This will allow the application of standard procedures to create composite measures for the domains of executive function, social cognition and verbal learning. These domains were initially prioritized given their relevance to schizophrenia vulnerability.[51,56–59] Also, the majority of sites used comparable measures within these domains.

GENOMIC APPROACH

The IBBC genomic approach to identify variants that may contribute to expression of schizophrenia and related conditions in the 22q11DS population is outlined in Figure 2. The approach envisions testing four genetic models. One model is that variants on the haploid (single copy) 22q11.2 deletion region segment are enriched in those with psychotic illness and related conditions. The other three models will test for enrichment of genome-wide

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variants outside this 22q11.2 region: in common sequence variants (that is, single-nucleotide polymorphisms (SNPs), in a traditional genome-wide association study (GWAS) paradigm), in rare sequence variants and in rare structural variants.

To test these models the IBBC plans to use both existing and new genotypic data. Indeed, a major focus of the IBBC is to generate WGS data for this valuable cohort. In comparison to whole exome sequencing, WGS permits more complete analysis of sequence and structural variation including coding and non-coding regions of the genome, while not suffering from capture and coverage biases.[67] The rapid drop in price increased feasibility, and thus the bulk of the grant funding has supported the generation of WGS data for >80% of subjects in the IBBC 22q11DS cohort (estimated $n = 1576$, before quality control measures, Figure 2). DNA samples were submitted for WGS (average depth 30 ×) using two platforms: for the first ~100 samples the Illumina HiSeq 2000,[68] and thereafter the Illumina HiSeq X Ten. Initially, participants with schizophrenia ('cases'), or with no psychotic disorder and age ≥25 years ('controls'), were prioritized.

Mapping and variant calling of single-nucleotide variants (SNVs; including SNPs) includes use of novel software tools (PEMapper/PECaller) developed at Emory.[69] Cleaned and annotated (reference genome assembly GRCh38) sequence data are placed into variant call format and annotated using ANNOVAR,[70] enabling standard analytic approaches to be applied, including variant classes (that is, exonic, intronic), rarity and functional effects.[71–73] Annotation of SNVs from coding regions uses standard pipelines and bioinformatic filters for functional impact.[15,74–78] Similarly, variants from non-coding regions are annotated using the most up-to-date methods available in this evolving area of genomic study.[15,74]

WGS data allow detection of small structural variants beyond the resolution of microarray data. Calling of structural variants across the size spectrum from WGS data requires a combination of complementary methods. The analytic strategy for the IBBC includes interrogating read depth data, and discordant pair/split read data needed to call both balanced (inversions and translocations) and unbalanced (CNV) variants.[79–82] CNV calls will provide a comprehensive genome-wide structural map for each subject, ready for further computational analyses.

Complementing the WGS approach, the study takes advantage of existing data from Affymetrix 6.0 microarrays (Figure 2) available for many participating 22q11DS subjects from previous studies of cardiac phenotypes,[83,84] with comparable array data generated for remaining subjects. Arrays provide both SNP data for use in GWAS analyses and calculation of schizophrenia polygenic scores,[85] and for genome-wide studies of structural variants (CNV) in addition to the 22q11.2 deletion.[86,87] These common sequence and rare and common structural variant data also serve an important role in quality control, for example, for comparison with variants identified by WGS.

The potential power of the proposed IBBC genomic approach and the enhanced effect sizes in 22q11DS are supported by the results to date reported in the Early Findings section below for additional rare structural variants.[87] For genome-wide rare sequence variants, published power calculations in a proof-of-principle study using a WGS method to assess patients with

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22q11DS with and without schizophrenia are available for gene-set burden tests.[15] For n = 100 subjects per group, power for plausible functional gene-sets was >0.90 for various types of coding sequence variants, with Cohen's d effect size estimates of, for example, 1.90, 0.88 and 0.55, based on the nine genomes investigated.[15] With respect to aggregate common variants, there were also promising though non-significant results reported for the schizophrenia polygenic risk score in this study.[15] Estimates for detecting individual common variants with relative risk of two to three using a typical GWAS genome-wide significance threshold (5×10^{-8}) show power >0.80 in the IBBC cohort (sample sizes as in Table 2) but, as expected, very low power for individual rare variants.

Variants in the 22q11.2 deletion region that increase likelihood of expressing schizophrenia and related phenotypes

Individuals with 22q11DS have just a single copy of the genes within the 22q11.2 deletion region, a region of the genome well known for its complexity, partly due to the multiple LCR22s flanking and within this region (Figure 1).[19–21] Comprehensive analysis of genotype-phenotype associations in 22q11DS mandates special expertise and consideration of this region. The IBBC study thus involves initial analysis of the haploid allele on the intact chromosome 22 separately from WGS data from the rest of the genome (Figure 2).[88,89] To call hemizygous SNVs, PEcaller is rerun for this region in a haploid mode. 22q11.2 deletion region variants are then annotated and analyzed as for variants from other regions, but with special consideration of the hemizygosity and its potential effects on phenotype.[13] Detailed annotation and investigation of the complex architecture of the LCR22s,[89–91] and analysis of breakpoints and corresponding 22q11.2 deletion extent, including for rare nested 22q11.2 deletions, provide further unique data. These will allow regional and haplotype-based analyses of the deleted or non-deleted allele, effects related to coding and non-coding sequence variants within and flanking the 22q11.2 deletion region, and the potential for identifying hotspots for meiotic chromosomal rearrangements. Collectively, these data will enable the development of a morbid/benign variation map of the entire 22q11.2 region for testing with phenotypic expression.[88]

Genome-wide variants that increase likelihood of expressing schizophrenia and related phenotypes

Planned analyses of SNV data include logistic-regression modeling to compare allele frequencies between the 22q11DS schizophrenia and no psychotic illness groups, accounting for covariates such as sex, ethnicity, genotyping platform and read length. Statistical significance is established via permutation. These sequence-based variant analyses will proceed in a logical fashion, using standard genome-wide approaches. These will include GWAS for common variants of >5% minor allele frequency, gene-based analyses for other SNPs (1–5% minor allele frequency) and modified Sequence Kernel Association Test or other burden tests for rare SNVs with <1% minor allele frequency.[91] Rare coding and non-coding variants will be grouped by type (for example, missense, lincRNAs) and prioritized using factors such as functional scores (for example, CADD) and conservation.[15,76] Inspection of expected variant frequencies using public databases (for example, ExAC, gnomAD) will be important.[75] Both individual variants and groups of variants at the gene or pathway (for example, functional gene-set) level will be tested, using such aggregate-type

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tests as polygenic risk scores (for common variants) and burden tests (for rare variants).[15,92] Notably, a test like Sequence Kernel Association Test can detect genes or pathways that contain causal variants that act in different directions on phenotypes (for example, some variants may increase risk, while others decrease risk). Also, as noted above, there is some published evidence for the utility of functional gene-set based burden analyses for rare functional variants and for polygenic risk scores in 22q11DS.[15,87] For the rapidly evolving area of analyzing non-coding variants, including intragenic enhancers distal to coding sequence, the most up-to-date resources and functional-based variant scoring methods available will be used, appropriate to common or rare variant analyses.[15,71]

To test the generalizability of individual and aggregate rare and common, coding and non-coding, top hits will be evaluated in available general population schizophrenia samples.[85] Other resources will also be used to prioritize the schizophrenia-related variants identified in 22q11DS, including in silico analyses of potential function (including regulatory function) using animal model data and human tissue expression data.[16] To adjust for multiple testing while avoiding overcorrection in analyses that involve inherently correlated data (for example, testing two pathways that may have genetic overlap), various methods may be applied, for example, using permutation or other standard methods (for example, Benjamini–Hochberg false discovery rate).[15,74] To appreciate the overall genomic architecture of schizophrenia in 22q11DS will require eventual integration of all genomic variant findings with the phenotypic data, and other downstream analyses to investigate potential disease mechanism and function[16] using actual animal models, tissue expression profiles and spatiotemporal expression profiles during brain development (Figure 2).

Alternative phenotype approach

Initial analyses prioritize comparisons between participants with 22q11DS and schizophrenia and those with no psychotic disorder over age 25 years (Table 2).[87] Other analyses involving data from youths where numbers are far greater (Table 2) will test whether various definitions of subthreshold psychosis and related phenotypes, including IQ decline,[54] produce similar genetic findings. Thus, all of the analyses described above will also be performed using alternative schizophrenia-related phenotypes. Notably, the vast majority of the 22q11DS sample with WGS results from this cross-sectional IBBC study comprises youths with their years of greatest risk for psychotic illness ahead of them.

EARLY FINDINGS

The IBBC has provided an unprecedented large sample, establishing a database for 22q11DS with common phenotypes that enable data integration. Initial published findings illustrate the promise of this consortium. In the largest study of psychiatric disorders in 22q11DS (n = 1,402; ages 6–68 years) using validated diagnostic instruments, psychotic disorders were present in 41% of adults over age 25 years. Autism spectrum disorders and attention deficit hyperactivity in children and adolescents, and anxiety disorders across all age ranges, were also prevalent with sex differences in attention deficit hyperactivity and anxiety disorders similar to those reported in the general population.[22]

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To evaluate the relationship between psychosis and cognitive functioning, clinical assessment and IQ measures were examined longitudinally in 411 participants with 22q11DS.[54] Across the sample, mild decline in IQ, especially in the verbal domain, was noted with increasing age.[53] However, in youth who developed psychotic illness, this decline was significantly steeper, similar to observations in schizophrenia in the general population.[94]

In another recent Consortium-based paper, individuals with 22q11DS (n = 692) were assessed for subthreshold psychotic symptoms. Nearly one-third of the participants met criteria for positive subthreshold psychotic symptoms and almost a quarter met criteria for negative/disorganized subthreshold symptoms. Adolescents (aged 13–17 years) showed the highest rates of subthreshold psychotic symptoms. Cognitive deficits were associated with subthreshold psychosis.[95]

To investigate the role of additional rare genome-wide CNVs in expression of schizophrenia in 22q11DS,[87] high-quality genome-wide CNV from the available microarrays were annotated using stringent methods and adjudicated for rarity using independent control microarray data as in previous studies.[12,86,96–101] Compared to participants aged 25 years and older with no psychotic illness, the schizophrenia group was significantly enriched for genome-wide rare CNVs that implicated known and novel schizophrenia risk genes and loci.[87] Evidence of interactions at a network level of these genes with 22q11.2 deletion region genes is consistent with the threshold-lowering effect of the deletion, highlighting the importance of an integrated genome-wide approach.[92]

A novel data mining strategy that integrates biological information from gene association, gene network and disease/trait phenotypes has been developed to help prioritize potential schizophrenia risk genes and networks.[93] This method is to be applied in the IBBC WGS analyses. Other upcoming IBBC publications include findings on novel structural polymorphisms that predispose to chromosome 22q11.2 rearrangements, and analysis of the extent to which schizophrenia polygenic risk score predicts outcome in 22q11DS.

LIMITATIONS AND FUTURE DIRECTIONS

The IBBC has capitalized on existing global collaborations in an effort to assemble the largest sample available of a highly informative neurogenetic syndrome. The consortium includes retrospective data. While carefully assessed, the IBBC data do not have the depth and scope that prospective studies can attain. Importantly, prospective studies can use the same standardized validated and efficient measures for deep phenotyping. Such future studies could use novel tools, such as computerized assessment, which can be administered in multisite large-scale studies across a broad age range, and have been validated in 22q11DS. To investigate the possible contribution of environmental non-genetic factors to schizophrenia will require recon-tacting subjects or collection of new data. Prospective longitudinal studies would help address these limitations and enhance the contributions to the field. Although the current work of the IBBC is focused on schizophrenia, the data set and findings might also be of interest to researchers working in other areas, such as anxiety

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in neurodevelopmental disorders and cognitive development in young people with intellectual disabilities.

The genomic efforts were also built on the expertise of established investigators and the promise of WGS to help delineate the genomic architecture of human disease. Nevertheless, other molecular approaches may well be needed to fully understand the changes wrought by a 22q11.2 deletion, including risk for schizophrenia and the variable expression of 22q11DS in general. The potential roles of transcriptional regulation, post-translational modification and other yet to be identified mechanisms will be important to pursue with emerging technologies. These investigations are likely to need expanded sample sizes. This would also be the case for the current study which is underpowered, despite the large sample amassed, for lower effect size individual variants or variant groups, and for analyses of interaction effects.

With advances in technology and reduction in costs, future studies can also consider informative replication samples and the best methods to address hypotheses generated in the field, particularly those that focus on relevant genotype-phenotype associations. Novel strategies to identify potential protective factors, and develop possible preventive and therapeutic interventions, are also promising future directions.

CONCLUSION

The IBBC has demonstrated the feasibility and utility of large-scale collaborations to examine an informative rare CNV that can contribute to advancing the mechanistic understanding of schizophrenia spectrum disorders. The rich phenotypic data set amassed for individuals with 22q11DS and schizophrenia spectrum disorders, compared to those without psychotic features, as well as at-risk youth is unprecedented and buttresses the generalizability to schizophrenia and clinical risk for the disorder in the general population. Integration with the broad genomic approach, combining whole-genome sequencing, genome-wide microarray technologies and novel emerging technologies can contribute to advancing the pathogenesis of schizophrenia and ultimately to targeted preventive and therapeutic efforts. Likely outcomes include identifying genetic modifiers from both the intact 22q11.2 region alleles and genome-wide[87] that will not only address the key goal of delineating schizophrenia vulnerability but could also inform other important aspects of expression in this syndrome and beyond. The implications for other molecular models, for understanding development and for neuropsychiatric research in general are potentially profound.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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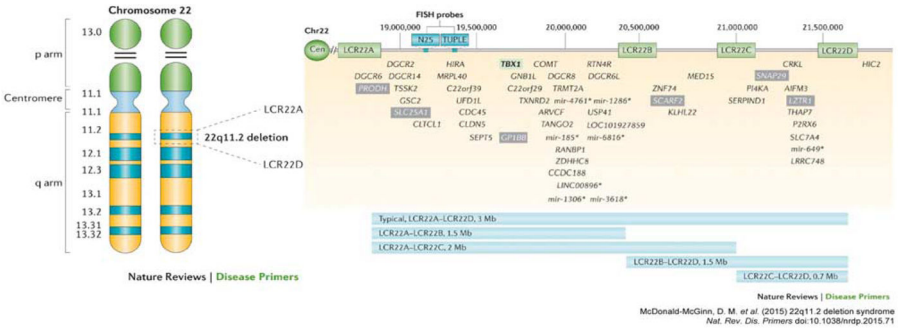

Figure 1.

The 22q11.2 region. Cytogenetic representation of chromosome 22 showing the short (p) and long (q) arms with the centromere, which functions to separate both arms. Chromosome 22 is an acrocentric chromosome, as indicated by the two horizontal lines in the p arm. The 22q11.2 deletion occurs on the long arm of one of the two chromosomes, depicted by dashed lines in the 22q11.2 band. The position of the two low-copy repeats (LCRs) on 22q11.2 (LCR22A and LCR22D), which flank the typical 3-Mb deletion, LCR22B and LCR22C and genes (protein-coding and selected non-protein-coding) within the 22q11.2 deletion region, as well as the three typical 22q11.2 deletions that include LCR22A and two nested 22q11.2 deletions that do not include LCR22A (all indicated by blue bars below), are depicted in the inset.[13]

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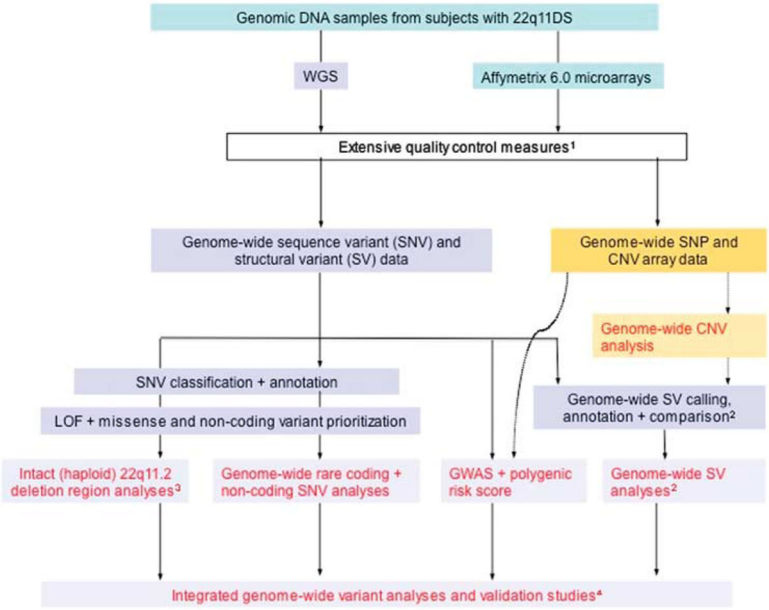

Figure 2.

Genomics flow diagram. The figure provides an overview of the ongoing and planned genomics analyses in the IBBC study, beginning with genomic DNA samples available from subjects with 22q11DS from participating international centers. Samples were sent to one laboratory (Albert Einstein College of Medicine, NY) that serves as a central clearinghouse, and provides a unique IBBC identification number to enable linking of genotypic and phenotypic data. Data primarily from studies that pre-dated the IBBC are indicated on a cyan background. The mauve and yellow backgrounds indicate data generated from the IBBC study from WGS and microarray data, respectively. Dotted lines indicate the work flow for microarray data. These provided data for initial analyses, and available for comparison with WGS data, albeit at lower resolution. Red font indicates analyses using the phenotypic data available for subjects with 22q11DS from participating international centers. Main analyses involve comparisons of subjects with 22q11DS and schizophrenia vs those with no psychotic illness at age 25 years and older. See text for details. [1] Quality control measures applied to both WGS and microarray data include checking for duplicate and related samples, for sex and ethnicity using phenotypic data to help detect sample mix-ups, and for 22q11.2 deletion size (assisted by available multiplex ligation dependent probe amplification (MLPA) data and heat-map data generated from microarrays). Additional quality control measures, following transfer of raw WGS data (fastq files) generated at HudsonAlpha Genome Sequencing Center (Huntsville, AL) to the Human Genetics Computational Cluster (Emory U), include checking for mixing of samples, level of genetic variation, base transition to transversion ratios, too many variants in particular regions, and variants that departed from Hardy–Weinberg equilibrium at $P<10^{-4}$. [2]The intact haploid 22q11.2 allele requires special computational considerations, thus is analyzed separately from data from the rest of the genome.[82,833]Annotation of structural variation from WGS data is a pioneering area of genomics. Availability of CNV data from standard microarrays in this study will be valuable for comparison purposes.[74–774]Integrated analysis using all variant data, common and rare, sequence and structural, will provide an overview of the

genomic architecture of schizophrenia in 22q11DS. Validation studies, for example, polymerase chain reaction for SNV, quantitative polymerase chain reaction for SV, will proceed according to results. CNV, copy number variation; LOF, loss of function; SNP, single-nucleotide polymorphisms (common sequence variants); SNVs, single-nucleotide variants; SV, structural variants; WGS, whole-genome sequencing.

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Table 1

IBBC sites, roles and samples

IBBC site (by Group)	Samples submitted per site
Toronto[a,b]	155
Australia	28
Santiago	73
Total by group CAN[c]	256
Leuven[a,b]	125
Geneva	119
Maastricht	95
Marseilles	25
Tel Aviv	103
Utrecht[a]	151
Total by Group EUA	618
Cardiff[a]	122
Dublin	64
London	22
Madrid	34
Mallorca	27
Rome	89
Total by Group EUB	358
Albert Einstein NY[c]	NA
CHOP-Penn[a,b]	335
Duke	70
Emory[a,b]	29
SUNY	101
UCLA	81
UC Davis	69
Total by Group USA	685
Total samples submitted[d]	**1,917**

Abbreviations: CAN, Canada; EUA, Europe group A; EUB, Europe group B; IBBC, International 22q11.2 Brain Behavior Consortium; NA, not applicable; NY, New York; SUNY, State University of New York; UCLA, University of California Los Angeles.

[a]Indicates group organizing site.

[b]Indicates both phenomic and genomic site.

[c]Indicates genomic only site.

[d]Total samples submitted will be greater than the number of those with usable genomic and phenotypic data, after extensive data cleaning.

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Table 2

Demographic and genotypic characteristics of the 22q11DS IBBC sample with typical 22q11.2 deletions (c 15 February 2017)[a]

	Main diagnostic groups (*n* = 582)							
	Psychotic illness[b] (*n* = 292)		No psychotic illness at age ≥25 y (*n* = 290)		At risk group (*n* = 1034)		Total (*n* = 1616)	
	n	%	n	%	n	%	n	%
Females	143	49.0	177	61.0	513	49.6	833	51.6
Youths (< age 21 y)	97	33.2	–	–	915	88.5	1012	62.6
European ethnicity	241	86.7	258	91.5	809	78.2	1308	80.9
A to D 22q11.2 deletion	268	91.8	263	90.7	953	92.2	1484	91.8
WGS data available	271	92.8	275	94.8	918	88.8	1464	90.6
	Mean	*s.d.*	*Mean*	*s.d.*	*Mean*	*s.d.*	*Mean*	*s.d.*
Mean age	29.3	13.0	35.6	9.8	14.4	4.5	20.9	11.8
Age at onset	22.3	8.9	–	–	–	–	–	–

[a]All numbers are best estimates for subjects with typical 22q11.2 deletions, subject to data checking and cleaning; individuals (total *n* = 301 of 1917) without available 22q11.2 deletion data as yet, and a small number (<20) with atypical 22q11.2 deletions, are not included (see text for details).

[b]The psychotic illness designation comprises schizophrenia (66.1%), schizoaffective or psychotic disorder not otherwise specified (25.3%), or other psychotic spectrum disorders (8.6%), but excludes a small number of subjects with psychotic mood disorders.