A large schizophrenia family with a highly penetrant, rare, nonsynonymous functional variant in the NRXN1 interacting Caskin 1

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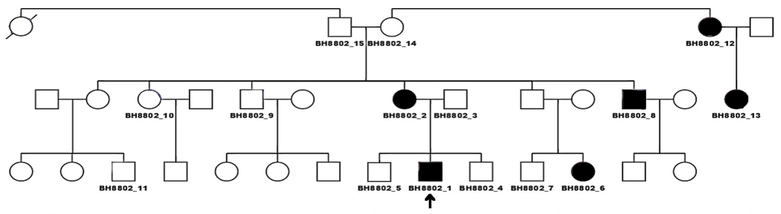
Introduction

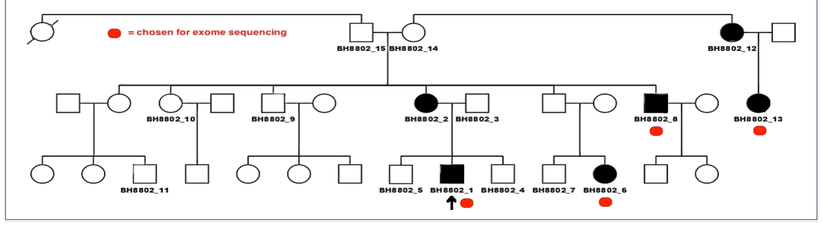
- A large family with history of psychiatric illness presented with what appears to be an autosomal dominant inheritance pattern with high penetrance (< 100% including 2 obligate carriers).
- DNA is available from individuals with identifying numbers.
- Proband is marked with an arrow.



- Clinical evaluation: MINI International Neuropsychiatric Interview and detailed medical records review.
- Best estimate diagnoses using DSM-IV criteria after a 2 stage consensus diagnosis (SD, SB and NS).

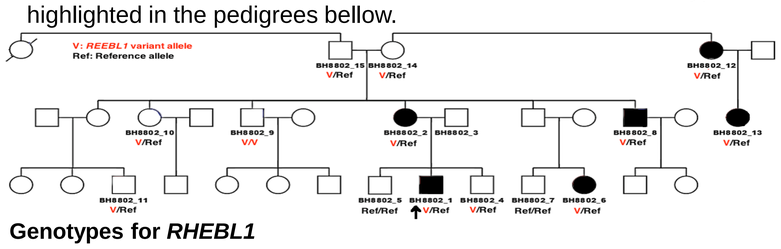
Code	Sex	Age (2016)	Diagnosis (MINI Interview)	DSM IV Diagnosis
BH8802_12	F	74	Psychotic Disorder	Schizophrenia
BH8802_13	F	49	Psychotic Disorder	Schizophrenia
BH8802_14	F	82	Healthy	Healthy
BH8802_15	M	86	Healthy	Healthy
BH8802_10	F	58	Healthy	Healthy
BH8802_9	M	56	Healthy	Healthy
BH8802_8	M	55	Psychotic Disorder	Schizophrenia
BH8802_2	F	50	Psychotic Disorder	Schizophrenia
BH8802_11	M	30	Healthy	Healthy
BH8802_5	M	31	Healthy	Healthy
BH8802_1	M	30	Mood Disorder with psychotic features	Bipolar Disorder
BH8802_6	F	27	Psychotic Disorder	Schizoaffective Disorder (bipolar type)
BH8802_4	M	26	Healthy	Healthy
BH8802_7	M	23	Healthy	Healthy

- Most distant affected relatives to the proband (marked with red circles) were whole exome sequenced to minimize shared alleles that are only due to relatedness.
- Sequencing was done by the Baylor Hopkins Center for Mendelian Genomics.



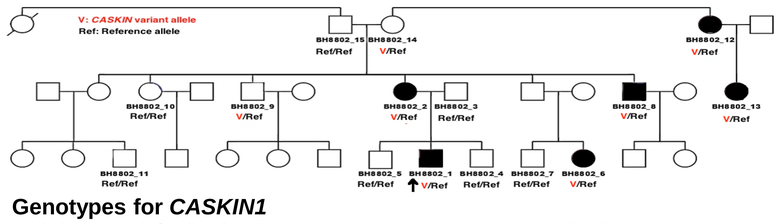
Exome sequencing identified 2 variants in genes expressed in brain that pass initial filtering

- Criteria for initial variant filtering:
  - Allele frequency <1% in public data bases.
  - Present in a gene expressed in brain.
- 2 variants identified are in the genes *RHEBL1* and *CASKIN1.*
- All available DNA was then sequenced for both variants. Genotypes highlighted in the pedigrees bellow.



Genotypes for *RHEBL1*

- This variant was excluded because there is a homozygote and multiple heterozygous individuals who were unaffected.



Genotypes for *CASKIN1*

- Linkage analysis shows perfect segregation of the *CASKIN1* variant with a LOD score of 2.2, the family's maximum score.

Variant in *CASKIN1* predicted to be damaging

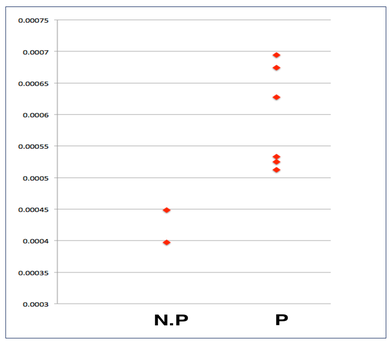
	Variant	pLI	missense Z	Presence in databases	VEST	fathmm	LJB-all	SIFT
RHEBL1	Valine 114→ Leucine	0	0.96	N/A in dbSNP, ExAC, gnomAD	0.6	0.98511	0.56	0.2
CASKIN1	Aspartate 1204 → asparagine	1	2.65	N/A in dbSNP, ExAC, gnomAD	0.025	0.94836	0.77	0.05

- **pLI:** Probability of loss of function intolerance.
- **missense Z:** + Z scores indicate intolerance to variation.
- **VEST:** Predicts the functional significance of a missense mutation based on the probability that it's pathogenic. Scores near 1 indicate a functional prediction.
- **SIFT:** Predicts an amino acid substitution's affect on protein function. Close to 0 scores mean substitution is intolerable.
- **fathmm:** Predicts the consequences of coding variants. Variant is considered deleterious at scores > 0.5.

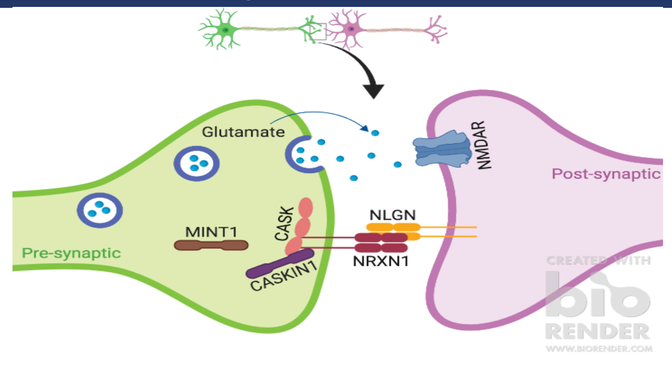
Hypothesis: CASKIN1 (Aspartate 1204 → asparagine) is the causative SZ highly penetrant variant in this family

Low SZ polygenic risk scores in non-penetrant individuals

- Polygenic Risk Scores (PRS) for all individuals were calculated using the PGC2 GWAS as reference.
- All possible permutations were calculated for this family and this perfect separation can be observed by chance ~21% of the time.
- Albeit weak, this is consistent with polygenic scores being important for the penetrance of other damaging variants

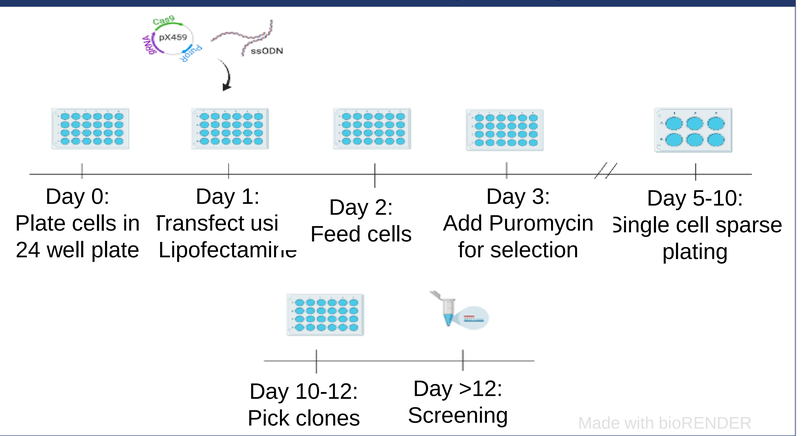


CASKIN1 competes with MINT1 for binding CASK, and directly interacts with NRXN1



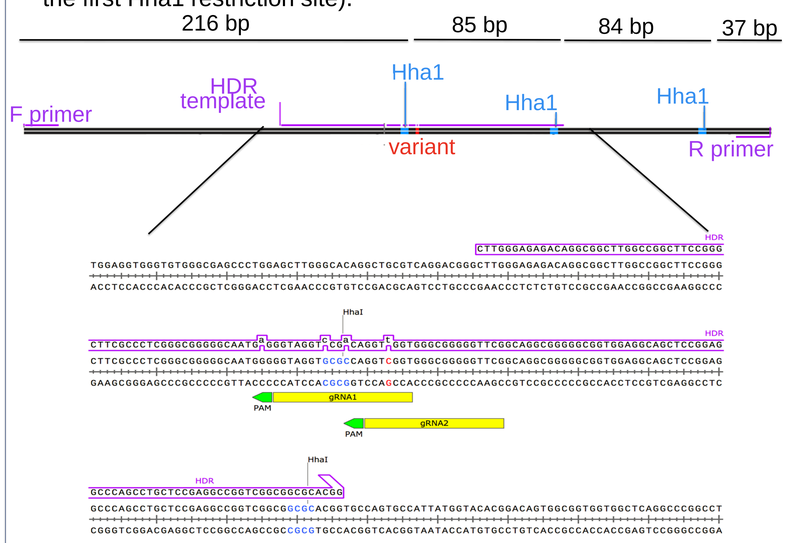
- The simplified graph shows 2/3 of the proteins involved in tripartite complexes that include CASK, and either MINT1 or CASKIN1 (as the compete for CASK interaction) and their interaction with neurexin at the synapse.

CRISPR/Cas9 used to introduce *CASKIN1* variant into hiPSCs to asses functionality in isogenic lines

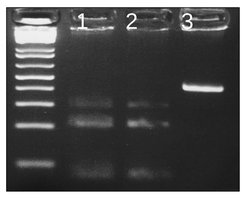


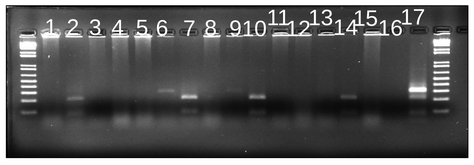
CRISPR/Cas9 used to introduce *CASKIN1* variant into hiPSCs to asses functionality in isogenic lines

- We introduced 4 bp changes in *CASKIN1*. The Variant and 3 synonymous changes (abolishment of gRNA1 and 2 PAM sites and the first Hha1 restriction site).

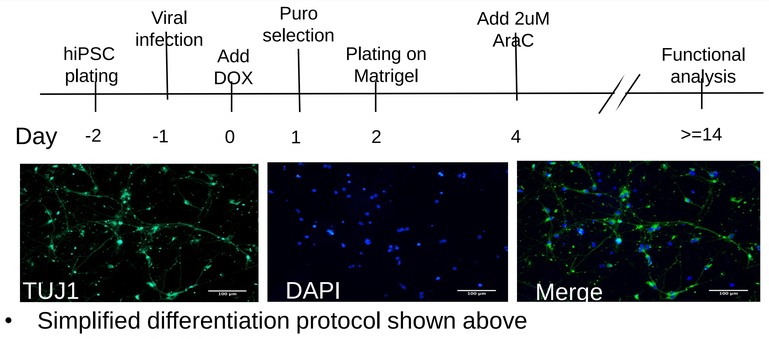


- Hha1 digestion of bulk cells derived from gRNA1 transfection shows high editing efficiency (lane 1 and 2). Lane 3 is an undigested control.
- Representative gel of Hha1 digestion of single clones. Lane 6 and 9 show positive edited clones. Lane 7 and 10 show unedited clones. Lane 17 is digested bulk as control. Sanger sequencing confirmed the clone in lane 9. Clone in lane 6 was lost due to contamination. More negative clones were picked and confirmed.





NGN2 directed induction generates homogeneous populations of glutamatergic neurons



- Simplified differentiation protocol shown above
- TUJ1 and DAPI staining shows that most cells have successfully differentiated into neurons.

Summary and Conclusions

- Screening a family with multiple schizophrenia affected individuals and an apparent autosomal dominant inheritance, we identified 1 variant that perfectly segregates with the disease. The variant is in *CASKIN1* which is expressed in brain and has not been previously observed. It is involved in the neurexin-neuroligin signaling pathway at the synapse and interacts specifically with CASK and neurexin 1 which had been shown to be involved in schizophrenia development.
- We have introduced this variant into hiPSCs. Further follow up for the functionality of this variant is pending.

References

- Dachtler, J., Ivorra, J. L., Rowland, T. E., Lever, C., Rodgers, R. J., & Clapcote, S. J. (2015). Heterozygous deletion of α-neurexin I or α-neurexin II results in behaviors relevant to autism and schizophrenia. *Behavioral Neuroscience*, *129*(6), 765–776
- Grayton, H. M., Missler, M., Collier, D. A., & Fernandes, C. (2013). Altered Social Behaviours in Neurexin 1α Knockout Mice Resemble Core Symptoms in Neurodevelopmental Disorders. *PLoS ONE*, *8*(6).
- Kirov, G., Rujescu, D., Ingason, A., Collier, D. A., Odonovan, M. C., & Owen, M. J. (2009). Neurexin 1 (NRXN1) Deletions in Schizophrenia. *Schizophrenia Bulletin*, *35*(5), 851–854.
- Stafford, R. L., Ear, J., Knight, M. J., & Bowie, J. U. (2011). The Molecular Basis of the Caskin1 and Mint1 Interaction with CASK. *Journal of Molecular Biology*, *412*(1), 3–13.
- Tabuchi, K., Biederer, T., Butz, S., & Südhof, T. C. (2002). CASK Participates in Alternative Tripartite Complexes in which Mint 1 Competes for Binding with Caskin 1, a Novel CASK-Binding Protein. *The Journal of Neuroscience*, *22*(11), 4264–4273.

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