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Mancini, Valentina; Sandini, Corrado; Padula, Maria; Zoeller, Daniela; Schneider, Maude; Schaer, Marie; Eliez, Stéphan

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ARTICLE

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Positive psychotic symptoms are associated with divergent 2 developmental trajectories of hippocampal volume during late 3

adolescence in patients with 22g11DS 4

Valentina Mancini 1. Corrado Sandini¹ · Maria C. Padula^{1,2} · Daniela Zöller 1.³ · Maude Schneider^{1,4} · 5 Marie Schaer¹ · Stephan Eliez^{1,5} 6

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Abstract 9

Low hippocampal volume is a consistent finding in schizophrenia and across the psychosis spectrum. However, there is a 10 lack of studies investigating longitudinal hippocampal development and its relationship with psychotic symptoms. The 11 22q11.2 deletion syndrome (22q11DS) has proven to be a remarkable model for the prospective study of individuals at high 12 risk of schizophrenia to unravel the pathophysiological processes predating the onset of psychosis. Repeated cerebral MRIs 13 were acquired from 140 patients with 22q11DS (53 experiencing moderate-to-severe psychotic symptoms) and 135 healthy 14 controls aged from 6 to 35 years and with up to 5 time points per participant. Hippocampal subfield analysis was conducted 15 using FreeSurfer-v.6 and FIRST-FSL. Then, whole hippocampal and subfield volumes were compared across the groups. 16 Relative to controls, patients with 22q11DS showed a remarkably lower volume of all subfields except for CA2/3. No 17 divergent trajectories in hippocampal development were found. When comparing patients with 22q11DS exhibiting 18 19 psychotic symptoms to those without psychosis, we detected a volume decrease during late adolescence, starting in CA1 and spreading to other subfields. Our findings suggested that hippocampal volume is consistently smaller in patients with 20 22q11DS. Moreover, we have demonstrated that patients with 22q11DS and psychotic symptoms undergo a further decrease 21 in volume during adolescence, a vulnerable period for the emergence of psychosis. Interestingly, CA2/3, despite being 22 affected in patients with psychotic symptoms, was the only area not reduced in patients with 22q11DS relative to controls, 23 thus suggesting that its atrophy exclusively correlates with the presence of positive psychotic symptoms. 24

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🖂 Valentina Mancini valentina.mancini@unige.ch

- 1 Developmental Imaging and Psychopathology Laboratory, University of Geneva School of Medicine, Geneva, Switzerland
- 2 Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland
- 3 Institute of Bioengineering, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland
- 4 Department of Neuroscience, Center for Contextual Psychiatry, Research Group Psychiatry, KU Leuven, Leuven, Belgium
- 5 Department of Genetic Medicine and Development, University of Geneva School of Medicine, Geneva, Switzerland

Introduction

26 Q1 It is widely acknowledged that the hippocampus plays a crucial role in learning, memory retrieval and imagination [1]. Beyond its involvement in memory, converging lines of 28 evidence supported by MRI [2-7] and postmortem studies 30 Q2 [8, 9] has suggested that patients with schizophrenia are characterized by a smaller hippocampus. In fact, a lower hippocampal volume has been found in patients across the 32 psychosis spectrum [3, 10, 11], comprising first-episode 33 psychosis patients (FEP) [12, 13] and subjects at high/ ultrahigh (UHR) risk for psychosis [14, 15]. However, a meta-analysis has recently shown that there was no evi-36 dence for a significant reduction in the whole hippocampal 37 38 Q3 volume in patients at clinical high risk for psychosis [16], suggesting that only subtler changes might be detectable 39 early in disease progression. Indeed, a dose-response rela-40 tionship depending on the stage of the disease has been found in individuals at UHR for psychosis [10].

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To date, many studies have reported the involvement of 43 different combinations of hippocampal subfields-CA1, 44 CA2/3, CA4, dentate gyrus and subiculum-in patients 45 with clinical high risk for psychosis [12, 17, 18]. Conse-46 quently, several theories aimed at explaining the relation-47 ship between psychotic symptoms and hippocampal volume 48 reduction have devoted great attention to the functional 49 anatomy of the hippocampus [19]. In fact, each hippo-50 campal subfield has a different density of pyramidal neurons 51 [20], diverse synaptic architecture and distinct patterns of 52 connectivity with cortical areas [21]. Notably, dentate 53 gyrus, CA3 and CA1 are part of the trisynaptic circuitry 54 responsible for encoding episodic memory [22], whereas 55 the subiculum extends the persistence of such information 56 conveying it to the neocortex [23]. 57

Even though there is no consensus on which subfield is central to the development of psychosis, CA3 and CA1 are very likely to be involved [24].

One theory posits that CA3 hyperactivity is instrumental to the onset of psychotic symptoms [25]. CA3 has a pivotal role in the hippocampal autoassociative network responsible for memory encoding and retrieval [26], so it might be involved in the generation of false memories, perceived as hallucinations by schizophrenic patients [25, 27].

On the other hand, CA1 appeared to be the earliest 67 68 affected area in UHR [28, 29] and FEP [18] patients. One study demonstrated that increased cerebral blood volume 69 (CBV) in the left CA1 of UHR patients predicted its atrophy 70 and the development of psychosis [28]. Similarly, in mouse 71 models of ketamine-induced schizophrenia, CA1 exhibited 72 the highest CBV, paralleled by an increase in extracellular 73 glutamate concentration [30]. Therefore, elevated hippo-74 campal activity and subsequent excitotoxicity might have a 75 mechanistic role in the development of atrophy [31]. 76 Finally, another study showed that UHR patients who 77 developed schizophrenia had, at the first assessment, a 78 lower right hippocampal and CA1 volume and a steeper 79 CA3 volume decline over time [29], suggesting that CA1 80 and CA3 might have different roles in the development of 81 82 psychosis.

Overall, a volume decrease in specific subfields has been 83 demonstrated cross-sectionally [10-12, 15] and long-84 itudinally [13, 14, 29, 30] in patients at clinical risk of 85 86 psychosis. Subjects with a genetic risk, such as siblings of psychotic patients, also presented hippocampal abnormal-87 ities [32-36], and hippocampal volume was demonstrated to 88 be highly heritable [37]. Therefore, lower hippocampal 89 volume is considered to be a putative endophenotype for 90 psychosis [38]. 91

However, even though the clinical high risk phase of psychosis has been extensively studied, there is a lack of studies investigating the previous period [11], i.e., that premorbid phase when possible brain dysfunctions are not

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vet accompanied by overt symptoms [39]. It is still debated 96 as to whether a lower hippocampal volume is a cause or a 97 consequence of psychotic symptoms [11], or whether this 98 relationship is even more complex, with psychosis and 99 volume decreases being bounded by reciprocal causation. In 100 this regard, the study of populations with a genetic risk of 101 psychosis provides a unique opportunity to evaluate patients 102 from childhood to clarify the temporal relationship between 103 hippocampal development and the onset of psychosis. 104

The 22q11.2 deletion syndrome (22q11DS)-a neuro-105 developmental disorder caused by a 1.5-3 Mb deletion on 106 the long arm of chromosome 22-is considered to be 107 among the most important genetic risk factors for psychosis 108 [40]. As up to 41% of patients with 22q11DS will develop a 109 psychotic disorder by adulthood [41], the syndrome has 110 been recognized as a valuable model for detecting early 111 psychosis biomarkers. Furthermore, predictive measures of 112 conversion to psychosis, such as UHR status, have been 113 validated in patients with 22q11DS [42]. Deletion carriers 114 have cognitive and learning deficits and are more prone to 115 developing psychiatric disorders, such as attention-deficit/ 116 hyperactivity disorder (ADHD), anxiety and obsessive-117 compulsive disorder (OCD) [43]. A wide range of medical 118 conditions, including congenital heart defects (CHD) and T-119 cell immunodeficiency, can accompany neuropsychiatric 120 manifestations [43]. Brain abnormalities are also a common 121 feature of the syndrome, as patients have an average 11% 122 total brain volume decrease [44] and reduced gyrification in 123 the frontal and parietal lobes [45]. Moreover, lower hip-124 pocampal volume-either driven by the hippocampal head 125 [46] or body [47–49]—has been found [50], and the size of 126 the hippocampal head has been positively correlated to the 127 onset of hallucinations [49]. However, it remains unclear at 128 which point during development the hippocampal volume 129 of patients with 22q11DS diverges from healthy subjects, 130 i.e., if hippocampal volume is already reduced in patients 131 during childhood or whether it occurs later, during adoles-132 cent brain maturation. 133

Consequently, the first objective of the present study was 134 to investigate the developmental trajectory of hippocampal 135 volume in a large cohort of patients with 22q11DS over a 136 wide timespan using a longitudinal design. The second aim 137 was to analyze the association between hippocampal 138 development and the onset of positive psychotic symptoms. 139 A longitudinal approach was chosen to provide insights into 140 whether a smaller hippocampus at baseline and/or a further 141 volume decrease is specific to 22q11DS patients experien-142 cing symptoms of psychosis. 143

We employed a recently developed automated segmentation technique from FreeSurfer v6.0, which allows a better delineation of the hippocampal subfields [51] than its previous version [52]. Furthermore, given previous studies highlighting the existence of selective morphological 148

abnormalities in the anterior and mid-body hippocampus 149 across the psychosis spectrum [7, 13, 15, 53] and in patients 150 with 22q11DS [46-49], we complemented the analysis of 151 subfields with FIRST-FSL [54], another widely used tech-152 nique, which provides information about the shape of the 153 hippocampus along the anteroposterior axis. 154

According to previous studies [46-50], we hypothesized 155 that by using these techniques, we would detect a global and 156 robust difference between patients with 22q11DS and 157 healthy controls. In light of the findings reported with UHR 158 patients [10, 13, 14, 29], we further proposed that 22g11DS 159 patients with moderate-to-severe psychotic symptoms 160 would have a volume reduction in critical subfields, such as 161 CA1 and CA3. Understanding the timing of hippocampal 162 development in patients with 22q11DS could help to predict 163 the emergence of psychotic symptoms in at-risk 164 populations. 165

Materials and methods 166

Participants 167

One hundred forty individuals with a genetically confirmed 168 diagnosis of 22q11DS and 135 healthy controls (HC) were 169 170 recruited in the context of an ongoing longitudinal study being carried out in Geneva since 2001 (additional details in 171 Supplementary Information and Supplementary Table 1). 172

The age of the patients and HC ranged from 6 to 35 173 years, and the two groups were matched for age and sex. On 174 average, each participant was assessed at 2.14 time points, 175 which varied from 1 to 5 across participants (Table 1). The 176 presence of axis I disorders according to DSM-IV criteria 177 and current use of psychotropic medication in the group of 178 patients with 22q11DS are listed in Table 2. 179

Written informed consent was obtained from participants 180 and/or their parents. The study was approved by the can-181 tonal ethics committee and conducted according to the 182 Declaration of Helsinki. 183

Psychiatric assessment 184

Patients with 22q11DS experience subthreshold psychotic 185 186 symptoms to a greater extent than the general population; [55] therefore, they are a compelling model to explore the 187 underlying neurobiology. The presence of moderate-to-188 severe psychotic symptoms was assessed at each time point 189 by means of the Structured Interview for Psychosis-Risk 190 Syndromes (SIPS), as the SIPS is a well-validated diag-191 nostic tool for assessing psychotic symptoms in deletion 192 carriers [56, 57]. Patients with 22q11DS were categorized 193 as experiencing positive symptoms of psychosis, using a 194 cutoff score of 3 or higher in at least one of the 195

corresponding items. Together with time and frequency 196 criteria, this intensity threshold has been proven by several 197 studies to be the most sensitive at detecting prodromal risk 198 syndromes [58]. Negative symptoms of psychosis, with a 199 score of 3 or higher in at least one negative SIPS subscale, 200 were taken into account separately to enable clarification of 201 the relative contribution of positive and negative symptoms 202 to hippocampal development. 203

Due to their young age, 33 patients were unable to 204 complete the SIPS, thus reducing the sample group to 107 205 patients. Negative symptoms were present in 72 patients, 206 while positive symptoms were present in 52 patients, 207 including 13 with a diagnosis of schizophrenia and 2 with 208 schizoaffective disorder. Specifically, 15 patients had a 209 score of 6 on one or more positive subscales at one or more time points.

The inclusion of a heterogeneous group of deletions carriers with various degrees of positive psychotic symp-213 toms allowed us to compare larger subgroups to discover 214 putative brain abnormalities underlying the presence of such 215 symptoms. From now onwards, all the patients with 216 moderate-to-severe positive psychotic symptoms will be 217 referred to as 22q11DS psy+ patients. 218

MRI acquisition

Due to the wide timespan of this study, the scans were 220 acquired with three different scanners: a 1.5T Philips Intera 221 scanner was used for the first 151 scans, a 3T Siemens Trio 222 for the subsequent 294 scans and a 3T Siemens Prisma for 223 the remaining 138 scans. T1-weighted images were 224 acquired at the Center for Biomedical Imaging (CIBM) in 225 Geneva with a three-dimensional volumetric pulse. The 226 1.5T scanner parameters were TR = 35 ms, TE = 6 ms, flip 227 angle = 45° , NEX = 1, matrix size = 256×192 , field of 228 view = 24 cm2, slice thickness = 1.5 mm, 124 slices. The 229 parameters for both 3T scanners were TR = 2500 ms, TE =230 3 ms, flip angle = 8° , acquisition matrix = 256×256 , field 231 view = 23.5 cm, slice thickness = 3.2 mm,of and 232 192 slices.

To avoid possible confounding factors, the scanner 234 model was entered as a covariate in all the statistical 235 analyses. 236

T1-weighted images underwent fully automated image 237 processing with FreeSurfer version 5.3.0, comprising skull 238 stripping, intensity normalization, reconstruction of the 239 internal and external cortical surface and parcellation of 240 subcortical brain regions [59]. 241

Hippocampal segmentation

A recently developed automated segmentation technique 243 published with FreeSurfer version 6.0 was used to label the 244

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	22q11DS patients	Healthy controls	T-test/ Chi square
Number of subjects (female%)	140 (51.4%)	135 (48.1%)	p = 0.934
Number of subjects with 5 visits	8	3	p = 0.124
Number of subjects with 4 visits	16	11	p = 0.313
Number of subjects with 3 visits	21	19	p = 1.000
Number of subjects with 2 visits	42	47	p = 0.613
Number of subjects with 1 visit	57	65	p = 0.785
Number of scans (total)	308	275	N/A
Number of 1.5 T scans	71	80	p = 0.09
Number of 3 T (Trio) scans	162	132	p = 0.23
Number of 3 T (Prisma) scans	75	63	p = 0.75
Age range	6–35 y.o.	6–35 y.o.	N/A
Mean age	16.24 ± 6.44	15.48 ± 5.87	p = 0.089
Mean age at first visit	13.53 ± 6.44	13.26 ± 5.33	p = 0.713
Mean distance between visits	3.80 ± 1.07	3.72 ± 1.55	p = 0.724

Table 2 Medical history comprising psychiatric disorders and medications in the whole group of patients with 22q11DS and in the subgroups with and without SIPS positive score > 3

Table 1 Demographic

information

	All 22q11DS	22q11DS SIPS positive > 3	22q11DS SIPS positive < 3	<i>p</i> -value
Number of subjects (%f)	140 (51.4%)	53 (50.94%)	54 (48.15%)	0.7725
Mean age	16.33 ± 6.44	17.64 ± 6.25	17.43 ± 6.18	0.7882
Number of scans (total)	308	134	125	N/A
Number of 1.5 T scans	71	33	27	0.564
Number of 3 T (Trio) scans	162	72	70	0.7139
Number of 3 T (Prisma) scans	75	29	28	0.883
Subjects medicated	67 (47.85%)	32 (60.38%)	26 (48.15%)	0.0767
Methylphenidate	37 (26.43%)	12 (22.64%)	17 (31.48%)	0.2089
Antidepressants	26 (18.57%)	14 (26.41%)	9 (16.7%)	0.0728
Antipsychotics	15 (10.71%)	15 (28.3%)	0	< 0.001
Anxiolytics	17 (12.14%)	10 (18.87%)	6 (11.11%)	0.0742
Antiepileptic drugs	7 (5%)	4 (7.55%)	3 (5.56%)	0.5210
More than one class of medication	21 (15%)	13 (24.53%)	7 (12.96%)	0.0120
Subjects meeting criteria for psychiatric diagnosis	97 (69.28%)	45 (84.9%)	33 (61.11%)	0.0010
ADHD	52 (37.14%)	24 (45.28%)	20 (37.04%)	0.2153
Anxiety disorders	46 (33.57%)	23 (42.59%)	17 (31.48%)	0.0784
Mood disorders	28 (20%)	13 (24.53%)	9 (16.67%)	0.1266
Psychotic disorders	20 (14.28 %)	20 (37.75%)	0	< 0.001
OCD	12 (8.57%)	4 (7.55%)	3 (5.56%)	0.5210
More than one diagnosis	40 (28.57%)	22 (41.51%)	13 (24.07%)	0.0025

NB: due to the lack of SIPS data in younger patients, the sum of the two sub-groups does not correspond to the whole group

hippocampal subfields. This algorithm employs a prob-245 abilistic atlas built from a combination of ex vivo 7T MRI 246 data from autopsied brains and in vivo 3T images of the 247 neighboring structures in a Bayesian framework [51]. 248 Compared to the previous version [52], this technique 249 provides a higher resolution and the segmentation of a 250

larger number of structures, including the cornu ammonis 251 regions (CA1, CA2/3, CA4 and their molecular layer 252 (ML)), the granule cell layer of dentate gyrus (GC-DG), the 253 hippocampal tail and fissure. Surrounding regions, such as 254 subiculum, parasubiculum, presubiculum, the 255 hippocampus-amygdala-transition-area and fimbria, are also 256

included. Given the purpose of the present study, the 257 volume of the whole hippocampus and 7 relevant subfields, 258 CA1, CA2/3, CA4, GC-DG, ML, tail and subiculum, were 259 analyzed. For an example of FreeSurfer segmentation in a 260 patient and an HC, see Fig. 1. 261

Because we used FreeSurfer v5.3 to preprocess the data 262 and v6.0 to perform hippocampal subfield segmentation, we 263 tested and confirmed the reliability of using different ver-264 sions of FreeSurfer by means of intraclass correlation 265 coefficient analysis (Supplementary Table 5). 266

To understand whether the difference between patients 267 with 22q11DS and HC has a specific distribution along the 268 anteroposterior axis, a shape analysis via the FSL software 269 FIRST [54] was also performed. This technique provides a 270 surface mesh of the hippocampus for each subject in a 271 common 3D space, modeled on intensity distribution and 272 vertex analysis. Then, an average mask was created by 273 concatenating all the hippocampal meshes of patients with 274 275 22q11DS and controls.

All the obtained images were visually inspected and then 276 excluded from the analysis if the quality of the segmenta-277 tion was inappropriate. Specifically, we carefully checked in 278 each subject that the hippocampal mask as whole was 279 correctly placed, with no portions of the hippocampus were 280 cut off or shifts of the mask beyond the borders of the 281 282 hippocampus. Then, we verified that there were no mislabeling of hippocampal subfields and extrahippocampal 283 regions; in this regard, as suggested by the quality control 284 procedure provided by the ENIGMA protocol (https:// 285 pgcptsd.com/wpcontent/uploads/2017/08/PTSD 286

Instructions_Subfields_part_IR_II.pd), any mislabeling of 287 single subfields was sufficient to exclude the whole seg-288 mentation. We therefore excluded 2 scans of patients with 289 22q11DS from FreeSurfer segmentation and 5 scans (3 290 patients and 2 controls) from FIRST-FSL analysis. 291

Statistical analyses 292

Mixed modeling has proven to be an ideal method for 293 handling nested data, such as multiple time points [60]. 294 295 Considering that participants had a variable number of time points, with an inconstant time interval and age distribution 296 (Supplementary Fig. 1), a mixed model regression analysis, 297 298 described in previous papers [61, 62], was used to analyze the longitudinal data from FreeSurfer. Briefly, population 299 parameters (age and diagnosis) were modeled as fixed 300 effects and within-subject factors as random effects by 301 using the nlmefit function in MATLAB R2017a (Math-302 Works). The normal distribution of data in each group was 303 required and therefore evaluated by our statistical analysis 304 approach. Total intracranial volume, sex, scanner model and 305 antipsychotic medications were included as covariates. 306 Developmental trajectories were estimated by fitting 307

random-slope models (constant, linear, quadratic or cubic, 308 each corresponding to a different relationship between age 309 and hippocampal volume) to our data, taking into account 310 both within-subject and between-subject effects. Then, the 311 most suitable model order was selected using the Bayesian 312 information criterion, obtaining, e.g., a full quadratic model 313 as follows: 314

$$Y_{ij} = \beta_0 + \beta_{g1} \cdot g_i + \beta_{a1} \cdot a_{ij} + \beta_{ag1} \cdot g_i \cdot a_{ij} + \beta_{a2} \cdot a_{ij}^2$$
$$+ \beta_{ag2} \cdot g_i \cdot a_{ij}^2 + u_{i0} + u_{i1} \cdot a_{ij} + \epsilon_{ij}$$

Y: hippocampal volume *i*, *j* : [subjects, scan]index β_{xn} : fixed effects g : grouping variable a:age

u : normally distributed random effect ϵ_i : normally distributed error term

The significance of the between-group differences in the intercept and in the slope were evaluated by means of a loglikelihood ratio test between the full model and any of the 321 following reduced models: 322

Reduced group	effect model
$Y_{ii} = \beta_0 + \beta_{a1} \cdot$	$a_{ii} + \beta_{a2} \cdot a_{ii}^2 + u_{i0} + u_{i1} \cdot a_{ii} + \epsilon_{ii}$

Reduced slope model

 $Y_{ij} = \beta_0 + \beta_{g1} \cdot g_i + \beta_{a1} \cdot a_{ij} + \beta_{a2} \cdot a_{ij}^2 + u_{i0} + u_{i1} \cdot a_{ij} + \epsilon_{ij}$

Hence, we obtained a comparison between the intercept (group effect) and the slope of developmental trajectories $(\text{group} \times \text{age interaction effect})$ of the hippocampal volume of each group. Finally, the results were adjusted for multiple testing with the false discovery rate correction. Where appropriate (i.e., in quadratic models), the age corresponding to the inflection point of each developmental trajectory was estimated at the intersection between the derivative of the curve in that point and the x-axis.

We further tested whether the degree of psychotic symp-337 toms as measured by the SIPS for each positive subscale (P1: 338 unusual thought content/delusional ideas, P2: suspiciousness/ 339 persecutory ideas, P3: grandiose ideas, P4: perceptual 340 abnormalities/hallucinations, P5: disorganized communica-341 tion) was correlated with hippocampal volume in patients 342 with 22q11DS by using the fitlme function in MATLAB 343 R2017a (MathWorks). The results were covaried for age, 344 age², sex, ICV, antipsychotics and scan type and finally 345 adjusted for multiple comparisons with FDR correction. 346

FSL data were analyzed cross-sectionally, selecting the 347 first time point for each participant. Statistical maps and 348 analyses were included in FIRST-FSL and obtained fol-349 lowing the pipeline described on the FSL website (https:// 350

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Right hippocampus



Fig. 1 Comparison between patients with 22q11Ds and HC. Upper panel: an example of Freesurfer v.6.0 hippocampal segmentation with coronal (\mathbf{a}, \mathbf{b}) and sagittal (\mathbf{c}, \mathbf{d}) sections in a healthy control (\mathbf{a}, \mathbf{c}) and in a patient with 22q11DS (\mathbf{b}, \mathbf{d}) of the same age. Lower panel: mixed

fsl.fmrib.ox.ac.uk/fsl/fslwiki/FIRST/UserGuide). With the 351 'randomise' function, significant differences between the 352 two groups were computed with a cluster-based multiple-353 comparison correction, covarying for total intracranial 354 volume, sex, scanner model, age and antipsychotic medi-355 cations. The output was a 3D mask showing a selective 356 inward or outward displacement in the affected regions, 357 depending on whether the hippocampus of the patients was 358 359 smaller or larger than the controls.

360 **Results**

Patients with 22q11DS have widespread reductionsin hippocampal volume

A smaller hippocampal volume was demonstrated bilaterally in patients with 22q11DS in comparison to HC by using data from FreeSurfer and FIRST-FSL segmentations.

The mixed model analysis revealed strong group differences in the whole right and left hippocampal volume (p < 1

Left hippocampus





model analysis of the developmental trajectories showing a marked smaller hippocampal volume in patients with 22q11DS without differences in the shape of the two curves

0.001), with no difference in developmental trajectories 368 (Fig. 1). Similarly, a consistently lower volume was 369 detected bilaterally in all subfields (p < 0.001), except for 370 CA2/3 (Table 3). As in a previous study on healthy participants [63], all the trajectories had a second-order model, 372 meaning that the relationship between age and hippocampal 373 volume was quadratic (Fig. 2). 374

The statistical map obtained using FIRST-FSL confirmed 375 a diffuse inward displacement in the hippocampus of 376 patients with 22q11DS involving the head, the body and the 377 tail of the hippocampus. In particular, the medial and lateral 378 surfaces of the right and left hippocampi were more con-379 sistently affected, whereas the upper and lower hippo-380 campal surfaces showed some unaffected areas along the 381 midline, irrespective of anatomical boundaries (Fig. 3). 382

22q11DS psy+ patients have altered developmental 383 trajectories of specific hippocampal subfields 384

To further assess the association between the development 385 of hippocampal volume and psychotic symptoms, 386

		22q11DS pat	ients vs HC		SIPS positive	symptoms		SIPS negativ	e symptoms	
		Model order	p-value group effect	<i>p</i> -value interaction	Model order	<i>p</i> -value group effect	<i>p</i> -value interaction	Model order	<i>p</i> -value group effect	<i>p</i> -value interaction
Whole	Left	2	<0.001	0.1788	2	0.0035	0.9481	2	0.4426	0.2647
	Right	2	<0.001	0.2463	2	0.0020	0.0054	2	0.2793	0.2657
Subiculum	Left	2	<0.001	0.2206	2	0.0114	0.4436	2	0.5947	0.3988
	Right	2	<0.001	0.0994	2	0.0900	0.2322	2	0.2260	0.1974
CA1	Left	2	<0.001	0.3251	2	0.0065	0.8213	2	0.7209	0.5478
	Right	2	<0.001	0.7646	2	0.0535	0.0574	2	0.2294	0.3190
CA2/3	Left	2	0.3554	0.7290	2	0.0179	0.8566	2	0.4540	0.2767
	Right	2	0.0470	0.6536	2	0.0035	0.0063	2	0.1311	0.2739
CA4	Left	2	<0.001	0.2197	2	0.0126	0.7546	2	0.1541	0.0760
	Right	2	<0.001	0.9006	2	0.0022	0.0024	2	0.2362	0.4083
GC-DG	Left	2	<0.001	0.1667	2	0.0136	0.9187	2	0.2106	0.1148
	Right	2	<0.001	0.7589	2	0.0023	0.0028	2	0.2387	0.3901
Molecular lay	er Left	2	<0.001	0.2253	2	0.1183	0.6901	2	0.2365	0.6374
	Right	2	<0.001	0.7880	2	0.1125	0.0633	2	0.2396	0.1986
Tail	Left	2	<0.001	0.9312	2	0.0736	0.4467	2	0.9263	0.8581
	Right	2	<0.001	0.0143	2	0.0537	0.0721	2	0.8912	0.9998

22q11DS and HC



387 patients with moderate-to-severe positive or negative symptoms were compared to patients with low symptom 388 scores. 389

The hippocampal volume of participants with at least one 390 SIPS negative symptom score ≥ 3 did not differ from the 391 group without negative symptoms (Table 3). In contrast, 392



R-hippocampus surface



Fig. 3 Results of FIRST-FSL vertex analysis of group differences between patients with 22q11DS and HC. The orange overlay indicates the hippocampal regions displaying significant inward displacement in

patients with 22q11DS. **a** medial surface; **b** lateral surface; **c** upper surface; **d** bottom surface

22q11DS psy+ patients had a decreased hippocampal volume (p = 0.002 on the right side and p = 0.0035 on the left side) (Fig. 4) compared to patients without positive symptoms. Moreover, 22q11DS psy+ patients had lower volumes of distinct subfields: bilateral CA2/3, CA4 and GC-DG and left CA1 and subiculum (Table 3). The right CA1 approached significance (p = 0.053).

Slope differences were only detected for the right hip-400 pocampus, with a developmental trajectory showing a 401 volume decrease starting from 18.5 years in 22q11DS psy+ 402 patients (Supplementary fig. 4). Right-side subfields dis-403 played similar developmental trajectories across subfields 404 with an inflection point corresponding to late adolescence: 405 CA1: 16.5 years, DG: 17.3 years, CA4: 18 years, CA2/3: 406 18.8 years (Supplementary Fig. 2). 407

408 22q11DS patients with hallucinations have aberrant 409 developmental trajectories

We evaluated whether hallucinations as measured by SCID-410 411 I or DICA (see Supplemental Information and specifically Supplementary Table 2 for further details) were specifically 412 associated with a decreased hippocampal volume. Patients 413 414 with 22q11DS who experienced hallucinations had a bilaterally reduced volume of the whole hippocampus and 415 of all the subfields, except for the left tail, in comparison to 416 those without hallucinations. Regarding all the right-side 417 subfields, except for CA1 and GC-DG, 22q11DS patients 418 with hallucinations exhibited a significantly different inter-419 action effect (Supplementary Fig. 3), comparable to that 420 found in the group of patients selected according to 421 SIPS score. 422

The degree of positive symptoms is not correlated423with hippocampal volume424

We did not find a significant correlation between any of the positive SIPS subscales and volume of the hippocampal subfields (Supplementary Table 3). Only the correlation with the P5 subscale (disorganized communication) approached significance (left hippocampus p = 0.06, R = 429 -0.247; right hippocampus p = 0.06, R = -0.217). 430

22q11DS patients with CHD have a smaller hippocampus

The 22q11DS patients with major CHD who underwent 433 heart surgery (22q11DS CHD+) had a smaller hippo-434 campus than those without cardiac malformations (see 435 Supplementary Table 4 for demographic information in the 436 two groups). All the subfields, except for CA2/3, were 437 bilaterally decreased in 22q11DS CHD+. No interaction 438 effect was detected, except for the right CA2/3. The left 439 CA2/3 area did not show a group or interaction effect 440 (Supplementary Fig. 4). 441

Discussion

A smaller hippocampal volume is an anatomical trait of patients with 22q11DS

Our findings pointed to a smaller global hippocampal 445 volume in patients with 22q11DS than HC, broadening the 446 evidence of a reduced hippocampal head [46] and body 447 [47–49] previously demonstrated in smaller samples. 448

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Fig. 4 Comparison between developmental trajectories of 22q11DS psy+ and psy- patients. Upper panel: developmental trajectories of the whole right and left hippocampal volume. Lower panel: each arrow

specifies the subfield showed in the plot according to the color displayed in the legend

According to the FIRST-FSL shape analysis, a significant volumetric difference without an anteroposterior gradient was detected, thus confirming a diffuse volume reduction in the group of patients with 22q11DS. Our results are therefore in line with previous MRI studies demonstrating a lower hippocampal volume in nonsyndromic UHR patients [14] and healthy relatives of schizophrenic patients [32, 33].

Similarly, all the subfields displayed a consistently 456 smaller volume except for CA2/3. The reason why CA2/3 is 457 458 the only subfield that was not affected remains elusive. However, from the observation that CHD could influence 459 hippocampal volume in 22q11DS [64], we can formulate 460 461 some hypotheses. CA3 is the most ischemia-resistant area in the hippocampus because of its more efficient vasculariza-462 tion, provided by the large dorsal intrahippocampal arteries 463 [65]. If CHD had a pivotal role in determining a smaller 464 hippocampal volume in 22q11DS, then CA3 vasculariza-465 tion would be a protective factor towards hypoperfusion due 466 to hemodynamic instability. Our data partially support this 467

hypothesis, as all the subfields, except for CA2/3, were 468 bilaterally reduced in 22q11DS patients with CHD. More-469 over, one study employing magnetic resonance angiography 470 reported hypoplasia of the right posterior cerebral artery 471 (PCA) in more than half of a sample of patients with 472 22q11DS [66]. Notably, PCA is the major source of hip-473 pocampal vascularization [65], so minor vascular anomalies 474 might explain why the whole group of patients with 475 22q11DS had a smaller hippocampal volume than the 476 HC group. 477

In patients with 22q11DS, the whole hippocampus and 478 its subfields showed no divergent volume trajectories. 479 Hence, a lower hippocampal volume already characterized 480 the group of patients from the age of 6. Developmental 481 studies on healthy subjects have documented that the most 482 significant increase in hippocampal volume occurs during 483 the first years of life [67], especially between the ages of 1 484 and 2 [68], while from 4 to 25 years of age, no global 485 volume change was detected [69]. Likewise, the notion of 486

adult hippocampal neurogenesis in humans has recently 487 been disputed [70]. Unfortunately, the timespan of our 488 longitudinal dataset starts from the age of 6, so we cannot 489 exclude a different maturation of the hippocampus before 490 this period. However, patients with 22q11DS are known to 491 carry a wide range of brain abnormalities with prenatal 492 origin, such as cortical folding alterations [45]. A recent 493 study posits the DGCR2 gene, located in the 22a11.2 494 region, as a pivotal regulator of early stages of cortico-495 genesis in utero [71], implying early embryonic pathologi-496 cal processes conferring vulnerability to schizophrenia. 497 Interestingly, even TBX1, another gene haploinsufficient in 498 22q11DS and related to CHD, can alter proper neuronal 499 migration and disrupt corticogenesis [72]. However, CHD 500 could have alternatively acted through hemodynamic 501 mechanisms, since the presence of CHD in fetuses inhibited 502 the autoregulation mechanism aimed at maintaining con-503 stant cerebral perfusion [73]. 504

In conclusion, we demonstrated a consistently lower hippocampal volume from the age of 6 in patients with 22q11DS, which might, therefore, be an anatomical trait of the syndrome. The results with those with CHD offer exploratory evidence for the role of cardiovascular anomalies in determining a smaller hippocampal volume, presumably during corticogenesis.

512 22q11DS psy+ patients have a further hippocampal 513 volume decrease in specific subfields

We observed that positive but not negative psychotic 514 symptoms are related to a smaller hippocampal volume in 515 patients with 22q11DS. Regarding positive symptoms, 516 several studies have demonstrated a correlation with 517 decreased hippocampal volume [74-77]. However, the 518 results have been conflicting with regard to negative 519 symptoms [17, 74, 78]. This lack of an association between 520 hippocampal volume and negative symptoms could depend 521 on the absence of shared pathophysiological mechanisms 522 involving the hippocampus or on confounding factors 523 peculiar to our sample, such as the high rate of psychiatric 524 comorbidities. 525

Strikingly, CA2/3 was the only hippocampal subfield 526 that did not differ in the group of 22q11DS psy+ patients 527 when compared with HC, but CA2/3 underwent progressive 528 atrophy in the patients. As such, its later involvement could 529 be directly related to the appearance of positive symptoms. 530 The relationship between positive psychotic symptoms and 531 the hippocampus—especially the CA3 area—lies at the core 532 of theoretical frameworks connecting memory and halluci-533 nations [25, 27] and has been further corroborated by 534 empirical evidence. Tamminga et al. proposed that hallu-535 cinations arise from the imbalance between the independent 536 mechanisms of pattern separation and pattern completion, 537

respectively, involved in distinguishing new sensory inputs 538 that differ slightly from previously stored memories and in 539 the retrieval of memories from fragmented sensory cues 540 [27]. The CA3 area is responsible for both of these pro-541 cesses. If there is a DG hypofunction, which is heavily 542 involved in pattern separation [79], then hyperactivity in 543 CA3-driven pattern completion [80] leads to wrong asso-544 ciations and false memories, possibly resulting in halluci-545 nations [27, 81]. In keeping with this theory, it has been 546 demonstrated that FEP and chronic schizophrenia patients 547 have a selective impairment in pattern separation with 548 respect to healthy controls [82–84]. Subsequently, as shown 549 in other studies, hippocampal hyperactivity can later lead to 550 atrophy [28, 85]. Consistent with this, only those patients 551 with Parkinson's disease experiencing hallucinations 552 exhibited hippocampal atrophy [86]. Furthermore, 21% of 553 patients with selective hippocampal stroke experienced 554 transient hallucinations [87]. Hence, the connection 555 between positive symptoms, especially hallucinations, and a 556 decreased hippocampal volume becoming increasingly 557 accepted in the literature. 558

Nevertheless, the quest to understand the relationship 559 between hippocampal dysfunction and psychosis is yet to be 560 completed, as we still do not know at what point of 561 development it occurs. Our findings showed that several 562 right-side hippocampal subfields atrophied over time start-563 ing from late adolescence in 22q11DS psy+ patients. 564 Accordingly, a multisite study demonstrated the highest rate 565 of subthreshold positive symptoms in patients with 566 22q11DS during adolescence [56], suggesting that hippo-567 campal volume decreases occurred in a period that was 568 sensitive to the emergence of psychotic symptoms. 569

Overall, in 22q11DS psy+ patients, atrophy started in 570 the CA1 area and subsequently involved the DG, CA4 and 571 CA2/3 subfields. Interestingly, a similar pattern of pro-572 gression has been previously described in the early phases 573 of schizophrenia, with hippocampal volumetric deficits 574 spreading over time from CA1 to CA2/3 and DG [18]. Our 575 results not only confirmed such a progression but also 576 specifically associated CA3 volumetric loss with the pre-577 sence of psychotic symptoms. Therefore, our findings add 578 to the effort of defining the timeframe in which preclinical 579 pathophysiological processes occur, leading to the onset of 580 the first psychotic symptoms. 581

On the other hand, the left-side hippocampus group 582 differences started from the age of 6 without divergent 583 trajectories. We do not know whether these asymmetrical 584 trajectories were due to inadequate sample size or depend 585 on some functional hemispheric specialization. Hippo-586 campal asymmetry, with a larger right side, has been 587 demonstrated at every stage of healthy development in 588 adults [88], children [89] and infants [90], as well as in 589 patients with schizophrenia [91]. Likewise, in other studies, 590

the right hippocampal volume had a different develop-591 mental trajectory from the left [67], increasing more quickly 592 from childhood to adolescence [92]. Our findings of later-593 alized trajectories might, therefore, reflect a different speed 594 of the pathophysiology of schizophrenia within the right 595 hippocampus and left hippocampus. A captivating hypoth-596 esis is that the asymmetry of carotid and vertebral arteries 597 reported in deletion carriers [93] and related to the abnormal 598 development of the derivatives of the third and fourth 599 branchial arches might partly explain our lateralized find-600 ings. However, future studies in larger samples are needed 601 to test whether asymmetrical trajectories of hippocampal 602 development are related to the risk of psychosis. 603

Lower hippocampal volume is associated with the presence rather than the degree of positive symptoms

607 We tested whether the severity of positive symptoms was correlated with the degree of hippocampal volume loss in 608 each subfield. Although there is evidence for such a cor-609 relation in independent samples of patients with schizo-610 phrenia [74, 75], in our group of patients with 22q11DS, we 611 did not find any significant result. Nonetheless, we 612 demonstrated subfield-specific progressive involvement 613 starting in late adolescence, suggesting that there is indeed a 614 link between disease progression and hippocampal volume 615 decrease. 616

This discrepancy might be explained by the fact that positive psychotic symptoms have an inherently fluctuating nature that does not necessarily parallel the general progression of the disease [94], especially in patients with 22q11DS [42, 95].

Hippocampal volume as a vulnerability factor for psychosis: hypotheses and perspectives

Considering our findings, it is worth noting that the volumes 624 of most of the areas expected to have a role in psychosis, 625 such as CA1, DG, CA4 and subiculum, were already lower 626 in the entire 22q11DS group compared to the HC group. 627 Therefore, we cannot ignore the fact that 22q11DS is per se 628 a risk factor for the development of psychosis. Furthermore, 629 630 22q11DS psy+ patients had a smaller left-side hippocampus starting from the age of 6. Taking into account 631 hallucinations instead of the SIPS score (Supplementary 632 Information), allowed us to include more patients between 633 the ages of 6 and 10, showing that patients with halluci-634 nations had a smaller right-side hippocampus even during 635 childhood. Taken together with the observation that these 636 areas undergo a further decrease during late adolescence, it 637 is conceivable that a smaller hippocampal volume at 638

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baseline could be a vulnerability factor for developing 639 positive psychotic symptoms and hippocampal atrophy. 640

We, therefore, propose a framework that could explain 641 our results in light of some recent findings. Increased hip-642 pocampal activity, either in cerebral blood flow [28] or 643 glutamatergic tone [30, 80], has been shown to precede 644 atrophy in psychotic patients and mouse models 645 [28, 30, 96]. The most commonly accepted interpretation is 646 that enhanced glutamatergic activity requires an increased 647 blood supply and can lead to atrophy through excitotoxicity 648 mechanisms [31]. Interestingly, a continuum of increased 649 glutamate levels has been found from controls to psychotic 650 22q11DS patients using MRS [97], suggesting that pre-651 morbid 22q11DS patients might have an excitatory/inhibi-652 tory imbalance that worsens along with the progression of 653 psychosis. We can speculate that reduced hippocampal 654 volume at baseline could lead to compensatory mechanisms 655 involving enhanced glutamatergic transmission. Then, 656 environmental factors known to interfere with hippocampal 657 physiology, such as stress [98] and neuroinflammation [99], 658 may act as a second hit in some patients, resulting in an 659 abnormally increased demand, which would, in turn, lead to 660 psychotic symptoms and additional atrophy. 661

Further research is required to disentangle the relationship between hippocampal morphology and the excitatory/ inhibitory imbalance in relevant subfields in patients with 22q11DS.

Limitations and conclusion

Several limitations need to be taken into consideration when 667 interpreting our results. First, compared to HC, patients with 668 22q11DS had many psychiatric comorbidities. However, 669 the aim of this analysis was to explore the overall effect of 670 the 22q11.2 deletion on hippocampal development, irre-671 spective of phenotypic manifestations. Second, 22q11DS 672 psy+ were comparable to those without positive symptoms 673 regarding each psychiatric comorbidity, except for having 674 more than one psychiatric diagnosis and taking anti-675 psychotic medications (Table 2). However, covarying for 676 antipsychotic medications, which were shown in some 677 studies to decrease hippocampal volume [100], did not 678 affect any of our findings. Moreover, many other studies 679 failed to demonstrate a direct relationship between hippo-680 campal volume and antipsychotics [2, 10, 17, 18]. To rule 681 out any interference of CHD and psychiatric comorbidities 682 as anxiety and mood disorders, we added those variables as 683 covariates, and there was still a strong effect of psychotic 684 symptoms, both regarding the group effect and the inter-685 action with age (Supplementary Table 6). Furthermore, we 686 separately took into consideration anxiety and mood dis-687 orders and estimated the hippocampal developmental tra-688 jectories according to the diagnosis of each of these 689

comorbidities. However, we did not find any evidence of an 690 effect on hippocampal volume (Supplementary Table 7). 691 Third, only 15 patients formally met the criteria for a 692 diagnosis of schizophrenia: therefore, we lacked the power 693 to predict the development of a full-blown disorder. Indeed, 694 the psychosis literature would greatly benefit from long-695 itudinal investigations of hippocampal development pre-696 dicting the conversion to psychosis in patients with 697 22q11DS. 698

Although three different scanners were employed over 699 time in data collection, the number of 22q11DS patients and 700 HC acquired with each scanner was comparable (Tables 1 701 and 2), and the results were covaried according to the 702 scanner model. Finally, the lack of data before the age of 6 703 and after 35 prevented us from obtaining a broader picture 704 of hippocampal development, although adolescence is 705 considered the most sensitive period for psychosis. 706

In summary, we demonstrated in a large sample of 707 708 patients with 22q11DS a decreased hippocampal volume compared to HC, suggesting that this could be an anato-709 mical trait of the syndrome. A progressive decrease in the 710 volume of the right hippocampus starting from late ado-711 lescence was found in 22q11DS psy+ patients. With regard 712 to hippocampal subfields, CA1 was the first affected area, 713 while CA3 was the last, and its atrophy was exclusively 714 715 correlated with positive symptoms.

As far as we are concerned, no study in the general 716 population has ever longitudinally evaluated the occurrence 717 of psychotic symptoms and hippocampal volume changes 718 over such a broad timespan. Therefore, in light of our 719 findings and considering that healthy relatives of schizo-720 phrenia patients carry hippocampal malformations [32–36]. 721 future studies should address whether a smaller or abnormal 722 hippocampus is also present from childhood in non-723 syndromic subjects who will later develop schizophrenia. 724

Code availability 725

The code employed to model hippocampal developmental 726 trajectories is available upon request. 727

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