Genetic association with prefrontal glutathione deficit: a preliminary 3T ^{1}H MRS study in early psychosis

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Introduction Schizophrenia is a major psychiatric disorder which results from a complex interplay between genetic, environmental and developmental risk factors. Increasing evidence suggests that oxidative stress and redox dysregulation play a role in the development of the disease. Glutathione (GSH) as the major cellular redox regulator and antioxidant protects cell from damages induced by oxidative stress. Impairment of GSH metabolism has been reported in schizophrenia patients, including decreased GSH levels in cerebro-spinal fluid and medial prefrontal cortex of chronic patients[1] and its correlation with negative symptoms[2]. However, the available information about brain GSH levels in patients in the early phase of psychosis (EP) is still limited[3]. Moreover, the GAG trinucleotide repeat (TNR) polymorphisms in the gene coding for the catalytic (GCLC) subunit of the glutamate-cysteine ligase (GCL), the rate-limiting enzyme for GSH synthesis, are associated with schizophrenia in case-control studies[4]: the GAG-high-risk genotypes (7/8, 8/8, 8/9 and 9/9) were more frequent in patients (30%) and were associated with lower GCLC protein expression, GCL activity and GSH fibroblasts contents as compared with low-risk genotypes (7/7 and 7/9). The *in vivo* measurement of brain GSH levels has been demonstrated using short TE ^{1}H magnetic resonance spectroscopy at 3T[5] and 4T[6]. In this study, we studied EP patients and controls to 1) quantify brain GSH and other metabolites levels using short TE ^{1}H MRS; 2) test the association of the brain GSH content with the GAG-TNR of GCLC gene; and 3) assess other metabolites levels.

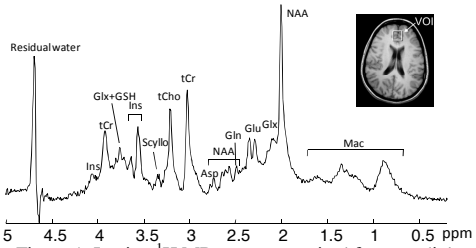

Figure 1. In vivo ^{1}H MR spectra acquired from medial prefrontal cortex of a EP patient. (TE/TR=6/4000ms, VOI=20×20×25mm^3, NEX=148)

Methods 19 EP patients (Caucasian, male, age = 25 ± 6), having met the threshold for psychosis (according to the CAARMS criteria) and recruited from the Treatment and Early Intervention in Psychosis Program[7], and 20 control subjects (Caucasian, male, age = 28 ± 8) participated in this study. All MRS measurements were performed on a 3T Trio MR scanner (Siemens Healthcare, Erlangen, Germany) with a TEM volume coil. B_0 field homogeneity was optimized using first- and second-order shimming with FAST(EST)MAP. ^{1}H MR spectra were obtained in a voxel located in the medial prefrontal cortex using the SPECIAL[5,8] localization sequence. Tissue composition inside the VOI was calculated based on the segmentation of 3D T_1-weighted images (MPRAGE) using an in-house software. Metabolite concentrations were quantified with LCModel[9] using unsuppressed water MR spectra as an internal reference and then corrected for cerebrospinal fluid contribution in the VOI. The GAG-TNR polymorphism in GCLC was genotyped as previously described[4]. The association of GSH levels with the GCLC GAG-TNR polymorphism (low-risk vs. high-risk) and EP (controls vs. patients) was investigated using two-way ANOVA. The GSH levels were further compared in four subgroups (low-risk controls, low-risk patients, high-risk controls and high-risk patients) using one-way ANOVA with Tukey's multiple comparison test. The effect of disease (controls vs. patients) and age on other metabolites and ratios of WM, GM and CSF was evaluated using general linear model with Bonferroni correction. The potential effect of medication was assessed by correlating antipsychotic doses (chlorpromazine equivalent) with metabolites levels, using the Spearman rank correlation (two-tailed).

Results 1) The minimal signal loss when using short TE MRS combined with excellent shimming performance (FWHM = 4.3 ± 1.4Hz) yielded high quality ^{1}H MR spectra in human prefrontal cortex at 3T (Fig.1), which in turn allowed the quantification of GSH with Cramer-Rao Lower bounds (CRLBs) of 11 ± 5% and other 11 metabolites with CRLBs below 20% including glutamate (Glu) and glutamine (Gln). 2) Subjects with the GAG-TNR high-risk genotype had lower GSH levels (p=0.004) as compared to those with low risk genotype, independent of the disease status (p=0.06); GSH levels were also decreased in high-risk EP patients as compared with low-risk genotype patients (- 33%, p<0.05) and with low-risk genotype controls (-36%, p<0.05) (Fig.2a). 3) The comparison of other metabolites in controls and EP patients showed a reduction of Glu (-12%, p<0.001), N-acetylaspartate (NAA, -9%, p<0.01), myo-inositol (Ins, -14%, p<0.05), total creatine (tCr, -11%, p<0.01) levels in EP patients (Fig.2b). No correlation was found between medication and metabolites levels.

Discussion and conclusions Our study showed for the first time that the GAG-TNR high-risk genotype of the GCLC gene predicts lower prefrontal GSH levels. These data extended to central nervous system is in line with findings from peripheral tissue[4]: in fibroblasts under oxidative stress conditions of high-risk subjects, GCLC protein expression, GCL activity and GSH levels were decreased as compared with low-risk genotypes. However, this preliminary study didn't show decreased GSH levels in early psychosis patients. This may reflect the recruitment bias related to the presently studied small sample. Indeed, in contrary to the larger cohorts previously studied in which the high-risk GAG-TNR genotypes were associated with the disease status[4], this is not the case for the present small sample in which the percentage of high-risk controls subjects (high-risk: n=6; low-risk: n=14) were higher than that of EP patients (high-risk: n=4; low-risk: n=15). Further study with a larger sample is needed. The reductions of Glu, NAA, Ins, and tCr levels in EP patients in the present study are consistent with previous observations in the anterior cingulate cortex of male chronic patients[10] suggesting the onset of neurochemical alterations already in the early phase of the disease.

In conclusion, GAG TNR polymorphisms of GCLC gene predicts prefrontal GSH levels and the decrease in Glu, NAA, Ins and tCr reported in chronic schizophrenia can also be observed in the early phase of the disease.

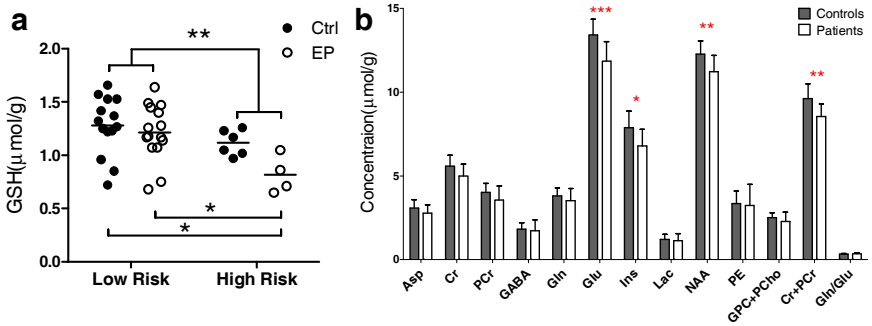

Figure 2 a). Relevance of brain GSH concentration with GCLC GAG TNR polymorphism and early phase of psychosis. The plot shows brain GSH level in subjects with low-risk genotypes (ctrl: n=14, EP: n=15) and with high-risk genotypes (ctrl: n=6, EP: n=4). b). Concentrations of other metabolites (mean ± sd) in controls and early psychosis patients. *p<0.05,**p<0.01,***p<0.001.

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Acknowledgements Supported by Centre d'Imagerie BioMédicale (CIBM) of the UNIL, UNIGE, HUG, CHUV, EPFL and the Leenaards and Jeantet Foundations, the National Center of Competence in Research (NCCR) "SYNAPSY - Swiss National Science Foundation (n° 51AU40_125759), SNSF n° 320000-122419, Fondation Damm-Etienne.