Hippocampal Metabolite Concentrations in A Schizophrenia Case Series Vary in Association With Rare Gene Variants For Schizophrenia Versus Autism

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Research

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Abstract

Background

Rare variants in the TRIO gene are associated with schizophrenia and autism spectrum disorders (ASD), which are commonly comorbid. ASD may define a specific schizophrenia subtype. This study examined person-specific hippocampal metabolite concentrations for 4 schizophrenia cases harboring rare variants in TRIO or its interaction partner ARMS/KIDINS220 and 5 cases with other rare variants.

Methods

Nine of 19 cases from a prior imaging study underwent targeted exome sequencing. Multi-voxel ^{1}H-MRS imaging of the entire 3-dimensional hippocampus found only increased Creatine [Cr] (cellular energy use) concentration, distinguished cases at the group level. However, concentrations of N-acetyl-aspartate [NAA] (neuronal integrity) and choline [Cho] (membrane turnover/myelination) concentrations had a greater variance in cases than controls.

Results

Four cases with rare, missense-coding mutations or non-frameshift deletions in TRIO or ARMS/KIDINS220 had significantly lower [Cho] than the other 5 (1.78 ± 0.18 mM versus 2.67 ± 0.8 mM: t = 3.55, p = 0.005) but similar [NAA]. Two cases harboring rare variants in the SLC39A1 zinc transporter had the lowest [NAA]. (8.41 ± 0 80 mM versus 10.35 ± 2.03 mM, t = 4.52, p = 0.001). The highest [Cho] accompanied rare variants in SORCS2 and SORT, associated with schizophrenia and Alzheimer's Disease.

Limitations

This preliminary study of a small sample of exceptionally well characterized cases requires replication in larger samples for clinical utility.

Conclusions

The hippocampus is vulnerable to more than one pathology causing schizophrenia. TRIO rare variants predicted significantly lower Cho, indicating reduced myelin. [Cho] and [NAA] may have importance for choosing and monitoring schizophrenia treatment.

Background

Schizophrenia is highly comorbid with autism spectrum disorder (ASD) with a recent meta-analysis finding the prevalence of ASD in individuals with schizophrenia ranged from 3.4 to 52% (1). Both conditions are associated with rare gene variants in the Rho guanine nucleotide exchange factor (RhoGEF) TRIO gene, which is considered to be a hotspot for mutations associated with ASD (2, 3). A

phenotype for this comorbidity could define an important biological subtype of schizophrenia for research and treatment.

This study examined rare variants in TRIO and other genes with respect to hippocampal metabolite concentrations, obtained by multivoxel proton MR spectroscopic imaging (^{1}H-MRSI) of the entire structure. The different metabolites it quantitates are closely associated with cellular processes, including NAA (*N*-Acetyl-Aspartate and *N*-acetyl-aspartyl-glutamate) for neuronal integrity, Cr (creatine and phosphocreatine) for cellular energy metabolism; Cho (choline, phosphocholine, and glycerophosphocholine) for membrane turnover and myelin, and myo-inositol (mIns) to measure glial cells (4, 5). Accumulating evidence suggests these metabolites are altered in patients with schizophrenia (6), but the results are highly inconsistent in distinguishing groups of schizophrenia cases versus comparisons groups (7–10).

Using a novel multi-voxel, whole hippocampal ^{1}H-MRSI to assess metabolite concentrations in the entire bilateral 3-dimensional structure (as opposed to single voxel approaches), our prior study demonstrated only elevated Cr concentrations [Cr] significantly distinguish the group of cases from the control groups (11). Notably, concentrations of Cho and NAA in this study were significantly more variable within the group of cases than controls, consistent with heterogeneous underpinnings among schizophrenia cases.

The current study examined if variability in [Cho] and [NAA] was related to the harboring of rare variants for TRIO or its interaction partner, ARMS/KIDINS220 compared to those only harboring rare variants in other genes for psychosis. Our panel included the zinc transporter SLC39A13, which was identified as harboring a *de novo* mutation in a sporadic case from an Israeli Cohort and replicated in a New York study, as well as other genes associated with schizophrenia, some of which are also associated with Alzheimer's Disease.

Methods

The imaging and subsequent genetic study were approved by the Institutional Review Boards of NYU and Bellevue Hospital Center and all subjects provided written informed consent. Participant assessments and ^{1}H-MRSI procedures were previously described in 19 schizophrenia cases (11), with nine of them further agreeing to participate in genetic studies. Targeted exome sequencing of 38 CNS signaling genes was done by targeted exome capture, also previously described (3, 12). Rare or novel variants of these genes were identified according to Genome Aggregation Database (gnomAD v 2.1.1) and 1000Genomes Project databases (13, 14). The whole-bilateral hippocampal [Cho] and [NAA] were measured, as shown in Fig. 1. In the parent study, the mean [Cho] and [NAA] concentrations of the control group were 2.1 mM and 8.7 mM (11). All schizophrenia cases were stable and maintained on steady medication regimens for at least a month prior to the imaging study.

Results

The nine participants' ages (mean = 41.2 years; SD = 10.3, 5 women, 4 men), disease duration (mean = 19.9 years; SD = 11.9), genetic information, hippocampal [Cho] and [NAA], are compiled in Table 1, identified by #1 - #9, and sorted in descending order of their [Cho]. Alongside each gene are annotations indicating if it is associated through a genome-wide association study (GWAS) with (a) schizophrenia or bipolar disorder, (b) Alzheimer's disease (AD) or other dementias, and (c) autism or intellectual disability.

#	Age/Sex	Illness Duration (yrs)	Genes	Position; AA exchange	dbSNP	Frequency (gnomAD) [Average MAF]	1000 Genomes Phase 3 ALL [Average MAF]	[Cho] (mM)]	[NAA] (mM)	[Cr] (mM)]
1	43/F	20	*SORCS2 (a,b)*	T778M	rs199516236	0.0001	0.0004	3.31	9.62	10.60
2	48/M	23	*PTPRG (a)*	R323C	rs142366357	0.0007	0.003	2.76	10.57	10.91
			MAGI2 (a)	R738Q	rs145722885	0.0019	0.001			
			SORT1 (b)	E307Q	rs2228606	0.0133	0.004			
			DISC1 (a)	V71L	rs149444280	0.0012	0.004			
3	23/M	3	*NPTX2 (b)*	G307S	Novel mutation			2.76	10.57	10.91
4	29/F	8	*FAAH*	A356V	rs77101686	0.003	0.008	2.37	10.60	10.51
				A476G	rs75429705	0.003	0.009			
			OXTR	L206V	rs150746704	0.0019	0.009			
5	51/F	34	*SLC39A13/ ZIP13*	R40Q	rs35741412	0.01297	0.008	2.14	8.38	8.70
			IL1A	G412A	rs3783581	0.0016	0.006			
			SORCS2 (c)	R795Q	rs145113042	0.004705	0.015			
			DISC1 (a)	I798T	Novel Mutation					
6	34/M	5	*PTPRG (a)*	G523S	rs149885804	0.0001	0.001	2.03	11.31	10.77
			SORT1 (b)	T424M	rs149226217	0.0005	0.002			
			TRIO (b,c)	K2816R	rs73749289	0.0015	0.004			
7	52/M	32	*DISC1 (a)*	V71L	rs149444280	0.0012	0.004	1.88	8.43	8.68
			SLC39A13	A180G	rs147227015	0.0006	0.002			
			ARMS/ KIDINS220	A1299G	rs76164009	0.0014	0.005			
8	49/F	31	*TRIO (a,c)*	2486_2490del	rs140308852	0.0027	0.014	1.75	10.00	12.01
9	42/F	23	*TRIO (a,c)*	2486_2490del	rs140308852	0.027	0.014	1.46	9.81	7.72

TABLE 1 Subject Specific Demographics, Exome Data and Hippocampal Metabolite Concentrations

Legend:

Superscripts:

a) associated with schizophrenia or bipolar with genome wide significance

b) associated with Alzheimer's or other dementia with genome wide significance

c) associated with autism or intellectual disability with genome wide significance

Abbreviations: AA: amino acid; MAF: minor allele frequency; yrs: years

Strikingly, each of the nine subjects harbored at least one rare (minor allele frequencies (MAF) ≤ 1%) and/or ultra-rare (MAF ≤ 0.1%) missense coding variant or had a deletion in a coding region of a gene. Four cases (#1, 3, 8, 9), had only a single rare variant. Most of the polymorphisms were single amino acid exchanging variants. Two unrelated cases (#8, #9) had the same rare, non-frameshift deletion in the Trio Rho Guanine Nucleotide Exchange Factor (*TRIO*) gene. Ultra-rare single nucleotide variants (SNVs) were identified in cases #1, #2, #3, and #6. Two cases, (#2, #5) had four different missense coding variants in four different genes; two other cases (#6, #7) had three rare variants in three different genes; one case (#4) harbored two missense mutations in the Fatty Acid Amide Hydrolase gene (*FAAH*); and one (#5) had a novel mutation in the disrupted-in-schizophrenia (*DISC1*, I798T) gene. Notably, four (44%) of these unselected cases had a rare variant in the ASD associated TRIO gene or its interaction partner ARMS/KIDINS220.

The first three cases (#1, #2, #3) with the highest [Cho], 2.8–3.3 mM (all exceeding the controls' mean), also had 20% higher [NAA], 9.4–10.6 mM than the controls group Two of them harbored missense-coding mutations in endocytosis-related genes (*SORT1*, *SORCS2*) and the third had a variant in the Neuronal Pentraxin II regulation gene (*NPTX2*). Other rare/novel gene variants identified these three included Protein Tyrosine Phosphatase Receptor Type G (*PTPRG*) and its related synaptic scaffolding molecule (*MAGI2*) and *DISC1*. The highest [Cho] accompanied rare variants in SORCS2 and SORT, associated with schizophrenia and Alzheimer's Disease.

The next three cases (#4, #5, #6, [Cho] = 2.0- 2.4 mM), had similar concentrations as controls. Two had 21% and 30% higher [NAA] than the controls, but case #5, who had lower [NAA], harbored the greatest number of rare variants in the panel of CNS signaling genes. These included a variant in *SORCS2* (R795Q), a neurotrophic signaling gene that plays a role in Brain Derived Neurotrophic Factor (BDNF)-dependent hippocampal synaptic plasticity, the pro-inflammatory Interleukin 1A (*IL1A*), a novel mutation in *DISC1* (I798T) and the zinc transporter *SLC39A13*. Other variants in these cases included a gene for anandamide metabolism (*FAAH*) and the oxytocin receptor (*OXTR*). The two cases harboring rare variants in the SLC39A1c zinc transporter had significantly lower NAA than all other cases (8.41 ± 0,17 versus 10.35 ± 2.03 mM, t = 4.52, p = 0.001).

The final three cases (#7, #8, #9) had 15%-33% lower [Cho] and [NAA] than the controls. Cases #8 and #9, with the lowest [Cho], both harbored a non-frameshift mutation in *TRIO*, a Rho guanine nucleotide exchange factor. Case #7 had a mutation in *ARMS/KIDINS220* which is a scaffold protein and an interaction partner of *TRIO*, and also the lowest [NAA] in the group at 15% less than the controls. In addition to the variant in *ARMS/KIDINS220*, case #7 harbored ultra-rare genetic variants in the zinc

transporter, *SLC39A13* and a rare missense-coding variant in *DISC1.* Cases 6, 7, 8 and 9, who all harbored rare, missense-coding mutations or non-frameshift deletions in TRIO or ARMS/KIDINS220, had significantly lower [Cho] than the other 5 cases (1.78 ± 0.18 versus 2.67 ± 0.8 mM: t = 3.55, p = 0.005), although the [NAA] was similar in these groups.

Discussion

Schizophrenia cases with rare missense coding variants in Trio Rho Guanine Nucleotide Exchange Factor (*TRIO*) or its interaction partner *ARMS/Kidns220*, had significantly lower [Cho] than other schizophrenia cases in this series and compared to the healthy control group in the parent study (11). TRIO is associated with autism and intellectual impairment in addition to schizophrenia (15–17), and this analysis suggests that these cases have significantly reduced hippocampal myelin. If confirmed, the findings indicate a distinct pathophysiology for some schizophrenia cases, presumably some with comorbid ASD, indicating the need for distinct approaches for prevention and treatment.

TRIO codes for a rho-guanine nucleotide exchange factor, a major regulator of neuronal development downstream of neurotrophin signaling and also plays roles in cell adhesion, neuronal spine formation and other signaling pathways (18). Its protein is involved in the formation of a ternary complex with the neurotrophin p75 and SORCS2. The dissociation of TRIO from this ternary complex occurs upon binding of the proneurotrophin pro-NGF to the p75-SORCS2 complex, which leads to growth cone retraction in hippocampal neurons (19). Lower [Cho] in these cases may reflect reduced dendritic spine assembly and neurite outgrowth in the hippocampus, leading to less membrane turnover. The *ARMS/KIDINS220* rare variants impacted the region between transmembrane domains 2 and 3. These transmembrane regions facilitate the functional interactions of the gene with tropomyosin receptor kinase (Trk) receptors. It promotes prolonged mitogen-activated protein kinase (MAPK) signaling, enhanced neuronal maintenance, and modulates spine formation.

Differences in [NAA], which is specific for neuronal integrity, did not distinguish subjects with and without rare TRIO or *ARMS/KIDINS220* variants. Lower [NAA] were only previously demonstrated in a meta-analysis for children with ASD, but not in adults (20). A meta-analysis of antipsychotic naive schizophrenia cases also found no significant group level differences in hippocampal [NAA] compared with controls (21). Nonetheless, there were two persons in this series with significantly reduced [NAA] than the other cases. Each of them harbored rare variants in *SLC39a13* (zinc transporter 13) and *DISC1* (“disrupted in schizophrenia 1”), which regulates adult neurogenesis, controlling dendritic development and synapse formation. It was one of the earliest identified mental-illness genes using linkage analysis and is now well-replicated for association with schizophrenia (22). It codes for a scaffold protein that is highly expressed in the hippocampus and is responsible for multiple aspects of neurogenesis, perhaps through Wnt/β-Catenin signaling (23). Both *DISC* and *SLC39A13* are essential for neurodevelopment and have behavioral phenotypes. *SLC39A13* transports vesicular Zinc to the NMDA receptor where Zinc has an inhibitory action. The gene is associated with the BMP/TGFβ pathway that is differentially expressed

in pyramidal neurons in schizophrenia cases from *post-mortem* studies. SLC39a13 is significantly associated with alcohol use disorders in a GWAS study of European Americans (24).

Two cases in this series had very elevated [Cho] which is essential for the synthesis and turnover of the membrane phospholipid bilayer [and predicted more severe psychotic and manic symptoms in this cohort (25)], suggesting suggests increased membrane turnover and demyelination/remyelination. The gene variants in these cases included rare/novel mutations in genes associated with dopamine regulation, including the endocytosis related genes (*SORT1*, *SORCS2*), which are also associated with AD (26), protein tyrosine phosphatase (*PTPRG*) and its related synaptic scaffolding molecule (*MAGI2*), and *DISC*1, the latter three of which are identified in association with schizophrenia (27–29). *SORT1* promotes apoptosis by endocytosis of the pro-neurotrophic factors pro-BDNF and pro-Nerve Growth Factor (pro-NGF). SORCS2 interacts with pro-BDNF and is involved in growth cone collapse, Schwann cell apoptosis and dopaminergic hyperinnervation (19). *PTPRG* has trans-synaptic actions, is the mRNA target of FMR1 and regulates neurotransmission. MAGI2 is a synaptic scaffolding molecule which maintains the glutamate receptor subunits of AMPA receptors. The genetic variants in these genes support our finding that elevated [Cho] is significantly associated with the severity of psychosis, a required symptom domain for the diagnosis (25).

Indeed, case #1, with the highest [Cho], also harbored a rare *SORCS2* variant. In addition to its association with AD (above), *SORCS2* is a converging point of linkage, genetic association, and GWAS findings on chromosome 4p for bipolar disorder and schizophrenia (30). Its deficiency in a mouse model is accompanied by reduced NMDA-dependent hippocampal plasticity, impaired pre-pulse inhibition, as well as stress sensitivity, risk taking and stimuli seeking (31). Another high [Cho] case harbored a *SORT1* variant, a member of the VPS10-related sortilin family which influences dendritic spine formation and apoptosis through neurotrophin signaling, acting as a receptor for pro-BDNF and pro-NGF. It is involved in a host of biological processes–including glucose and lipid metabolism, cardiovascular conditions and AD—contributing to the overlap of diabetes and AD risk. The case with the rare *SORT1* variant also harbored a rare *DISC1* variant. Notably, a *DISC1* knockout in rats shows disrupted morphogenesis of adult-born hippocampal neurons in the dentate gyrus. Another variant among the three cases with increased [Cho] were *OXTR*, which modulates the frequency of interneuron spiking in the hippocampus in addition to social bonding (32), and the neuronal pentraxin gene (*NPTX2)*. The latter is associated with excitatory synaptogenesis, AMPA receptor aggregation, and the onset of the critical period. It is a direct transcriptional target of *BDNF* and is key to the *BDNF*-mediated modulation of glutamatergic synapses and mossy fiber plasticity (33). NPTX2 has reduced expression in mouse models of AD and is reduced in the cerebrospinal fluid (CSF) of persons with AD in association with cognitive deficits and hippocampal volume reduction (34).

The three cases with intermediate [Cho] harbored several potentially influential variants that could act through other pathways. One had two rare missense-coding variants in the *FAAH* gene, which encodes the degradation enzyme for the endogenous endocannabinoid anandamide, which is highly expressed in the hippocampus where it modulates short-term synaptic depression (35). Reduced activity is associated

with working memory deficits in a rat model (33). The mouse knockout displayed increased numbers of activated microglia and cytokines (IL-6 and IL-1β) predicting an inflammatory state (35, 36). Another case carried the greatest number of rare variants and a unique novel mutation in *DISC1* (I798T) that localized to the region interacting with *Lis1*, which is related to neurodevelopment (37). Also in this group were rare variants of *SORCS2*, a proneurotrophin receptor essential for BDNF and neurogenesis, and of *IL1A*, which is linked to schizophrenia in numerous genetic studies (38). *IL1A* is a cytokine that interacts with numerous pathways relevant to psychosis, including transforming growth factor beta (TGF-β), MAPK and Protein Kinase B (Akt) signaling. These findings support the contention that deficient myelination of hippocampal parvalbumin GABAergic interneurons could underlie the pathophysiology of schizophrenia (39). Reduced numbers and function of GABAergic interneurons are well supported in models that bridge hippocampal pathology with schizophrenia (40). Their reduced function increases the activity of the far more numerous NMDA glutamatergic neurons, proposed to produce psychosis by deregulating dopamine-induced firing (41, 42).

These findings support the use of ^{1}H-MRSI concentrations of [Cho] for the treatment selection and monitoring of potentially distinct pathologies for schizophrenia. But notably, the genetics of schizophrenia are complex. As only a fraction of the population attributed risk is explained by any of the hundreds of common gene variants identified through GWAS, we have focused on the novel and rare gene variants. We propose that rare missense coding variants could plausibly be more important in explaining risk and particular illness features, *e.g.*, symptom severity, cognitive involvement, and medical and psychiatric comorbidity (12, 43).

Limitations

As a first-of-its-kind study, it has two main limitations. First, it is a rather small cohort comprising patients with varying disease durations and treatments. Second, the approach of targeted exome capture on a set of pre-selected genes does not have the informational benefit of whole exome sequencing. Consequently, we could not determine the exact effect-size of these genes in relation to information that could be derived from whole exome variants on the schizophrenia-related pathologies. However, we are convinced that the co-occurrence of rare missense coding variants and deletions in the gene, in combination with the observed variability in [NAA] and [Cho], point the way to a novel categorization method of patients and thereby a method of dissecting schizophrenia into biologically relevant subtypes.

Conclusions

Rare variants in genes associated with autism (TRIO or its interaction partner ARMS/KIDINS220) were identified in four of nine cases with schizophrenia (44%) undergoing multi-voxel ^{1}H-MRSI of their entire 3-dimensional hippocampus and targeted exome sequencing. These preliminary findings, if replicated, suggest that a person's specific ^{1}H-MRSI metrics may be employed to select and monitor treatments for psychosis. Specifically, those with ^{1}H-MRSI evidence of inflammation, for example, may benefit from anti-inflammatory treatments. Further research may show that certain metabolic patterns suggest disruptions

in certain genetic pathways that drive pathophysiologies indicating targeted treatments; for instance, patients with rare missense coding SNVs in the zinc transporter SLC39A13 may benefit from supplementation.

Abbreviations

AD: Alzheimer's disease; Akt: Protein kinase B; BDNF: Brain Derived Neurotrophic Factor; Cho: Choline; CNS: central nervous system; Cr: Creatine; CSF: Cerebrospinal fluid; GWAS: genome-wide association study; ^{1}H-MRS: Proton magnetic resonance spectroscopy; ^{1}H-MRSI: Proton magnetic resonance spectroscopy imaging; MAF: minor allele frequencies; mIns: myo-inositol; MAPK: Mitogen-activated protein kinase; NAA: *N*-Acetyl-Aspartate; Pro-NGF: Pro-Nerve Growth Factor; SNV: Single nucleotide variant; TGF-β: Transforming growth factor beta; Trk: Tropomyosin receptor kinase.

Declarations

Acknowledgements

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Authors' contributions

DM and TMK oversaw all aspects of the study; OD designed and conducted the imaging components, KWH conducted research, KWH and HR contributed to writing of the final manuscript, AH and MVC designed and oversaw the genetic sequencing of cases. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All study components were approved by the NYU and Bellevue Hospital Center, as described, and all subjects signed written informed consent for the imaging and genetic studies.

Consent for publication

Not applicable

Competing interests

The authors declare no competing interests.

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Figures

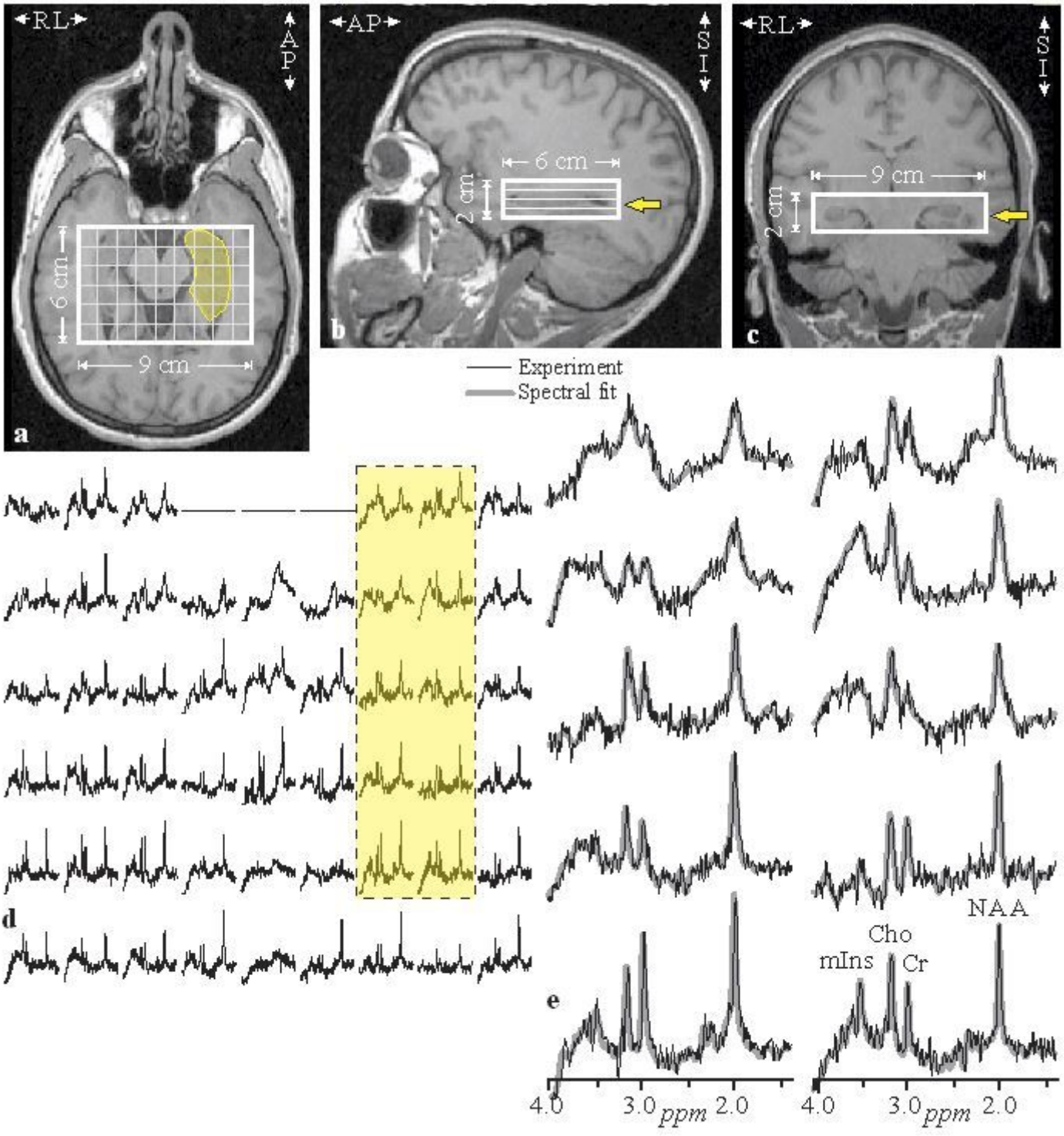

Figure 1

Hippocampus MRI and 3D 1H-MRSI of the entire structure: Position, Size and Analysis. Top: Axial (a) coronal (b) and sagittal (c) T1-weighted MRI from a 23 year old female SCZ patient (#3 in Table 1), superimposed with the 9×6×2 cm3 (LR×AP×IS) VOI and 1H-MRSI grid (thick and thin white frames) and the left hippocampus outline (transparent yellow on a). Yellow arrow on b and c indicates the level of a. Bottom, left, d: Real part of the 9×6 axial 1H spectra matrix from the VOI on a on common frequency and

intensity scales. Spectra within left hippocampus on a are marked by the dashed yellow-filled frame. Bottom, right e: The six spectra in the frame in d expanded for greater detail (thin black lines) and superimposed with the spectral-fitting simulation (thick gray lines). With the main quantified peaks: N-acetyl-aspartate, creatine, choline and myo-inositol (NAA, Cr, Cho, mIns) indicated on the bottom right spectrum. Note the good signal-to-noise-ratio, spectral resolution (8.1±3.0 Hz line-width) from these 0.5 cm3 voxels and the quality of the spectral fit.