Gene expression analysis in blood of ultra-high risk subjects compared to first-episode of psychosis patients and controls

MARCOS LEITE SANTORO1,2, ARY GADELHA2,3, VANESSA K OTA1,2, GRACCIELLE R CUNHA3, ELSON ASEVEDO3, CRISTIANO S NOTO3,4, LETICIA M SPINDOLA1,2, PEDRO M PAN2,3, FERNANDA TALARICO1, RODRIGO B MANSUR2,3, PATRICIA N SILVA1,2, ELISA BRIETZKE2,3, QUIRINO CORDEIRO4, RODRIGO A BRESSAN2,3 & SINTIA IOLE BELANGERO1,2

1Genetics Division, Department of Morphology and Genetics, Universidade Federal de Sao Paulo (UNIFESP), Sao Paulo, Brazil, 2LiNC – Interdisciplinary Laboratory of Clinical Neurosciences, UNIFESP, Sao Paulo, Brazil, 3PRISMA – Program of Recognition and Intervention in subjects At-Risk Mental States, UNIFESP, Sao Paulo, Brazil, and 4Department of Psychiatry, Irmandade da Santa Casa de Misericordia de Sao Paulo (ISC MSP), Sao Paulo, Brazil

Abstract
Objectives. This study aimed to investigate peripheral blood gene expression in ultra-high-risk subjects (UHR) compared to first-episode psychosis individuals (FEP) and healthy controls (HC).

Methods. We enrolled 22 UHR, 66 FEP and 67 HC and investigated the expression of 12 genes using Taqman assays. We used the Univariate General Linear Model, as well as Bonferroni correction for multiple comparisons.

Results. We found that UFD1L (ubiquitin fusion degradation 1 like (yeast)) gene was upregulated in UHR group compared to HC and FEP (P/H11005 3.44/H11003 10-6; P/H11005 9.41/H11003 10-6). MBP (myelin basic protein) was downregulated in UHR compared to FEP (P/H11005 6.07/H11003 10-6). DISC1 (disrupted in schizophrenia 1) was also upregulated in UHR compared to FEP but lost statistical significance when corrected for age.

Conclusions. These genes are directly related to neurodevelopmental processes and have been associated to schizophrenia. Recent findings described that DISC1 overexpression can disrupt MBP expression, thus, we think that these alterations in UHR individuals could be associated with a common process. UFD1L showed a different pattern of expression only for UHR group, suggesting that they can be under an acute endoplasmatic reticulum stress, demanding elevated levels of Ufd1. Further studies can improve knowledge on disease progression and putative targets to preventive strategies.

Key words: schizophrenia, gene expression, UFD1L, MBP, DISC1

Introduction
The prodromal period for schizophrenia (SCZ), operationally defined in prospective studies as a state called ultra-high risk (UHR), is characterized by the presence of attenuated psychotic symptoms accompanied by a cognitive and functional decline (McGlashan and Johannessen, 1996; Yung and McGorry, 1996). Transition to psychosis ranges from 18% at 6-month to 36% after 3-year follow-up (Fusar-Poli et al. 2012). Schizophrenia is a neurodevelopmental disorder with major contribution of genetic factors (estimated heritability around 80%) (Sullivan et al. 2003). Thus, the comparison of peripheral blood gene expression in individuals at UHR and in the first episode of psychosis (FEP) has the potential to provide new insights about the neurobiology of the critical moment when someone transits from an at-risk state to a full-blown and potentially severe mental illness. In addition, investigation of potential biomarkers associated with development of psychosis may enhance predictive power of current UHR criteria. In this way, we selected 12 genes with known or potential relation with schizophrenia involved in neurodevelopment, drug abuse or neurotransmitter metabolism. The aim of this study was to characterize
peripheral blood expression of genes involved in the early stages of schizophrenia, including UHR and FEP individuals and to compare them with a healthy control group (HC).

Methods

We recruited 22 UHR, 66 FEP and 67 HC aged 14 to 44 years. All the subjects signed a consent prior to the inclusion in the study.

UHR individuals were recruited in the “Program of Recognition and Intervention in subjects At-Risk Mental States” (PRISMA) and they were help-seeking individuals or subjects referred by primary and secondary care services. The definition of UHR was confirmed using the Comprehensive Assessment of At-Risk Mental States (CAARMS). Individuals were classified into three possible groups: (a) brief and intermittent psychotic symptoms (BLIPS); (b) attenuated positive symptoms (APS); and (c) family history of psychosis or schizotypal personality plus impairment in functioning in the last year (HDec).

FEP patients were recruited in the “Centre for Integrated Mental Health of Santa Casa de São Paulo” (CAISM) and diagnosed according to DSM-IV criteria, using SCID-I. All individuals were antipsychotic-naïve and had no previous history of: psychotic episodes due to a general medical condition, substance-induced psychotic disorder, mental retardation and psychotic episode related to bipolar disorder or depression.

HC were recruited in UNIFESP and in the “Centro de Solidariedade ao Trabalhador” and evaluated in the “Programa de Esquizofrenia da UNIFESP”. They did not meet criteria for any Axis I DSM-IV mental disorder, according to SCID-I and had no family history of psychotic or mood disorders in first-degree relatives.

Blood samples preparation

RNA samples were collected via vein puncture using PAXgene tubes for mRNA stabilization and isolated using PAXgene blood RNA kit (Qiagen) according to the manufacturer’s protocol. Quantity and quality of the extracted RNA were measured to ensure that samples were not degraded. cDNA was synthetized using the High-Capacity cDNA reverse transcription (RT) kit (Life Technologies) with a standard RNA input of 400ng.

Quantitative PCR

We investigated the expression of 12 candidates genes (AKT1, COMT, DGCR2, DGCR8, DICER1, DISC1, DROSHA, MBP, NDEL1, PAFAH1B1, TNF and UFD1L), two endogenous genes (ACTB and GAPDH) using Taqman Low Density Array (TLDA) technology (Life Technologies), which is preconfigured in a 384-well format and spotted on a microfluidic card in duplicate assays. We selected 12 genes based on Gene Cards website information regarding gene expression in blood and the relevance of each gene in different nervous system functions and to schizophrenia. We then validated if expression was detectable by TLDA.

We followed the manufacturer’s protocol for all steps using standard TaqMan Universal Master Mix without UNG and ViiA 7 Real-Time PCR System (Life Technologies).

Statistical analysis

Using the Comparative Crt method (for further information about this method, please see: http://abcommunity.lifetechnologies.com/docs/DOC-1492 or Leal et al. (2015)), geometric mean of the endogenous genes was calculated in order to obtain the Crt values for each gene of interest. We performed a Univariate General Linear Model to verify gene expression differences comparing UHR group with FEP and HC groups. We used the Bonferroni test for post hoc and correction of multiple comparisons, considering significant P values /m, where m is the number of genes tested and α = 0.05).

We used the Pearson correlation test to verify if the genes differentially expressed could be involved in a common or convergent processes.

Results

Clinical and demographic variables are shown in Table I. Mean age of UHR was 18.3 years and, as expected, significantly lower than FEP mean age of 25.9 years (p < 0.001) (Supplementary Figure 1, available online at http://informahealthcare.com/doi/abs/10.3109/15622975.2015.1048724). There were no gender differences among all groups. Among the 22 individuals in UHR group, there were 14 fulfilling criteria for APS, 4 fulfilling criteria for BLIPS and 4 for HDec.

As we found a significant difference in age between groups, we tested if this variable could influence our significant results using it as covariate in the GLM test. We found that UFD1L gene was upregulated in UHR group compared to HC and FEP and that MBP was downregulated in UHR compared to FEP (Supplementary Figures 2 and 3, available online at http://informahealthcare.com/doi/abs/10.3109/15622975.2015.1048724) DISC1 was also upregulated in UHR compared to FEP, but after including age as a covariate it lost the statistical significance.
Table I. Description of UHR, FEP and HC groups concerning gender, mean age, antipsychotic use and subgroup diagnosis.

| Group | N  | Mean age (SD) | Sex   | Antipsychotic-naïve | Diagnosis                                                                 |
|-------|----|---------------|-------|---------------------|---------------------------------------------------------------------------|
| UHR   | 22 | 18.3 (3.52)   | 14M; 8F | 17/22               | 4BLIPS; 15APS; 3HDec                                                      |
| FEP   | 66 | 25.9 (7.44)   | 39M; 27F| 66/66               | First episode of schizophrenia confirmed after 8 weeks of treatment      |
| HC    | 67 | 26.3 (8.07)   | 40M; 27F| 67/67               | No familial history of psychosis                                          |

SD: Standard deviation; UHR: Ultra High Risk for psychosis subjects; FEP: First episode of psychosis patient; HC: Healthy control; BLIPS: Brief Limited Intermittent Psychotic Symptoms; HDec: Familial history of psychosis + functional decline.

Table II presents our significant findings. We did not observe any statistical differences between FEP and HC gene expression and no significant correlation between the genes differentially expressed as well (P > 0.004).

Table II. Gene expression results of the 12 genes analysed. Fold change refers to the relative expression ratio between groups.

| Gene   | N  | Comparison | Mean difference | Standard error | P value | Fold change* |
|--------|----|------------|-----------------|----------------|---------|--------------|
| AKT1   | HC = 67 UHR/HC | -0.140 | 0.191 | 1 | 1.00 |
|        | UHR = 21 UHR/FEP | 0.009 | 0.191 | 1 | 0.96 |
|        | FEP = 66 FEP/HC | -0.149 | 0.132 | 0.784 | 1.05 |
| COMT   | HC = 67 UHR/HC | -0.475 | 0.222 | 0.103 | 1.17 |
|        | UHR = 22 UHR/FEP | -0.625 | 0.227 | 0.020 | 1.25 |
|        | FEP = 64 FEP/HC | 0.149 | 0.151 | 0.978 | 0.93 |
| DGCR2  | HC = 66 UHR/HC | 0.226 | 0.237 | 1 | 1.01 |
|        | UHR = 21 UHR/FEP | 0.180 | 0.237 | 1 | 1.01 |
|        | FEP = 66 FEP/HC | 0.046 | 0.164 | 1 | 1.00 |
| DGCR8  | HC = 67 UHR/HC | -0.139 | 0.228 | 1 | 1.07 |
|        | UHR = 22 UHR/FEP | -0.430 | 0.229 | 0.187 | 1.17 |
|        | FEP = 64 FEP/HC | 0.291 | 0.162 | 0.223 | 0.92 |
| DICER1 | HC = 66 UHR/HC | -0.236 | 0.226 | 0.897 | 1.06 |
|        | UHR = 22 UHR/FEP | 0.102 | 0.227 | 1 | 0.96 |
|        | FEP = 64 FEP/HC | -0.338 | 0.161 | 0.113 | 1.10 |
| DISC1† | HC = 63 UHR/HC | -0.330 | 0.191 | 0.259 | 0.75 |
|        | UHR = 22 UHR/FEP | -0.608 | 0.193 | 0.006 | 1.37 |
|        | FEP = 64 FEP/HC | 0.278 | 0.131 | 0.106 | 0.55 |
| DROSHA | HC = 67 UHR/HC | -0.235 | 0.219 | 0.856 | 1.21 |
|        | UHR = 21 UHR/FEP | -0.449 | 0.220 | 0.130 | 1.32 |
|        | FEP = 64 FEP/HC | 0.214 | 0.153 | 0.495 | 0.92 |
| MBP*   | HC = 65 UHR/HC | 0.664 | 0.216 | 0.008 | 0.71 |
|        | UHR = 22 UHR/FEP* | 1.077 | 0.218 | 6.07x10^-6 | 0.48 |
|        | FEP = 62 FEP/HC | -0.413 | 0.156 | 0.027 | 1.46 |
| NDEI   | HC = 67 UHR/HC | 0.016 | 0.228 | 1 | 0.97 |
|        | UHR = 21 UHR/FEP | 0.548 | 0.229 | 0.054 | 0.83 |
|        | FEP = 65 FEP/HC | -0.532 | 0.159 | 0.005 | 1.18 |
| PAFAHIB1 | HC = 66 UHR/HC | -0.507 | 0.219 | 0.066 | 1.13 |
|        | UHR = 22 UHR/FEP | -0.411 | 0.220 | 0.192 | 1.11 |
|        | FEP = 66 FEP/HC | -0.096 | 0.154 | 1 | 1.02 |
| TNF    | HC = 59 UHR/HC | 0.212 | 0.238 | 1 | 0.92 |
|        | UHR = 21 UHR/FEP | 0.076 | 0.235 | 1 | 0.95 |
|        | FEP = 64 FEP/HC | 0.137 | 0.169 | 1 | 0.97 |
| UFDIL* | HC = 66 UHR/HC* | -1.131 | 0.223 | 3.44x10^-6 | 1.38 |
|        | UHR = 22 UHR/FEP* | -1.080 | 0.223 | 9.41x10^-6 | 1.39 |
|        | FEP = 66 FEP/HC | -0.051 | 0.158 | 1 | 1.00 |

*Fold change were calculated using 2^-ΔΔCt values.
* P < 0.004.
†DISC1 lost statistical significance after using age as covariable (P = 0.008).

Discussion

We found two genes differentially expressed in peripheral blood of UHR subjects when compared to HC and FEP patients. These genes are known to
have specific functions in processes related to neurodevelopment.

MBP is critical for myelin membrane biogenesis, entry regulation of proteins into membrane sheets (Boggs 2006) and is the major constituent of the myelin sheath of oligodendrocytes and Schwann cells. It is suggested that defects in myelin insulation may lead to reduced nerve impulse propagation and, consequently, compromise of neuronal and glial functions (Martins-de-Souza 2011). Moreover, MBP is a known marker for neurodegenerative diseases, such as multiple sclerosis, demonstrating its close relation to neurodevelopment (Lieberman, 1999). Conversely, dysfunction of oligodendrocytes has been considered a pivotal feature of SCZ pathogenesis, mainly due to its impact on brain connectivity (Davis et al. 2003; Kubicki et al. 2005).

Intriguingly, Hattori et al. (2014) described that DISC1 overexpression in in vitro neurons disrupts not only induction of MBP expression, but also transformation of oligodendrocytes to a complex morphology. In the same way, we observed an upregulation of DISC1 that can be disrupting MBP expression, suggesting that these alterations may reflect a common process, or convergent functional sequelae. However, we must highlight that DISC1 upregulation did not remain significant after correcting for age, indicating that its expression could be influenced only by age.

Supporting these disturbances in myelination of SCZ, transcriptome and proteome studies found MBP differentially expressed in several brain regions of patients, however, most of them found a decreased expression of MBP in patients (Parlapani et al. 2009; Martins-de-Souza, 2010; Martins-de-Souza et al. 2010; Matthews et al. 2012). In this study, we found a downregulation of MBP expression in UHR compared to FEP; however, one might say that MBP is actually upregulated in FEP compared to UHR. Indeed, its expression is also upregulated in FEP compared to controls, although we did not find statistical significance (Table II). Similarly, other studies suggested that antipsychotics could downregulate MBP expression (Kumarasinghe et al. 2013; Narayan et al. 2007). Similarly to our results, Kumarasinghe et al. (2013) reported an upregulation of MBP in a sample of antipsychotic-naive schizophrenia patients compared to controls that was normalized after 6–8 weeks of antipsychotic treatment.

In this way, we propose that this upregulation of MBP in FEPs blood can be a key turning point in the disorder, a potentially psychosis-state marker, and that the downregulation observed previously in SCZ patients might be a treatment effect. However, new longitudinal studies should be conducted to understand if UHR individuals present an upregulation of MBP during transition to psychosis and also if MBP expression decreases in FEP after chronic antipsychotic treatment.

Ubiquitination is a post-translational modification that plays a central role in regulating protein half-life (Mysagyi et al. 2001). In eukaryotes, post-translational conjugation to ubiquitin is an obligatory preliminary step for degradation of many proteins (Ciechanover 1994). UFD1L gene encodes the human homolog of yeast ubiquitin-fusion-degradation 1 protein and is located in 22q11.2 region. In humans, UFD1L is expressed from 10 weeks of gestational age and continues to be transcribed throughout foetal and post-natal life (Novelli et al. 1998), thus, it was suggested that this gene was involved in neurodevelopment.

Ufd1 acts together with Npl4 as an adaptor protein, which recognizes mono and poly-ubiquitinated proteins (Park et al. 2005) for p97 specific activity (AAA-ATPase). This p97-Ufd1-Npl4 complex acts in endoplasmatic reticulum (ER) associated degradation (ERAD), extracting misfolded proteins marked with ubiquitin from ER to be degraded in cytosol by the proteasome (Ye et al. 2001). Thus, it was suggested that Ufd1 is directly associated with stress response in ERAD (Chen et al. 2011).

UFD1L SNP, rs5992403, has been associated with schizophrenia (De Luca et al. 2001), with early-onset of the disorder (Ota et al. 2010) and with a deficit in the set-shifting task (which represents a deficit in fundamental dimensions of cognition in schizophrenia) (Ota et al. 2013). Moreover, its genomic region (22q11.2) is deleted in DiGeorge syndrome, which is considered one of the main genetic risk factors, beside monozygotic twins, for schizophrenia (~25% displays psychotic symptoms in early adulthood) (Bassett et al. 2005). Likewise, deletions in 22q11.2 are more common in schizophrenia subjects than in the general population (Christofolini et al. 2011).

In this study, we found a significant upregulation of UFD1L in blood of UHR subjects when compared to HC and to FEP patients. This result suggests that this upregulation is specific for the UHR group, indicating a good potential to be a future biomarker specific for the UHR group.

Accordingly to UFD1L function, we could suggest that UHR subjects are under an acute ER stress, demanding elevated levels of UFD1. In this way, future studies should focus on the ERAD system and investigate the differences between individuals before and after transition to psychosis.

The results of this study should be interpreted at light of some limitations. One of them is the sample size, especially in the UHR group. It is a consequence of the difficulty in identifying early signs of psychosis in adolescents and young adults and properly refer them. Although our main aim was to find biomarkers
to identify the UHR group, it would be very valuable to understand how these alterations in blood could influence brain tissue, which is not possible in humans. Another point to consider is that UHR subjects are being followed for a short period of time (mean follow-up period < 1 year) and only one patient developed psychosis (not included in the FEP group). We used standardized criteria and instrument (CAARMS) and, so far, our transition rate is similar to what was recently reported (Demjaha et al. 2012; Ziermans et al. 2011).

Accumulating evidence indicates that SCZ involves subtle cytoarchitectural abnormalities that arise during neurodevelopment. Nevertheless, the underlying molecular mechanisms are still unclear. In this study we found two genes differentially expressed in blood of UHR group compared to HC (UFD1L) and FEP (UFD1L and MBP). These genes have been previously studied in SCZ and are considered to be directly related to neurodevelopment. Moreover, these genes displayed a great potential as biomarkers, which in the future could enhance identification of UHR subjects. Here, we highlighted UFD1L, which showed a different pattern of expression in the UHR group compared to HC and FEP. Concerning MBP and DISC1, they seem to be involved in a common process; however, new studies should be conducted to understand this mechanism.

Statement of Interest

This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2010/08968-6; 2011/50740-5; 2012/12686-1; 2010/19176-3), Brazil, and this agency did not participate in the study design, data collection and analysis, manuscript preparation, or decision to submit this paper for publication.

Dr Noto has received a scholarship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and has served as a consultant or advisory board member for Janssen. Dr Gadilha was on the speakers’ bureau and/or has acted as a consultant for Janssen-Cilag in the last 12 months and has also received research support from Brazilian government institutions (CNPq). Dr Brietzke has been supported by CNPq, CAPES and FAPESP. Dr Bressan has received research funding by FAPESP, CNPq, CAPES, Fundação Safra, Fundação ABADS, Janssen, Eli Lilly, Lundbeck, Novartis and Roche, served as a speaker for Astra Zeneca, Bristol, Janssen, Lundbeck and Revista Brasileira de Psiquiatria, and is a shareholder of Radiopharmaceutics Ltda and Biomedical Technology Ltda. The other authors have no conflicts of interest to disclose.

Acknowledgements

None.

References

Bassett AS, Chow EW, Husted J, Welsberg R, Caluseriu O, Webb GD, et al. 2005. Clinical features of 78 adults with 22q11 Deletion Syndrome. Am J Med Genet A 138:307–313.

Boggs JM. 2006. Myelin basic protein: a multifunctional protein. Cell Mol Life Sci 63:1945–1961.

Chen M, Gutierrez GJ, Ronai ZA. 2011. Ubiquitin-recognition protein Ufd1 couples the endoplasmic reticulum (ER) stress response to cell cycle control. Proc Natl Acad Sci USA 108:9119–9124.

Christofolini DM, Belluccio FT, Ota VK, Belangero SI, Cernach MC, Gadilha, A, et al. 2011. Assessment of 22q11.2 copy number variations in a sample of Brazilian schizophrenia patients. Schizophr Res 132:99–100.

Ciechanover A. 1994. The ubiquitin-proteasome proteolytic pathway. Cell 79:13–21.

Davis KL, Stewart DG, Friedman JI, Buchsbaum M, Harvey PD, Hof PR, et al. 2003. White matter changes in schizophrenia: evidence for myelin-related dysfunction. Arch Gen Psychiatry 60:443–456.

De Luca A, Pasini A, Amati F, Botta A, Spalletta G, Alimenti S, et al. 2001. Association study of a promoter polymorphism of UFD1L gene with schizophrenia. Am J Med Genet 105:529–533.

Demjaha A, Valmaggia L, Stahl D, Byrne M, McGuire P. 2012. Disorganization/cognitive and negative symptom dimensions in the at-risk mental state predict subsequent transition to psychosis. Schizophr Bull 38:351–359.

Fusar-Poli P, Bonoldi I, Yung AR, Borgwardt S, Kempton MJ, Valmaggia L, et al. 2012. Predicting psychosis: meta-analysis of transition outcomes in individuals at high clinical risk. Arch Gen Psychiatry 69:220–229.

Hattori T, Shimizu S, Koyama Y, Emoto H, Matsumoto Y, Kumamoto N, et al. 2014. DISC1 (disrupted-in-schizophrenia-1) regulates differentiation of oligodendrocytes. PLoS One 9:e88506.

Kubicki M, Park H, Westin CF, Nestor PG, Mulkern RV, Maier SE, et al. 2005. DTI and MTR abnormalities in schizophrenia: analysis of white matter integrity. Neuroimage 26:1109–1118.

Kumarasinghe N, Beveridge NJ, Gardiner E, Scott RJ, Yasawadene S, Perera A, et al. 2013. Gene expression profiling in treatment-naïve schizophrenia patients identifies abnormalities in biological pathways involving AKT1 that are corrected by antipsychotic medication. Int J Neuropsychopharmacol 16:1483–1503.

Leal MF, Belangero PS, Figueiredo EA, Cohen C, Loyola LC, Andreoli CV, et al. 2015. Identification of suitable reference genes for gene expression studies in tendons from patients with rotator cuff tear. PLoS One 10:e0118821.

Lieberman JA. 1999. Is schizophrenia a neurodegenerative disorder? A clinical and neurobiological perspective. Biol Psychiatry 46:729–739.

Martins-de-Souza D. 2010. Proteome and transcriptome analysis suggests oligodendrocyte dysfunction in schizophrenia. J Psychiatr Res 44:149–156.

Martins-de-Souza D. 2011. Proteomics as a tool for understanding schizophrenia. Clin Psychopharmacol Neurosci 9:95–101.

Martins-de-Souza D, Maccarrone G, Wobrock T, Zerr I, Gorrmanns P, Reckow S, et al. 2010. Proteome analysis of the
thalamus and cerebrospinal fluid reveals glycolysis dysfunction and potential biomarkers candidates for schizophrenia. J Psychiatr Res 44:1176–1189.
Matthews PR, Eastwood SL, Harrison PJ. 2012. Reduced myelin basic protein and actin-related gene expression in visual cortex in schizophrenia. PLoS One 7:e38211.
McGlashan TH, Johannessen JO. 1996. Early detection and intervention with schizophrenia: rationale. Schizophr Bull 22:201–222.
Myung J, Kim KB, Crews CM. 2001. The ubiquitin-proteasome pathway and proteasome inhibitors. Med Res Rev 21:245–273.
Narayan S, Kass KE, Thomas EA. 2007. Chronic haloperidol treatment results in a decrease in the expression of myelin/oligodendrocyte-related genes in the mouse brain. J Neurosci Res 85:757–765.
Novelli G, Mari A, Amati F, Colosimo A, Sangiuolo F, Bengala M, et al. 1998. Structure and expression of the human ubiquitin fusion-degradation gene (UFD1L). Biochim Biophys Acta 1396:158–162.
Ota VK, Belangero SI, Gadelha A, Bellucco FT, Christofolini DM, Mancini TI, et al. 2010. The UFD1L rs5992403 polymorphism is associated with age at onset of schizophrenia. J Psychiatr Res 44:1113–1115.
Ota VK, Berberian AA, Gadelha A, Santoro ML, Ottoni GL, Matsuzaka CT, et al. 2013. Polymorphisms in schizophrenia candidate gene UFD1L may contribute to cognitive deficits. Psychiatry Res 209:110–113.
Park S, Isaacson R, Kim HT, Silver PA, Wagner G. 2005. Ufd1 exhibits the AAA–ATPase fold with two distinct ubiquitin interaction sites. Structure 13:995–1005.
Parlapani E, Schmitt A, Erdmann A, Bernstein HG, Breunig B, Gruber O, et al. 2009. Association between myelin basic protein expression and left entorhinal cortex pre-alpha cell layer disorganization in schizophrenia. Brain Res 1301:126–134.
Sullivan PF, Kendler KS, Neale MC. 2003. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry 60:1187–1192.
Ye Y, Meyer HH, Rapoport TA. 2001. The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol. Nature 414:652–656.
Yung AR, McGorry PD. 1996. The prodromal phase of first-episode psychosis: past and current conceptualizations. Schizophr Bull 22:353–370.
Ziermans TB, Schothorst PF, Spong M, van Engeland H. 2011. Transition and remission in adolescents at ultra-high risk for psychosis. Schizophr Res 126:58–64.

**Supplemental material available online**

Supplemental Figures 1-3 available online at http://informahealthcare.com/doi/abs/10.3109/15622975.2015.1048724.