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Single-cell long-read sequencing in human cerebral organoids uncovers cell-type-specific and autism-associated exons

人脑类器官的单细胞长读长测序揭示细胞类型特异性和自闭症相关的外显子

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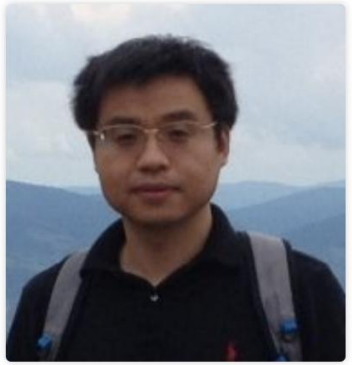
Anguo Liu

2025-5-23

Introduction

Lead Contact Overview



首席研究员

贺信博士是芝加哥大学人类遗传学副教授。Xin 的职业生涯始于实验生物学家：他在中国科学技术大学获得生物化学学士学位，随后在西北大学接受了两年的研究生培训。之后，Xin 转向计算生物学领域，并于 2009 年在伊利诺伊大学厄巴纳-香槟分校获得计算机科学博士学位，师从 Saurabh Sinha 博士。在担任现职之前，Xin 在 UCSF 师从李浩博士，在卡内基梅隆大学师从Kathryn Roeder博士和Ziv Bar-Joseph博士。Xin 于 2014 年加入芝加哥大学。

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张晓昌

Xiaochang Zhang 是芝加哥大学人类遗传学系的助理教授，也是神经科学研究所的成员。他的小组专注于新皮层发育，并试图了解脑细胞类型如何随着时间的推移而指定并在神经系统条件下发生变化。他于 2009 年在上海复旦大学获得博士学位，在韩敏教授的指导下研究大脑和神经肌肉发育中的 SUN-KASH 核包膜蛋白。他继续与科罗拉多大学博尔德分校和霍华德休斯医学研究所的 Han 教授合作，在那里他发现了 microRNA 在压力反应中的关键作用。作为博士后研究员，他与哈佛大学和波士顿儿童医院的 Christopher A. Walsh 教授合作（2011-2017 年），在那里他研究了皮层神经发生的选择性 RNA 剪接和结构性脑畸形的遗传学。他是 NIH 职业发展奖 （K01）、SFARI 飞行员奖和 NIH 主任新创新者奖 （DP2） 的获得者。

Xiaochang Zhang

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brain development neurological disorders alternative splicing

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Upregulation of SYNGAP1 expression in mice and human neurons by redirecting alternative splicing R Yang, X Feng, A Arias-Cavieres, RM Mitchell, A Polo, K Hu, R Zhong, ... Neuron 111 (10), 1637-1650. e5	38	2023
Orgo-Seq integrates single-cell and bulk transcriptomic data to identify cell type specific-driver genes associated with autism spectrum disorder ET Lim, Y Chan, P Dawes, X Guo, S Erdin, DJC Tai, S Liu, JM Reichert, ... Nature Communications 13 (1), 3243	19	2022

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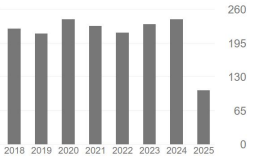

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- Neuroscience
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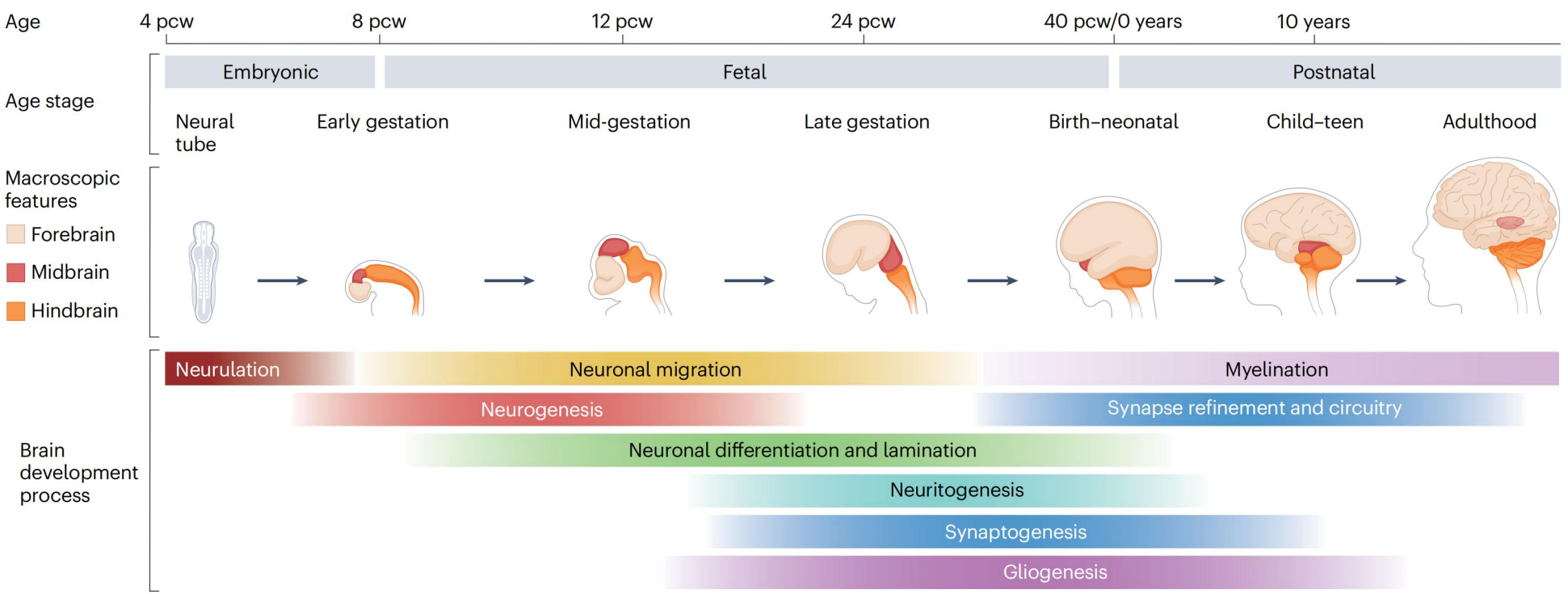
- RNA biology

Human brain development

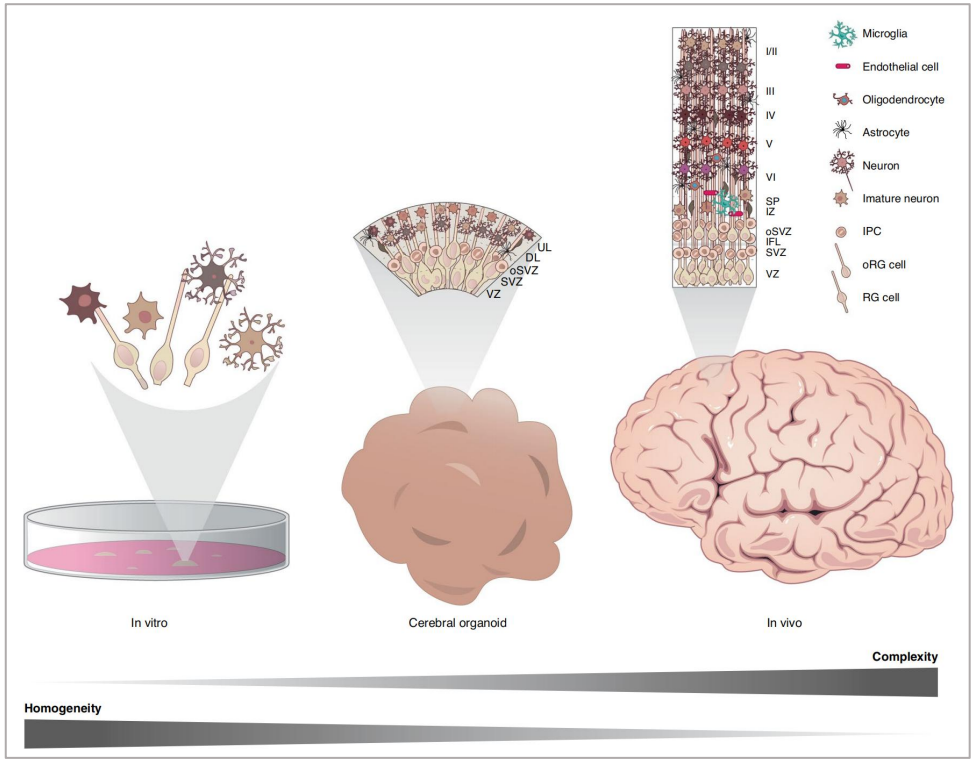


Zhou et al. ***Nat Rev Genet*** 2024

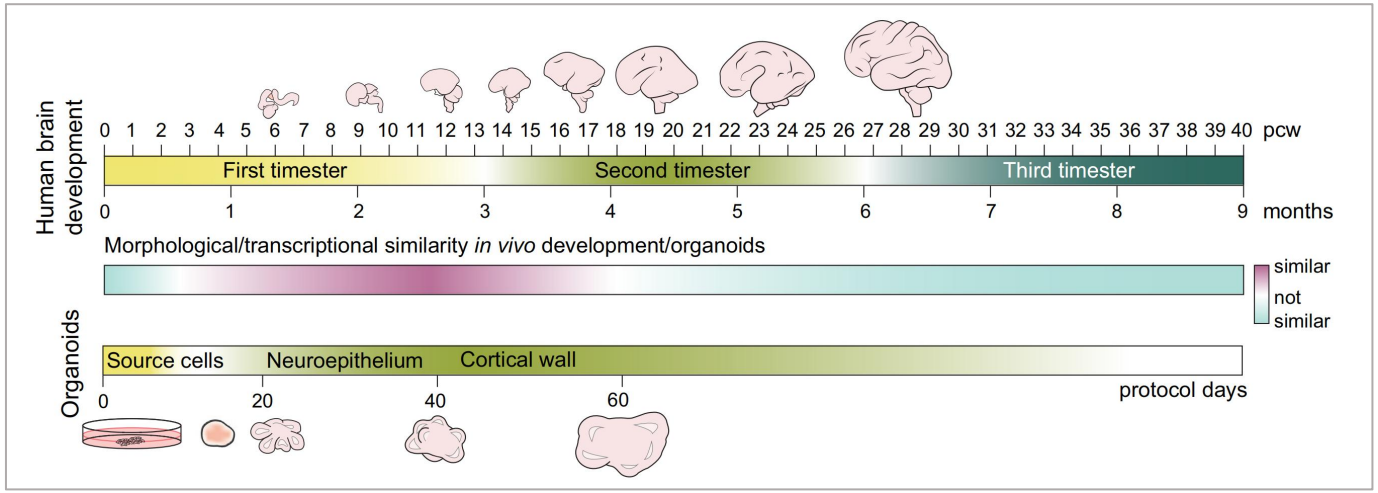
- **Dysregulation** (调控异常) or **perturbation** (扰动) of each procedure can pontentially lead to **neurological disorders**.

Human brain organoids

Ethic permission **Sample availability** **Reproducibility**



Chiaradia & Lancaster. ***Nat. Neurosci.*** 2020

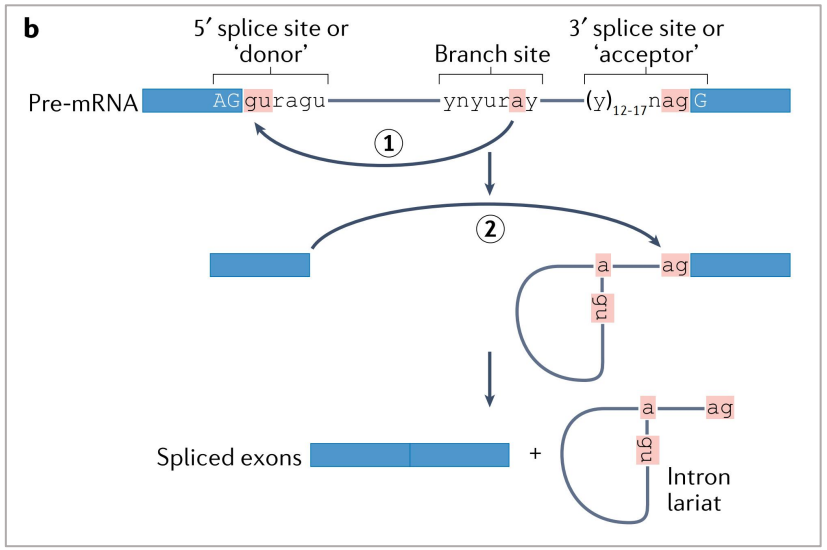


Kelava & Lancaster. ***Dev. bio.*** 2016

- The developing human brain has been increasingly modeled by **cerebral (大脑) organoids** for <u>developmental mechanisms</u> and <u>human diseases</u>, such as autism.

Alternative splicing

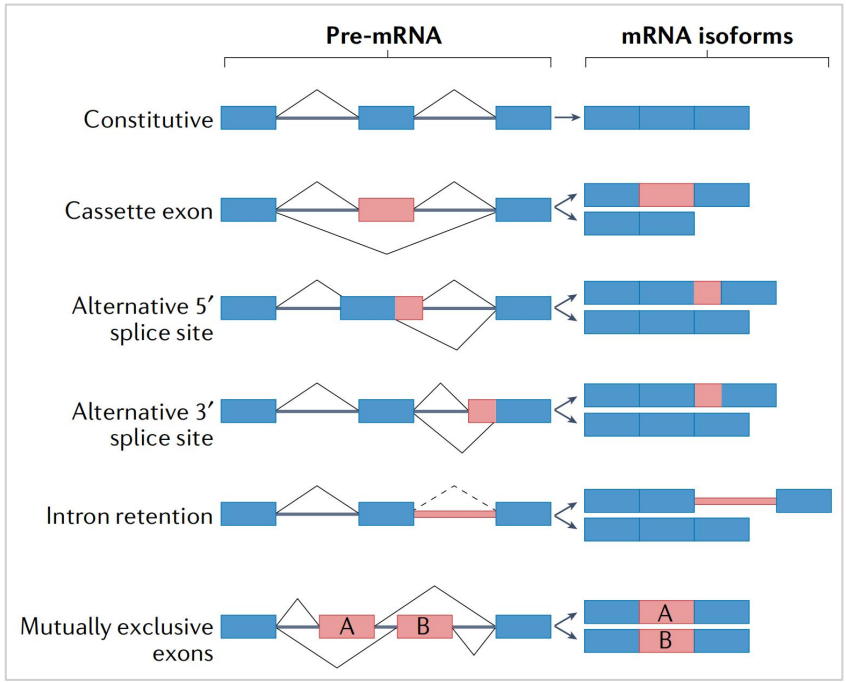
RNA splicing



Marasco & Kornblihtt ***Nat. Rev. Mol. Cell Biol*** 2023

Multiple exons

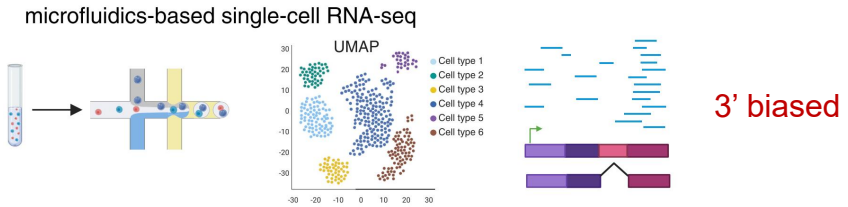
Alternative splicing (AS)



- AS regulates **>90%** of human genes.
- As is a major mechanism for generating **protein diversity**.
- The human **brain** displays the **most distinctive AS pattern** compared to other tissues.
- Mutations disrupting **alternative exons** can cause human brain malformation (畸形) and epilepsy (癫痫).

Current limitations & Research gaps

Limitations:



Belchikov et al. ***Genome Res.*** 2024

Current short-read scRNA-seq platforms:

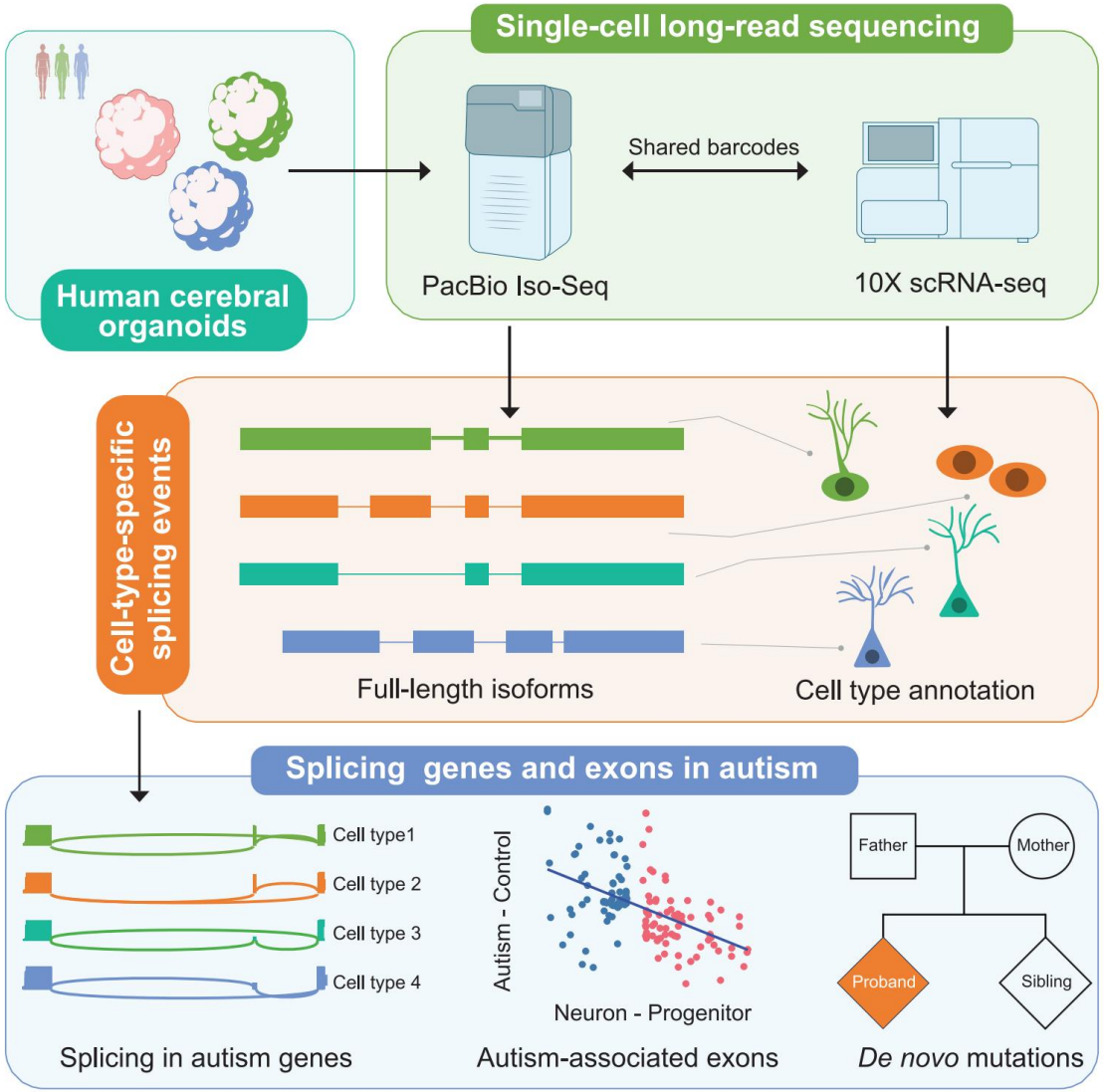
- predominantly built on read counts at the **3'** or **5'** end of polyA transcripts, do not generate sufficient coverage for splice junctions;
- misses the opportunity to uncover coordinated splicing events.

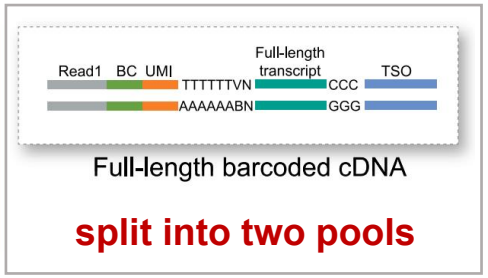
Gaps:

What we don't know:

- The expression of full-length splice isoforms in human neural development;
- The extent of cell-type-specific pre-mRNA splicing;
- How human genes are differentially spliced between cell types in neural development.

Abstract & Framework



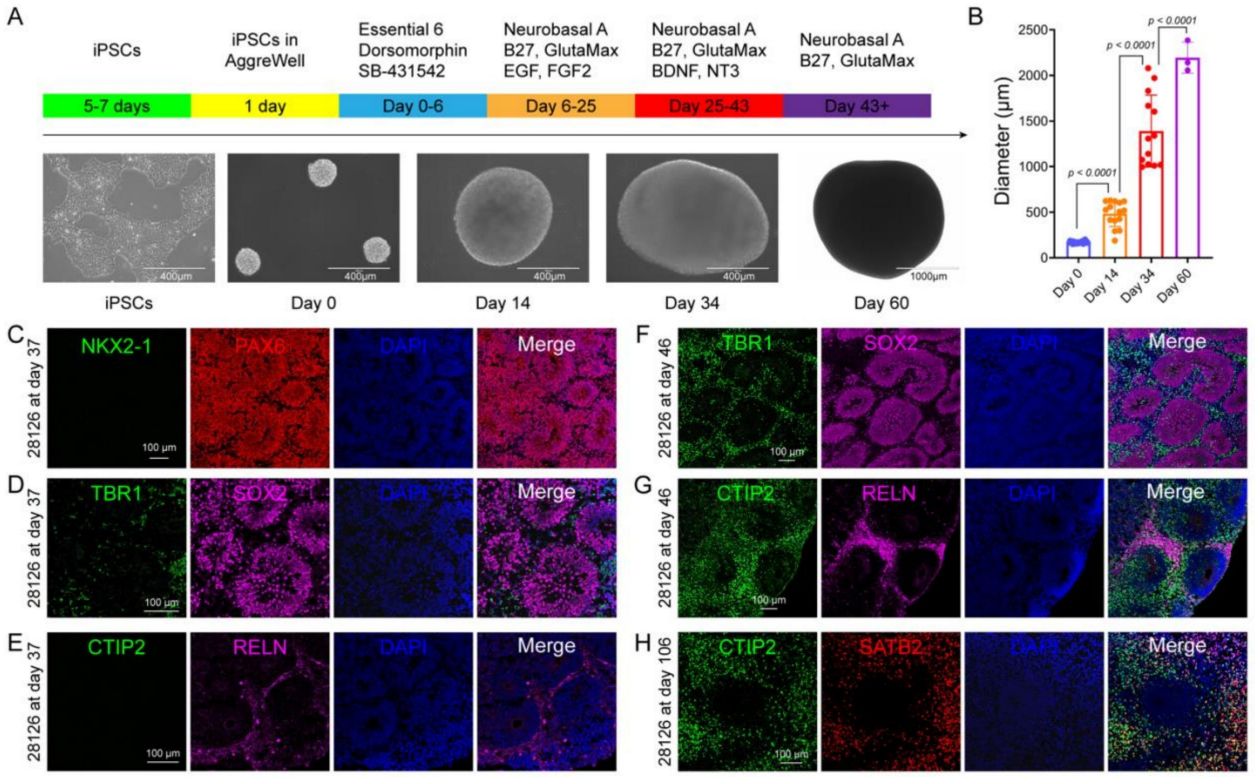


Aim:

Fill the gaps by **single-cell long-read sequencing** in human **cerebral organoids**.

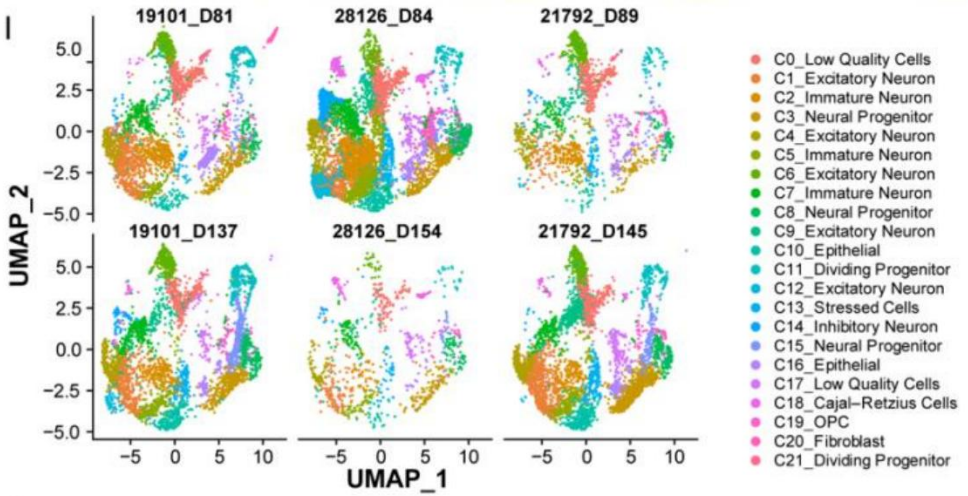
- Identifiy previously unannotated exons and splice isoforms.
- Uncover pervasive splicing changes across neural cell types.
- Find autism-associated exons and *de novo* mutations that are enriched in cell-type-specific exons.

Generate human cerebral organoids



scRNA-seq:

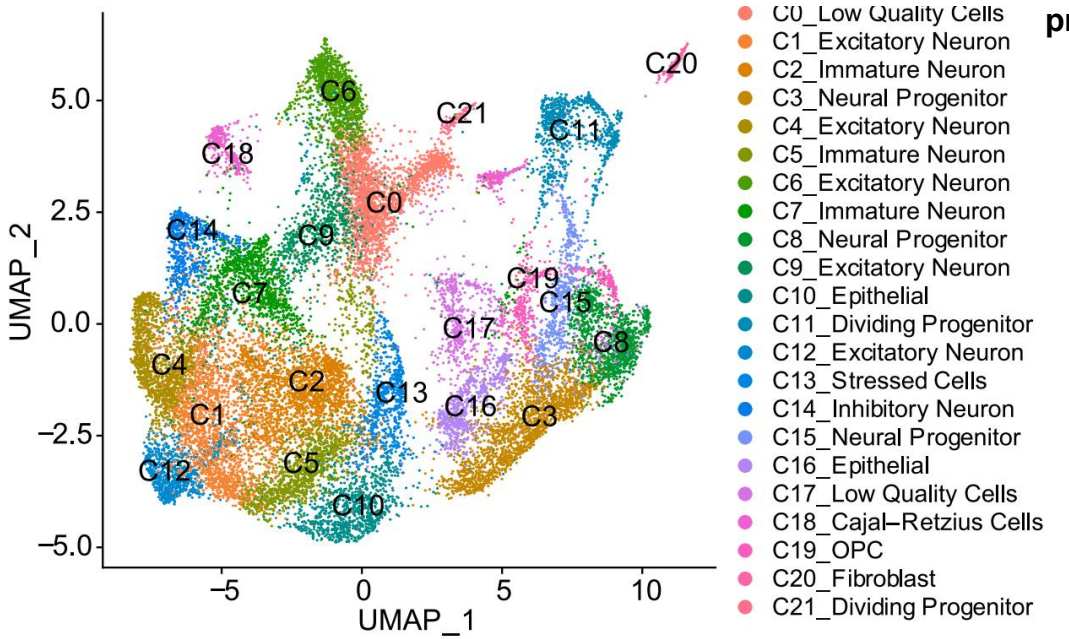
- **6 batches** of cerebral organoids,
- at **3–5 months** of growth

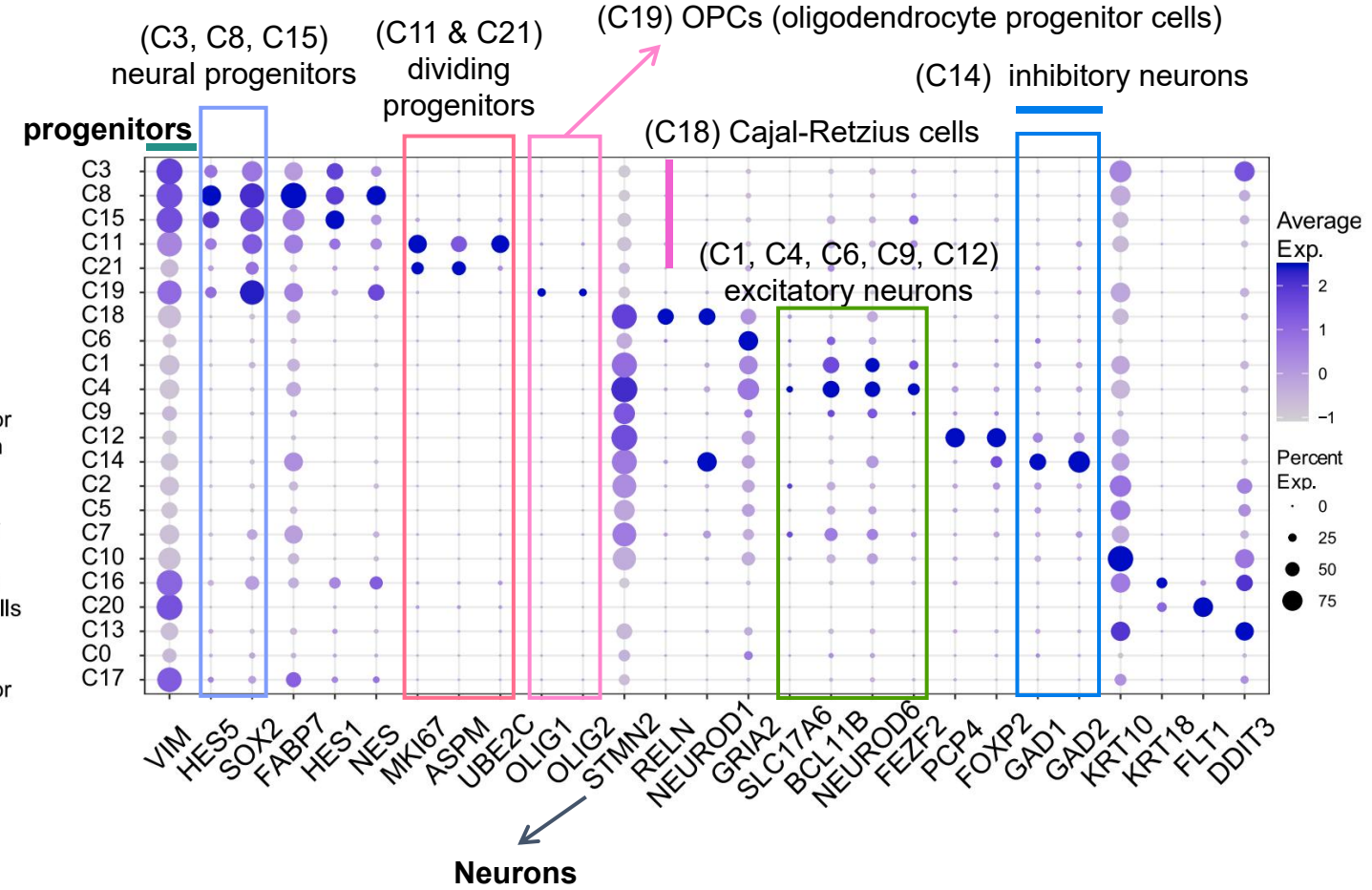


3 independent human iPSCs → Dorsal (背侧) forebrain (前脑) organoids

Single-cell and long-read RNA sequencing of human cerebral organoids

28,290 cells - 22 clusters (Seurat)



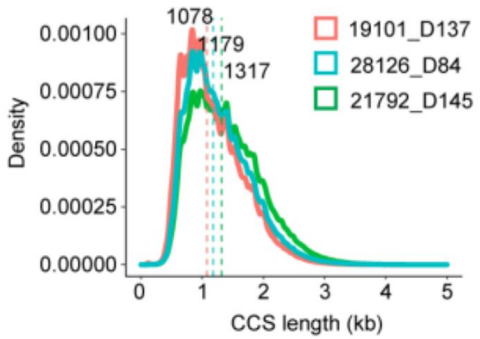


(C0) low-quality cells

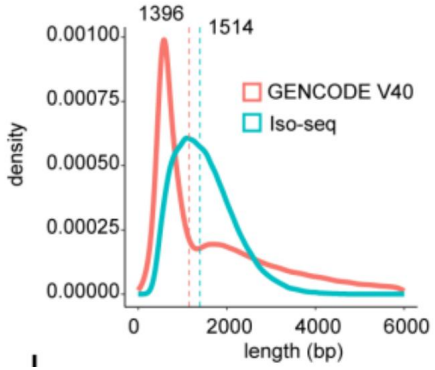
- >5,000 or < 300 detected genes;
- over 5% mitochondrial gene counts.

3/6 organoid samples showing balanced cell types and cell numbers were selected for Iso-seq.
(19101_D137 [day 137], 28126_D84, and 21792_D145)

Long-read RNA sequencing of human cerebral organoids



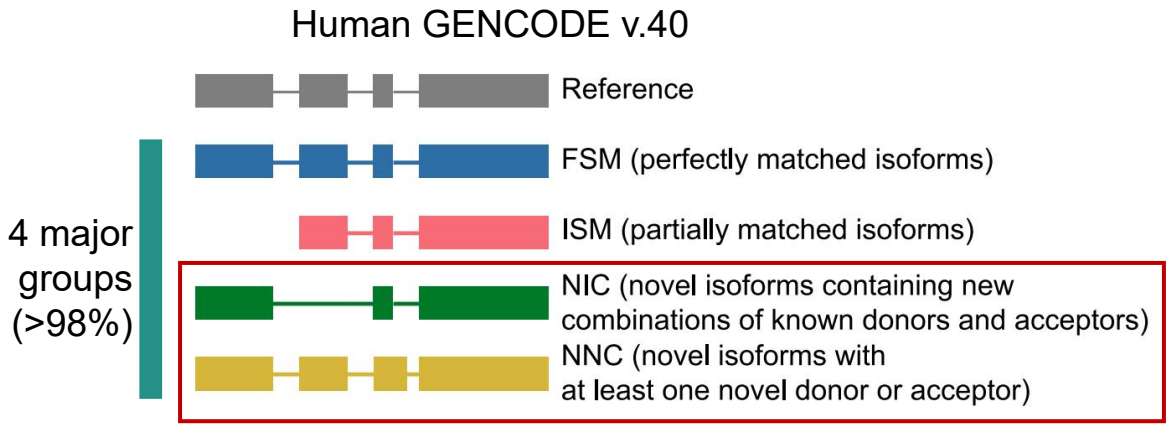
- 2-3 SMRT cells (PacBio Sequel II)
- 26.0 million CCS reads
- **Read median length: >1 kbp**

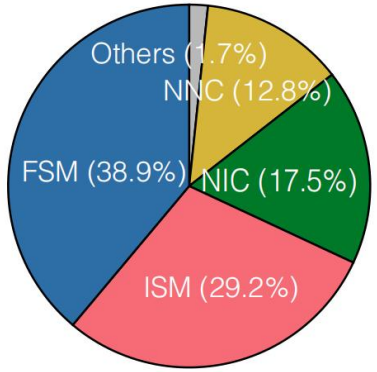


Isoform median length

- 1,514 bp (this study)
- 1,396 bp (GENCODE ref)

103,007 non-redundant isoforms





New isoforms

4,820 previously unannotated exons

4,137 coordinated splicing events

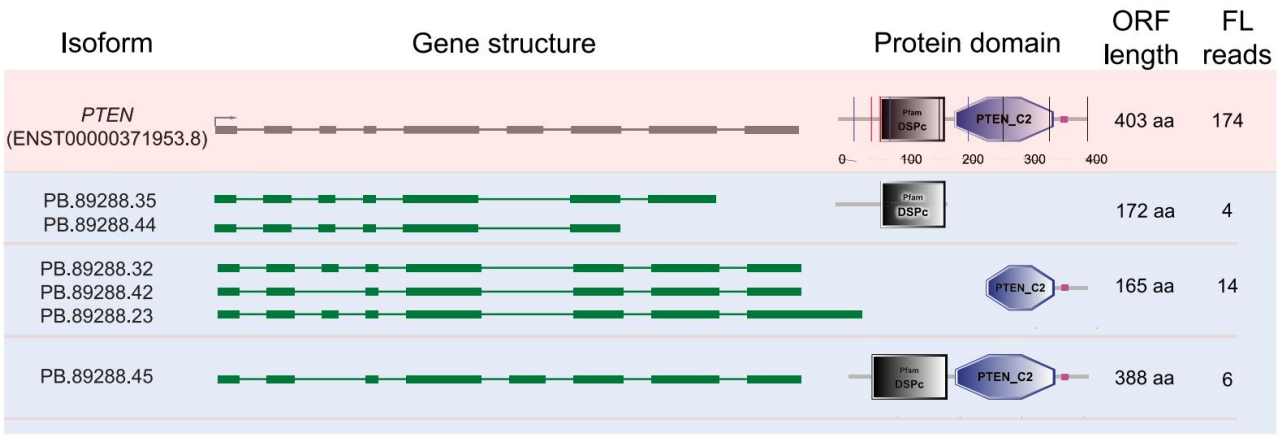
31,181 previously unannotated splice isoforms

Long-read sequencing captured **Full-Length** transcripts.

Long-read RNA sequencing of human cerebral organoids

PTEN
a tumor suppressor
one of the most extensively studied autism (ASD, 自闭症) genes.

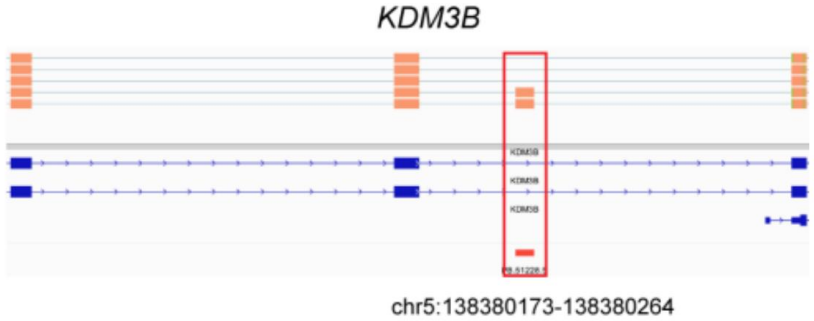
New isoform



13 FSM, 8 NIC, and 8 ISM isoforms

unannotated PTEN splice forms may lead to:
1) the deletion of the catalytic N-terminal phosphatase domain
2) the shortening of the C2 domain.

New exon

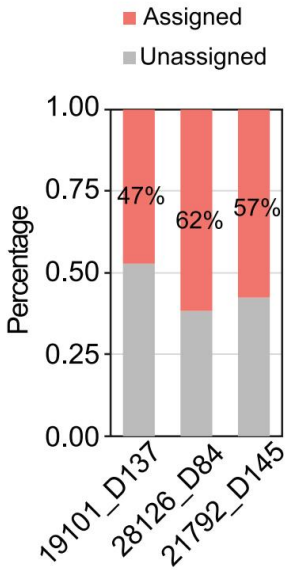


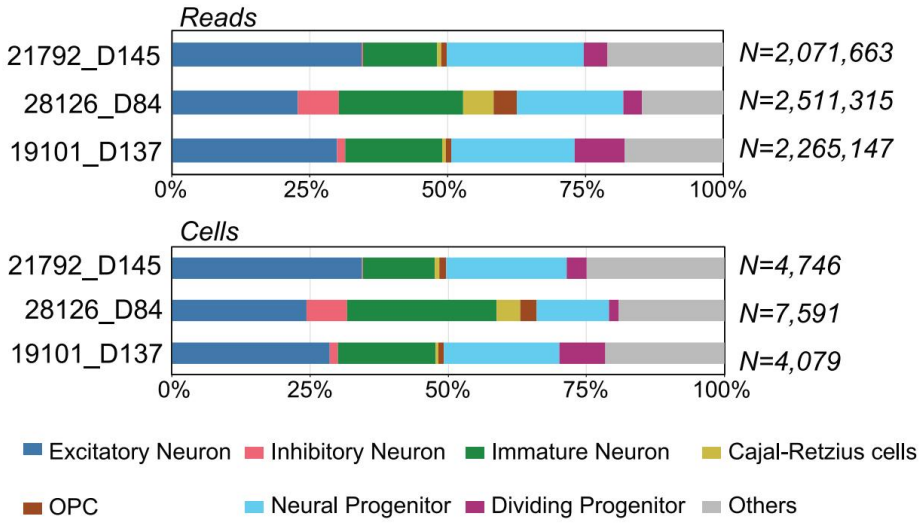
111 previously unannotated exons in SFARI Autism Genes

- The FL transcriptome data greatly expanded the isoform catalog for cell types in the human cerebral organoids.
- the scIso-seq uncovers previously unannotated splice isoforms that are biologically relevant.

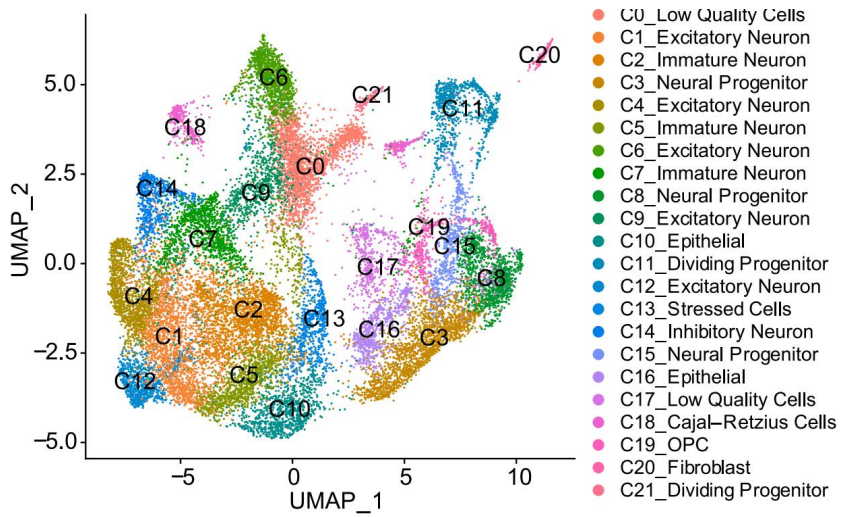
Combining Single-cell and long-read RNA-seq data

54.9%
12.47 million FLNC reads → assign cell barcodes → annotated cell types





Iso-Seq reads → assign → 7 major cell types

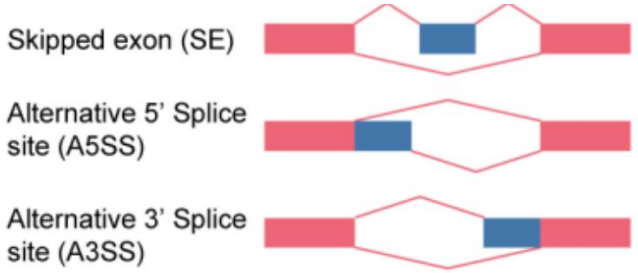


Extensive alternative exon usage in human cerebral organoids

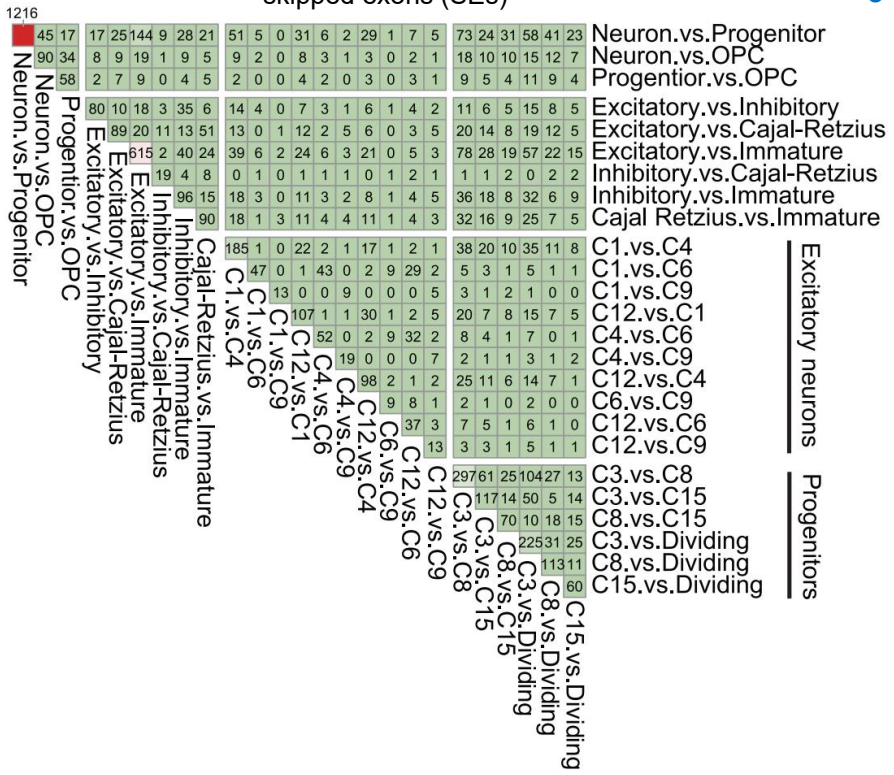
Differential splicing exons (DSEs) between cell types

FLAIR (commonly-used in long-read RNA-seq data)

3 types



skipped exons (SEs)



3 levels

(1) among neurons, progenitors, and OPCs

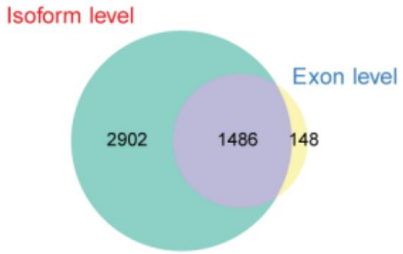
(2) between neuronal types

(3) between subtypes of:

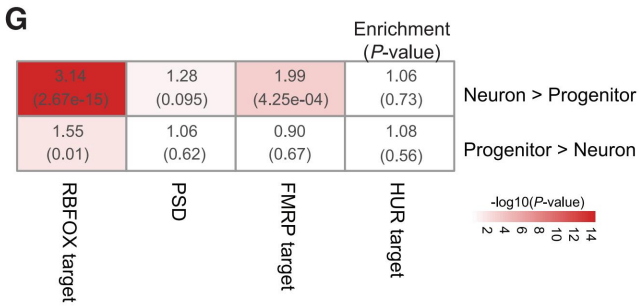
- excitatory neurons;
- progenitors

25 pairwise comparisons

2,393 DSEs
(|△PSI(percent spliced in) | ≥ 5% and [adj.p] < 0.05)

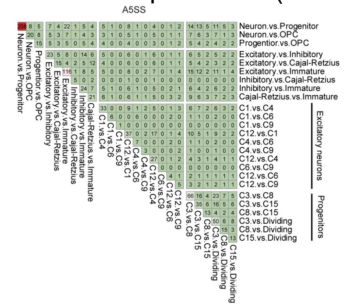


Most of the genes (1,486/1,634 = 90.9%) that contained DSEs were also differentially expressed at the isoform level.

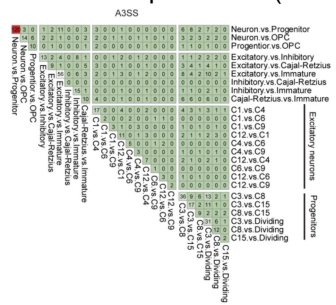


Genes harboring neuron-specific exons were enriched for postsynaptic density (PSD) proteins (Figure 2G).

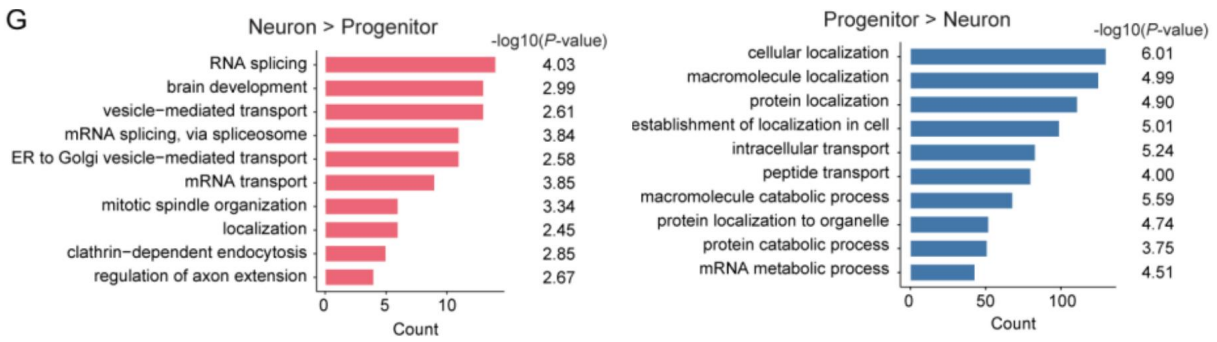
alternative 5' splice sites (A5SSs)



alternative 3' splice sites (A3SSs)

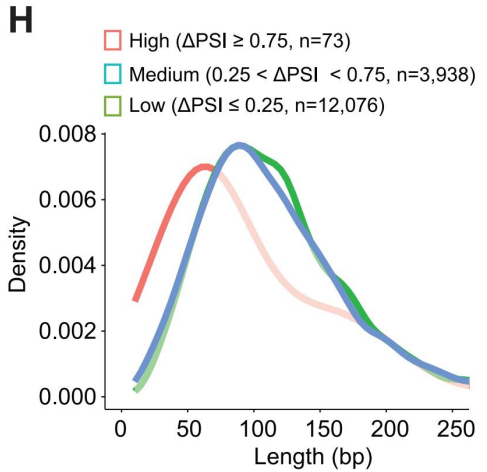


Extensive alternative exon usage in human cerebral organoids



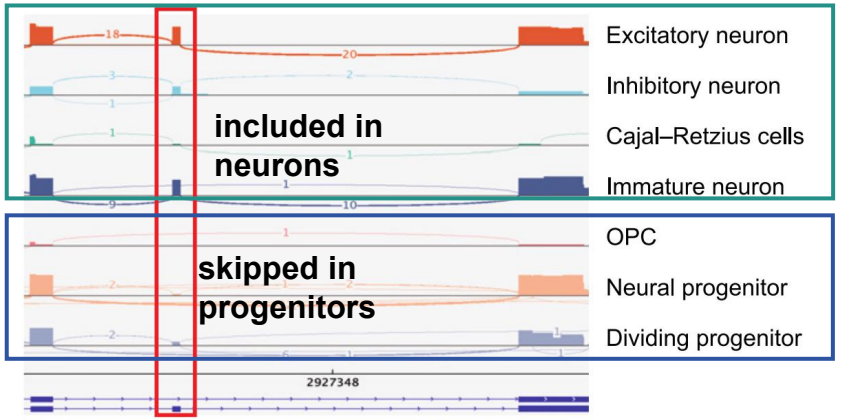
Genes with DSE genes enriched in:

- biological processes for RNA splicing
- brain development
- cellular localization and transport.



most variable exons:
significantly **shorter** lengths.

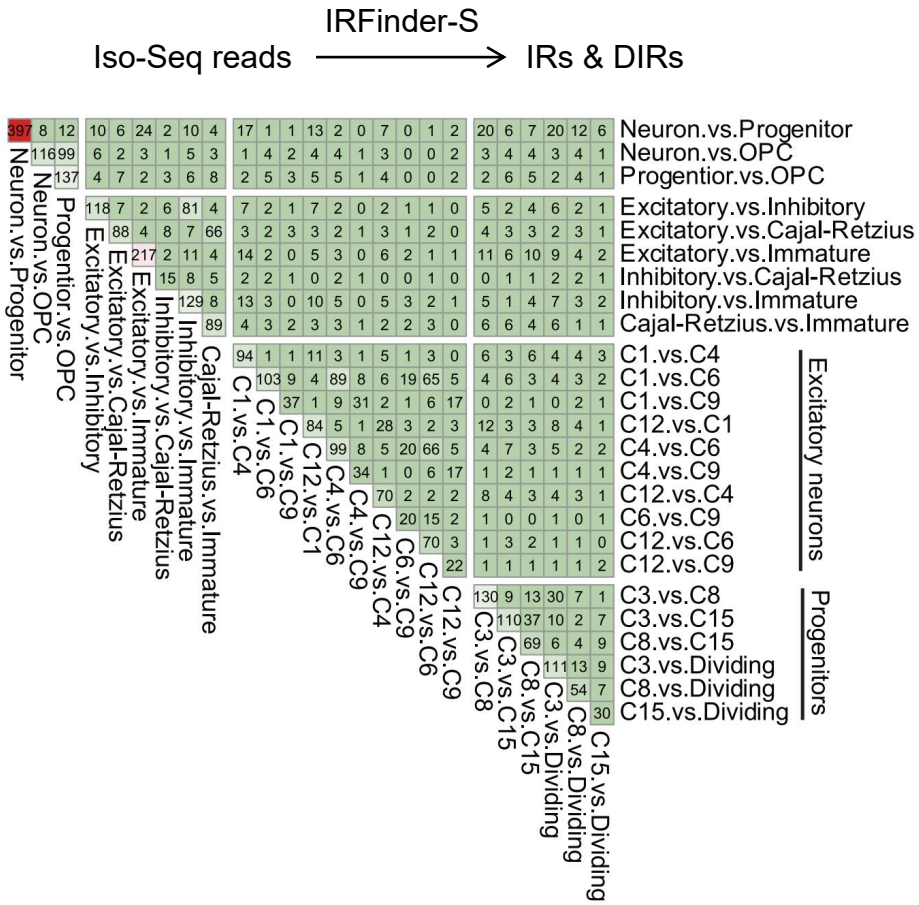
73 highly variable switch-like exons



ADD1 (chr4:2,926,641-2,926,675)

- Cell-type-specific exons are prevalent in neural development.

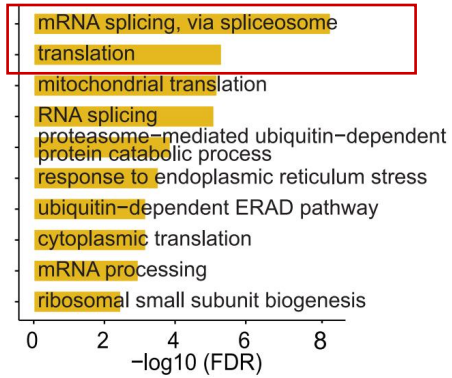
Differentially retained introns (DIRs) between neural cell types

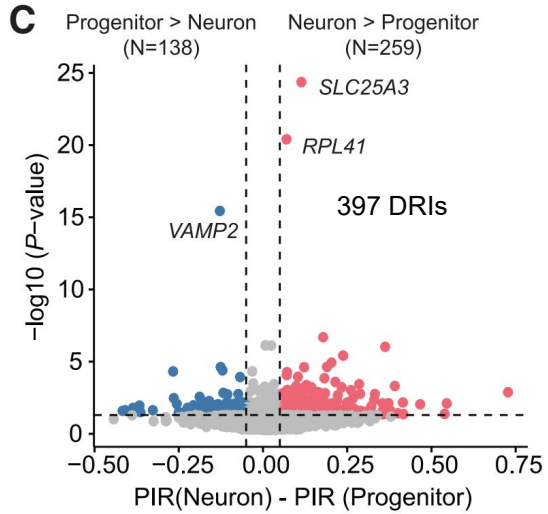


1,427 differentially retained introns (DRIs)
(|△PIR| ≥ 5% and FDR < 0.05)

percent intron retention

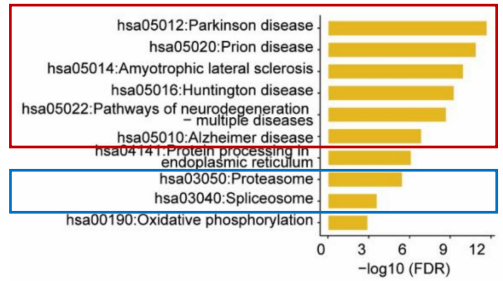
DRI genes GO enrichment:





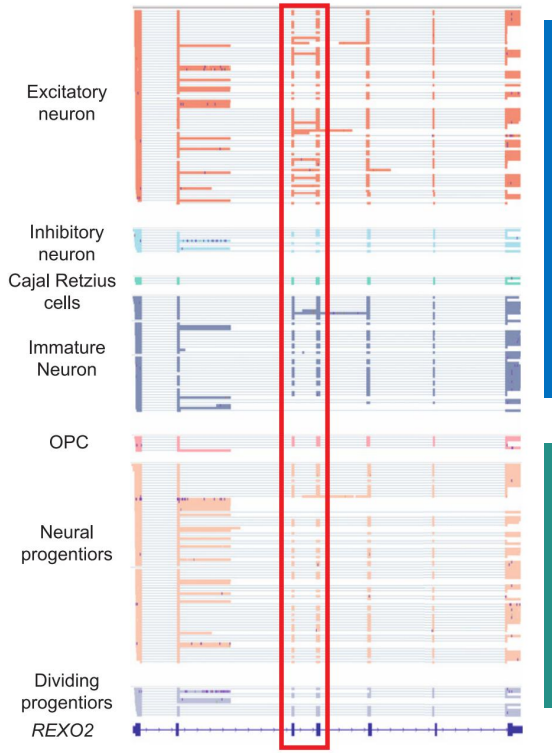
More prevalent in neurons

DRI genes KEGG enrichment:



neurological diseases

mRNA processing



partially retained in neurons

spliced out in progenitors

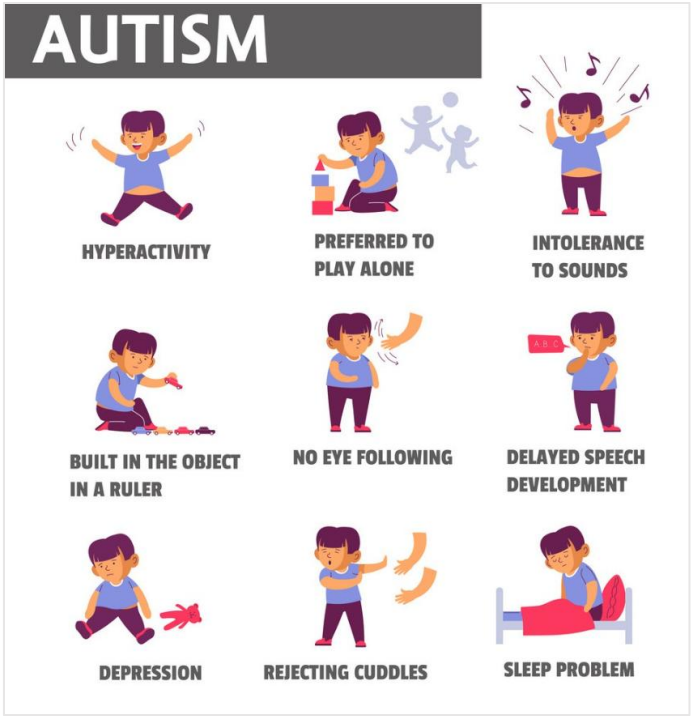
AS of ASD risk genes

Autism spectrum disorder (ASD) **impairs social interaction** and affects about 2.8% of 8-year-old children in the US.

自闭症，又称孤独症，是一种较为严重的神经发育障碍性疾病[1]。典型自闭症，其核心症状就是所谓的“三联症”，主要体现为在社会性和交流能力、语言能力、仪式化的刻板行为三个方面同时都具有本质的缺损。

其主要症状为： 1、社交障碍：一般表现为与他人交往困难或不愿意交往，严重者甚至与父母缺乏情感依恋；

2、语言交流障碍：完全无语言、语言发育落后、语言能力倒退，或者鹦鹉学舌式重复语言； 3、重复刻板行为：兴趣狭窄、异常动作频繁、性格固执不愿意接受改变。 不典型自闭症则在前述三个方面不全具有缺陷，只具有其中之一或之二。



On the other hand, **ASD genes** have been reported to **express different isoforms** that are important for neural development and synaptic connectivity.

SFARI GENE

SFARI Gene is an evolving database for the autism research community that is centered on genes implicated in autism susceptibility.

1 CATEGORY 1 (High Confidence)

2 CATEGORY 2 (Strong Candidate)

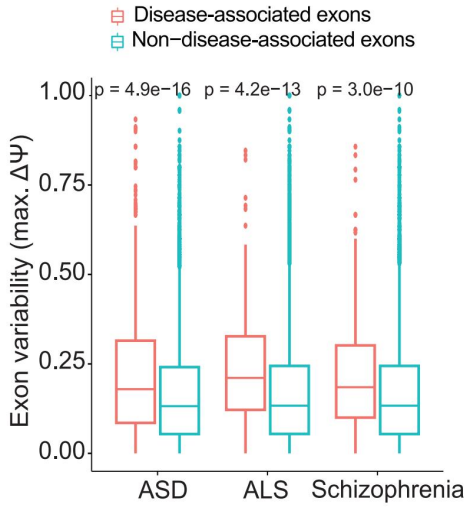
S SYNDROMIC

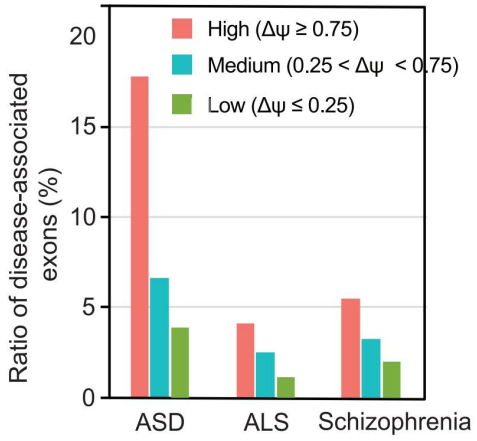
A comprehensive database that includes any **gene associated** with autism risk.

DSEs inform the splicing disruption and genetics of autism

Inclusion variability across cell types for exons associated with ASD, ALS (肌萎缩侧索硬化), and schizophrenia (精分).

Disease-associated exons *VS* non-disease-associated exons

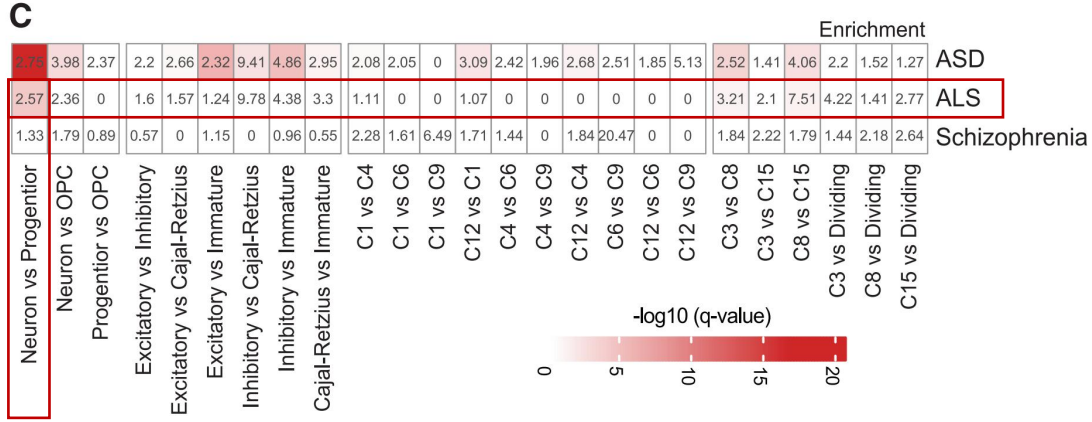




- Significantly higher variability across cell types
- The most significant difference observed in ASD

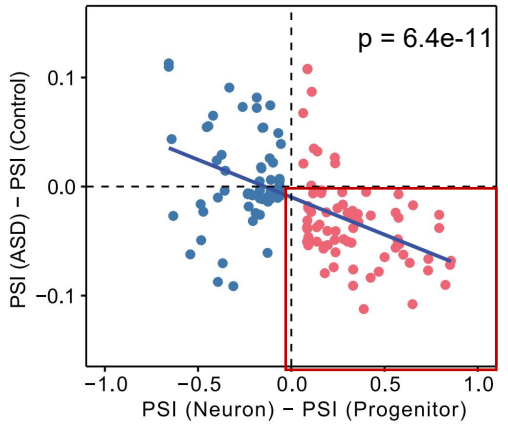
Disease-associated exons:
defined based on differential splicing between postmortem brain samples from affected individuals and controls.

C



significant enrichment in ASD-associated exons.

AS pattern



144 differentially spliced exons

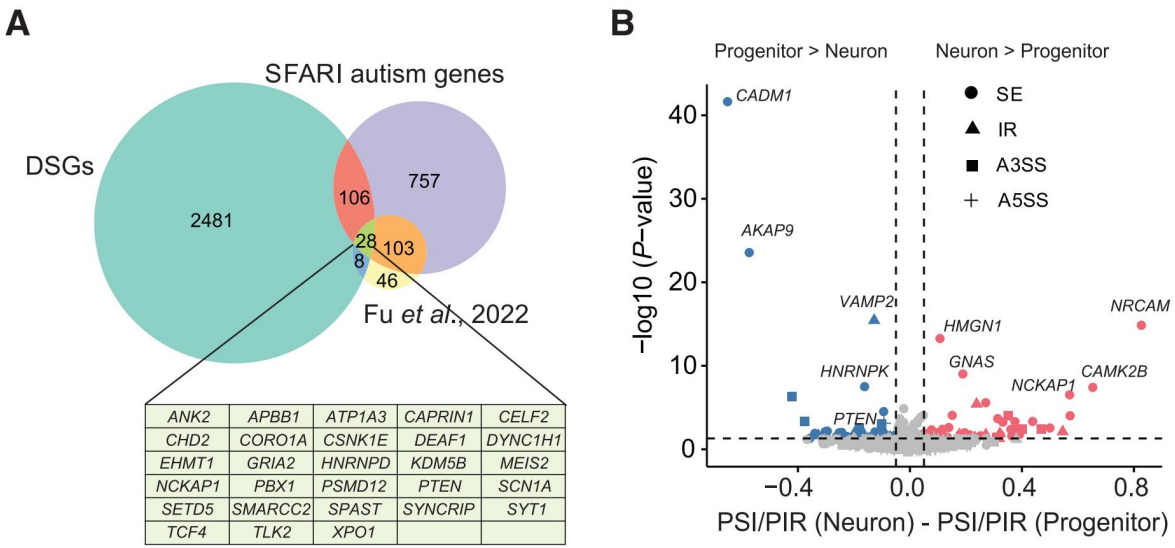
(1) between neurons and progenitors

(2) between individuals with ASD and control subjects

Individuals with ASD appears closer to the splicing state in progenitors than in differentiated neurons.

AS of ASD risk genes

Examined the splicing pattern of specific ASD genes cataloged in (1) the SFARI database, and (2) a recent study.

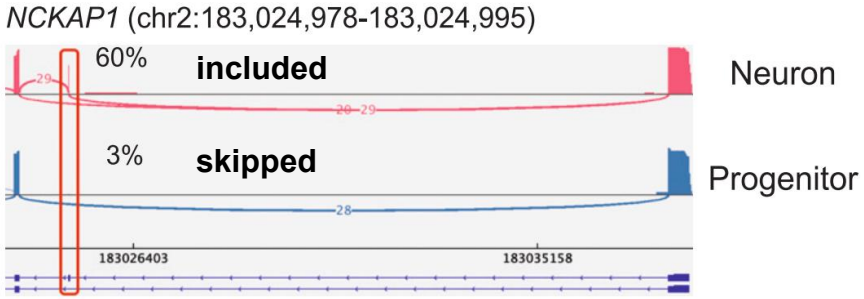


ANK2	*APBB1*	*ATP1A3*	*CAPRIN1*	*CELF2*
CHD2	*CORO1A*	*CSNK1E*	*DEAF1*	*DYNC1H1*
EHMT1	*GRIA2*	*HNRNPD*	*KDM5B*	*MEIS2*
NCKAP1	*PBX1*	*PSMD12*	*PTEN*	*SCN1A*
SETD5	*SMARCC2*	*SPAST*	*SYNCRIP*	*SYT1*
TCF4	*TLK2*	*XPO1*		

142 ASD genes were differentially spliced between neural cell types

PSD (postsynaptic density, 突触后密度) proteins

constitute ~1/4 ASD genes (33/142)



18-bp microexon

ASD gene; regulates neuronal cytoskeletal dynamics

Also lower in the neocortices of ASD individuals

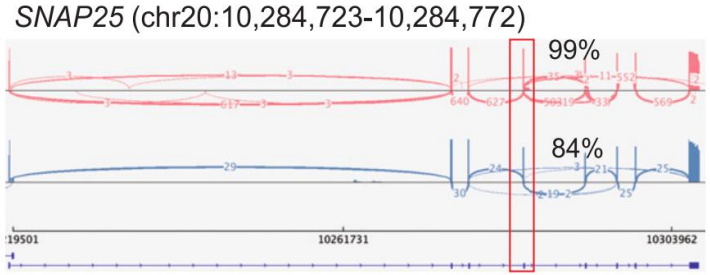
PSI, percent spliced in
PIR, percent intron retention

AS of ASD risk genes

5 ASD genes

SNAP25, *HNRNPD*, *SF3B1*, *METTL26*, *JMJD1C*

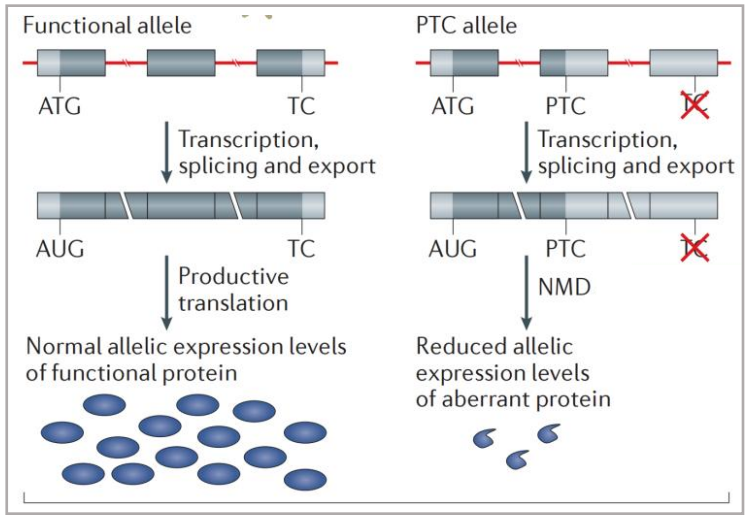
AS potentially coupled with NMD (nonsense-mediated mRNA decay)



Neuron

v

Progenitor



deleterious *de novo* mutation (DNM)

c.142G>T, p.Val48Phe

SIFT value = 0.01
PolyPhen value = 0.997

The exclusion of the *SNAP25* DSE

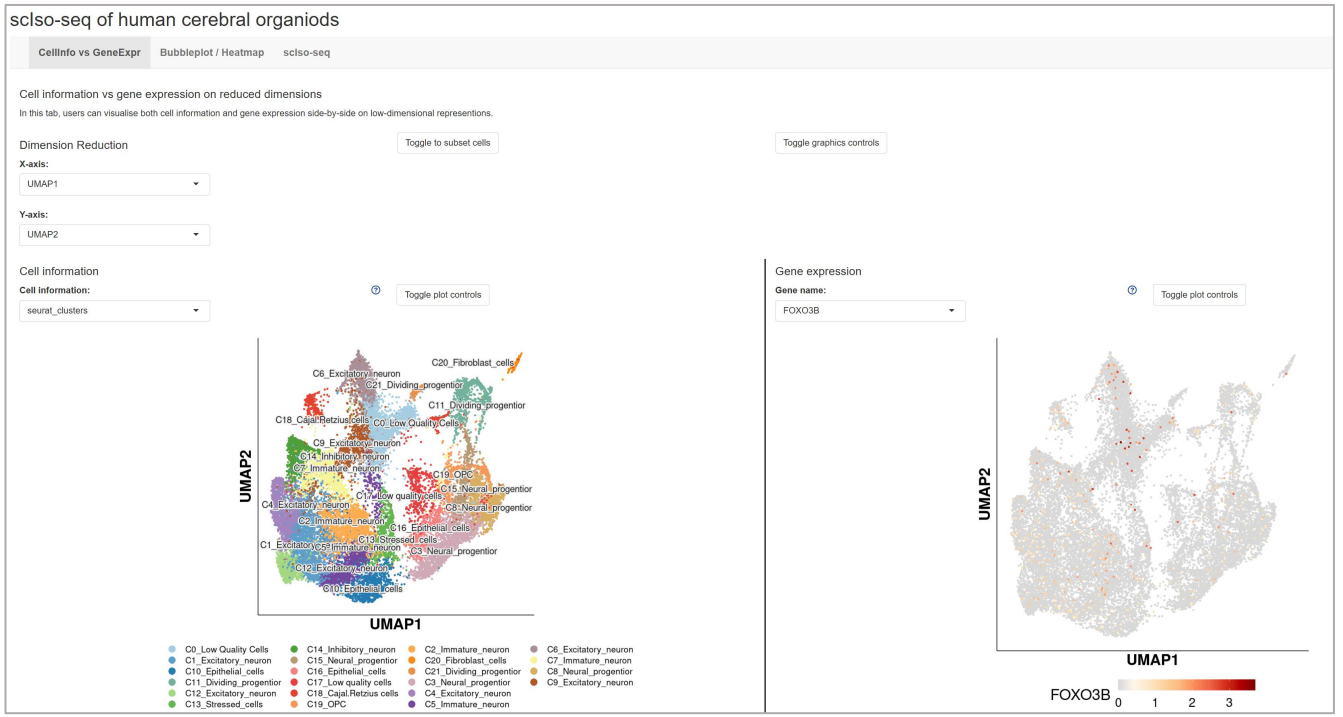
frameshift
premature stop codon

Cell-type-specific NMD exons regulate gene expression and may contribute to autism pathogenesis.

Summary & Discussion

Novelty & Significance:

- scIso-seq analysis of cerebral organoids (data & resource);
- Expanded transcript annotation & splicing landscape;
- Provide a cell-type-specific full-transcript reference of human cerebral organoids;
- analyzed the regulatory mechanism of cell-type-specific cassette exons during neurogenesis.



Limitations:

(1) variations in the cell-type proportions were frequently seen in different organoid batches;

(2) the in vitro culture of cerebral organoids is different from the developing brain, its lack of vasculature and limited neuronal differentiation, brain samples are needed !

(3) involving **patient-derived** iPSCs and organoids would be much more convincible.

Thanks for your attention!

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