NPCs and neurons[11] with a transcriptional profile most similar to fetal forebrain tissue,[12] whereas data presented here is from neurons derived from SHH/FGF8-treated EBs (Supplementary Table 1). Although this report[10] (and ours) utilized the very same control and SZ hiPSC lines,[11] direct comparisons are difficult, given that the TH-positive neurons have different spatial patterning.

It is critical to note that the field still lacks a full electrophysiological characterization confirming that TH-positive neurons derived from SZ patients are in fact functionally mature DA neurons. Others have rigorously demonstrated DA characteristic features, such as overshooting action potentials with prominent K+ currents,[13] after-spike hyperpolarizations,[13] tonical firing patterns[13,14] and DA release,[7,14] in control hiPSC-differentiated or fibroblast-induced DA neurons. Pharmacologically, the repetitive firing pattern of mature DA neurons is reversibly inhibited following the addition of DA (or a DA receptor agonist such as quinpirole).[13] In addition, some populations of DA neurons are susceptible to the toxin 1-methyl-4-phenylpyridinium (MPP+).[14] Moreover, because diverse neuronal populations express TH,[3,15–17] these functional validations need to be accompanied by demonstration of markers associated with DA production and release, such as AADC and DAT.

So what could explain the different findings in these reports? One explanation may relate to the heterogeneity of SZ patients used to derive hiPSC lines, Robicsek *et al.*[1] derived lines from three patients with paranoid SZ, whereas we and Hook *et al.*[10] derived lines from three clinically heterogeneous SZ patients (Supplementary Table 2). In addition, the reprogramming technique and somatic cell source, as well as the demographic status and treatment history may also represent confounding variables[1,11,18] (Supplementary Table 2); however, as the particulars of the later are unknown, it is difficult to assess what contribution this may have had. Another possibility is that simple methodological differences, such as media composition, patterning protocols, neuronal density and/or length and extent of neuronal maturation, may account for the vastly different final compositions of the neuronal populations obtained in these reports. Ultimately, many of these methodological variables could lead to differences in oxidative stress, which has been increasingly linked to SZ in animal models[19–21] and human studies.[22] Moreover, increased reactive oxygen species and oxidative stress, impaired mitochondrial function and sensitivity to sub-threshold environmental stresses are among the phenotypes reported in a number of recent hiPSC-based[1,12,23,24] and olfactory neural stem cell-based[25] studies of SZ.

To conclusively resolve whether SZ hiPSC-derived DA neurons have specific defects in patterning, maturation or survival relative to controls, researchers need to not just utilize larger cohort sizes with known clinical and treatment history, but couple this to a more rigorous phenotypic, biochemical and functional characterization of neuronal fate, particularly on neurons derived from protocols that generate midbrain DA neurons,[7,9] the DA subtype currently hypothesized relevant to SZ. Only in this way can we begin to identify neuronal subtype-specific defects contributing to SZ.

CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

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Altered mTORC1 signaling in multipotent stem cells from nearly 25% of patients with nonsyndromic autism spectrum disorders

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Recent autism spectrum disorders (ASD) research supports a model wherein disinhibited mTORC1 signaling and dysregulated protein synthesis in neurons contribute to the clinical features of

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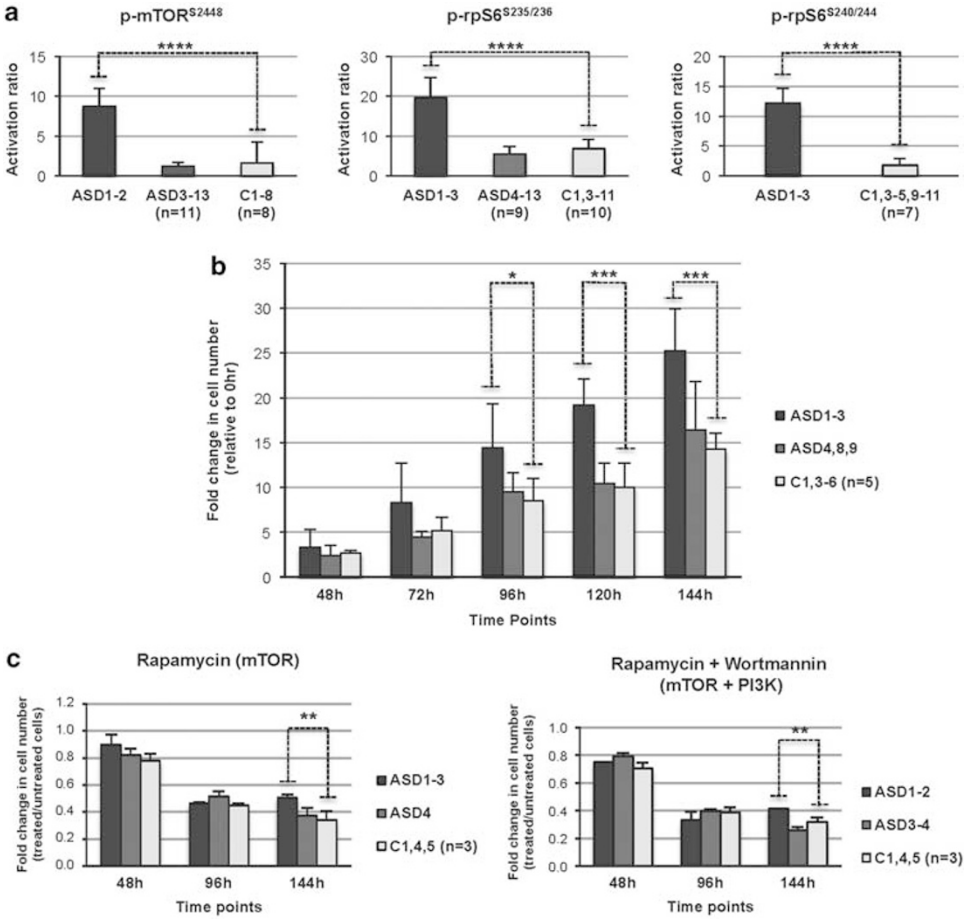

Figure 1. (**a**) mTORC1-signaling pathway analysis in SHEDs derived from patients (ASD1–13) and controls (C1–11)**.** Western blot analysis of p-mTOR^{S2448}, p-rpS6$^{S235/236}$ and p-rpS6$^{S240/244}$ was conducted in serum-deprived and serum-stimulated cells and the bar graphs represent the fold changes in the phosphorylation levels of these proteins in the fetal bovine serum (FBS)-stimulated condition as compared with the FBS-deprived condition (the activation ratio, see Supplementary Information). (**b**) Proliferation of SHEDs derived from patients and controls. SHEDs were cultured in growth medium with 20% FBS and were collected and counted at the indicated time points. Results are expressed as the fold increase in cell number relative to 0 h. Similar results were obtained using 0.5, 5 and 10% FBS (Supplementary Figure 1b). (**c**) Effect of mTOR and PI3K-mTOR inhibition on the proliferation of SHEDs derived from patients and controls**.** SHEDs were cultured in growth medium (20% FBS) supplemented with either the vehicle or 100 nM rapamycin, and with either the vehicle or 100 nM rapamycin plus 1 uM wortmannin, collected and counted at the indicated time points. Bar graphs represent the fold change of the number of drug-treated versus untreated cells (vehicle only). Patient samples were divided into two groups according to the phosphorylation state of mTORC1-signaling components (**a**), and response to serum (**b**) or drug treatment (**c**). It is noteworthy that, as we have not analyzed stem cells from two different teeth of the same individual, the extent of intra-individual variation is unknown. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$; see Supplementary Information.

ASD, mainly through disturbances in synaptic connectivity and plasticity.[1,2] Most insights into this pathomechanism came from ASD-related monogenic syndromes caused by loss of function mutations in negative regulators of PI3K-mTOR pathway (such as TSC1/TSC2, tuberous sclerosis syndrome; FMR1, fragile-X syndrome).[3,4] In addition, it has been recently reported aberrant mTORC1-dependent translation in a mouse model for nonsyndromic ASD (eIF4E).[5] However, functional studies addressing mTORC1-signaling activity in accessible sources of cells from patients with nonsyndromic ASD are lacking. In this study we have made use of the cultured stem cells from human exfoliated deciduous teeth (SHEDs) derived from nonsyndromic ASD patients, a model system that, albeit non-neural, we have recently shown to be suitable to explore dysregulated pathways and biological processes in ASD,[6] to investigate this important and yet poorly explored question.

Because mTORC1 signaling functions as an environmental sensor of nutrient availability, we took advantage of the use of cultured SHEDs derived from 13 patients (ASD1–13) and 11 age- and sex-matched controls and examined the status of mTORC1 activity in serum-deprived and serum-stimulated (after starvation) cells (see Supplementary Information). Phosphorylation analysis of key mTORC1 cascade components (p-mTOR^{S2448} and its downstream effector p-rpS6$^{S235/236 \text{ and } S240/244}$) suggested hyperfunction of mTORC1 signaling in SHEDs derived from three patients (ASD1–3), 23% of our patient sample (Figure 1a, Supplementary Figure 1a). Notably, no differences in the phosphorylation levels of mTOR and rpS6 between ASD1–3 and control cells were observed when the cells were maintained in standard growth medium without a prior step of serum starvation followed by serum stimulation (Supplementary Figure 1a), suggesting that the extracellular environment has an important role in determining ASD cell phenotype.

In accordance with the hyperresponsive mTORC1 signaling, growth curves of ASD1–3 SHEDs in response to different fetal bovine serum concentrations showed that these cells could be

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clearly distinguished from the other samples analyzed, and displayed a 1.5–2-fold greater increase in cell number, mainly at the later time points of the curve (Figure 1b, Supplementary Figure 1b), and reduced response to the antiproliferative effect of rapamycin, a specific mTOR inhibitor (Figure 1c, Supplementary Figure 1c). Curiously, whereas dual PI3K-mTOR inhibition (wortmannin+rapamycin treatment) continued to be less effective in reducing to a similar extent the proliferation of ASD1–2 and control cells, it was able to restore the enhanced proliferation of ASD3 cells (Figure 1c, Supplementary Figure 1c), suggesting a possible different regulatory mechanism in ASD3 cells. Knockout mouse models of both syndromic and nonsyndromic ASD with disinhibited mTOR signaling and dysregulated protein synthesis present dysplastic and enlarged neurons with increased spine density in several brain regions.[5,7,8] Although we did not notice increased ASD1–3 SHED volumes, it will now be important to determine and explore further whether the altered proliferative phenotype observed in these cells may also be observed in neuronal cell types, which could be a potential additional mechanism whereby disrupted mTORC1 signaling contributes to ASD neuropathology.

Conventional karyotyping and analysis of copy number variations at 15q11-q13, 16p11 and 22q13, found to occur more often in ASD, did not reveal any genomic aberrations in all patients except ASD3, who presents an inverted duplication of 15q11-q13. It is possible that genes located at this region may contribute to the aberrant molecular and cellular phenotypes observed in ASD3 cells. In addition, *TSC1/2*, *FMR1*, *PTEN, NF1* and *MeCP2* genes (ASD-associated genes known to be negative regulators of PI3K-mTOR signaling pathway) were screened for coding and splice-site mutations in ASD1–13 patients and no potentially deleterious variants were identified. Therefore, the causative genetic architecture underlying mTORC1 overactivity in ASD1–3 cells remains to be determined. It is also noteworthy that patients ASD1–3, who seem to some extent share overlapping pathophysiological mechanisms, show different degree of cognitive and social impairments: ASD1 was diagnosed with Asperger syndrome and ASD2–3 with low-functioning autism (Supplementary Table S1), suggesting that other genetic and environmental modifier factors may also have a role in cognitive development in these patients.

In conclusion, our results suggest that dysregulation of mTORC1 signaling has an important role in the pathogenesis of a subgroup of nonsyndromic ASD, and that mTOR pathway components might be promising therapeutic targets for these patients. Importantly, during the revision process of this manuscript, it was reported hyperactivated mTOR signaling in postmortem brain tissue from both adolescent patients with idiopathic ASD[9] and 15q11-q13 duplication patients with ASD,[10] which provide further strong support for this hypothesis. Finally, our results suggest that SHEDs are an alternative and more readily accessible source of patient material to study disease pathophysiology and to refine treatment approaches for individual patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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OPEN

Resilience after 3/11: structural brain changes 1 year after the Japanese earthquake

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Stressful events can have both short- and long-term effects on the brain.[1,2] A recent investigation by our lab identified regional grey matter volume (rGMV) changes in people in the months following the Japanese earthquake.[3] These findings indicated that smaller anterior cingulate cortex volume was a preexisting vulnerability factor for posttraumatic stress disorder (PTSD) symptoms and that decreased volume of the orbitofrontal cortex (OFC) was a result of these acquired symptoms.[3] These types of symptoms were regarded as manifestations of the short-term effects of post-earthquake stress. However, the long-lasting effects of stressful events on brain structures remain unclear. Thus, this study examined the 1-year prognoses of subjects after a stressful event to clarify the long-term effects of stress on structural brain changes.

Of the 42 subjects included in our previous study,[3] 37 subjects (male/female (M/F) = 28/9, age = 21.0 ± 1.6 years) were recruited for a third time, and their structural magnetic resonance imaging (MRI) scans were evaluated 1 year after the earthquake. The optimized voxel-based morphometry (VBM) method for a brain structural data set (for greater detail, see Sekiguchi *et al.*,[3]) was applied, and rGMVs from before (Pre), soon after (Post) and at the 1-year follow-up (Follow-up) of the earthquake were compared using conjunction analyses. In addition, we also assessed the subjects' psychological characteristics, including anxiety, depression, posttraumatic growth and self-esteem. Furthermore, we

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