

# Neurodevelopmental copy-number variants: A roadmap to improving outcomes by uniting patient advocates, researchers, and clinicians for collective impact

Commission on Novel Technologies for Neurodevelopmental Copy Number Variants

## Summary

Copy-number variants and structural variants (CNVs/SVs) drive many neurodevelopmental-related disorders. While many neurodevelopmental-related CNVs/SVs give rise to complex phenotypes, the overlap in phenotypic presentation between independent CNVs can be extensive and provides a motivation for shared approaches. This confluence at the level of clinical phenotype implies convergence in at least some aspects of the underlying genomic mechanisms. With this perspective, our Commission on Novel Technologies for Neurodevelopmental CNVs asserts that the time has arrived to approach neurodevelopmental-related CNVs/SVs as a class of disorders that can be identified, investigated, and treated on the basis of shared mechanisms and/or pathways (e.g., molecular, neurological, or developmental). To identify common etiologic mechanisms among uncommon neurodevelopmental-related disorders and to potentially identify common therapies, it is paramount for teams of scientists, clinicians, and patients to unite their efforts. We bring forward novel, collaborative, and integrative strategies to translational CNV/SV research that engages diverse stakeholders to help expedite therapeutic outcomes. We articulate a clear vision for piloted roadmap strategies to reduce patient/caregiver burden and redundancies, increase efficiency, avoid siloed data, and accelerate translational discovery across CNV/SV-based syndromes.

## Introduction

Genomic disorders represent a significant public health burden. Copy-number variants and structural variants (CNVs/SVs) comprise moderate-to-large-sized chromosomal variants that include deletions, multiplications, insertions, inversions, translocations, mosaicisms, and ring chromosomes. Pathogenic CNVs/SVs often cause a spectrum of complex phenotypes in neurodevelopmental disorders, including intellectual disability (ID), developmental delay (DD), and autism spectrum disorder (ASD), and epilepsy and other neurological phenotypes, congenital anomalies, and psychiatric disorders.<sup>1,2</sup> For example, up to 35% of congenital disabilities are attributable to the combined effect of chromosomal imbalance (25%) and CNVs (10%).<sup>3</sup>

In the past, the complexity of CNVs/SVs was seen as an insurmountable barrier to effective therapies and this class of disorders was largely ignored by translational researchers and primary funding agencies. However, improvements in technology have accelerated the discovery of disease-causing CNVs/SVs, and now chromosome disorders are recognized as the most common diagnosis among neurodevelopmental-related disorders.<sup>1</sup> Additionally, innovations in therapeutic approaches such as small molecules, antisense oligonucleotide (ASO), and gene therapy, coupled with pathway discoveries and modification techniques, show promise. These diagnostic and therapeutic advancements have incentivized patient communities to organize, fund, and participate in translational research. However, CNV/SV research is costly, and the tactical

funding provided by patient communities should not substitute for strategic funding by federal agencies (e.g., National Institutes of Health [NIH]).

In this perspective, we assert that the time has arrived to rethink traditional research approaches, dismantle old silos, and highlight neurodevelopmental-related CNVs/SVs as a class of disorders that can be identified and investigated with novel approaches where patient communities are integral partners. To that end, we introduce the patient-led "Commission on Novel Technologies for Neurodevelopmental CNVs" to convene relevant stakeholders and facilitate funding. We propose a collaborative and integrated approach that (1) engages patients and their families alongside scientists and clinicians; (2) provides economies of scale for research expenditures; and (3) leverages recent advances and creates new approaches, including "omic," stem-cell technologies and novel therapy development. There may be no single unifying approach to address CNV/SV neurobiology, but here we describe specific demonstrable strategies to increase efficiency and reproducibility while accelerating translational discovery across CNV/SV-based syndromes.

## The CNV Commission

The Commission on Novel Technologies for Neurodevelopmental CNVs (the "CNV Commission," <https://www.cnvcommission.org/>) was established with funding from the Chan-Zuckerberg Initiative "Rare As One" Project. Our mission is to establish a patient-led effort to rapidly

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and collectively tackle scientific and financial resources to prioritize treatment for those living with neurodevelopmental chromosome disorders. Our vision is to advance the treatment of neurodevelopmental disorders by focusing on copy-number variants and structural variants through team science and open data sharing.

The CNV Commission was initiated by patient communities, which quickly engaged a diverse group of stakeholders, including patient advocates, basic scientists, clinicians, and biopharma leaders, to work collaboratively on CNV/SV disorders. Commission members were incentivized not by initial funding, which was limited and utilized as described below, but by patient communities uniting people of a similar mindset to identify gaps in scientific initiatives and plot a course into a largely novel landscape. Patient advocates partnered with researchers and clinicians to establish goals and structure and to create a culture of open dialogue with the plight of the patients and their families front and center.

More specifically, the patient-led CNV Commission convened a series of working group meetings in 2020–2021 consisting of key patient advocates, researchers, clinicians, program managers, and directors from NIH centers including the National Center for Advancing Translational Sciences. The members were selected on the basis of interests and expertise in the following: (1) *in vitro* modeling, (2) animal models, (3) phenotyping, (4) bioinformatics/genomics, and (5) patient/community engagement. The CNV Commission formalized guiding principles with a team-science structure reflected in a charter (see [supplemental information](#)). Benefits of this team-science approach include (1) the creation of a collaborative research environment supported by robust and standardized data collection infrastructure that is scalable; (2) the opportunity to attract additional resources in support of proposed projects; (3) access to larger samples and datasets; (4) better analytical tools; and (5) synergies emerging from complementary skill sets. The charter reflected equity and inclusion of young investigators in authorship and created a defined collaborative focused on CNVs/SVs. In our meetings and various forums, we shared our successes and failures, discussed emerging science, identified ideas with the most potential impact, and collectively produced a roadmap to advance effective therapies associated with CNVs/SVs. Finally, we acknowledged a shared responsibility to attract the required funding and resources to achieve the consensus goals.

The CNV Commission recognizes that including patients and families from minority populations, most of whom have been historically excluded or marginalized in research, is critical for building trust in the community and promoting equitable access to the testing, treatments, and general benefits arising from research. Our inclusive model aims to dissolve barriers between researchers and affected individuals to help build a more equitable and patient-focused research environment where the goals of researchers and stakeholders are aligned. The CNV Commission is dedicated to emphasizing patients as partners so

that everyone better understands the daily impact of these disorders and treatments on quality of life for the affected individuals and their families.

This paper is divided into two sections. Part 1 details the building blocks needed to collaboratively address CNV/SV research by integrating all stakeholders and creating a shared, scalable infrastructure ([Figure 1A](#)). The aim of part 1 is to simplify the real-life logistics of data and bio-sample collection, management, and accessibility to decrease this stakeholder burden to empower research progress. Part 2 details the current innovative approaches and model systems that are emerging or needed. Taken together, part 1 and part 2 define our research roadmap ([Figure 1B](#)), which targets potential therapeutic approaches for neurogenetic CNVs/SVs by starting with a small number of representative disorders with the idea that scaling to comparable conditions will be possible in the future. The framework, infrastructure, and ideas presented here arose out of the CNV Commission convenings; data collection was initiated at the Moving Mountains Conference, a joint family conference between Project 8p, Dup15q Alliance, and Ring14 USA in July 2021.

## Part 1: Creation of building blocks to accelerate CNV/SV studies

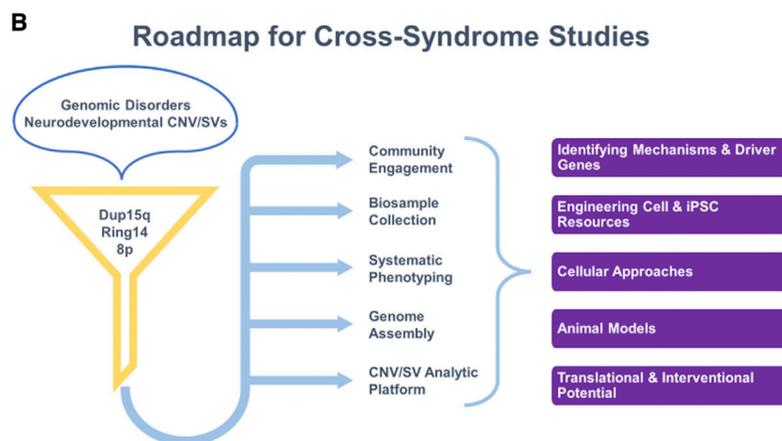
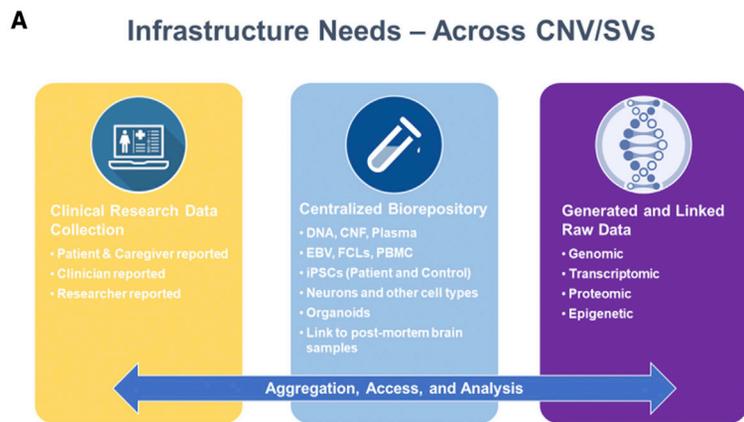
An effective roadmap that integrates researchers, advocates, and clinicians requires the following building blocks:

- community engagement and patient recruitment;
- biosample collection and establishment of cell lines;
- systematic phenotyping;
- patient-based genotyping and genome sequencing; and
- CNV/SV analytic platform for aggregation, access, and analysis.

A guiding principle of the Commission's roadmap is to develop platform technologies with the same protocols for collecting resources that are widely inclusive, broadly applicable, and agnostic to genotype and to address the need for logistical centralization of data and biospecimens.

### Community engagement and patient recruitment

The CNV Commission selected three pilot conditions that represent disease-associated classes of complex neurodevelopmental-related CNVs/SVs. Each of the three conditions presents with the core neurodevelopmental phenotype, has an engaged patient and family organization, and poses unconventional investigational challenges. These are dup15q (15q11.2-13.1 duplications<sup>4</sup>); 8p (8p deletion, duplication, and inversion/duplication/deletion<sup>5</sup>); and ring 14 (ring chromosome 14<sup>6</sup>). We focused on these three conditions, but other CNVs/SVs organizations were also engaged in roadmapping, including 3q29 deletion,<sup>7</sup> chromosome 18 conditions,<sup>8</sup> and ring chromosome 20.



We recruited participants through patient/family support/advocacy groups. The CNV Commission wanted to identify affected individuals but also to (1) promote dialog between families and researchers that lead to shared research priorities; (2) inform families about research, associated terminology, and how patient and family needs can be addressed in research; and (3) disseminate relevant information to individual families and to the broader CNV/SV communities. We recognized there is a critical need to increase representation from ethnically and socioeconomically underserved communities and to explore social determinants of health that shape the phenotypic diversity and quality-of-life outcomes. Thus, we created and implemented logistics that support data collection in flexible formats to meet and recruit participants from diverse communities. This includes remote/online data collection, data collection at patient community events, point of clinical care, and remote biosample collection (in-home/local collection). These methods endeavor to include a diverse population in a biobank of biological samples that are paired with clinical data to ensure that advances in translational neurogenetics are available to the broadest community. Data and sample sharing preferences were indicated at the time of consent to help expedite collaborative research.

nors that can serve as a comparison group. The collection, processing, banking, and sharing of control lines are often overlooked. However, this cohort of neurotypical lines will serve as a typical comparison cohort to benchmark findings across multiple labs and improve reproducibility.

The CNV Commission prioritizes the use of optimal and consistent reprogramming protocols done at the same facility with the same level of quality control to minimize variation and promote reproducibility. For neurodevelopmental CNVs/SVs, cell lines should be capable of efficient differentiation. While this process is variable across individuals and even clones derived from the same individual,<sup>9</sup> gene expression signatures in induced pluripotent stem cells (iPSCs) can be used to robustly predict the efficiency of neuronal differentiation.<sup>10</sup> Routine monitoring of cell line karyotypes is essential to studying neurodevelopmental-related CNVs/SVs *in vitro*. Thus far, 102 biospecimens have been collected across several CNVs/SVs supported by a comprehensive consent and governance process to enable accessibility of samples, the connection of existing data, and data sharing in a robust and scalable infrastructure.

#### Systematic phenotyping

When the clinical presentation is heterogeneous, biosamples must be accompanied by robust phenotypic

**Figure 1. Infrastructure and roadmap to facilitate cross-syndrome studies**

(A) Shared infrastructure to support aggregation, access, and analysis of shared data through open access.

(B) Research roadmap to translation for neurodevelopmental-related CNVs/SVs starting with three distinct yet similar CNVs/SVs (dup15q, 8p, and ring 14). Abbreviations include EBV (Epstein-Barr virus DNA), FCLs (fibroblast cell lines), and PBMCs (peripheral blood mononuclear cells).

#### Biosample collection and establishment of cell lines

The CNV Commission recommends that biological samples from participants maximize research utility and minimize participant burden (i.e., reduce the need for recollection of samples from participants). The Commission contracted an independent biorepository to adhere to a processing protocol focused on banking plasma and isolated peripheral blood mononuclear cells of probands and family members (parents, siblings). These specimens can be used for DNA or RNA extraction, biomarker identification, and generation of cell lines as a resource for the CNV/SV research community. In addition, we plan to establish and bank cell lines from neurotypical control do-

data. The collection of detailed phenotype data is essential for a better understanding the natural history/care-as-usual of CNV/SV disorders from both patient/family and clinician perspectives. In addition, these data are crucial to (1) define the prognosis, (2) determine the extent of phenotypic overlap among CNVs/SVs, and (3) establish meaningful clinical trial endpoints. To this end, we implemented a scalable centralized collection of phenotypic data by using a data platform (see “Clinical Research Data Platform” below) that will improve our understanding of disease progression and the capture of FDA-defined real-world evidence (RWE) and support the development of disorder-specific surveys and tools, including severity scales for clinical trial staging and outcome measures. The common data collection domains identified by our working groups were cognition and development, social communication, ASD, receptive language, motor, gastrointestinal (GI), epilepsy, and sleep.

It is critical to align research efforts with patient-centered concerns. Key considerations include intervention priorities, developmental and behavioral challenges, disease burden, and informational needs.<sup>11</sup> Such collaborative efforts help build strong partnerships between patients/families and researchers and maximize potential to achieve positive outcomes. We favor a layered approach to phenotypic data collection where a minimal set of standardized evaluations is initially completed and subsequent data collection is contingent upon that minimal dataset. This layered approach helps ensure that longitudinal data are relevant to families while reducing caregiver stress and patient burden. In addition to patient-reported data, we support clinician-reported data at the point of care that can be structured and coded systematically among investigators and institutions.

### **Patient-based genotyping and genome sequencing**

One challenge with neurodevelopmental-related CNVs/SVs is understanding the genetic basis of extreme phenotypic diversity. Considerable variation exists in size and breakpoints of each CNV/SV and the number of affected genes. Differences in precise breakpoints among affected individuals may contribute to neurodevelopmental phenotypes, but they have been challenging to detect, especially when breakpoints are within segmental duplications or other repetitive sequences. Advances in long-read sequencing and optical genome mapping have greatly improved CNV/SV breakpoint determination and the identification of structural variants, including within segmental duplications and simple repeats that might have positional effects on gene expression.<sup>12</sup> These new technologies can also help resolve structural variation classes in these loci, including inversions and complex rearrangements. The improved delineation of CNV/SV breakpoints may provide insight into variable expressivity across these disorders. Unfortunately, the lack of complete human genome reference sequences containing

biomedically relevant SVs has impeded efforts to identify pathogenic CNVs/SVs.

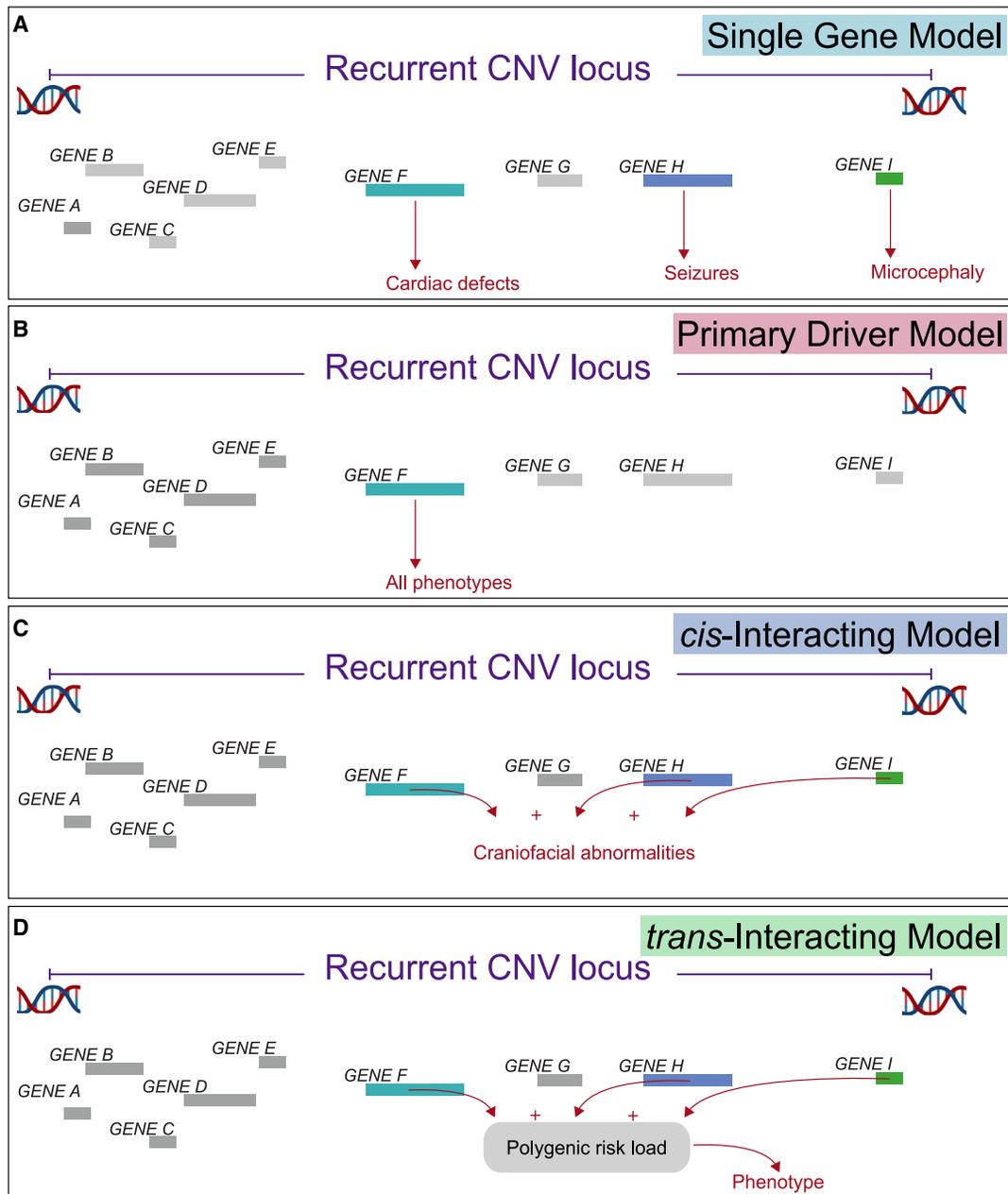
To fill this knowledge gap, the CNV Commission is generating high-quality, haplotype-resolved human sequence reference genomes that contain entirely resolved complex CNVs/SVs associated with neurodevelopmental-related disorders. The initial focus is on genomes containing neurodevelopmental-associated CNVs/SVs from the three charter CNVs (i.e., dup15, 8p, and ring 14) and genomes from associated iPSC models. Furthermore, high-quality phased genome assemblies from reference families will be generated with a suite of experimental and computational techniques<sup>13,14</sup> to identify potentially pathogenic rearrangements. We will integrate genetic, transcriptional, and epigenetic data to understand the genes and pathways affected in each patient. Ultimately, a comprehensive meta-analysis of these data will reveal pathways of genomic pathogenesis.

These three new reference genomes from each population will be immensely beneficial for the greater scientific, research, and medical communities, as they will reveal never-before-seen complex SVs, provide the most detailed view of the genetic content of genomes associated with neurodevelopmental-related disorders, and help explain the genetic basis for phenotypic variation associated with these disorders. In addition, this work may spur the development of new methods to better characterize families at the genetic and genomic level and, importantly, suggest new hypotheses for the diagnosis and treatment of these disorders. Finally, this set of reference genomes may allow for the development of imputation-based methods to more broadly screen individuals, thereby improving the scalability of complex SV and CNV detection.

### **CNV/SV analytics platform for aggregation, access, and analysis**

A repository of patient-derived samples that includes genomic and phenotypic data is required to enable (1) precise genotyping of CNV/SV aberrations and (2) derivation of iPSCs to study gene expression and pathway disruption in relevant cell types (induced neurons, organoids, etc.) and to test potential therapeutic approaches. A centralized repository established by the CNV Commission will provide samples to approved investigators under a standard Universal Material Transfer Agreement.

The integration of phenotypic, genomic, transcriptomic, and proteomic studies along with experimental data across CNV/SV disorders is essential for performing cross-disorder studies to identify shared phenotypes, molecular pathways, and potential therapeutics effective for various neurodevelopmental-related CNVs/SVs. Unfortunately, clinical, genetic, and molecular data about CNVs/SVs are currently siloed across disparate laboratories, registries, databases, and the literature. Therefore, the CNV Commission has established a “Clinical Research Data Platform” to collect and link new and existing data elements from consented individuals to the CNV/SV Biobank that is



**Figure 2. Mechanistic models of pathogenic CNVs**

(A) Primary driver model: dosage sensitivity of a gene or genes encompassed within the CNV is the leading hypothesis underlying CNV pathogenicity. In the simplest scenario, altered dosage of a single gene may contribute to all or many phenotypes. For example, in 22q13 (Phelan-McDermid)<sup>18</sup> and 15q11 (Angelman syndrome),<sup>19</sup> the majority of defects seem to be due to haploinsufficiency of *SHANK3*<sup>20</sup> and *UBE3A*, respectively. Emerging data from a systematic approach testing constraints on haploinsufficiency and triplosensitivity across the genome suggests that the phenotypes associated with roughly 1/3 of recurrent CNVs are produced by a single primary driver gene.<sup>21</sup> (B) Multiple driver model: one or more genes at a CNV locus are each responsible for discrete phenotypes. For example, in Williams syndrome (7q11.23Del), *LIMK1* is proposed to be responsible for visuospatial deficits whereas *ELN* has been linked to cardiovascular phenotypes.<sup>22,23</sup> Importantly, in both this paradigm and the primary driver model, restoration of expression levels of only one gene should be sufficient to ameliorate acute phenotypes. (C) *Cis*-interaction model: haploinsufficiency of multiple genes within a CNV locus may be required to produce a single given phenotype (“*cis*-interaction model”).<sup>24</sup> This seems to be the case at 16p11.2, where multiple genes are involved in craniofacial abnormalities.<sup>35</sup> (D) *Trans*-interaction model: a fourth possibility is *trans*-interaction. In one scenario, *trans*-interactions could imply that phenotypes associated with a CNV only emerge in specific genetic backgrounds, most likely because of polygenic risk load or the presence of secondary rare disruptive gene variants.<sup>25</sup> This scenario is observed in some cases of inherited CNVs where the full phenotype is not expressed and can even go undetected in the parent carrier. In another scenario, the change in dosage or arrangement of a gene regulatory element within the CNV locus impacts the expression of genes outside the locus. In this case, the manifestation of phenotypes is not dependent on a change in the dosage of a protein-coding gene. This model may explain ring chromosome or complex inversions and deletions/duplications found on chromosome 8p. These four models are not mutually exclusive, and it is likely that complex interactions are a feature of many CNVs that show variable phenotypic expressivity.

interoperable and has value for patients, families, clinicians, and researchers. This data platform facilitates genetic diagnosis interpretation, research, and education for CNVs/SVs. Importantly, this platform structure also facilitates the sharing of relevant information back to the participant. Robust governance and machine-readable sharing preferences enable expedited sharing of data on the basis of patient consent.

To simplify access to the integrated data, we have launched the interactive and user-friendly neurodevelopmental-related CNV (“NDD-CNV”) portal, a free web resource application that displays expert-curated CNV/SV datasets alongside biomedical annotations, user-friendly analytics, and educational resources. We are aggregating clinical and genomic data from affected individuals with pathogenic CNV/SV disorders from the patient advocacy collaboratives. In addition, we are collecting and curating additional pathogenic and population-control CNVs/SVs from publicly available databases such as the UK Biobank,<sup>15</sup> gnomAD,<sup>16</sup> and ClinVar.<sup>17</sup> To enable exploration of one or multiple CNVs simultaneously, we will overlap with reference CNV databases, regulatory elements, disease-associated genes, SNVs, and genome-wide association study (GWAS) hits. Multiple gene-level features such as inter- and intraspecies sequence constraint metric and dosage sensitivity will be annotated. By combining gene-level features, candidate genes can be ranked as most likely to contribute to phenotypes. Moreover, enrichment analyses for phenotype, functional, and pathway annotations can be performed.

We designed the NDD-CNV portal for three user scenarios: (1) educational videos for patients, families, and clinicians; (2) expert-level variant interpretation with guideline-based pathogenicity classification tools; and (3) research tools that explore the rich source of interconnected data for investigators. In addition, the NDD-CNV portal infrastructure is scalable and can integrate diverse datasets and data types. This can transform variant interpretation, research, and education for neurodevelopmental CNV/SV disorders.

With these building blocks from part 1 in place, we now explore proposed research initiatives that these building blocks facilitate in part 2.

## Part 2: Innovative approaches and relevant model systems for CNVs/SVs

In part 2 of our paper, we briefly highlight key technologies or systems that have great potential for the study and treatment of CNVs/SVs. These include

- identifying mechanisms and driver genes,
- engineering cell and iPSC resources,
- critical developmental windows and cell types,
- animal models, and
- translational and interventional potential.

## Identifying mechanisms and driver genes

Mechanisms to explain pathogenesis within large CNVs vary from a simple driver gene to multifactorial models that involve many genes (Figure 2). Until recently, the general research approach for CNVs/SVs was to separate contiguous gene syndromes, where the identification of one single gene driving the neurodevelopmental phenotype is feasible from deletion presentations, versus deletion/duplication presentations where the effects of gene-gene interactions become more complicated, making it hard to identify the driving mechanism. With this general approach, there have been notable successes in some well-established CNVs/SVs, most recently 22q13 (Phelan-McDermid syndrome)<sup>18</sup> and 15q11 (Angelman syndrome),<sup>19</sup> where most phenotypes seem to be caused by haploinsufficiency of *SHANK3*<sup>20</sup> and *UBE3A*, respectively. However, unlike single-gene disorders, disease-causing CNVs/SVs often involve many (sometimes hundreds) of genes. Thus, it is challenging to determine how many and which gene(s) contribute to the phenotype and whether there is a single driver gene. There is evidence in several CNV/SV-based syndromes that multiple genes might contribute to congenital anomalies, and in some cases, it might be pleiotropic. For instance, recent organoid work in 16p (an example of the *cis*-interaction model depicted in Figure 2C) suggests disruption of specific pathways in the absence of a single driver gene.<sup>26</sup> In addition, some disease-associated CNVs/SVs are balanced (i.e., without appreciable copy-number change), suggesting the mechanisms by which they affect a phenotype may be due to differences in gene expression/regulation,<sup>27</sup> perhaps through chromatin conformation.<sup>28</sup> There are also examples where the chromosomal breakpoint disrupts the function of a single gene.<sup>29</sup> Taken together, these studies illustrate the utility of investigating CNV/SV disorders with emerging technologies to understand the molecular basis of each condition and potential overlaps between conditions.

One approach is to leverage the variability in CNV breakpoints to determine the minimal genomic region linked to phenotypic outcomes.<sup>30</sup> While many eukaryotic genes are not sensitive to the loss or gain of a single copy, large-scale population genetic studies are deciphering dosage sensitivity maps spanning the entire human genome.<sup>21,31</sup> By examining CNVs in apparently healthy individuals, the tolerance of copy-number changes for each human gene can be empirically computed. Genes intolerant to loss or gain are prioritized as driver genes of pathogenic CNVs. In a large-scale analysis, over half of pathogenic CNVs associated with specific human phenotypes were predicted to contain one or two driver genes.<sup>21,32</sup> Thus, driver gene identification nominates targets for therapeutic intervention in CNV disorders. Bioinformatic approaches have been developed to narrow the list of driver genes through analysis of gene expression patterns in publicly available databases.<sup>33</sup> In addition, proteomic methods based on proximity labeling offer insight into the signaling

networks regulated by driver genes and how the haploinsufficiency of driver genes within the CNV leads to dysfunctional signaling, leading to opportunities for therapeutic intervention.

In most cases of CNVs/SVs, clinical phenotypes of interest are most likely driven by multiple genes within the CNV locus.<sup>34,35</sup> A central task for CNV/SV researchers is to identify such genes, the underlying cellular and molecular phenotypes, and how they correspond to the clinical presentation. The use of directed and unbiased approaches will be necessary to completely characterize and identify the effects of all genes contributing to a phenotype within a CNV/SV. Although several examples were noted in the previous paragraphs, further methods will need to be developed. Understanding the molecular pathogenesis of CNV/SV syndromes is a critical step toward the rational design of therapeutics. Toward that end, determining the constituent genes that contribute to the disorder when the dosage is disrupted is fundamental.

To emphasize this point, many neurodevelopmental-associated CNVs/SVs give rise to complex phenotypes that are not neatly described by existing diagnostic categories, and phenotypic overlap across seemingly unrelated CNVs/SVs can be extensive<sup>36</sup>. This convergence at the level of clinical phenotype across heterogeneous CNVs/SVs, especially concerning neurodevelopmental phenotypes, implies convergence in at least some aspects of the mechanisms and underlying biological processes. These shared impairments open the door to investigate a poly/omnigenic model for clinical presentation.<sup>37</sup> For example, despite the locus heterogeneity of CNVs identified in individuals with ASD, the affected gene networks converge on neuronal signaling, synapse function, and the regulation of gene expression.<sup>38</sup> This creates the potential for shared research approaches and common therapeutic strategies across multiple types of CNV/SV-based syndromes, as opposed to siloed approaches that investigate one disorder at a time, which can be slow and costly.

### Engineering cell and iPSC resources

Advances in cellular engineering have ushered in a new era for *in vitro* disease modeling and translational cell biology. Complex CNVs/SVs are now ripe for mechanistic investigation. iPSC technology has been a breakthrough for neurogenetic research, as previously inaccessible cell types such as neurons can now be generated with iPSCs from individuals harboring rare pathogenic gene variants.<sup>39,40</sup> The variants of interest and the complete genomic sequence of the donor are retained as iPSCs differentiated to cell types of interest. Importantly, iPSCs can be cultured while generally maintaining genomic stability—a crucial point when studying unstable chromosomes (e.g., rings).

Nevertheless, these molecular tools have not been easily adapted for studying CNVs/SVs. While engineering a single-nucleotide variant into a cell line with CRISPR-Cas-based technology is now routine, generating a large deletion, duplication, or inversion—let alone a complex

rearrangement—is much more challenging. In the case of ring chromosomes, developing iPSC models is problematic due to the unstable nature of the ring during mitosis, which can lead to dynamic mosaicism,<sup>41</sup> but reducing the number of cell divisions reduces the likelihood of ring loss. Fortunately, recent studies report the production of pluripotent stem cells with a variety of ring chromosomes (including 8, 13, 18, and 22), and CRISPR-Cas-based approaches have been developed to engineer large chromosomal variants into iPSC lines.<sup>42,43</sup> Inducing rings in pluripotent or differentiated cells may be a complementary method to investigate these disorders in neuronal lineages.<sup>44</sup>

In parallel to the proband-derived lines, we envision introducing duplications, deletions, and ring chromosomes into a few well-characterized iPSC lines. For example, the SCORE strategy targets the repetitive elements in the genome to engineer microdeletions and microduplications.<sup>45</sup> By mechanistically mimicking non-allelic homologous recombination, SCORE was able to efficiently introduce reciprocal deletions and duplications in iPSCs at multiple loci. This strategy may also be used in directed and/or unbiased screens to identify genes contributing to cellular phenotypes of particular CNVs/SVs that may be of relevance to the clinical phenotype. Another approach, using single guide RNAs targeting repetitive regions, achieves efficient chromosome deletion *in vitro* and *vivo*.<sup>43</sup> One benefit of using a CRISPR-Cas-based approach is that it can be parallelized to engineer multiple to large chromosomal changes in iPSCs simultaneously.<sup>46</sup> Ultimately, these studies are yielding technical and mechanistic insights that one day may lead to effective therapeutic interventions.

### Critical developmental windows and cell types

A key problem in CNV neurobiology is determining the cell type(s) and developmental timing of phenotypic etiology. Unfortunately, even for well-studied CNV syndromes such as 22q11.2 deletion syndrome, we do not know the cell type or developmental window most critical for neurodevelopmental sequelae.<sup>20</sup> Fortunately, advances in 2-dimensional (2D) and 3-dimensional (3D) tissue culture approaches have made these questions more tractable. From an *in vitro* disease modeling perspective, iPSC lines can be differentiated to neural tissues in both formats.<sup>47–49</sup> Human forebrain cortical progenitors and excitatory neurons are now commonly produced from iPSC lines and are often the first-line cell type for the investigation of neurodevelopmental cellular phenotypes. Additionally, robust cellular phenotypic endpoints that offer medium to high throughput, including neural induction efficiency and progenitor proliferation, neuronal morphology, synaptogenesis, and organoid/neurosphere size, can be assessed. 3D brain organoids have been developed and optimized in various forms, including cortical-region-specific spheroids<sup>50–52</sup> and several types of cerebral organoids.<sup>48</sup> These cultures can mature for many months,

allowing the constituent cell types to resemble the perinatal period both transcriptionally<sup>53</sup> and functionally.<sup>54</sup> Such cultures will provide excellent opportunities to test chromosomal engineering techniques that could lead to therapeutic interventions.

Gene expression profiling and image-based morphology are cellular phenotyping strategies that can capture unbiased, high-dimensional data.<sup>55</sup> Microfluidic systems (single-cell sequencing) now provide large-scale multi-omics approaches and *in silico* developmental reconstruction.<sup>56</sup> Similarly, arrayed or pooled CRISPR screens can be used across multiple CNVs/SVs in gene agnostic approaches to identify and validate a phenotypic target. These approaches could be leveraged to determine the cell types most impacted by a CNV/SV during specific developmental windows. Imaging platforms can now be used to trace and quantify neurite number, length, and branching in dozens to hundreds of neurons from each patient-derived and isogenic cell line. Quantified phenotypes can be normalized to neurotypical control cells, and cell lines can then be hierarchically clustered by phenotypic distance.<sup>57</sup> Morphometric signatures can reveal functional relationships across patient-derived lines and serve as robust, quantitative, and screenable phenotypes for drug discovery.<sup>58</sup>

Transcriptional profiling (RNA sequencing) can be used to infer cell state and compare typical with atypical cells. In iPSC-derived neuronal cells, this method can determine the efficiency, timing, and progression of neuronal differentiation and regional specification.<sup>59</sup> Transcriptional profiling enables quantitative assessment of expression, prioritizing genes that might drive specific neurological aspects of CNV/SV disorders. Elegant computational approaches such as weighted gene co-expression network analysis can identify sets of genes that may comprise functional modules.<sup>60</sup> Critically, module preservation across cell lines from individuals with diverse neurodevelopmental CNVs/SVs may point to shared and distinct neurobiological underpinnings.

Neuronal function is obviously a crucial phenotypic dimension. There is yet no substitute for single-cell patch-clamp electrophysiology, but high-throughput electrophysiology using multi-electrode arrays (MEAs) can provide a robust, disease-relevant readout of neuronal activity across many cultures without extensive training requirements.<sup>61,62</sup> Optimized protocols provides MEAs with the power and reliability to discover disease-specific and/or convergent neuronal properties.

To discover convergent molecular and cellular mechanisms across diverse neurodevelopmental CNVs/SVs, an ideal experiment would compare many iPSC lines in parallel. Current obstacles to this approach are the significant labor, cost, and heterogeneity associated with neuron generation and phenotyping. Pooling tens to hundreds of iPSCs lines together before differentiating them to neurons sidesteps these barriers, enabling the simultaneous profiling and direct comparison of patient-derived

lines.<sup>10,63</sup> In addition, single-cell profiling modalities, including transcriptomics, chromatin accessibility, protein quantification, and transcription factor binding, can be readily integrated into this approach to identify cell-autonomous features of a CNV.

A teratoma assay combined with single-cell sequencing could reveal impacts on developmental lineages outside the brain. The teratoma is a recognized standard for validating pluripotency in stem cells and is a promising platform for studying human developmental processes.<sup>64</sup> Moreover, the inter-teratoma cell type heterogeneity can be compared to organoid systems and other cell types corresponding to similar fetal cell types.

### Animal models

Animal models have been used to investigate monogenic disorders and some CNV/SV-based syndromes; however, substantial challenges remain, especially for complex rearrangements. Neurodevelopmental disorders are complex, and animals may not recapitulate all the symptoms or phenotypes. Therefore, it is important to identify animal phenotypes (e.g., social/communication, repetitive behaviors, motor issues, and cognition) that are relevant to human phenotypes. Close collaborations among animal modelers, cell modelers, and clinical/translational researchers—including insights provided by patient families or their support groups—will enable the design of more accurate and relevant phenotypic batteries. Simpler model organisms such as zebrafish, *Drosophila*, and *C. elegans* may not display all the relevant phenotypes of neurodevelopmental disorders, but they are amenable to high-throughput screening and can be invaluable for drug discovery. Ultimately, the development of animal models for CNVs and SVs will most likely be critical to the development of the efficacious therapies needed.

Evolutionary diversification poses a significant roadblock to recapitulating a CNV/SV in an animal model, as the organization of genes on chromosomes in other species often does not parallel genomic organization in humans. Furthermore, creating viable, construct-valid animal models is not trivial and maintaining animals is expensive. Our primary recommendation is to find ways to incentivize the creation of well-characterized, valid animal models of CNVs/SVs. In disorders that involve multiple/complex genes, one approach may be to create an animal model for each of the genes affected to evaluate their contributions because the technical challenges of modeling the exact mutation are often not possible. Unfortunately, the cost of developing and maintaining animal models for CNVs/SVs that span syntenic boundaries in the model species may be hard to justify until techniques for chromosome-level editing are developed, although sophisticated chromosomal engineering and even maintenance of human chromosome 21 in mouse and rat has been reported.<sup>65</sup>

The prioritization and development of animal models should be based on findings from patient and associated

cell lines. Given the cost and complexity of generating models with large CNVs/SVs, we recommend against such models for first-line discovery. Instead, we suggest creating targeted animal models supported by evolutionarily informed bioinformatic predictions and *in vitro* data. In parallel, these analyses can assist in understanding potential driver genes or regions, which can then be used to create targeted animal models. Such validated animal models will be essential for testing future therapeutic interventions *in vivo*.

## Translational and interventional potential

The ultimate goal of neurodevelopmental CNV/SV research is to translate discoveries into clinical benefits. Such benefits can come in many forms, including acute symptomatic relief, chronic interventional therapies, surgical interventions, and disease-modifying treatments. Research efforts toward these goals should move forward in parallel for optimal efficiency but also so that individuals who participate in research studies realize near-term benefits. Our cross-disorder approach, shared infrastructure, and robust data sharing model will facilitate future basket trials for shared phenotypic symptom targets. Furthermore, the cellular models we develop will be valuable for drug and small molecule screening and testing potential new therapies. Finally, relevant animal models will allow *in vivo* testing of promising treatments.

Novel therapeutic approaches under active investigation (and in some cases in clinical use) for single-gene disorders may also benefit CNV/SV syndromes. Promising examples include gene replacement, RNA therapies for modulation of gene expression, and CRISPR-based approaches for gene editing or modulating gene expression. We hypothesize that convergent neurodevelopmental phenotypes reflect convergent biological mechanisms. If correct, a single mechanistic target may benefit multiple, genetically distinct CNV/SV syndromes such as dup15q, 8p, and ring 14.

The rational design of therapeutic interventions will depend on the molecular underpinnings of the disorders. Gene replacement therapies offer hope for restoring driver genes deleted by CNVs/SVs,<sup>66</sup> and in some cases, the re-expression of deleted genes in adulthood can rescue molecular and behavioral outcomes.<sup>67</sup> Unfortunately, these technologies are designed to modulate the expression of one or a few genes and are not well-suited to megabase-size portions of a chromosome, but emerging methods have potential for larger-scale gene regulation. Tools such as small activating RNAs (saRNAs)<sup>68</sup> and non-cutting CRISPR-Cas9 methods (dCas fusions) can be programmed to upregulate target genes enabling simultaneous transcriptional activation of up to ten genes.<sup>69</sup> Likewise, causal genes with dominant mutations or excessive expression are being targeted by ASOs in monogenic neurodevelopmental and neurodegenerative conditions,<sup>70</sup> and viral delivery of RNA-target-

ing CRISPR to the nervous system may also have the potential to target multiple genes in a duplicated interval.<sup>71</sup> Correction of chromosomal aberrations in iPSCs may allow the differentiation of healthy cells that may ultimately be useful for cellular transplantation. For example, transplantation of GABAergic neurons has ameliorated seizures in animal models.<sup>19</sup> In addition, technical advances in iPSC differentiation enable nearly unlimited generation of excitatory and inhibitory neurons that may be suitable for cellular replacement.<sup>40,72</sup> Much work is needed to improve and refine these methods in animal models before they can be considered in humans. Regardless of the exact methodology, it is clear that new and emerging research is promising with respect to the ultimate treatment CNVs/SVs, and an integrative and inclusive approach to tackle these disorders will expedite therapeutic interventions.

## Conclusions

CNVs/SVs syndromes are overwhelmingly associated with neurodevelopmental sequelae that collectively impose a significant burden on affected individuals and their families. Furthermore, these disorders are complex, life-long, and they require an interdisciplinary team-science approach to connect genotype to clinical and cellular phenotypes and to identify disease-modifying interventions. We believe that the time is ripe to investigate CNVs with the same urgency used to analyze single-gene disorders. Unfortunately, funding agencies have not yet prioritized such studies.

In this perspective, the CNV Commission provides a roadmap for cross-disorder CNV/SV research while keeping the patient voice at the center by supporting dynamic relationships between researchers and the patient/family community. As more stakeholders engage, a major goal should be to define knowledge and resource gaps, share the latest research, and identify a shared approach motivated by focusing on the convergence of clinical phenotypes. This roadmap provides a strategic direction to address neurodevelopmental-related CNVs/SVs (as exemplified by dup15q, 8p, and ring 14) but with tools and methods that encourage expansion to all other CNV disorders. In addition, we have created critical infrastructure (neurogenetics data platform, biorepository, and a CNV/SV portal) that is scalable, interoperable, and provides value for patients, families, clinicians, and researchers.

Funding is required to recruit a diverse assemblage of neurodevelopmental-related CNV/SV patients, drive and expand research, and translate this research into efficacious clinical interventions. We think a team-science approach with open-data access in the CNV/SV space should be championed by current funding and academic structures, similar to the Human Genome Project, the Genes to Mental Health Consortium,<sup>2</sup> the Psychiatric Genomics Consortium,<sup>2</sup> and the Cancer Moonshot. Investigation of complex CNV/SV problems should not be buried as

exploratory aims in a larger grant focused elsewhere, and we hope that funding agencies recognize that shared approaches to treating diverse neurodevelopmental-related disorders may emerge from comprehensive research into CNVs/SVs. If so, this would be an outstanding return on investment.

Converging molecular pathways underlying diverse CNVs/SVs may suggest common solutions or therapies with broader impact for more than one CNV/SV. Recent advances in genome engineering, sophisticated *in vitro* and animal-based disease modeling, and clinical phenotyping protocols can help address these syndromes. The CNV Commission hopes to inspire and enable more collaborative, synergistic team-science approaches to CNV/SV research that will ultimately improve the health and well-being of patients and their caregivers.

## Consortia

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## Supplemental information

Supplemental information can be found online at <https://doi.org/10.1016/j.ajhg.2022.07.003>.

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## Declaration of interests

The authors declare no competing interests.

## Web resources

3q29deletion Registry, <https://3q29deletion.patientcrossroads.org/>

Chromosome 18 Clinical Research Center, <https://wp.uthscsa.edu/chrome-18/>

Dup15q Alliance, <https://dup15q.org/>

FDA guidance on RWE, <https://www.fda.gov/science-research/science-and-research-special-topics/real-world-evidence>

NDD-CNV Portal, <https://ndd-cnv-portal.broadinstitute.org/>

Project 8p, <https://project8p.org/chromosome8p/>

Ring 14 International Onlus, <http://www.ring14.org/>

Ring 14 USA, <http://www.ring14usa.org/>

Ring 20 research and support UK, <https://ring20researchsupport.co.uk>

The Chromosome 18 Registry and Society, [www.chromosome18.org](http://www.chromosome18.org)

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