

1 **Title Page:**

2 **Elevated serum adenosine deaminase levels in neuroleptic-naïve patients with recent-**
3 **onset schizophrenia**

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30 **Abstract**

31 Schizophrenia is characterized by pathophysiological alterations of multiple neurotransmitter
32 systems such as dopaminergic, glutamatergic, GABA-ergic and serotonergic pathways.
33 Adenosine, a homeostatic neuromodulator that mediates signaling through multiple
34 neurotransmitter pathways, is an emerging candidate neurobiological substrate of
35 schizophrenia. The present study examined peripheral blood levels of adenosine deaminase,
36 an adenosine metabolizing enzyme, in 16 neuroleptic-naïve patients with recent-onset
37 schizophrenia (mean age = 25.59 years (range: 16-35)) and 18 age-matched healthy
38 comparison subjects (mean age = 25.17 years (range: 18-28)). Serum adenosine deaminase
39 levels were assayed at two time points; before (7 p.m.) and after (7 a.m.) sleep. The
40 adenosine deaminase levels were compared between groups and were correlated to positive
41 and negative symptom severity measures. Adenosine deaminase levels were found to be
42 higher at both evening ($p=0.013$) and morning ($p<0.001$) time points in our sample of
43 patients with recent-onset schizophrenia who were never exposed to neuroleptic medications.
44 Correlational analysis revealed evidence for a possible link between evening rise in adenosine
45 deaminase and severity of auditory hallucinations ($p=0.003$) as well as morning rise in
46 adenosine deaminase and severity of avolition-apathy in patients with schizophrenia
47 ($p=0.013$). The results of the study provide strong support to the adenosine hypothesis of
48 schizophrenia and highlight the potential utility of serum adenosine deaminase as a peripheral
49 biomarker of schizophrenia.

50 **Keywords:**

51 Schizophrenia; adenosine deaminase; neuroleptic-naïve; hallucination; avolition

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53 **1. Introduction**

54 The pathophysiology of schizophrenia is understood to involve dysfunction of the
55 dopaminergic (Carlsson, 1988), serotonergic (Meltzer and Massey, 2011), glutamatergic
56 (Coyle, 2006) and GABA-ergic (Gonzalez-Burgos and Lewis, 2008) neurotransmitter
57 systems. The diverse psychopathology of schizophrenia cannot be explained by dysfunction
58 of neurotransmitter systems when considered in isolation (Keshavan et al., 2011). Adenosine,
59 a homeostatic neuromodulator, is an emerging candidate neurobiological substrate with
60 effects on multiple neurotransmitter pathways (Boison, 2008; Cunha and Cunha, 2001). In
61 addition, adenosine plays an important role in early brain development and regulation of
62 brain immune responses (Cunha and Cunha, 2001), and thereby could also contribute to the
63 neurodevelopmental deviations implicated in schizophrenia (Lara et al., 2006).

64 Adenosine deaminase (ADA) is a purine-inactivating endoenzyme that irreversibly
65 deaminates adenosine to inosine (Yegutkin, 2008), leading to its final degradation to uric
66 acid. Like adenosine, ADA too is ubiquitously found in the human body (Franco et al., 1997)
67 and hence implicated in diverse physiological functions. Thus, serum ADA level has been
68 suggested as an important peripheral biomarker of adenosine signaling in neuropsychiatric
69 disorders (Elgün et al., 1999; Herken et al., 2007; Stubbs et al., 1982) especially in
70 schizophrenia, given that a hypoadenosinergic state has been linked to its pathogenesis (Lara
71 et al., 2006).

72 In support of the above, Dutra et al. (Dutra et al., 2010) demonstrated lower frequency of
73 occurrence of an ADA variant with decreased enzymatic activity (G/A genotype) among
74 patients with schizophrenia, suggesting increased levels of ADA and reduced levels of
75 ambient adenosine. Another study reported significantly higher serum ADA in patients with
76 schizophrenia on antipsychotic monotherapy (more so with atypical antipsychotic), when
77 compared to healthy control subjects (Brunstein et al., 2007), but found no correlation with
78 clinical psychopathology. However, Ghaleiha et al. (Ghaleiha et al., 2011), reported that in
79 patients with chronic schizophrenia, antipsychotic therapy (particularly with clozapine) was
80 associated with an increase in serum ADA and symptomatic improvement. Therefore, it is
81 unclear whether the increased serum ADA reported in the above studies was the consequence
82 of treatment with antipsychotics or a marker of the disorder per se.

83 Therefore, we examined whether serum ADA levels were significantly different in patients
84 with schizophrenia who have never been exposed to antipsychotic medications in comparison
85 to matched healthy comparison subjects. In accordance with the proposed adenosine theory
86 of schizophrenia pathophysiology, we hypothesized that the serum ADA will be significantly
87 higher in patients with schizophrenia. We also aimed at exploring the hitherto unreported
88 relationship between serum ADA and symptom severity scores.

89 **2. Materials and Methods**

90 The study was carried out at the National Institute of Mental Health and Neurosciences
91 (NIMHANS), Bangalore, India, with due approval from the Institute Ethics Committee thus
92 conforming to the ethical standards laid down in the 1964 Declaration of Helsinki. Written
93 informed consent was obtained from all the participants prior to enrolment into the study.

94 **2.1. Participants**

95 20 patients with schizophrenia (SZ) or schizophreniform disorder and 20 age-matched
96 healthy comparison subjects (HS) participated in the study. Of the above subjects, the blood
97 samples of four SZ and two HS were of inadequate quantity and/or poor quality, and
98 therefore had to be omitted from the study. The remaining participants (SZ=16; HS=18) were
99 of similar age group (SZ: mean=25.59 years (range: 16-35); HS: mean =25.17 years (range:
100 18-28)). Patients with schizophrenia/schizophreniform disorder had a mean illness duration
101 of 21 months (range: 1-96). Of these, 11 SZ and 17 HS had ADA samples collected at two
102 time points, i.e., at 7p.m. on day1 and 7a.m. on day2. Five SZ had only their morning
103 samples collected, since they did not abstain from consuming food for 3 hours prior to the
104 evening sample collection. The sample collection could not be rescheduled to the next day for
105 ethical reasons, since the patients were neuroleptic naïve and had to be started on medications
106 at the earliest. In one of the healthy subjects also, the morning sample alone could be
107 collected due to certain logistic constraints.

108 The diagnosis of schizophrenia/schizophreniform disorder was arrived at using criteria from
109 the Diagnostic and Statistical Manual for Mental Disorders – Fourth Edition (DSM-IV)
110 (American Psychiatric Association, 2000) based on the consensus of a research psychiatrist
111 who conducted a semi-structured interview and a trained research assistant who used the
112 Mini International Neuropsychiatric Interview (MINI) for DSM-IV (Sheehan et al., 1998).
113 Positive and negative symptoms were rated using the Scale for Assessment of Positive
114 symptoms (SAPS) (Andreasen, 1983) and Scale for Assessment of Negative symptoms
115 (SANS) (Andreasen, 1982) respectively by one rater (S.K.) for all subjects after undergoing
116 adequate training and establishment of inter-rater reliability. None of the patients had history
117 of exposure to neuroleptic medications.

118 The healthy comparison subjects were recruited from the community through word-of-mouth.
119 They were screened to exclude past history of Axis I psychiatric disorders, personal history
120 of psychoactive medication use and family history of schizophrenia spectrum disorders in
121 first-degree relatives using a study-specific proforma.

122 All participants were screened to exclude history of significant head injury, neurological
123 disorders, medical conditions including acute infections, autoimmune disorders, endocrine
124 disorders, specific sleep disorders, and substance abuse including caffeine (daily intake of
125 caffeine-containing beverages and food substances exceeding 300mg/day). All were free of
126 any drugs known to affect immune or endocrine function. All participants underwent clinical
127 screening to rule out any unstable medical conditions. The participants were instructed to
128 avoid food and caffeine for at least 3 hours before the evening blood sample, following which
129 they had their dinner; overnight fasting was ensured before drawing the morning blood
130 sample. The above precautionary measures were adopted to avoid any confounding effects,
131 including that of food and caffeine on the measured ADA levels.

132 **2.2. Measurement of ADA**

133 Blood samples were collected from participants before and after their sleep as there was no
134 available evidence from the literature regarding the most appropriate time to collect serum for
135 ADA assay. The first sample was collected around 7p.m. (before dinner and before sleep)

136 similar to a previous schizophrenia study (Brunstein et al., 2007) and the second sample
137 around 7a.m. the following morning (after sleep and before breakfast) similar to earlier
138 studies in major depression (Elgün et al., 1999; Herken et al., 2007). The participants slept in
139 the sleep cabin of the Sleep Research Laboratory at the Department of Neurophysiology. 5ml
140 of venous blood samples were collected in vacuum tubes (BD Vacutainer®) without
141 anticoagulants on both occasions. The 7p.m. samples were allowed to clot overnight at
142 4°Celsius whereas the 7a.m. samples were allowed to clot at room temperature for two hours.
143 Later both the samples were centrifuged for 10 minutes at 4000rpm to extract the serum. The
144 serum samples were then coded and stored in microtubes (Eppendorf Inc.) at -80°Celsius for
145 the assay.

146 The ADA assay from serum was performed using a previously validated (Al-Rubaye and
147 Morad, 2012, 2013) colorimetric sandwich-enzyme immunoassay kit E91390Hu96 (USCN
148 Life Science Inc., Wuhan), following the instructions provided in the manual (“SEB390Hu-
149 96 ELISA Kit for Adenosine Deaminase (ADA) - Instruction manual,” 2012). All samples
150 were analyzed in duplicate and the intra-assay variability was less than 10%. Assays were
151 conducted at the Department of Neurochemistry under the supervision of S.S. The trained
152 neurochemist who carried out the assays was blinded to the study group status of the blood
153 samples.

154 **2.3. Genotyping of ADA polymorphism**

155 The ADA 22G>A polymorphism (rs73598374) was genotyped using allele-specific
156 polymerase-chain reaction under the supervision of M.P and S.J. Genotyping was done for 32
157 (HS=16; SZ=16) out of the 35 subjects, all of whom were found to have the G/G genotype.
158 Therefore, any further examination of the relationship between the ADA rs73598374
159 genotypes and serum ADA levels and the differences between patients with schizophrenia
160 and healthy comparison subjects was not possible in our limited sample.

161 **2.4. Statistical analysis**

162 Statistical analysis was performed using GraphPad Prism 5.0 (GraphPad Software, Inc.) and
163 Statistical Toolbox of MATLAB 2012b (Mathworks, USA). D'Agostino and Pearson
164 omnibus test and visual inspection of histogram plot was used to determine the normality of
165 data distribution. As ADA levels of both groups did not follow normal distribution, statistical
166 analyses were done on their log transformed values which followed a normal distribution. All
167 other values, except age, followed normal distribution. Mann–Whitney U-test was used to
168 test the group difference in age. Between-group comparisons for ADA levels of the 7p.m.
169 (ADA_{even}) and of the 7a.m. (ADA_{morn}) samples as well as their difference ($ADA_{\text{diff}(\text{even-morn})}$),
170 were done using the Student's t-test, after conducting a permutation-based two-way ANOVA
171 that found no group vs time interactions (**Table 1**). Comparisons between ADA_{even} and
172 ADA_{morn} levels within each group were carried out using paired t-test. The significance level
173 for these tests was set at $p < 0.05$. Pearson's test was used to test correlations of ADA levels
174 (ADA_{even} , ADA_{morn} and $ADA_{\text{diff}(\text{even-morn})}$) with duration of illness, positive symptom scores
175 and negative symptom scores within the schizophrenia group. Spearman rank order
176 correlation test was used for the within-group correlations between ADA levels and age. All
177 the SAPS and SANS sub-scores (including their total scores) were included in the correlation
178 analysis, and thus 11 correlations were made for each ADA level (ADA_{even} , ADA_{morn} and

179 ADA_{diff(even-morn)}). To exclude accidental significance associated with multiple correlations, a
180 threshold value of 0.735 was set for the absolute correlation co-efficients ($\alpha=0.05$; $n=11$)
181 using G*power 3.1 software (Faul et al., 2009, 2007) in order to obtain a statistical power
182 greater than 80% (Sabri et al., 1997). All tests were assessed using two-tailed p values.

183 3. Results

184 In the two-way ANOVA, significant main effects were noted only for the group factor, while
185 there were no time effects or group x time interactions (**Table 1**). On further exploring the
186 group difference, patients with schizophrenia showed significantly higher ADA_{even} (**Fig.1A**;
187 $t=2.66$, $p=0.013$) and ADA_{morn} (**Fig.1B**; $t=3.79$, $p<0.001$) levels, when compared to healthy
188 comparison subjects. ADA_{even} and ADA_{morn} showed significant correlation in both HS
189 ($n=17$ pairs, $r=0.706$, $p=0.002$) and SZ ($n=11$ pairs, $r=0.760$, $p=0.007$) groups. ADA_{even}
190 appeared to be higher than ADA_{morn} in both SZ (meanADA_{even}=15.38ng/mL, SD=9.13, $n=11$;
191 meanADA_{morn}=14.08ng/mL, SD=5, $n=16$), and HS (meanADA_{even}=8.85 ng/mL, SD=6.10,
192 $n=17$; meanADA_{morn}=7.16ng/mL; SD=4.18, $n=18$). But the paired t-test did not find
193 significant difference between ADA_{even} and ADA_{morn} in both HS ($t=1.43$, $p=0.1712$) and SZ
194 ($t=0.368$, $p=0.7203$). We performed correlational analyses between ADA levels and SANS
195 and SAPS sub-scores to explore the relationship, if any, between symptom severity and ADA
196 levels (**Table 2**). A significant positive correlation was noted between ADA_{diff(even-morn)} and
197 hallucination sub-score of SAPS (**Fig.2B**; $n=11$ pairs, $r=0.810$, $p=0.003$) at the a-priori-
198 decided significance threshold to account for multiple comparisons (see Statistical analysis
199 subsection). However, there was a high negative correlation nearing significance between
200 ADA_{diff(even-morn)} and avolition-apathy sub-score of SANS (**Fig.2C**; $n=11$ pairs, $r=-0.717$,
201 $p=0.013$). A positive trend was observed between ADA_{even} and hallucination sub-score of
202 SAPS ($n=11$ pairs, $r=0.601$, $p=0.050$) while ADA_{morn} showed a trend for a positive correlation
203 with avolition-apathy sub-score of SANS ($n=16$ pairs, $r=0.500$, $p=0.049$).

204 ADA levels were not found to be significantly correlated with age in HS (ADA_{even}:
205 $n=17$ pairs, $r=-0.092$, $p=0.727$; ADA_{morn}: $n=18$ pairs, $r=-0.363$, $p=0.139$) and in SZ (ADA_{even}:
206 $n=11$ pairs, $r=0.097$, $p=0.777$; ADA_{morn}: $n=16$ pairs, $r=-0.190$, $p=0.481$). ADA levels were also
207 not significantly correlated with duration of illness in the SZ (ADA_{even}: $n=11$ pairs, $r=-0.044$,
208 $p=0.897$; ADA_{morn}: $n=16$ pairs, $r=-0.047$, $p=0.862$).

209 4. Discussion

210 The present study found significantly higher serum ADA levels at two different time points
211 (7p.m. on day1 and 7a.m. on day2) in patients with recent-onset schizophrenia who had never
212 been exposed to neuroleptic medications, in comparison to matched healthy comparison
213 subjects. The study also provides evidence for a link between ADA levels and
214 psychopathology in patients with schizophrenia.

215 As described in the Introduction, similar observations have been reported in medicated
216 patients with chronic schizophrenia (Brunstein et al., 2007; Ghaleiha et al., 2011). The results
217 of our study on neuroleptic-naïve patients with recent-onset schizophrenia provide the initial
218 evidence towards considering increased serum ADA as a potential peripheral marker of a
219 hypoadenosinergic state in schizophrenia (Lara et al., 2006), and not just a secondary effect
220 of illness chronicity or neuroleptic treatment (Brunstein et al., 2007; Ghaleiha et al., 2011).

221 The higher ADA levels in patients would imply faster and greater degradation of adenosine,
222 leading to a hypoadenosinergic state. Adenosine inhibits the release of several
223 neurotransmitters, such as glutamate, dopamine, serotonin and acetylcholine, and decreases
224 neuronal activity by post-synaptic hyperpolarization, thereby serving an important
225 neuromodulatory function (Lara et al., 2006). Animal models with altered adenosine
226 signaling have provided important insights into the adenosine dependent changes in
227 neurotransmitter signaling associated with the genesis of schizophrenia (Ferré, 1997; Lara,
228 2002; Sills et al., 1999). Thus the increased ADA levels observed in our study could be the
229 marker of an adenosine deficit state, which results in impaired neuromodulation in multiple
230 brain networks leading on to the various symptoms of schizophrenia. Further support to the
231 adenosine hypothesis of schizophrenia comes from a genetic study (Dutra et al., 2010) that
232 has reported a lower proportion of ADA genotype with reduced activity (G/A) among
233 patients with schizophrenia.

234 Though, as a group, patients with schizophrenia had significantly higher ADA at both time
235 points, there was indeed wide variability of ADA values within and between the two time
236 points (**Fig. 1B**). This might reflect the within-group differences in severity of the various
237 psychopathological dimensions. Interestingly we found a significant positive correlation
238 between $ADA_{\text{diff}(\text{even-morn})}$ and auditory hallucinations and a trend towards an inverse
239 correlation between $ADA_{\text{diff}(\text{even-morn})}$ and avolition-apathy. The positive correlation between
240 higher ADA_{even} (relative to ADA_{morn}) and auditory hallucinations (**Fig. 2A and 2B**) might
241 reflect the inhibitory deficit in the fronto-temporo-parietal networks associated with
242 hyperglutamatergia (Schobel et al., 2013; Théberge et al., 2002) and hyperdopaminergia
243 (Heinz and Schlagenhauf, 2010) secondary to a hypoadenosinergic state (Lara et al., 2006)
244 during the day. Similarly, the positive correlation between higher ADA_{morn} (relative to
245 ADA_{even}) and avolition-apathy (**Fig. 2A and 2C**) might reflect the hypoadenosinergic state
246 during night causing disrupted sleep, leading on to lethargy and avolition during day time.
247 These findings would therefore provide additional support to the possibility of a
248 hypoadenosinergic state (Boison, 2011; Lara et al., 2006) mediating the predominant
249 symptom dimensions of schizophrenia.

250 As mentioned in the Introduction, adenosine deaminase (ADA) is primarily responsible for
251 catalyzing the irreversible deamination of adenosine to inosine. At least two isoenzymes of
252 ADA viz., ADA1 and ADA2 are identified in humans (Ratech et al., 1981). While these
253 isoenzymes are present in several tissues, they differ in their kinetic properties and tissue
254 distribution. ADA1 is mostly intracellular or on the cell membrane in the ecto-form, attached
255 to dipeptidyl peptidase 4 (Fan et al., 2012). On the other hand, ADA2 is the main isoenzyme
256 in the serum. While ADA1 is expressed in lymphocytes and macrophages, ADA2 is more
257 abundant in the blood, brain and liver (Rosemberg et al., 2007). Previous studies cited earlier
258 (e.g., Brunstein et al., 2007; Ghaleiha et al., 2011) have also assayed serum levels of ADA as
259 a surrogate marker for ADA levels in the brain.

260 It is pertinent to mention at this juncture that adenosine and other nucleosides are transported
261 across the blood-brain barrier via a saturable, carrier mediated mechanism (Kalaria and
262 Harik, 1988). The increased ADA activity in the periphery, as seen in patients with drug
263 naïve schizophrenia, may result in reduction of the circulatory levels of adenosine. This may
264 decrease the transport across the blood-brain barrier (BBB) leading to a hypoadenosinergic

265 state in the CNS. However, this speculation requires confirmation with additional
266 experiments involving the quantitation of adenosine and its metabolites (inosine,
267 hypoxanthine) in serum and CSF by HPLC method and measuring the isoform specific
268 enzyme activity in the serum and CSF.

269 It may be argued that the higher ADA found in our patient sample may be a compensatory
270 phenomenon secondary to a state-dependent increase in brain adenosine levels during the
271 psychotic state (Cunha and Cunha, 2001; Hirayama et al., 2011; Reddy et al., 1992).
272 However, this argument fails to explain the overall ADA rise noted even among patients with
273 the negative symptom of avolition-apathy. As our patient group comprised of subjects with
274 heterogeneous symptom dimensions (**Fig. 2A**), it is unlikely that the higher ADA could have
275 exclusively resulted from higher state-dependent adenosine levels. Thus, the increased ADA
276 levels detected in our sample of patients with schizophrenia may be considered as a
277 peripheral marker of a hypoadenosinergic state that characterizes the disorder. Similarly,
278 medication induced elevation of serum ADA reported by Ghaleiha et al. (2011) in patients
279 with schizophrenia could be compensatory to an improvement in adenosinergic tone
280 associated with successful treatment. It has to be stated that these are indeed preliminary
281 hypotheses generated from the results of the study, which need to be tested in future studies
282 with larger sample sizes.

283 Adenosine has been shown to serve a regulatory function in the immune system of brain
284 (Haskó et al., 2005). Though general immunological disorders were ruled out in all our
285 subjects through careful history and routine clinical hematological and biochemical screening
286 investigations, we did not carry out screening blood investigations to rule out autoimmune
287 conditions. Although remote associations between acute psychotic states, encephalitis and
288 NMDA auto-antibodies have been reported (Deakin et al., 2014), schizophrenia has not been
289 conclusively shown to be an autoimmune disorder (Coutinho et al., 2014). Therefore, it is
290 unlikely that the raised ADA levels observed in our sample of patients with schizophrenia
291 could be secondary to an autoimmune state.

292 A potential limitation of the study is the fact that only protein levels of ADA in serum were
293 measured, while enzymatic activity of ADA was not assayed. While measuring the enzyme
294 activity offers greater correlation with physiological response, the practical difficulties in
295 handling the samples prompted us to measure the ADA protein levels. In our earlier pilot
296 studies, we observed a gradual decrease in the ADA enzyme activity when the serum samples
297 were stored at 4°C or when subjected to two freeze-thaw cycles. However, as expected, the
298 ADA protein content remained unaltered upon storage/ freeze thawing. The present study
299 involved the recruitment of drug naïve subjects with schizophrenia over a substantial time
300 period. The samples were collected as and when the subjects were recruited and the serum
301 separated and stored. To avoid inter-assay variation, all the samples were analyzed at the
302 same time. In order to avoid any bias in the results due to varying lengths of sample storage,
303 it was considered appropriate to measure the protein content. Moreover, ADA also has
304 significant non-enzymatic actions (protein-protein interactions) with respect to desensitizing
305 and enhancing functionality of adenosine receptors (Ciruela et al., 2010; Gracia et al., 2008).
306 Therefore, a protein level assessment may be a better indicator of the full range of ADA
307 activity in comparison to enzyme activity.

308 Finally, in our limited sample of neuroleptic-naïve patients with recent-onset schizophrenia
309 and matched healthy comparison subjects, all the genotyped subjects (HS=16; SZ=16) were
310 found to have G/G genotype of the ADA 22G>A polymorphism (rs73598374); none of the
311 subjects had the G/A genotype that is associated with lower ADA activity (Bachmann et al.,
312 2012; Battistuzzi et al., 1981). Larger samples of schizophrenia and healthy subjects may be
313 needed to observe between-group differences, if any, of the G/G and G/A genotypes as
314 reported in one previous study (Dutra et al., 2010). Nevertheless, since all our subjects were
315 homogeneous with respect to the ADA 22G>A functional polymorphic variation, it may be
316 inferred that our observation of higher serum ADA levels in patients with schizophrenia is
317 unlikely to be confounded by genotypic variation between the study samples.

318 **5. Conclusion**

319 To the best of our knowledge, this is the first report of elevated serum ADA levels at two
320 time points in neuroleptic-naïve patients with recent-onset schizophrenia. Further, the study
321 also provides preliminary evidence for a link between ADA levels at different time points
322 with positive and negative symptoms of schizophrenia. These findings may be considered
323 strong evidences in support of the adenosine hypothesis of schizophrenia. In the background
324 of previous reports suggesting a possible genetic basis for the elevated ADA activity in
325 schizophrenia and the observation of alteration of ADA levels with successful treatment, the
326 potential utility of serum ADA levels as a biomarker or endophenotype of schizophrenia
327 should be researched in future studies.

328 **6. Acknowledgments:**

329 We would like to thank Mrs. Ammu Lukose (for recruitment and evaluation of subjects), Dr.
330 Mathew John (for serum extraction and storage), Dr. Meera Purushottam (for genetic
331 analysis), and all the participants who devoted their time and efforts to take part in this study.
332 This work was funded in part by Department of Biotechnology (DBT), Government of India
333 (Grant No. BT/PR/8363/MED/14/1252 & DST/CSI/100/1321 to J.P.J), and Department of
334 Science & Technology (DST), Government of India (Grant No. SR/SO/BB-27/2009 to S.S.).

335 **7. Funding:**

336 This work was funded in part by Department of Biotechnology (DBT), Government of India
337 (Grant No. BT/PR/8363/MED/14/1252 & DST/SR/CSI/79/2010 to J.P.J), and Department of
338 Science & Technology (DST), Government of India (Grant No. SR/SO/BB-27/2009 to S.S.).

339 **8. Contributors**

340 A.S and S.K. carried out the data acquisition and analysis; A.S., S.K., B.M.K. and J.P.J.
341 conceptualized the study; M.P. helped with statistical analysis; S.S. facilitated the ADA
342 assessments; S.J. facilitated the Genetic assessments; B.M.K. and J.P.J. critically evaluated
343 the study; A.S and J.P.J. wrote the manuscript and all other authors contributed to writing the
344 manuscript.

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492

493 **Table 1: Two-way ANOVA of ADA levels between groups and sample collection times**

	CNT (n=17 pairs)	SCZ (n=11 pairs)		
Evening sample	0.862 (0.280)	1.129 (0.227)	F=-2.782; p=0.009	F=-28.671; p<0.001
Morning sample	0.731 (0.240)	1.111 (0.243)	F=-3.419; p=0.002	
	F=0.787;p=0.432	F=0.181;p=0.853	F=0.226;p=0.699	
	F=0.650;p=0.528			

494 **NOTE:** Permutation-based two-way ANOVA was used with 10,000 re-samples on log-
 495 transformed serum ADA levels. Mean (SD) values are given in the central white cells. Outer
 496 dark grey cells show the F- and p-values. Note that significant main effects exist only for
 497 group factor, and no interaction effect is seen. CNT – healthy controls; SCZ – patients with
 498 schizophrenia.

499

500 **Table 2: Correlational analysis between ADA levels and symptom severity**

	Scale for Assessment of Positive symptoms (SAPS)					Scale for Assessment of Negative symptoms (SANS)					
	Hallucinations	Delusions	Bizarre behaviour	Positive formal thought disorder	Total score	Affective flattening	Alogia	Avolition-apathy	Anhedonia	Attention	Total score
ADA (Evening – Morning)	0.81 (0.003)	0.18 (0.591)	-0.64 (0.035)	-0.38 (0.250)	0.22 (0.506)	-0.17 (0.623)	-0.39 (0.240)	-0.72 (0.013)	-0.49 (0.130)	-0.08 (0.819)	-0.41 (0.206)
ADA (Evening)	0.60 (0.050)	0.19 (0.580)	-0.38 (0.253)	-0.25 (0.457)	0.21 (0.530)	-0.09 (0.796)	-0.09 (0.787)	0.10 (0.767)	-0.20 (0.547)	0.10 (0.777)	-0.07 (0.839)
ADA (Morning)	0.05 (0.878)	0.10 (0.772)	0.02 (0.959)	0.04 (0.917)	0.08 (0.807)	0.07 (0.837)	0.20 (0.546)	0.60 (0.049)	0.17 (0.623)	0.14 (0.673)	0.25 (0.464)

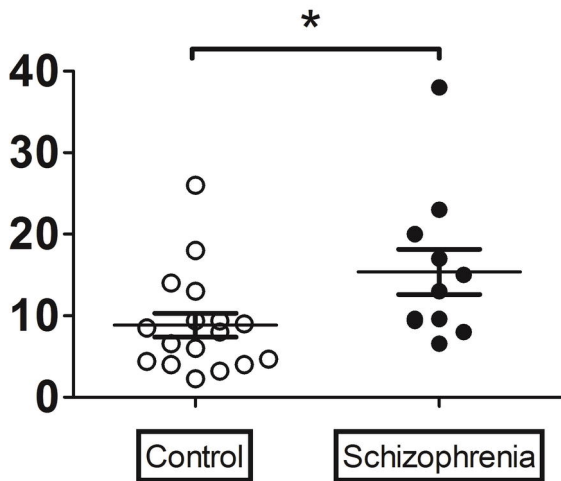
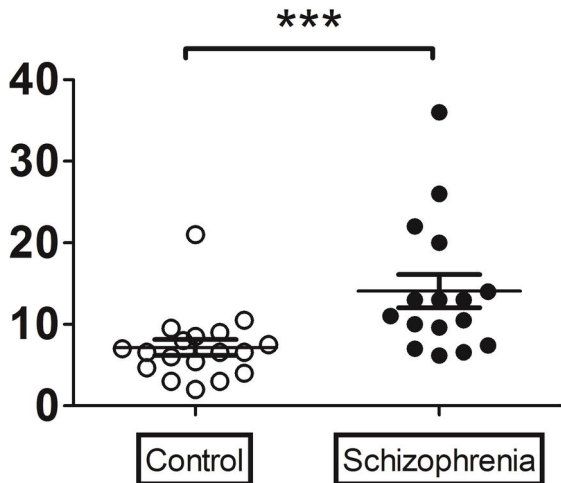
NOTE: Pearson’s Correlation test (two-tailed) was performed between log-transformed ADA levels and scores of SAPS and SANS. All data shown as: Correlation coefficient (p-value). Significant correlation is shown in bold and highlighted in dark grey. Trend correlations are shown in italics and highlighted in light grey.

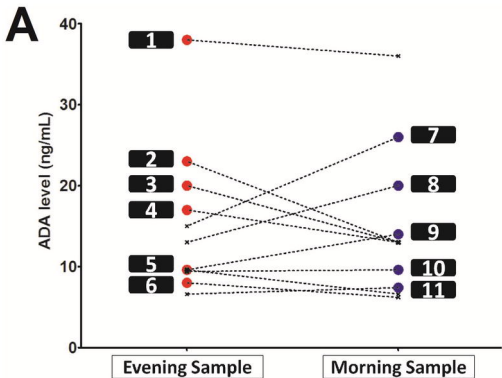
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502 **Figure legends**

503 **Fig. 1: Serum levels of Adenosine deaminase (ADA).** Scatter plots showing serum ADA
504 levels in: **A)** evening sample [control=17; schizophrenia=11], and **B)** morning sample
505 [control=18; schizophrenia=16]. Unpaired t test performed between log transformed ADA
506 values. * $p < 0.05$; *** $p < 0.001$.

507 **Fig. 2: Relation between Adenosine deaminase (ADA) levels and symptom severity. A)**
508 Line graph and table showing the relation between relatively higher ADA levels in the
509 evening samples (evening rise in ADA) or in the morning samples (morning rise in ADA),
510 with symptom severity of patients with schizophrenia [n=11]. Note that patients with higher
511 evening rise in ADA (shown in red) have higher hallucination scores, while those with higher
512 morning rise in ADA (shown in blue) have higher avolition-apathy scores. Line graph
513 showing the correlation of difference between evening and morning ADA levels versus: **B)**
514 Scale for Assessment of Positive symptoms (SAPS) hallucination sub-score [n=11 pairs,
515 $r = 0.8097$, $p = 0.0025$], and **C)** Scale for Assessment of Negative symptoms (SANS) avolition-
516 apathy sub-score [n=11 pairs, $r = -0.7165$, $p = 0.0131$]. Pearson's correlation test (two-tailed).
517 * $r > 0.735$ (see **Statistical Analysis section**).

A**ADA Evening (ng/mL)****B****ADA Morning (ng/mL)**



Evening rise in ADA level			
Subject code	ADA level (Evening) (ng/mL)	SAPS – Hallucination score	SANS – Avolition-Apathy score
1	38	17	12
2	23	16	12
3	20	20	6
4	17	8	13
5	9.6	13	8
6	8	3	9

Morning rise in ADA level			
Subject code	ADA level (Morning) (ng/mL)	SAPS – Hallucination score	SANS – Avolition-Apathy score
7	26	0	20
8	20	3	18
9	14	2	20
10	9.6	6	11
11	7.4	8	7

