European Neuropsychopharmacology (2016) **26**, 1150–1160

www.elsevier.com/locate/euroneuro

An acetylcholine alpha7 positive allosteric modulator rescues a schizophrenia-associated brain endophenotype in the 15q13.3 microdeletion, encompassing *CHRNA7*

Natalia Gass[a,*], Wolfgang Weber-Fahr[a], Alexander Sartorius[a,b], Robert Becker[a], Michael Didriksen[c], Tine Bryan Stensbøl[c], Jesper Frank Bastlund[c], Andreas Meyer-Lindenberg[b], Adam J. Schwarz[d,e,f]

[a]*Research Group Translational Imaging, Department of Neuroimaging, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Germany*
[b]*Department of Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Germany*
[c]*H. Lundbeck A/S, Valby, Denmark*
[d]*Tailored Therapeutics - Neuroscience, Eli Lilly and Company, Indianapolis, IN, USA*
[e]*Department of Psychological and Brain Sciences, Indiana University, Bloomington, IN, USA*
[f]*Department of Radiology and Imaging Sciences, Indiana University, Indianapolis, IN, USA*

Received 24 January 2016; received in revised form 20 March 2016; accepted 21 March 2016

KEYWORDS
15q13.3 Microdeletion;
Mouse model;
Schizophrenia;
Resting-state fMRI

Abstract
The 15q13.3 microdeletion copy number variation is strongly associated with schizophrenia and epilepsy. The *CHRNA7* gene, encoding nicotinic acetylcholine alpha 7 receptors (nAChA7Rs), is hypothesized to be one of the main genes in this deletion causing the neuropsychiatric phenotype. Here we used a recently developed 15q13.3 microdeletion mouse model to explore whether an established schizophrenia-associated connectivity phenotype is replicated in a murine model, and whether positive modulation of nAChA7 receptor might pharmacologically normalize the connectivity patterns. Resting-state fMRI data were acquired from male mice carrying a hemizygous 15q13.3 microdeletion ($N=9$) and from wild-type mice ($N=9$). To study the connectivity profile of 15q13.3 mice and test the effect of nAChA7 positive allosteric modulation, the 15q13.3 mice underwent two imaging sessions, one week apart, receiving a single intraperitoneal injection of either 15 mg/kg Lu AF58801 or saline. The control group comprised wild-type mice treated with saline. We performed seed-based functional

*Correspondence to: Department of Neuroimaging, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, J5, 68159 Mannheim, Germany. Tel.: +49 0621 17032966.
E-mail address: natalia.gass@zi-mannheim.de (N. Gass).

http://dx.doi.org/10.1016/j.euroneuro.2016.03.013

connectivity analysis to delineate aberrant connectivity patterns associated with the deletion (15q13.3 mice (saline treatment) versus wild-type mice (saline treatment)) and their modulation by Lu AF58801 (15q13.3 mice (Lu AF58801 treatment) versus 15q13.3 mice (saline treatment)). Compared to wild-type mice, 15q13.3 mice evidenced a predominant hyperconnectivity pattern. The main effect of Lu AF58801 was a normalization of elevated functional connectivity between prefrontal and frontal, hippocampal, striatal, thalamic and auditory regions. The strongest effects were observed in brain regions expressing nAChA7Rs, namely hippocampus, cerebral cortex and thalamus. These effects may underlie the antiepileptic, procognitive and auditory gating deficit-reversal effects of nAChA7R stimulation.

1. Introduction

The 15q13.3 microdeletion copy number variation (CNV) is strongly associated with schizophrenia (OR=11.5–17.9), idiopathic generalized epilepsy (OR=68) and autism spectrum disorders (OR=∞) in humans (Dibbens et al., 2009; Moreno-De-Luca et al., 2013; Sebat et al., 2009). Cognitive deficits are also prevalent: 15q13.3 deletion carriers have an average non-verbal IQ of 60, show behavior abnormalities such as attention problems, hyperactivity and impairments in functional communication (Ziats et al., 2016).

While 25% of this microdeletion occurs *de novo*, in the majority of cases (75%) it is inherited (Hoppman-Chaney et al., 2013). The microdeletion comprises a region of approximately 1.5 megabase (million base pairs of DNA), encompassing 7 genes (*MTMR15*, *MTMR10*, *TRPM1*, *MIR211*, *KLF13*, *OTUD7A*, and *CHRNA7*). Convergent evidence has led to the hypothesis that the *CHRNA7* gene, encoding nicotinic acetylcholine alpha 7 receptors (nAChA7Rs), is one of the genes in this deletion responsible for the neuropsychiatric phenotype. For example, carriers of smaller deletions, encompassing only *CHRNA7*, manifest similar phenotypes (Gillentine and Schaaf, 2015); *CHRNA7* promoter mutations downregulating transcription are associated with schizophrenia and auditory gating deficits (Leonard et al., 2002), although this mutation is a weaker predictor of the schizophrenia phenotype, thus suggesting that other 15q13.3 CNV genes also contribute to the phenotype. Schizophrenia patients have reduced levels of nAChA7Rs in the hippocampus, reticular nucleus of thalamus, dentate gyrus and frontal cortex (Rowe et al., 2015); and expression of *CHRNA7* is reduced in autism (Yasui et al., 2011).

Pharmacological stimulation of nAChA7Rs restores auditory gating deficits in schizophrenia models in rodents (Hajos and Rogers, 2010), and results in activation of the prefrontal cortex and shell of nucleus accumbens, similar to conventional antipsychotics (Hansen et al., 2007). nAChA7Rs rapidly (within just a few milliseconds) desensitize in response to a full agonist; therefore positive allosteric modulators (PAMs) seem to be an ideal approach, as they do not bind to the orthosteric receptor agonist binding site, but rather act via the allosteric binding site to increase the channel conductance without affecting receptor desensitization (Type I), or reducing receptor desensitization (Type II) (Hajos and Rogers, 2010). A novel brain-penetrant PAM, Lu AF58801, has been recently developed and shown to attenuate phencyclidine-induced deficits in a novel object recognition task in rats, a paradigm which has some relevance to cognitive deficits in schizophrenia (Eskildsen et al., 2014). It belongs to Type I PAMs which facilitate transition from resting to open channel state upon binding of an agonist, increasing agonist response amplitude without significant effect on response decay rate. The compound was tested in a number of *in vitro* safety toxicology and pharmacology assays and has been shown to be non-cytotoxic and not leading to formation of reactive oxygen species (Eskildsen et al., 2014). Also it has no effect on basal locomotion and exploratory activity in the novel object recognition task (internal Lundbeck data).

Increasingly, psychiatric disorders are being conceptualized in terms of dysregulation of extended brain networks, and aberrant connectivity between brain regions, as measured by electrophysiological or functional imaging methods, is emerging as an important biomarker (Dawson et al., 2015). Dysfunctional connectivity indicates a disruption of information flow and interaction between distinct brain regions and is thought to underlie specific symptoms. For example, auditory hallucinations in schizophrenia are hypothesized to result from disengagement of default-mode-network functional connectivity to the auditory cortex and the latter's association with the control executive network, which could assign an external origin to the hallucinated voices rather than relating them to the internal origin (Northoff, 2015). In addition, the hippocampal-prefrontal network has abnormal connectivity in schizophrenia (Esslinger et al., 2009), which may underlie the working memory deficits in that disorder.

Recently, a mouse model of the 15q13.3 deletion has been developed and described, in which mice have approximately 50% downregulation of nAChA7 receptors expression in the brain (Fejgin et al., 2014). Mice carrying 15q13.3 deletion display auditory processing deficits similar to schizophrenia, propensity to develop myoclonic seizures, impaired long-term spatial reference memory and reduced capacity to generate gamma oscillations in response to auditory stimulus (Fejgin et al., 2014).

In the current study we tested the brain connectivity profile of 15q13.3 mice to delineate systems-level brain changes that could represent a translational endophenotype and provide a platform for target validation and proof-of-mechanism studies in drug discovery. We hypothesized that positive modulation of nAChA7 receptor by the highly