Seeking Convergence and Divergence between Autism and Schizophrenia using genomic tools and patients' neurons

Eva Romanovsky[1,2,*], Ashwani Choudhary[1,*], Ahmad Abu Akel[3], Shani Stern[1,#]

[1] Sagol Department of Neurobiology, Faculty of Natural Sciences, University of Haifa, Haifa 3498838, Israel

[2] Institute of Pathology, Heidelberg University Hospital, 69120, Heidelberg, Germany

[3] Department of Psychology, Faculty of Social Sciences, University of Haifa, Haifa 3498838, Israel

*Equally contributed

#Correspondance: Shani Stern, *sstern@univ.haifa.ac.il*

ABSTRACT

Autism spectrum disorders (ASDs) are a highly heritable and complex group of psychiatric conditions resulting in abnormal repetitive behaviors and impairment in communication and cognitive skills in children. Recent efforts have led to unmasking some of the genetic and environmental risk factors that contribute to the manifestation of ASD.

Previous studies have focused on the genetic correlation between ASDs and other neuropsychiatric disorders but an in-depth understanding of the common variants and its correlation to other disorders is required. In this study, we have conducted an extensive analysis of the common variants identified in ASDs by Genome-wide association studies (GWAS) and compared it to the consensus genes and single nucleotide polymorphisms (SNPs) of Schizophrenia (SCZ) that we have recently reported to determine the shared genetic associations in both the disorders. Further, using a meta-analytical approach we have also probed the cellular phenotypes observed in ASD and compared it to the phenotypes observed in SCZ induced pluripotent stem cell (iPSC) models. Our collective analysis of the deficits in ASD in comparison to SCZ iPSC-derived neurons indicates the presence of developmental divergent trajectories of neuronal pathophysiology that converge to similar phenotypes after the neuronal cells have matured even though the disorders share a high percentage of genetic mutations.

INTRODUCTION

ASDs are a complex and heterogeneous group of neurodevelopmental disorders that affect approximately 1% of children globally and vary in degree of severity(1). According to the DSM-5 criteria, abnormal social interaction, repetitive behavior, and delayed language and cognitive skills are the main characteristic symptoms used to diagnose ASD at an early age(2). Previous epidemiological studies have consistently reported a predominant occurrence of ASD in males compared to females (approx. 4:1). Although existing evidence does not establish sex-specific risk factors that can account for the heightened susceptibility observed in males.(3)(4). There are several genetic and non-genetic risk factors associated with ASD. Recent studies suggest that ASD is one of the most highly heritable neuropsychiatric disorders (83%)(5). Large-scale genomic studies (e.g., SPARK) have led to the implications of hundreds of genes that might play an important role in the manifestation of ASD(6)(7). Besides the genetic risk factors, neonatal hypoxia, increased parental age, preterm birth, gestational diabetes, and the use of valproate during pregnancy are documented environmental (or non-genetic) factors that can relatively increase the risk of ASD(1)(8).

Clinical reports also suggest the co-occurrence of other developmental and neurological disorders like intellectual disability, attention-deficit hyperactivity disorder (ADHD), anxiety, depression, epilepsy, and psychosis, including schizophrenia (SCZ) in ASD(9). Interestingly, since the first description of "autism" by Leo Kanner in the 1940s, there wasn't a clear distinction between SCZ and autism until the 1970s when it was regarded as a distinct disorder due to its age of onset and clinical features(10). A meta-analysis by a cross-disorder group of the psychiatric genomics consortium studying eight psychiatric disorders involving subjects from European ancestry discovered genetic correlation between the disorders in GWAS data but also classified them into three different groups(11). ASD and SCZ were classified into different groups of "early onset neurodevelopmental disorders" and "mood & psychotic disorders" respectively(11). Moreover, increased parental age and maternal immune activation are the environmental risk factors that are common to both disorders.

Besides the evidence of genetic and environmental risk factor correlations, clinical symptoms based on DSM-5 also appear to overlap, especially the impairments in social interaction in ASD and the negative group of symptoms in SCZ like social withdrawal and reduced communication(12). The meta-analysis of neuroimaging studies has also suggested alteration of common brain regions and functional networks among neuropsychiatric disorders, including SCZ and ASD (13)(14).

Given the observed overlap of certain features of SCZ and ASD, it is pertinent to dissect the neurobiological pathophysiology and disease phenotypes and seek the commonalities between the disorders. Over the last decade, induced pluripotent stem cells (iPSC) based models of SCZ and ASD(15)(16)(17)(18)(19) have provided important clues about the pathophysiological processes affected in neural cells, especially since the animal models do not fully recapitulate the complex genetics and clinical features of neuropsychiatric disorders(20). We recently performed a quantitative meta-analytical study of the progress done in the field of SCZ genetics and the findings of SCZ phenotypes in iPSC-derived neural cells(21). Some prior studies have focused on the shared rare variants and their pleiotropic effects in the development of SCZ and ASD(22)(23)(24).

Here in this study, we have analyzed genome-wide association studies (GWAS) performed on ASD individuals to find the frequently reported consensus genetic variants. We have also compared the consensus gene variants of ASD (single nucleotide polymorphism, SNPs) with the frequently reported genetic variants in SCZ(21) to determine the shared and specific SNPs (gene variant) affected and prevalent in both disorders to have an in-depth understanding of the genomic correlation between ASD and SCZ. Furthermore, we have performed a comprehensive analysis of approximately 50 iPSC-based studies of ASD to uncover the highly common disease phenotypes and have attempted to correlate them with the commonly reported ASD genes by performing gene set enrichment analysis. Hence, in this study, using a quantitative meta-analytical approach to genomics and an iPSC-based disease modeling, we have probed both the converging and distinct features between ASD and SCZ.

METHODS

Genetic Variant Analysis and Standardization of Gene Nomenclature of Autism Spectrum Disorder (ASD) and Schizophrenia (SCZ) Genes

The genetic variant analysis and the standardization of gene nomenclature were performed according to Choudhary et al., 2022 (21).Trait statistics were retrieved from the NHGRI-EBI Genome-Wide Association Study (GWAS) Catalog(25) for the study of ASD (EFO_0003756) and SCZ (EFO_0004609). A total of 17 publications with 305 genes related to ASD and 86 publications with 1119 genes related to SCZ were reported. The *SNP_GENE_IDS* column was utilized to identify the Ensembl IDs of genes that contain ASD-related reference SNPs (rs, SNP-risk allele) within their sequence. When several Ensembl IDs were attributed to one gene in a single publication, they were unified and the gene symbol was counted once in each publication to reduce noise from the data because Ensembl IDs of the same gene symbol had overlapping loci in most cases. Furthermore, care was taken to ensure that each publication was counted only once, since some publications had multiple entries and thus the same Ensembl IDs. For each gene variant, the frequency was calculated by counting how often it was reported in a publication with a *p*-value of <0.01. The most frequently annotated genes for ASD are those counted in more than three publications (out of n=23) and for SCZ those counted in more than five publications (out of n=105).

The full dataset from the Developmental Brain Disorder Gene Database (DBD) (26) was downloaded to extract a list of genes associated with ASD. According to DBD, 672 genes have been linked to autism. The Ensembl Data (Biomart, 2022-08-25) (27) was accessed to acquire a list of Ensembl IDs and their corresponding gene name. The chosen dataset was *Human Genes (GRCh38.p13)* from *Ensembl Genes 107* and the attributes selected were *Gene Stable ID* and *Gene Name*. Additionally, the database of Human Gene Nomenclature Committee (HGNC) gene symbols from http://ftp.ebi.ac.uk/pub/databases/genenames/hgnc/tsv/hgnc_complete_set.txt (2022-08-25) was downloaded which contained approved gene symbols, previous gene symbols, alias gene symbols and the related Ensembl ID for each gene. The gene nomenclature was standardized

for GWAS and DBD gene symbols using HGNC as described in Choudhary et al. The method involves searching for the Ensembl ID in both the Biomart results and the HGNC database. If the Ensembl ID is found in the HGNC database, the matching approved symbol from the HGNC database is used. If the Ensembl ID is not found in the HGNC database, the Biomart symbol is compared to the list of approved HGNC symbols. If the symbol is an approved HGNC symbol, it is used. If the symbol is not an approved symbol, it is searched in the list of previous gene symbols in the HGNC database. If the symbol matches a previous symbol of a gene, the currently approved HGNC symbol is used. This method facilitates the matching of HGNC-approved symbols to all Ensembl identifiers with no potential for confusion.

For ASD genes of GWAS, the identifier *LINC02163* was replaced by the approved symbol *NIHCOLE*. Similarly, for ASD genes of DBD, the identifier *C12ORF57* was not located in either the HGNC nor the Biomart symbols, and the identifier *KIAA0100* was substituted by the approved symbol *BLTP2*. The gene symbols of SCZ were already standardized by Choudhary et al., 2022 (21)

Data analyses

All statistical analyses were performed by using R (version 4.1.2 (2021-11-01)) [R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2022. https://cran.r-project.org.] and MATLAB (version 9.12.0.2009381 (R2022a) Update 4, 2022-07-7) [The MathWorks Inc. (2022). Optimization Toolbox version: 9.12 (R2022a), Natick, Massachusetts: The MathWorks Inc. https://www.mathworks.com.].

Investigating the Commonalities between ASD and SCZ Genes; Single Nucleotide Variants (SNPs)

In order to find common genes, the intersection of ASD genes from GWAS and DBD was formed first (for n=30 genes), followed by most frequent ASD genes and SCZ genes from GWAS (n=17 genes). Overlapping genes were represented as word clouds using MATLAB.

Genes were color-coded so that their overlap with the corresponding gene list could be seen. The frequency of genes associated with ASD that appeared more than three times in publications was visualized using MATLAB.

Subsequently, the commonalities between ASD and SCZ were further investigated. For this purpose, the most frequent genes of ASD (>3 publications) and SCZ (>5 publications) were overlapped to create a common gene list. The SNPs of the common genes as reference SNP (rs) were taken from the GWAS catalogs of ASD and SCZ (*SNPS* column). The common genes were matched with the SNPs and merged into one heatmap. ASD (red) and SCZ (green) were marked when the SNP was detected. The *oncoPrint()* function from the R package *ComplexHeatmap*(28) was used to generate the heatmap. In addition, all chromosomes were represented as a circular layout to mark the genomic position of detected SNPs. The human reference genome assembly GRCh38 was used. The *RCircos* R package (29) was employed for this purpose.

The maturation and neuron development scheme in iPSC models of ASD and SCZ has been simplified and created with BioRender.com. The scheme is referred (18)(30)(31)(15)

Analysis of RNA Expression Levels of ASD Genes Across Brain Regions

RNA consensus tissue gene data (*rna_tissue_consensus.tsv*) was downloaded from The Human Protein Atlas (HPA, proteinatlas.org) (32). This data set provided a summary of normalized expression levels for genes across 54 different tissues, based on transcriptomics data from HPA and Genotype-Tissue Expression (GTEx). The data included an Ensembl gene identifier (*Gene*), analyzed sample (*Tissue*) and normalized expression value (*nTPM*) for each gene. It was based on the HPA version 22.0 and Ensembl version 103.38.

First, the data set was subset by the overlapping ASD genes of GWAS and DBD. The average gene expression was calculated for each tissue, filtered by brain regions including the amygdala, basal ganglia, cerebellum, cerebral cortex, hippocampal formation (hippocampus), hypothalamus, medulla oblongata (medulla), midbrain, pons, spinal cord, and thalamus. BrainNet (33) and Biorender.com were utilized to assign color-coding based on the ranking of

average expression level, with red signifying a high expression level and violet representing a low expression level. The illustration is not a precise representation of the anatomical structure of the brain but rather a schematic one. The colored areas were used to demonstrate the different brain regions.

The Genetic Pathways of ASD Explored Using Gene Set Enrichment Analysis

A gene set enrichment analysis was performed on the most commonly observed ASD genes and those ASD genes which overlapped in both GWAS and DBD in order to ascertain any biological connections between them and the genetic pathways correlated to ASD. The CytoScape STRING app (34) was used to create a gene set network and to perform the functional enrichment analysis. The terms of fifteen categories, including Gene Ontology Cellular Component (GO) and Monarch Phenotypes (EFO, HP), were analyzed. The results of the analysis were considered significant when the false discovery rate (FDR) adjusted *p*-value was less than 0.05 using the Benjamini-Hochberg procedure. Significant terms associated with ASD or SCZ were highlighted in the gene set network.

Disease Phenotypes in ASD-Induced Pluripotent Stem Cells (iPSC) Through Data Visualization

A total of 51 ASD-iPSC-related publications were evaluated (Supplemental Table 1). Listed studies by Chiffre et al. (35) were also included. The overview of numerous collected data was divided into several pie charts. Subsequently, the individual phenotypes and their morphological and functional changes during the differentiation of ASD-iPSCs were compiled as a heatmap. The regulation or effect strength has been color-coded. Green color represents low regulation (↓) and red color represents high regulation (↑) of reported disease phenotypes. The color bar represents the number of ASD-iPSC-based models in which the corresponding regulation was observed. The R programming language was used to create the pie charts and heatmap, and the R packages *ggplot2* [Hadley Wickham. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016. https://ggplot2.tidyverse.org]

and *ggpubr* [Alboukadel Kassambara. ggpubr: 'ggplot2' Based Publication Ready Plots. CRAN, 2023. https://CRAN.R-project.org/package=ggpubr] were used for this purpose.

RESULTS

Linking the genome-wide associated genes of ASD and SCZ

Using the GWAS Catalog(25), we searched the database for "Autism Spectrum Disorders" and identified the highly associated genes when collecting the data from 17 studies. We included the studies from diverse/different ethnic groups/ancestry as plotted in Supplementary Fig. 1A. A substantial number of studies were from European ancestry followed by East Asian ancestry.

Fig. 1A shows the genes of the significantly associated variants with ASD that appeared in publications with a *p*-value of <0.01; The genes are presented in a word cloud. The frequency of genes (the number of publications in which the gene was reported) was denoted by the size of the font. We then checked which of the genes that showed associations with ASD are also associated with SCZ using the GWAS Catalog as a reference database as analyzed in our previous study(21). These genes have been marked in green in Fig. 1A.

Out of all 305 reported ASD genes in GWAS, 239 genes (78%) were also associated with SCZ, and 78 were associated with the 105 (74%) most frequently reported SCZ genes in GWAS (Fig. 1A). Remarkably, 74% of the most associated genes in ASD are also associated with SCZ (Supplementary Fig 2 A). The eight most frequently reported genes (MAD1L1, CACNA1C, SORCS3, TCF4, ITIH3, STAG1, TSNARE1, MPHOSPH9) were reported in more than three publications and are also associated with SCZ (Supplementary Fig 2A). Some of the most associated genes are linked to neuron function. These include subunits of calcium channels (CACNA1C, CACNB2, CACNA1l), TSNARE1 (a part of the SNARE complex), GRIN2A (a subunit of the NMDA receptor), MAD1L1 (Mitotic Arrest Deficient 1 Like 1), CACNA1I, SORCS3, SPRK2, ITIH3, and more.

We then examined the functional linkage of the 23 genes with the highest prevalence in ASD GWAS and visualized a gene network as shown in Fig. 1B. For this purpose, we used the STRING functional enrichment analysis. Sixteen genes exhibited significant enrichment for "Self-reported educational attainment" (p = 4.3e-17), and fifteen genes demonstrated analogous enrichment for "Behavior" (p = 3.9e-16). CACNA1C was found to be directly connected to other 5 genes (CACNB2, GRIN2A, NRGN, RBFOX1, ITIH3). Other genes like TCF4 and NT5C2 also formed a part of the network. Most of these genes are known to be associated with calcium-mediated activity of neural cells(36). The common significant terms ascribed to these genes were “Intelligence”, “Mental or behavioral disorder biomarker”, “Risk taking behavior” etc. The genes MAD1L1, TSNARE1, FURIN formed another distinct network as they are part of secretory pathways including synaptic vesicle release and fusion. Some of the terms associated with these genes were “Risk-taking behavior”, "Intelligence”, “Mental or behavioral disorder biomarker”.

Specific SNPs that are common between SCZ and ASD

Subsequently, we aimed to investigate whether the type of mutation determines the specific brain disorder (ASD vs. SCZ) in the top associated genes that were common between ASD and SCZ (Fig. 1C). For example, for the TCF4 gene (a transcription factor), nine SNPS are associated with SCZ only, one with ASD only, and five SNPs are common. Similarly, for the CACNA1C gene, seven SNPs are associated with SCZ only, two with ASD only, and four with both ASD and SCZ. Overall, there are more SNPs that are associated with SCZ and common SNPs. There are also a few SNPs associated exclusively with ASD, possibly attributed to the comparatively fewer ASD GWAS conducted compared to SCZ. As mentioned earlier, ASD patients are known to have an increased risk of SCZ (8) and Fig. 1C provides additional support for this assertion, as the majority of SNPs linked to ASD are also associated with SCZ.

We also mapped the regions in the different chromosomes that are associated with ASD specific genetic variants (Suppl Fig. 3A) and both with ASD and SCZ (Suppl Fig. 3B).

Chromosome 3 (10 SNPs), 7 (9 SNPs) and 10 (9 SNPs) had the highest number of specific SNPs reported for ASD. Chromosome 7 and 10

(9 SNPs each) had the highest number of common SNPs shared in ASD and SCZ.

Common genes between ASD GWAS and Developmental Brain Disorder (DBD) database

Moreover, we sought to investigate the overlap between our catalog of genes identified in ASD GWAS and those present in the DBD database (Fig. 2 A & Supplementary Fig. 2 B). The DBD database includes information obtained through exome and genome sequencing, chromosome microarray analyses, and copy number variation studies. It covers six different developmental brain disorders, including ASD and SCZ, and provides comprehensive phenotypic details that aid in the detection of pathogenic loss of function variants. We identified 30 genes that were shared between ASD GWAS genes and DBD genes. Notably, the top reported ASD GWAS genes CACNA1C, TCF4, STAG1, RBFOX1, and RERE were among this shared gene list.

We next used the RNA consensus tissue gene data to determine the brain regions that may be severely impacted by ASD from HPA. With the overlapping gene list of genes shared between ASD GWAS and DBD database, the average gene expression was computed for each tissue. The findings were represented using color-coded visualization, demonstrating the distinct brain regions influenced by ASD (Fig. 2B). The cerebellum exhibited the highest expression level, while the spinal cord displayed the lowest expression level, with a 37% decrease compared to the cerebellum.

Furthermore, we analyzed which of the genes are associated with autistic vs. schizophrenic behaviors as illustrated in Fig. 2C. Sixteen genes exhibited significant enrichment for "Autistic behavior" (p = 9.7e-16). In contrast, a more limited enrichment was found for "Schizophrenia symptom severity measurement" (p = 0.0003), with only three genes displaying significant associations. Thus, it appears that the resulting gene list is more likely to be associated with autistic behavior. In addition, network analysis revealed the presence of three distinct gene clusters. In particular, two smaller clusters, consisting of three and four

genes, respectively, showed significant associations with autistic behavior. Conversely, the largest cluster, comprising sixteen genes, showed pronounced associations with autism and schizophrenia.

iPSC models and neuronal transcriptome in ASD

We further performed a meta-analysis of previously published work using iPSC models for ASD, as performed previously for SCZ (21). Initially, we segmented the data to see the different ASD types (Fig. 3A). The majority of the data is classified as "ASD" (29.4%), while a large portion of the data is Rett Syndrome (27.5%), and the next largest group is fragile X (FXS 11.8%). Approximately 39.2% of the iPSC models had isogenic lines for controls (Fig. 3B). The cell type distribution from which the reprogramming was performed is shown in Fig. 3C. The vast majority of the studies started by reprogramming fibroblasts (52.9%). The reprogramming was usually performed with a retrovirus (50.9 %), or a lentivirus (35.3%), or a Sendai virus (21.6%). In the context of ASD, most research is performed on neurons or neural progenitor cells (Fig. 3E). It is interesting to note that more than 25% of the ASD patients in the studies also have epilepsy (Fig. 3F). The distribution into neuronal types of the analyzed studies is shown in Fig. 3G.

Fig. 4A provides a summary of the phenotypes that were found using the iPSC models. The studies report changes such as differentiation rate, neurites' length, and differential gene expression. Out of 51 publications, 10 (19.6%) reported low synaptic and network activity in neurons derived through NPC from the ASD patients. In addition, 11.8% reported shorter dendrites and neurites and 6% reported increasing associations with mitochondrial function in the neurons derived from the ASD patients. However, the direction is reversed when specifically examining cortical neurons. Five out of seven publications rather reported increasing synaptic and network activity and likewise other publications could detect increasing spike frequency and increased activity in calcium imaging(31)(30)(18)(19). Differential gene expression analyses showed a trend toward increased regulation of genes(31)(18).

Fig. 4B summarizes the functional phenotypes that are usually reported. The neurons derived from the ASD patients usually start with an expedited maturation (19)(30) compared to the control neurons. This includes increased sodium and potassium currents, a hyperexcitability, more arborized neurites, and even more synaptic connections initially. However, as the neurons mature, they lose their synaptic connections, have reduced currents, are less excitable and are less arborized compared to the control neurons derived from individuals without the disorder(19)(31). Interestingly, the neurons derived from the SCZ patients have a different trajectory but in the later timepoints they end up with a similar phenotype(18). The SCZ neurons start less arborized, less excitable, have decreased sodium and potassium currents, and less synaptic activity compared to the control neurons. When the SCZ patient-iPSC derived neurons develop, they always lag behind the control neurons and when they are mature(18), many of their functional and morphological phenotypes are similar to the neurons derived from the ASD patients (Fig. 4B)

DISCUSSION

ASD and SCZ are neuropsychiatric disorders with high heritability estimates with a clear difference in the onset age(21)(5). DSM-5 has distinguished both disorders with characteristic symptoms but the clinical representation and pathophysiology have certain converging features(12). Prior studies have reported a correlation at the genetic level between the disorders(13)(14)(37). Rare structural variants like deletion of chromosome 22q11.2 and 15q13.3 or duplication of chromosome 16p11.2 are associated with both SCZ and ASD and also with other developmental disorders. But the majority of rare variants (or copy number variations) stretch across large genomic regions and hence the position of mutation at the genic or allelic level is difficult to infer. For instance, exonic deletions of NRXN1 are a known monogenic rare variation that increases the risk for ASD, SCZ, and other neurodevelopmental disorders but the reported exonic mutations vary among different disorders (38). A recent study investigated the pleiotropy of rare variant genes in SCZ and

neurodevelopmental disorders(38). But more understanding of the shared genetic and pathophysiological processes is required especially for the GWAS-discovered common variants. Until now, it has been difficult to determine by GWAS, the potential causal genetic variants of either ASD or SCZ. Moreover, most of the previous studies on genetic correlations of the two disorders have been focused on studies based on European ancestry. Hence, to gain better insights, we started this study with the extensive analysis of the GWAS data on ASD individuals from diverse ancestry/ethnicity and then compared them to GWAS of SCZ individuals (also from diverse ethnicity) to find the genes and the SNPs that are common or specific to both disorders.

We found approximately two-thirds of the highly reported GWAS genes to be common between ASD and SCZ. Our further analysis of the SNPs of the highly reported genes revealed that most of the SNPs that are associated with ASD are also associated with SCZ. This observation may explain why ASD patients have an increased risk of SCZ (8). Also, there were more specific SNPs for SCZ than ASD. One of the reasons for this could be the difference between the scale of GWAS between ASD and SCZ both in the number of studies (17 ASD studies vs. 86 SCZ studies) and also the sample size recruitment.

To understand the functional role of GWAS-identified genes, we also searched for the overlap of these genes with DBD genes which has an exhaustive list of potential causal genes from six disorders including Intellectual disability, ASD, ADHD, bipolar disorder, SCZ, and epilepsy along with phenotypic information. These overlapping critical genes were expressed in different brain regions but a high percentage of them were expressed in the cerebellum, cortex, basal ganglia, and hippocampus. The cerebellum and basal ganglia are known to coordinate motor-related functions. The hippocampus and cortex are important for cognition, memory, and learning as well as language and social communication. Hence from this analysis, we can infer that variation and mutations resulting in functional dysregulation of these genes could affect these brain areas and circuits resulting in ASD symptoms.

iPSC-based models offer an alternative to understanding the effect of genomic mutations and associations on neural cells(39)(16)(40)(41)(21)(42)(43). The majority of studies investigated

NPCs and neurons derived from ASD patient-specific iPSC *in vitro* and only a few have studied the role of glial cells from ASD patients using iPSC technology. The most prominent change that was common across many patient iPSC-derived neuron studies was the alteration in the synaptic activity. It may seem confounding that some of the studies reported an increase while some reported a decrease in synaptic activity. These differences could in part be explained due to the different types of neurons studied (cortical, hippocampal, gabaergic, mixed neuron cultures). Other critical reasons for the variability reported could be the timepoint of differentiation studied. Recently, Brant et al.,2021(19) and Hussein et al.,2023(30) have shown how neurons derived from ASD patients start with a higher network connectivity compared to a control network derived from individuals that do not have ASD, but end up when they are mature with a less connected network. From the iPSCs based studies of ASD and SCZ, we see different trend of phenotypes emerging for these two disorders (18)(21). The neurons from ASD patients mature earlier with increased arborizations (31) and exhibit electrophysiological properties earlier than controls in earlier timepoints studied however gradually they show deteriorating electrophysiological properties in later timepoints. In the case of SCZ, the neurons are morphologically less arborized as well as exhibit inferior electrophysiological properties throughout the entire differentiation time compared to controls (18) (Fig. 4 B). This means that originally the two disorders start with opposite phenotypes, but eventually the neurons derived from these two disorders have similar phenotypes when they are mature.

In conclusion, our analysis of common genomic variants and the neuronal pathophysiology of ASD in comparison to SCZ hints at the presence of an initial diverging neuronal pathophysiology that upon maturation converges into similar pathophysiology.

ACKNOWLEDGMENTS:

The Zuckerman STEM leadership program and Israel Science Foundation grants - 1994/21 and 3252 /21 for Dr. Shani Stern.

ETHICS DECLARATION:

Competing Interests

The Authors declare no competing financial or non-financial Interests.

AUTHOR CONTRIBUTION:

SS- Supervision, Conceptualization, Methodology, Data analysis, Drafting and editing – original manuscript. . AAA- Conceptualization, Editing –original manuscript. Data analysis. ER and AC-Methodology, Data analysis, drafting the original manuscript. All authors reviewed the manuscript. ER and AC contributed equally to the work

REFERENCES:

1. Lord C, Brugha TS, Charman T, Cusack J, Dumas G, Frazier T, et al. Autism spectrum disorder. Nat Rev Dis Prim [Internet]. 2020;6(1):5. Available from: https://doi.org/10.1038/s41572-019-0138-4
2. Grzadzinski R, Huerta M, Lord C. DSM-5 and autism spectrum disorders (ASDs): an opportunity for identifying ASD subtypes. Mol Autism [Internet]. 2013;4(1):12. Available from: https://doi.org/10.1186/2040-2392-4-12
3. Baron-Cohen S, Lombardo M V, Auyeung B, Ashwin E, Chakrabarti B, Knickmeyer R. Why Are Autism Spectrum Conditions More Prevalent in Males? PLOS Biol [Internet]. 2011 Jun 14;9(6):e1001081. Available from: https://doi.org/10.1371/journal.pbio.1001081
4. Werling DM, Geschwind DH. Sex differences in autism spectrum disorders. Curr Opin Neurol [Internet]. 2013;26(2). Available from: https://journals.lww.com/co-neurology/Fulltext/2013/04000/Sex_differences_in_autism_spectrum_disorders.6.aspx
5. Sandin S, Lichtenstein P, Kuja-Halkola R, Hultman C, Larsson H, Reichenberg A. The Heritability of Autism Spectrum Disorder. JAMA [Internet]. 2017 Sep 26;318(12):1182–4. Available from: https://doi.org/10.1001/jama.2017.12141
6. Zhou X, Feliciano P, Shu C, Wang T, Astrovskaya I, Hall JB, et al. Integrating de novo and inherited variants in 42,607 autism cases identifies mutations in new moderate-risk genes. Nat Genet [Internet]. 2022;54(9):1305–19. Available from: https://doi.org/10.1038/s41588-022-01148-2
7. Fu JM, Satterstrom FK, Peng M, Brand H, Collins RL, Dong S, et al. Rare coding variation provides insight into the genetic architecture and phenotypic context of autism. Nat Genet [Internet]. 2022;54(9):1320–31. Available from: https://doi.org/10.1038/s41588-022-01104-0
8. Jutla A, Foss-Feig J, Veenstra-VanderWeele J. Autism spectrum disorder and schizophrenia: An updated conceptual review. Autism Res [Internet]. 2022 Mar 1;15(3):384–412. Available from: https://doi.org/10.1002/aur.2659
9. Ivanović I. Psychiatric Comorbidities in Children With ASD: Autism Centre Experience [Internet]. Vol. 12, Frontiers in Psychiatry . 2021. Available from: https://www.frontiersin.org/articles/10.3389/fpsyt.2021.673169
10. Sasson NJ, Pinkham AE, Carpenter KLH, Belger A. The benefit of directly

comparing autism and schizophrenia for revealing mechanisms of social cognitive impairment. J Neurodev Disord [Internet]. 2011;3(2):87–100. Available from: https://doi.org/10.1007/s11689-010-9068-x

11. Lee PH, Anttila V, Won H, Feng Y-CA, Rosenthal J, Zhu Z, et al. Genomic Relationships, Novel Loci, and Pleiotropic Mechanisms across Eight Psychiatric Disorders. Cell [Internet]. 2019;179(7):1469-1482.e11. Available from: https://www.sciencedirect.com/science/article/pii/S0092867419312760
12. Chisholm K, Lin A, Abu-Akel A, Wood SJ. The association between autism and schizophrenia spectrum disorders: A review of eight alternate models of co-occurrence. Neurosci Biobehav Rev. 2015 Aug 1;55:173–83.
13. Ardesch DJ, Libedinsky I, Scholtens LH, Wei Y, van den Heuvel MP. Convergence of Brain Transcriptomic and Neuroimaging Patterns in Schizophrenia, Bipolar Disorder, Autism Spectrum Disorder, and Major Depressive Disorder. Biol Psychiatry Cogn Neurosci Neuroimaging. 2023 Jun 1;8(6):630–9.
14. Moreau CA, Raznahan A, Bellec P, Chakravarty M, Thompson PM, Jacquemont S. Dissecting autism and schizophrenia through neuroimaging genomics. Brain [Internet]. 2021 Jul 1;144(7):1943–57. Available from: https://doi.org/10.1093/brain/awab096
15. Sarkar A, Mei A, Paquola ACM, Stern S, Bardy C, Klug JR, et al. Efficient Generation of CA3 Neurons from Human Pluripotent Stem Cells Enables Modeling of Hippocampal Connectivity In Vitro. Cell Stem Cell [Internet]. 2018;22(5):684-697.e9. Available from: https://www.sciencedirect.com/science/article/pii/S1934590918301711
16. Stern S, Sarkar A, Galor D, Stern T, Mei A, Stern Y, et al. A Physiological Instability Displayed in Hippocampal Neurons Derived From Lithium-Nonresponsive Bipolar Disorder Patients. Biol Psychiatry. 2020/04/13. 2020;88(2):150–8.
17. Santos R, Linker SB, Stern S, Mendes APD, Shokhirev MN, Erikson G, et al. Deficient LEF1 expression is associated with lithium resistance and hyperexcitability in neurons derived from bipolar disorder patients. Mol Psychiatry [Internet]. 2021;26(6):2440–56. Available from: https://doi.org/10.1038/s41380-020-00981-3
18. Stern S, Zhang L, Wang M, Wright R, Cordeiro D, Peles D, et al. Monozygotic

twins discordant for schizophrenia differ in maturation and synaptic transmission. 2022;2022.05.13.491776. Available from: https://www.biorxiv.org/content/biorxiv/early/2022/05/13/2022.05.13.491776.full.pdf

19. Brant B, Stern T, Shekhidem HA, Mizrahi L, Rosh I, Stern Y, et al. IQSEC2 mutation associated with epilepsy, intellectual disability, and autism results in hyperexcitability of patient-derived neurons and deficient synaptic transmission. Mol Psychiatry [Internet]. 2021;26(12):7498–508. Available from: https://doi.org/10.1038/s41380-021-01281-0
20. Nayak R, Rosh I, Kustanovich I, Stern S. Mood Stabilizers in Psychiatric Disorders and Mechanisms Learnt from In Vitro Model Systems. Int J Mol Sci [Internet]. 2021/09/11. 2021;22(17). Available from: https://www.ncbi.nlm.nih.gov/pubmed/34502224
21. Choudhary A, Peles D, Nayak R, Mizrahi L, Stern S. Current progress in understanding schizophrenia using genomics and pluripotent stem cells: A meta-analytical overview. Schizophr Res. 2022/11/29. 2022;
22. van Haren NE, Schnack HG, Cahn W, van den Heuvel MP, Lepage C, Collins L, et al. Changes in cortical thickness during the course of illness in schizophrenia. Arch Gen Psychiatry. 2011/09/07. 2011;68(9):871–80.
23. Kushima I, Aleksic B, Nakatochi M, Shimamura T, Okada T, Uno Y, et al. Comparative Analyses of Copy-Number Variation in Autism Spectrum Disorder and Schizophrenia Reveal Etiological Overlap and Biological Insights. Cell Rep. 2018 Sep 11;24(11):2838–56.
24. Nguyen HT, Bryois J, Kim A, Dobbyn A, Huckins LM, Munoz-Manchado AB, et al. Integrated Bayesian analysis of rare exonic variants to identify risk genes for schizophrenia and neurodevelopmental disorders. Genome Med [Internet]. 2017;9(1):114. Available from: https://doi.org/10.1186/s13073-017-0497-y
25. Sollis E, Mosaku A, Abid A, Buniello A, Cerezo M, Gil L, et al. The NHGRI-EBI GWAS Catalog: knowledgebase and deposition resource. Nucleic Acids Res. 2022/11/10. 2023;51(D1):D977-d985.
26. Gonzalez-Mantilla AJ, Moreno-De-Luca A, Ledbetter DH, Martin CL. A Cross-Disorder Method to Identify Novel Candidate Genes for Developmental Brain Disorders. JAMA Psychiatry [Internet]. 2016 Mar 1;73(3):275–83. Available from: https://doi.org/10.1001/jamapsychiatry.2015.2692

27. Cunningham F, Allen JE, Allen J, Alvarez-Jarreta J, Amode MR, Armean IM, et al. Ensembl 2022. Nucleic Acids Res [Internet]. 2022 Jan 7;50(D1):D988–95. Available from: https://doi.org/10.1093/nar/gkab1049
28. Gu Z, Eils R, Schlesner M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. Bioinformatics [Internet]. 2016 Sep 15;32(18):2847–9. Available from: https://doi.org/10.1093/bioinformatics/btw313
29. Zhang H, Meltzer P, Davis S. RCircos: an R package for Circos 2D track plots. BMC Bioinformatics [Internet]. 2013;14(1):244. Available from: https://doi.org/10.1186/1471-2105-14-244
30. Hussein Y, Tripathi U, Choudhary A, Nayak R, Peles D, Rosh I, et al. Early maturation and hyperexcitability is a shared phenotype of cortical neurons derived from different ASD-associated mutations. Transl Psychiatry [Internet]. 2023;13(1):246. Available from: https://doi.org/10.1038/s41398-023-02535-x
31. Schafer ST, Paquola ACM, Stern S, Gosselin D, Ku M, Pena M, et al. Pathological priming causes developmental gene network heterochronicity in autistic subject-derived neurons. Nat Neurosci [Internet]. 2019;22(2):243–55. Available from: https://doi.org/10.1038/s41593-018-0295-x
32. Sjöstedt E, Zhong W, Fagerberg L, Karlsson M, Mitsios N, Adori C, et al. An atlas of the protein-coding genes in the human, pig, and mouse brain. Science (80-) [Internet]. 2020 Mar 6;367(6482):eaay5947. Available from: https://doi.org/10.1126/science.aay5947
33. Xia M, Wang J, He Y. BrainNet Viewer: A Network Visualization Tool for Human Brain Connectomics. Csermely P, editor. PLoS One [Internet]. 2013 Jul 4 [cited 2023 Aug 10];8(7):e68910. Available from: https://dx.plos.org/10.1371/journal.pone.0068910
34. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. Genome Res [Internet]. 2003 Nov 1;13(11):2498–504. Available from: http://genome.cshlp.org/content/13/11/2498.abstract
35. Cheffer A, Flitsch LJ, Krutenko T, Röderer P, Sokhranyaeva L, Iefremova V, et al. Human stem cell-based models for studying autism spectrum disorder-related neuronal dysfunction. Mol Autism [Internet]. 2020;11(1):99. Available from: https://doi.org/10.1186/s13229-020-00383-w

36. Pers TH, Timshel P, Ripke S, Lent S, Sullivan PF, O'Donovan MC, et al. Comprehensive analysis of schizophrenia-associated loci highlights ion channel pathways and biologically plausible candidate causal genes. Hum Mol Genet [Internet]. 2016 Mar 15;25(6):1247–54. Available from: https://doi.org/10.1093/hmg/ddw007
37. Crespi B, Stead P, Elliot M. Comparative genomics of autism and schizophrenia. Proc Natl Acad Sci U S A. 2010 Jan 26;107(SUPPL. 1):1736–41.
38. Rees E, Creeth HDJ, Hwu H-G, Chen WJ, Tsuang M, Glatt SJ, et al. Schizophrenia, autism spectrum disorders and developmental disorders share specific disruptive coding mutations. Nat Commun [Internet]. 2021;12(1):5353. Available from: https://doi.org/10.1038/s41467-021-25532-4
39. Brennand KJ, Simone A, Jou J, Gelboin-Burkhart C, Tran N, Sangar S, et al. Modelling schizophrenia using human induced pluripotent stem cells. Nature [Internet]. 2011;473(7346):221–5. Available from: https://doi.org/10.1038/nature09915
40. Repudi S, Kustanovich I, Abu-Swai S, Stern S, Aqeilan RI. Neonatal neuronal WWOX gene therapy rescues Wwox null phenotypes. 2021;13(12):e14599. Available from: https://www.embopress.org/doi/abs/10.15252/emmm.202114599
41. Stern S, Sarkar A, Stern T, Mei A, Mendes APD, Stern Y, et al. Mechanisms Underlying the Hyperexcitability of CA3 and Dentate Gyrus Hippocampal Neurons Derived From Patients With Bipolar Disorder. Biol Psychiatry. 2019/11/17. 2020;88(2):139–49.
42. Mizrahi L, Choudhary A, Ofer P, Goldberg G, Milanesi E, Kelsoe JR, et al. Immunoglobulin genes expressed in lymphoblastoid cell lines discern and predict lithium response in bipolar disorder patients. Mol Psychiatry [Internet]. 2023; Available from: https://doi.org/10.1038/s41380-023-02183-z
43. Räsänen N, Tiihonen J, Koskuvi M, Lehtonen Š, Koistinaho J %J T in neurosciences. The iPSC perspective on schizophrenia. 2022;45(1):8–26.

FIGURE LEGENDS

Figure 1: ASD and SCZ GWAS

(A) The word cloud of GWAS-reported genes associated with ASD. GWAS-reported genes associated with SCZ highlighted in green. The font size indicates the frequency of gene mentions in publications, with a larger font size indicating a higher number of reported occurrences. The most frequently reported genes associated with ASD have been plotted in Supplementary Fig. 2 A.

(B) Gene network of the most occurring genes in GWAS associated with ASD. Functional analysis revealed significant pathways indicating autistic behavior. Connected genes implying interactions.

(C) Color map for shared SNPs between ASD and SCZ in GWAS. Intersection of the most occurring genes in ASD and SCZ and their 122 SNPs been visualized.

Figure 2: ASD GWAS and DBD database genes

(A) The word cloud of GWAS reported genes associated with ASD. Genes cataloged in the DBD database are highlighted in red. The font size indicates the frequency of gene mentions in publications, with a larger font size indicating a higher number of reported occurrences.

(B) RNA expression levels of ASD genes across brain regions. Overlapping genes of GWAS and DBD were used for creating the gene list (n=30) and the average gene expression level of each brain region was calculated. The “hot” colors representing higher expression level of the specific brain region and “cold” colors lower expression levels. The graphical image was created with BrainNet (33) and Biorender.com

(C) Gene network of the common gene list of GWAS and DBD. Color labeled genes are significant enriched in either category of autism (p = 6.82e-19) or schizophrenia (p = 2.9e-04).

Figure 3: Metanalysis iPSC models of ASD

(A-F) Donut plots of the summary statistics of the collected 51 publications of iPSC models. Seven categories *(ASD, Control, Somatic cells origin, reprogramming technique, iPSC differentiation, Epilepsy, Neural cell types)* describe the frequency of specific patients and procedural methods in iPSC models.

Figure 4: Summary of iPSC studies on ASD

(A) Heatmap of the number of publications reported activities of specific phenotypes in iPSCs models. If the number of publications is higher, the assigned color becomes darker. With reported high regulation (↑), the color becomes redder and with low regulation, it becomes green (↓). The color white with the number "0" implies no indication.

(B) Schematic of neuron progression over months in reported iPSC models(18)(30)(31)(19).Time was divided into three phases (initiation, maturation, stabilization). As the neurons develop, the curve also increases. The control curve describes the normal course of neuronal development and serves as a comparison for the ASD and SCZ curves. The arrows (↑↓) next to the figures describe whether this development is increased or decreased. The graphical image was created with Biorender.com

Figure 1

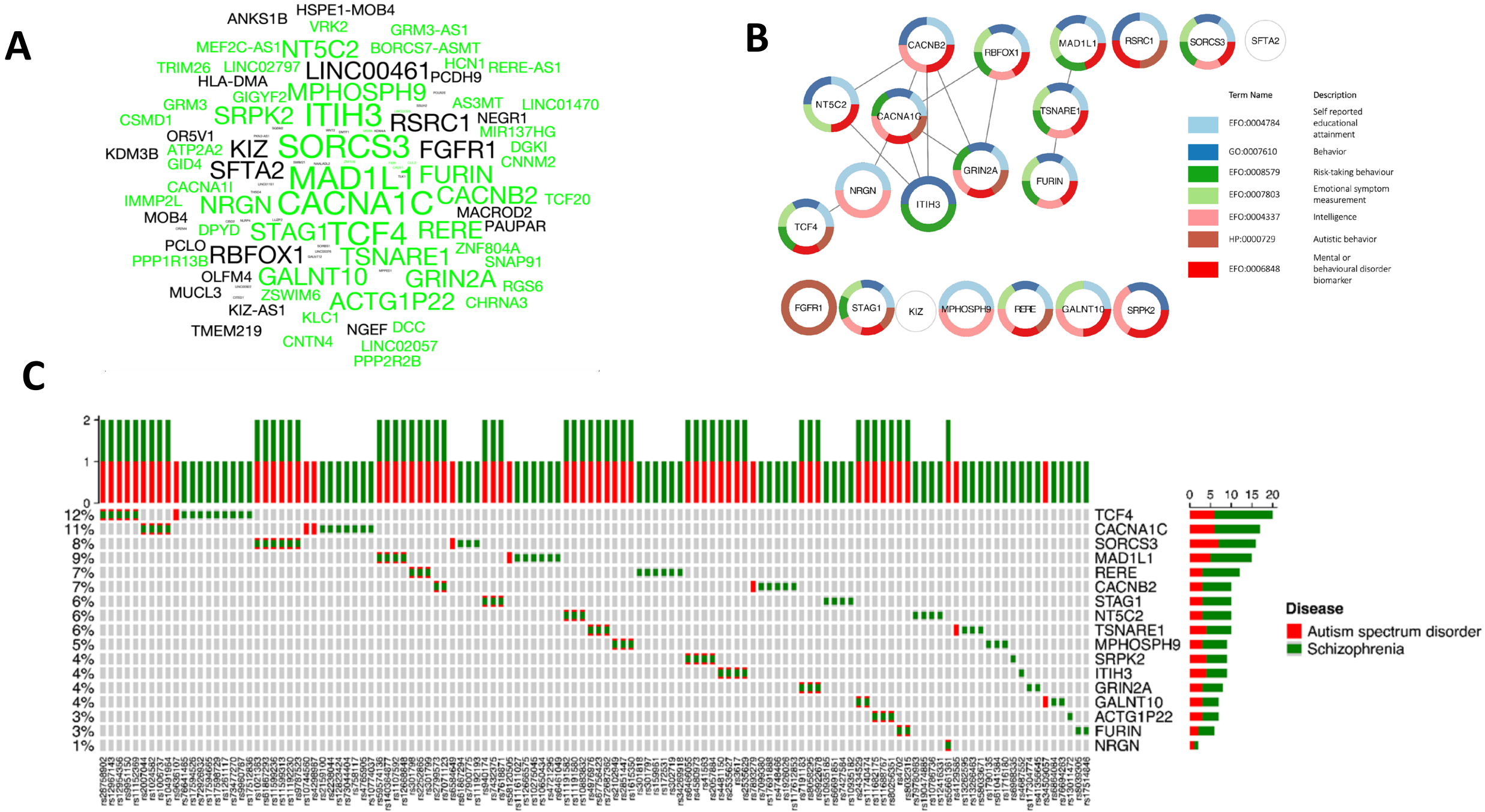
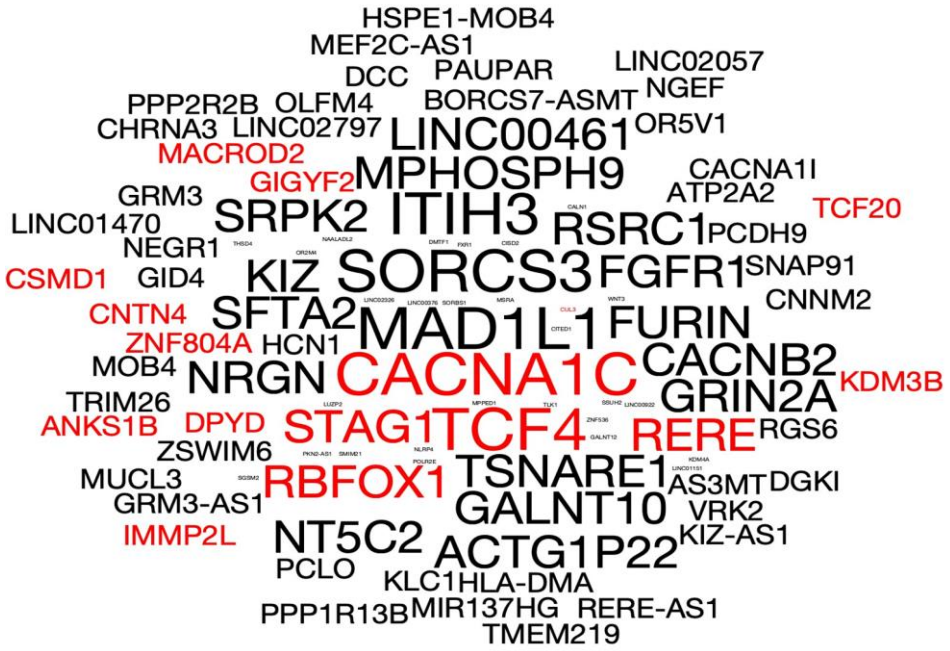
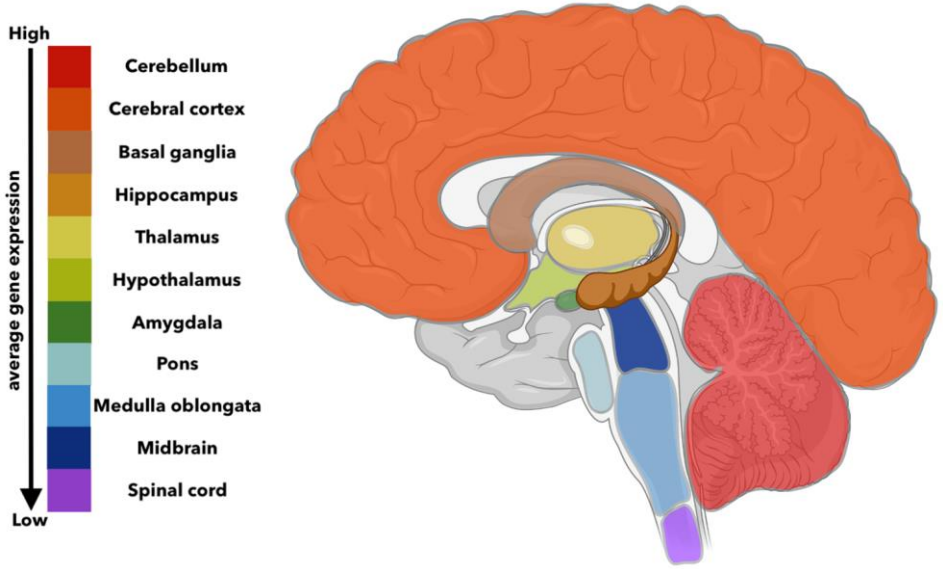

A
ANKS1B
HSPE1-MOB4
VRK2
GRM3-AS1
MEF2C-AS1
NT5C2
BORCS7-ASMT
TRIM26
LINC02797
LINC00461
HCN1
RERE-AS1
PCDH9
HLA-DMA
GIGYF2
MPHOSPH9
GRM3
AS3MT
LINC01470
CSMD1
SRPK2
ITIH3
RSRC1
NEGR1
MIR137HG
OR5V1
KDM3B
ATP2A2
KIZ
SORCS3
FGFR1
DGKI
GID4
CNNM2
SFTA2
MAD1L1
FURIN
CACNA1I
IMMP2L
NRGN
CACNA1C
CACNB2
TCF20
MOB4
MACROD2
DPYD
STAG1
TCF4
RERE
PAUPAR
PCLO
ZNF804A
PPP1R13B
RBFOX1
TSNARE1
SNAP91
OLFM4
GALNT10
GRIN2A
RGS6
MUCL3
ZSWIM6
ACTG1P22
CHRNA3
KIZ-AS1
KLC1
TMEM219
NGEF
DCC
CNTN4
LINC02057
PPP2R2B
B
CACNB2
RBFOX1
MAD1L1
RSRC1
SORCS3
SFTA2
NT5C2
CACNA1C
TSNARE1
GRIN2A
FURIN
NRGN
ITIH3
TCF4
FGFR1
STAG1
KIZ
MPHOSPH9
RERE
GALNT10
SRPK2
Term Name
Description
EFO:0004784
Self reported educational attainment
GO:0007610
Behavior
EFO:0008579
Risk-taking behaviour
EFO:0007803
Emotional symptom measurement
EFO:0004337
Intelligence
HP:0000729
Autistic behavior
EFO:0006848
Mental or behavioural disorder biomarker
C
2
1
0
12%
11%
8%
9%
7%
7%
6%
6%
6%
5%
4%
4%
4%
4%
3%
3%
1%
TCF4
CACNA1C
SORCS3
MAD1L1
RERE
CACNB2
STAG1
NT5C2
TSNARE1
MPHOSPH9
SRPK2
ITIH3
GRIN2A
GALNT10
ACTG1P22
FURIN
NRGN
0 5 10 15 20
Disease
Autism spectrum disorder
Schizophrenia

Figure 2

A


HSPE1-MOB4
MEF2C-AS1
DCC
PAUPAR
LINC02057
NGEF
PPP2R2B
OLFM4
BORCS7-ASMT
CHRNA3
LINC02797
LINC00461
OR5V1
MACROD2
GIGYF2
MPHOSPH9
CACNA1I
GRM3
ATP2A2
LINC01470
SRPK2
ITIH3
TCF20
RSRC1
PCDH9
NEGR1
CSMD1
GID4
KIZ
SORCS3
FGFR1
SNAP91
CNNM2
CNTN4
SFTA2
MAD1L1
FURIN
ZNF804A
HCN1
MOB4
NRGN
CACNA1C
CACNB2
KDM3B
TRIM26
GRIN2A
ANKS1B
DPYD
STAG1
TCF4
RERE
RGS6
ZSWIM6
MUCL3
RBFOX1
TSNARE1
AS3MT
DGKI
GRM3-AS1
GALNT10
VRK2
IMMP2L
NT5C2
ACTG1P22
KIZ-AS1
PCLO
KLC1
HLA-DMA
PPP1R13B
MIR137HG
RERE-AS1
TMEM219

B


High
Low
average gene expression
Cerebellum
Cerebral cortex
Basal ganglia
Hippocampus
Thalamus
Hypothalamus
Amygdala
Pons
Medulla oblongata
Midbrain
Spinal cord

C

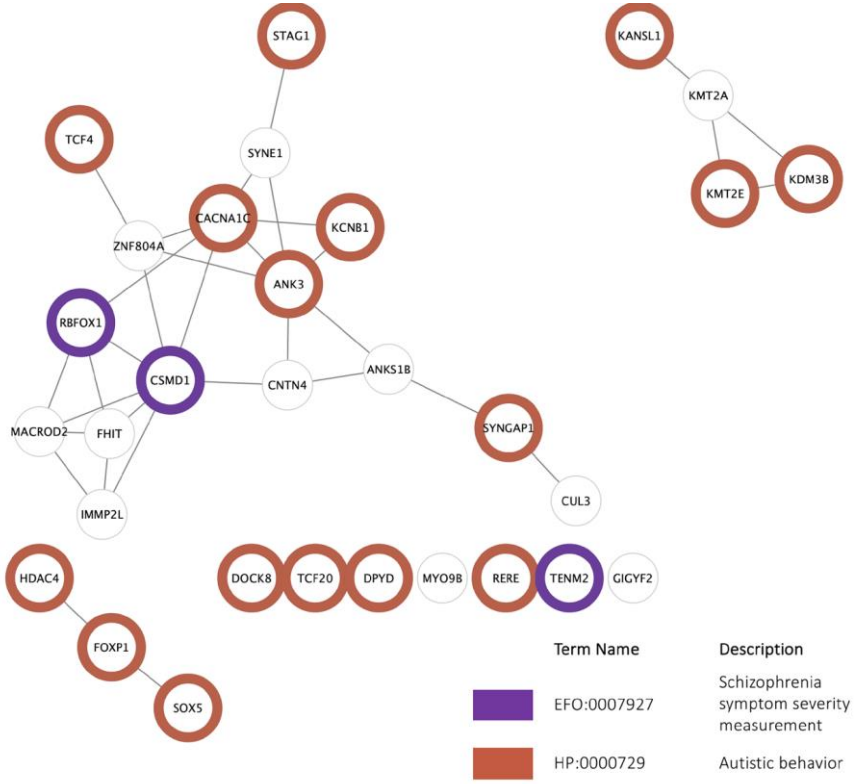
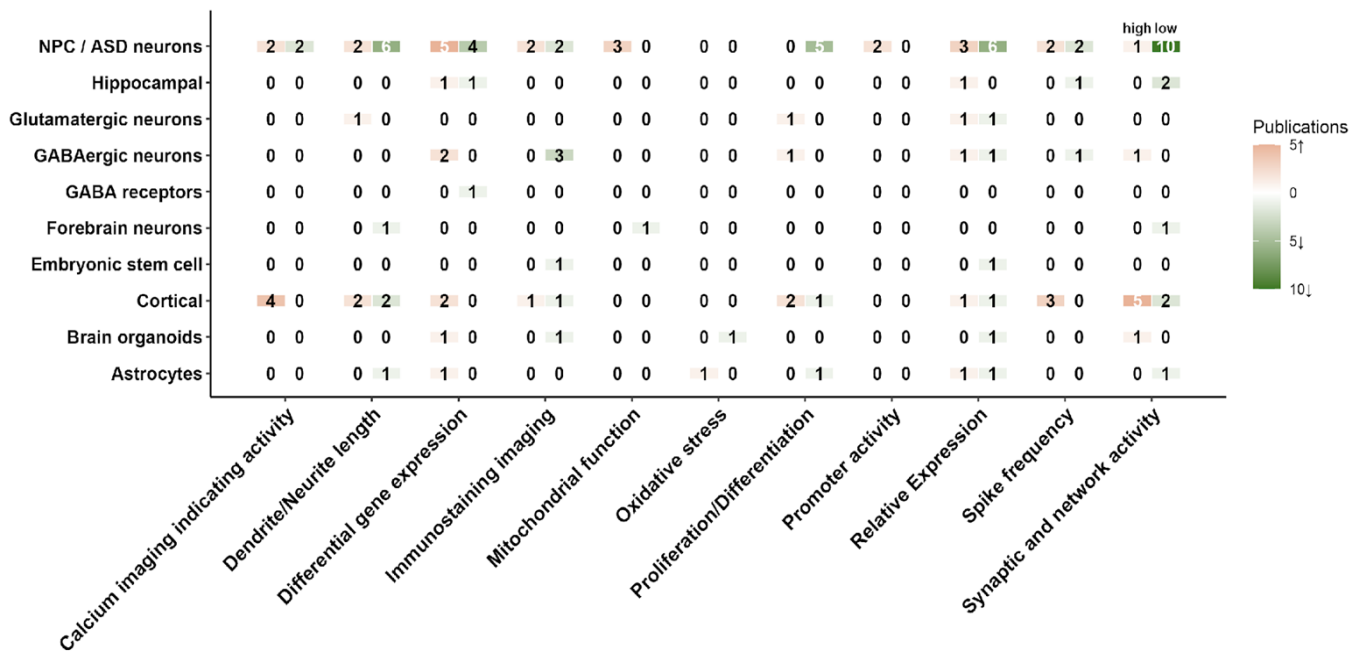

STAG1
KANSL1
KMT2A
TCF4
SYNE1
KMT2E
KDM3B
CACNA1C
KCNB1
ZNF804A
ANK3
RBFOX1
CSMD1
CNTN4
ANKS1B
MACROD2
FHIT
SYNGAP1
CUL3
IMMP2L
HDAC4
DOCK8
TCF20
DPYD
MYO9B
RERE
TENM2
GIGYF2
FOXP1
SOX5
Term Name
Description
EFO:0007927
Schizophrenia symptom severity measurement
HP:0000729
Autistic behavior

Figure 3

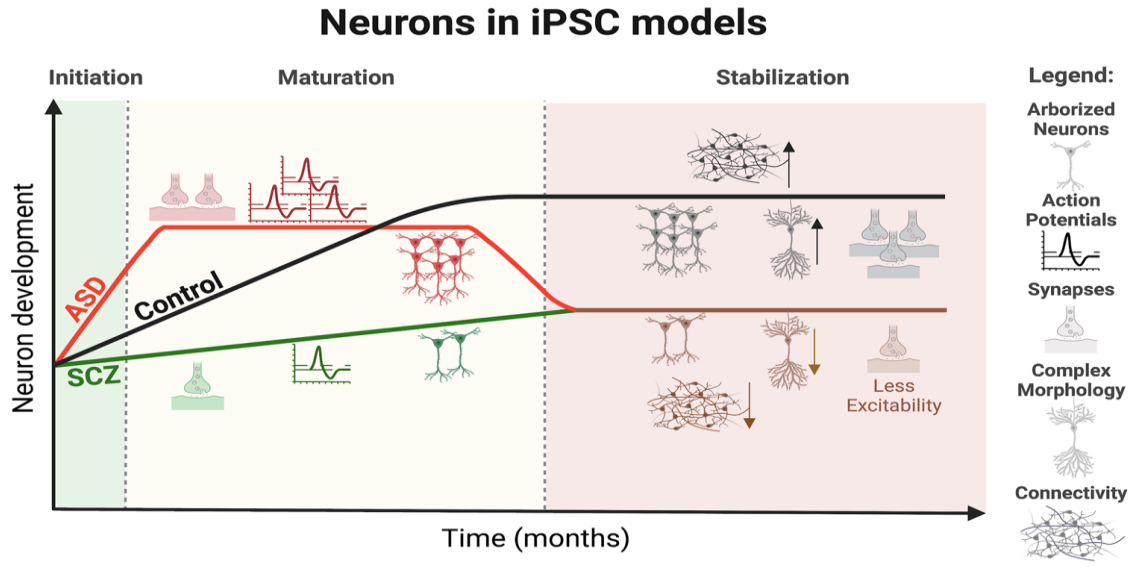
A
ASD type
AS
ASD and other
KS
other
PMS
RTT
ASD
FXS
NS
other, mouse model
PWS
TS
B
Control type
isogenic
isogenic and other
other
unknown
C
Somatic cells origin
Astrocytes
Dermal Fibroblasts
Embryonic fibroblasts
Fibroblasts
Human pluripotent stem cells and Cerebral organoids
Keratinocytes
Reference: Marchetto et al. 2010
shRNAs
Skin Fibroblasts (human embryonic stem cells)
unknown
Dental pulp stem cells
DPCs and Embryonic fibroblasts
Fibroblasts
Fibroblasts and Astrocytes
Human urine epithelium-derived cells
MEFs
SHED
Skin Fibroblasts
Somatic cells
D
Reprogramming technique
Lentivirus
Lentivirus and RNA
Retrovirus
Retrovirus and Lentivirus
Retrovirus and RNA
RNA processing and miRNA
Sendai virus and Lentivirus
unknown
Lentivirus and Retrovirus
Nucleofection and RNA processing
Retrovirus , Lentivirus and Sendai virus
Retrovirus and miRNA
RNA processing
Sendai virus
Sendai virus and Retrovirus
E
iPSC differentiation
Neurons
Neurons, NSCs
Neurons, Organoids
NPCs
NPCs, Neurons
NPCs, Neurons
NSCs
F
Epilepsy
No
Yes
G
Neural cell types
Cortical neurons
Hippocampal neurons
Neurons
Neurons (generic)
Neurons (generic), Astrocytes
Neurons (glutamatergic, GABAergic) , (Brain) Organoids
Neurons (mixed: glutamatergic and GABAergic), (Brain) Organoids
Neurons (mixed)
Neurons, Astrocytes
NPCs, Neurons
NPCs, Neurons (mixed)
Placodal neurons
Cortical Neurons (glutamatergic)
NEAs
Neurons (GABAergic), Astrocytes
Neurons (generic)
Neurons (glutamatergic, GABAergic)
Neurons (glutamatergic)
Neurons (mixed)
Neurons (post-mitotic), Glia
NPCs
NPCs, Neurons (mixed)
Organoids

Figure 4

A


high low
NPC / ASD neurons
Hippocampal
Glutamatergic neurons
GABAergic neurons
GABA receptors
Forebrain neurons
Embryonic stem cell
Cortical
Brain organoids
Astrocytes
Calcium imaging indicating activity
Dendrite/Neurite length
Differential gene expression
Immunostaining imaging
Mitochondrial function
Oxidative stress
Proliferation/Differentiation
Promoter activity
Relative Expression
Spike frequency
Synaptic and network activity
Publications
5↑
0
5↓
10↓

B


Neurons in iPSC models
Initiation
Maturation
Stabilization
Legend:
Arborized Neurons
Action Potentials
Synapses
Complex Morphology
Connectivity
Neuron development
ASD
Control
SCZ
Less Excitability
Time (months)