Published in final edited form as:

Drug Alcohol Depend. 2008 April 1; 94(1-3): 246–250.

Alpha Synuclein Protein Levels are Increased in Serum from Recently Abstinent Cocaine Abusers

Deborah C. Mash¹, Nikhil Adi¹, Linda Duque¹, John Pablo¹, Mahendra Kumar², and Frank R. Ervin³

¹Dept. Neurology, Miller School of Medicine, Miami, FL

²Dept. Psychiatry, Miller School of Medicine, Miami, FL

³Dept. Psychiatric and Behavioral Genetics, McGill University, Montreal, Canada

Abstract

Alpha synuclein is increased in dopamine neurons of cocaine abusers and in rats whose alcohol preference is inbred. Recent studies have shown increased alpha-synuclein protein expression in serum of alcoholic patients that is linked with severity of alcohol craving. The aim of this study was to analyze the serum levels of alpha synuclein in healthy subjects and in recently abstinent cocaine abusers. Alpha synuclein protein expression was measured by enzyme-linked immunosorbent assay in serum specimens obtained from 38 recently abstinent cocaine dependent patients and 14 control subjects. The presence of cocaine dependence disorder was based on the Structured Clinical Interview (DSM-IV). Drug severity was assessed by the Addiction Severity Index ratings and composite measures. Scores of the intensity and frequency of cocaine craving episodes were obtained from the Minnesota Cocaine Craving Questionnaire. The serum concentrations of alpha synuclein in cocaine dependent patients were significantly higher as compared with age-matched drug-free controls ($p < 0.001$). Alpha synuclein levels in blood were significantly correlated with the intensity ($r = 0.60$, $p < 0.001$) and frequency ($r = 0.64$, $p < 0.001$) of cocaine craving and with thirty days of cocaine use in the prior month before entry to treatment ($r = 0.56$, $p < 0.005$). However, there was no correlation between the serum protein levels of alpha synuclein and age in either group. This report is the first demonstration of altered alpha synuclein levels in peripheral blood from cocaine abusers. These data agree with previous reports in postmortem brain of cocaine abusers and provide support for an association between alpha synuclein and cocaine dependence.

Keywords

Cocaine; serum; dopamine; craving; synuclein

1. Introduction

Alpha synuclein is abundantly expressed in various brain regions and is particularly enriched in presynaptic terminals (Lavedan, 1998). Intracellular aggregates of the protein have been associated with synucleinopathies of Alzheimer's and Parkinson's disease (Eriksen et al., 2003; Sidhu et al., 2004). Animal models developed in mice and *Drosophila* have shown that

*Corresponding Author: Deborah C. Mash, Ph.D., Dept. Neurology (D4-5), 1501 N.W. 9th Avenue, Miami, FL 33136, Phone: (305) 243-5888 Fax: (305)243-3649, dmash@med.miami.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

pathological inclusions of alpha synuclein resulted in degeneration of dopaminergic neurons and motor deficits (Masliah et al 2000; Feany and Bender, 2000). In its normal context, alpha synuclein participates in diverse synaptic functions, including neuronal development, differentiation and neuroplasticity (Jenco et al., 1998; Maroteaux and Scheller, 1991; Papachroni et al., 2005). Alpha synuclein is expressed during development and maturation of dopaminergic neurons, and the protein regulates dopamine homeostasis in the adult brain by affecting neurotransmitter biosynthesis, the activity of the dopamine transporter, and presynaptic recruitment of dopamine for vesicular storage and release (Lee et al., 2001; Perez et al., 2002; Yavich et al., 2004).

Alpha synuclein is elevated in rats whose alcohol preference is inbred (Liang et al., 2003) and high levels are associated with an increase in alcohol craving in patients during withdrawal (Bonsch et al., 2005a; Bonsch et al., 2004). Peripheral blood alpha synuclein mRNA levels are elevated in cynomolgus monkeys that chronically self-administer ethanol (Walker and Grant, 2006). Alpha synuclein protein and mRNA is increased in midbrain dopaminergic neurons of cocaine abusers (Mash et al., 2003). Although the involvement of dopamine in reinforcement to drugs and alcohol is well established (Volkow et al., 1999), these findings link dysregulated alpha synuclein in addictions to cocaine and alcohol. The aim of this study was to determine whether the levels of alpha synuclein in blood were altered in recently abstinent cocaine addicts compared to non-drug using controls. We further correlated the serum levels of alpha synuclein with days of cocaine use in the prior month and with measures of the intensity and frequency of cocaine craving.

2. Methods and Materials

2.1 Subjects

Residual serum specimens were obtained from thirty-eight recently abstinent patients seeking treatment for cocaine dependence (34 male; 4 female). Each subject underwent a physical examination, blood tests and full psychiatric evaluation at treatment admission. Detailed demographic information was obtained and patients completed the Addiction Severity Index (McLellan et al., 1992). The average age was 36.8 ± 9.86 and the average duration of use of cocaine was 12.7 ± 7.1 (Table 1). All patients were diagnosed as cocaine dependent according to the DSM-IV criteria using a Structured Clinical Interview for DSM-IV diagnoses (SCID-IV; (Spitzer and First, 2005). The clinical interviews were carried out by a psychiatrist trained for application of the SCID-IV (F.R.E.). All of the subjects stopped cocaine and alcohol at program admission. Urinalysis, which tested for the presence of cocaine, opiates, amphetamine, and benzodiazepines was conducted at program intake. Urine specimens tested positive for cocaine in 90% of the patients. There was no other current substance dependence, but six subjects met DSM-IV criteria for alcohol dependence. None of the subjects had a history of a major medical illness or current use of prescription drugs. The intensity and frequency of cocaine craving was assessed at program entry using the Minnesota Cocaine Craving Scale (Halikas et al., 1991). The years of cocaine use and number of days of cocaine use in the last month were obtained by retrospective chart review. The retrospective chart review of patient clinical records was approved by the Institutional Review Board of the University of Miami Miller School of Medicine. Fourteen age-related control subjects (10 male; 4 female) were recruited from the general population. Control subjects were free from psychiatric or physical disturbances. The age range for the control subjects was 35.6 ± 5.2 years (male/female, 10/4).

2.2 Immunoassay of alpha synuclein in serum

Fasting blood was obtained at 8:00 AM from patients 24 to 72 hours after the last use of cocaine (28.8 ± 16.7 hours, mean $\pm$ S.D.). The blood samples were centrifuged after collection and residual serum specimens were stored at -80° C. Alpha synuclein was measured in serum by

an enzyme-linked immunosorbent assay performed according to the directions of the supplier (Biosource, Immunoassay Kit #KHB0061, Camarillo, CA 93012). The commercial kit uses a monoclonal antibody raised against full length human alpha synuclein to detect and quantify the level of total protein monomers. Purified recombinant protein standards of alpha synuclein were assayed in parallel with human serum. Standard curves with human recombinant alpha synuclein in control serum ($r^2 = 0.96$) were compared to the standard diluent buffer ($r^2 = 0.97$). The spiked sera from pooled controls gave results that were linear across the concentration range. Serum samples from patients and control subjects were analyzed together in batch runs. For some subjects, multiple assays were done on diluted serum from a single subject to verify that low or high concentrations were in the linear range of the assay. All measurements were repeated twice and the average value was determined.

2.3 Data analysis

Statistical calculations were carried out using SPSS Statistical Software Package (SPSS for Windows - version 14, Chicago, IL). Differences between cocaine dependent patients and control subjects were calculated using ANOVA and the Student's *t* test. Correlation studies were obtained with the Pearson correlation coefficient. Values are expressed as mean $\pm$ standard deviation and a significance level of $p < 0.05$ was used.

3. Results

A total of 38 cocaine dependent subjects with long-standing histories of cocaine use and 14 control subjects were evaluated. Table 1 shows the demographic and clinical data of the cocaine dependent group. All of the subjects met DMS-IV criteria for cocaine dependence. The groups of patients and control subjects were similar in age with male predominance. Psychiatric comorbidity was 21.6% and none of the participants met DMS-IV criteria for dependence to cannabis, opiates, sedatives or other drugs. By self-report, 84% of the cocaine addicts were drug users who smoked crack cocaine. None of the cocaine dependent subjects in this sample were injectors. The number of days of cocaine use in the 30 days prior to treatment admission was 9.0 (S.D. 7.9) days.

As shown in Figure 1A, alpha synuclein protein expression was significantly elevated in recently abstinent cocaine dependent patients (35.7 ng/ml; SD, 26.6 ng/ml) as compared to age-matched control subjects (4.1 ng/ml; SD, 2.5 ng/ml). These values agree with previous reported serum levels by Bonsch and coworkers (2005a) for alpha synuclein in healthy controls ($N = 50$; 5.92 ng/ml; SD, 9.72 ng/ml). Alpha synuclein concentrations in human plasma samples in 44 normal donors gave median of 5.6 ng/ml (Fjorback et al., 2007). These observations demonstrate a narrow concentration range for alpha synuclein in control subjects using a quantitative ELISA. In recently abstinent cocaine dependent patients, the serum levels ranged from 3.3 to 82.2 ng/ml with mean values elevated approximately 8-fold as compared to age-matched and drug-free controls (Fig. 1A). Patients who met criteria for cocaine and alcohol dependence had serum levels of alpha synuclein that were in the same range ($N = 6$; 32.4 ng/ml, SD 26.5). Age was not significantly correlated with the level of alpha synuclein in either controls ($r = 0.30$, $p = 0.31$) or abstinent cocaine dependent patients ($r = 0.16$, $p = 0.34$). The levels of alpha synuclein did not differ between male and female subjects in both the control and cocaine groups (data not shown; $p > 0.05$) in agreement with a recent study (Fjorback et al., 2007).

Independent variables at program admission were selected for determining their possible influences on craving measures (alpha synuclein protein, age, years of cocaine use, and days of use in the prior month). We observed a positive correlation between alpha synuclein protein levels and the intensity and frequency of cocaine craving. Individual patient scores on the reported intensity of craving were significantly associated with serum alpha synuclein levels

($r = 0.60$, $p < 0.001$). The individual values for alpha synuclein are shown as a function of the intensity of cocaine craving (Fig. 1B) and the days of use in the prior month (Fig. 1C). The mean intensity rating for craving at program admission was 45.8 (SD 31.2). The frequency of craving episodes was correlated with individual levels of alpha synuclein ($r = 0.64$, $p < 0.001$). Not surprisingly, a positive correlation was observed between serum levels of alpha synuclein and the number of days of cocaine use in the prior month ($r = 0.56$, $p < 0.005$).

4. Discussion

We have measured alpha synuclein in serum from recently abstinent cocaine dependent patients using the ELISA method. The expression of alpha synuclein protein concentrations was significantly elevated when compared with age-matched control subjects. Furthermore, there was a significant association between alpha synuclein and cocaine craving scores. The serum levels of alpha synuclein were correlated with days of cocaine use in the prior month, providing additional evidence for increased levels of alpha synuclein with cocaine exposure.

Bonsch et al (Bonsch et al., 2004) reported the first study evaluating the expression of alpha synuclein in blood in patients with alcoholism. Later work demonstrated a correlation between the levels of alpha synuclein in serum with the intensity of alcohol craving in addicted patients during withdrawal (Bonsch et al., 2005a; Bonsch et al., 2004). Monkeys that chronically self-administered ethanol for 18 months had 3-fold higher peripheral blood levels of alpha synuclein mRNA, suggesting that peripheral alpha synuclein upregulates with chronic exposure to alcohol (Walker and Grant, 2006). We observed an elevated expression of alpha synuclein in recently abstinent cocaine dependent patients that is linked to measures of craving and days of use in the past month. Cocaine and alcohol are frequently used together (Hearn et al., 1991) and this combination is associated with enhanced CNS toxicity and dependence severity (Bolla et al., 2000). These results suggest that alpha synuclein may be a biomarker of the intensity of withdrawal-induced cravings in both cocaine abusers and alcoholics.

The gene for alpha synuclein maps to chromosome position 4q21.3–22 (Chen et al., 1995; Shibasaki et al., 1995; Spillantini et al., 1995). This region has been associated with drug abuse through a genome-wide SNP genome scan (Uhl et al., 2001). Genomic variation in SNCA has been associated with susceptibility to alcohol (Bonsch et al., 2005b), methamphetamine psychosis/dependence (Kobayashi et al., 2004) and alcohol craving (Bonsch et al., 2005a; Bonsch et al., 2005b; Foroud et al., 2000; Foroud et al., 2007). Using a family based test of association in multiplex alcoholic pedigrees, analyses in the 3' end of SNCA identified a haplotype overestimated in European-American individuals who crave alcohol and a different haplotype, which was preferentially transmitted to noncravers (Foroud et al 2007). These observations are consistent with a previous report in alcohol preferring P rats, which proposed that SNPs in the 3'UTR of *Snca* contribute to alcohol preference (Liang et al., 2003). However, a more exhaustive analysis in two American Indian populations failed to find an association of alpha synuclein with alcohol and drug dependence (Clarimon et al., 2007). Although several SNPs initially returned significant p values, none of the results remained significant after correction for multiple testing, suggesting that SNCA was not a factor for addition vulnerability in these populations. Altered DNA methylation patterns in the alpha synuclein promoter have been associated with alcoholism, suggesting that epigenetic factors may contribute to differential expression of the protein in certain vulnerable populations (Bonsch et al., 2005c). Whether addicted individuals with high alpha synuclein concentrations are at increased risk for craving and relapse due to promoter polymorphisms requires further study.

Peripheral blood alpha synuclein is reactive with epitope specific antibodies and is indistinguishable from the human brain protein (Li et al., 2007). How alpha synuclein reaches serum from the brain is unknown, but blood levels of alpha synuclein may reflect the gene

dosage and/or altered protein expression, since triplication of the alpha synuclein gene causes increased brain expression and a doubling of blood alpha synuclein (Miller et al., 2004). Cocaine abuse leads to state dependent increases in the levels of alpha synuclein in postmortem brain (Mash et al., 2003; Qin et al., 2005) and peripheral blood as shown in this report. Dysfunction of central dopaminergic neurotransmission has been proposed as a mechanism for withdrawal severity and craving in drug and alcohol dependence (Self and Nestler, 1995) and changes in dopamine are associated with the level of craving reported by cocaine abusers with the largest effects seen in the most severely dependent (Volkow et al., 1999). Because alpha synuclein is involved in the modulation of dopaminergic activity, these preliminary results support a role for alpha synuclein gene expression in the intensity of craving for drugs or alcohol. The relationship between alpha synuclein and the risk for withdrawal-induced craving requires study in a large cohort. Further study is needed to determine if repeated measures of alpha synuclein in serum correlate with changes in cravings and rates of relapse in abstinent cocaine abusers.

References

- Bonsch D, Greifenberg V, Bayerlein K, Biermann T, Reulbach U, Hillemacher T, Kornhuber J, Bleich S. Alpha-synuclein protein levels are increased in alcoholic patients and are linked to craving. *Alcoholism, clinical and experimental research* 2005a;29:763–765.
- Bonsch D, Lederer T, Reulbach U, Hothorn T, Kornhuber J, Bleich S. Joint analysis of the NACP-REP1 marker within the alpha synuclein gene concludes association with alcohol dependence. *Human molecular genetics* 2005b;14:967–971. [PubMed: 15731118]
- Bonsch D, Lenz B, Kornhuber J, Bleich S. DNA hypermethylation of the alpha synuclein promoter in patients with alcoholism. *Neuroreport* 2005c;16:167–170. [PubMed: 15671870]
- Bonsch D, Reulbach U, Bayerlein K, Hillemacher T, Kornhuber J, Bleich S. Elevated alpha synuclein mRNA levels are associated with craving in patients with alcoholism. *Biological psychiatry* 2004;56:984–986. [PubMed: 15601610]
- Chen X, de Silva HA, Pettenati MJ, Rao PN, St George-Hyslop P, Roses AD, Xia Y, Horsburgh K, Ueda K, Saitoh T. The human NACP/alpha-synuclein gene: chromosome assignment to 4q21.3–q22 and TaqI RFLP analysis. *Genomics* 1995;26:425–427. [PubMed: 7601479]
- Clarimon J, Gray RR, Williams LN, Enoch MA, Robin RW, Albaugh B, Singleton A, Goldman D, Mulligan CJ. Linkage disequilibrium and association analysis of alpha-synuclein and alcohol and drug dependence in two American Indian populations. *Alcoholism, clinical and experimental research* 2007;31:546–554.
- Eriksen JL, Dawson TM, Dickson DW, Petrucelli L. Caught in the act: alpha-synuclein is the culprit in Parkinson's disease. *Neuron* 2003;40:453–456. [PubMed: 14642269]
- Foroud T, Edenberg HJ, Goate A, Rice J, Flury L, Koller DL, Bierut LJ, Conneally PM, Nurnberger JJ, Bucholz KK, Li TK, Hesselbrock V, Crowe R, Schuckit M, Porjesz B, Begleiter H, Reich T. Alcoholism susceptibility loci: confirmation studies in a replicate sample and further mapping. *Alcoholism, clinical and experimental research* 2000;24:933–945.
- Foroud T, Wetherill LF, Liang T, Dick DM, Hesselbrock V, Kramer J, Nurnberger J, Schuckit M, Carr L, Porjesz B, Xuei X, Edenberg HJ. Association of alcohol craving with alpha-synuclein (SNCA). *Alcoholism, clinical and experimental research* 2007;31:537–545.
- Halikas JA, Kuhn KL, Crosby R, Carlson G, Crea F. The measurement of craving in cocaine patients using the Minnesota Cocaine Craving Scale. *Comprehensive psychiatry* 1991;32:22–27. [PubMed: 2001617]
- Jenco JM, Rawlingson A, Daniels B, Morris AJ. Regulation of phospholipase D2: selective inhibition of mammalian phospholipase D isoenzymes by alpha- and beta-synucleins. *Biochemistry* 1998;37:4901–4909. [PubMed: 9538008]
- Kobayashi H, Ide S, Hasegawa J, Ujike H, Sekine Y, Ozaki N, Inada T, Harano M, Komiyama T, Yamada M, Iyo M, Shen HW, Ikeda K, Sora I. Study of association between alpha-synuclein gene polymorphism and methamphetamine psychosis/dependence. *Annals of the New York Academy of Sciences* 2004;1025:325–334. [PubMed: 15542733]

- Lavedan C. The synuclein family. *Genome research* 1998;8:871–880. [PubMed: 9750188]
- Lee FJ, Liu F, Pristupa ZB, Niznik HB. Direct binding and functional coupling of alpha-synuclein to the dopamine transporters accelerate dopamine-induced apoptosis. *FASEB J* 2001;15:916–926. [PubMed: 11292651]
- Li QX, Mok SS, Laughton KM, McLean CA, Cappai R, Masters CL, Culvenor JG, Horne MK. Plasma alpha-synuclein is decreased in subjects with Parkinson's disease. *Experimental neurology* 2007;204:583–588. [PubMed: 17258710]
- Liang T, Spence J, Liu L, Strother WN, Chang HW, Ellison JA, Lumeng L, Li TK, Foroud T, Carr LG. alpha-Synuclein maps to a quantitative trait locus for alcohol preference and is differentially expressed in alcohol-preferring and -nonpreferring rats. *Proceedings of the National Academy of Sciences of the United States of America* 2003;100:4690–4695. [PubMed: 12665621]
- Maroteaux L, Scheller RH. The rat brain synucleins; family of proteins transiently associated with neuronal membrane. *Brain research* 1991;11:335–343. [PubMed: 1661825]
- Mash DC, Ouyang Q, Pablo J, Basile M, Izenwasser S, Lieberman A, Perrin RJ. Cocaine abusers have an overexpression of alpha-synuclein in dopamine neurons. *J Neurosci* 2003;23:2564–2571. [PubMed: 12684441]
- McLellan AT, Kushner H, Metzger D, Peters R, Smith I, Grissom G, Pettinati H, Argeriou M. The Fifth Edition of the Addiction Severity Index. *Journal of substance abuse treatment* 1992;9:199–213. [PubMed: 1334156]
- Miller DW, Hague SM, Clarimon J, Baptista M, Gwinn-Hardy K, Cookson MR, Singleton AB. Alpha-synuclein in blood and brain from familial Parkinson disease with SNCA locus triplication. *Neurology* 2004;62:1835–1838. [PubMed: 15159488]
- Papachroni K, Ninkina N, Wanless J, Kalofoutis AT, Gnuchev NV, Buchman VL. Peripheral sensory neurons survive in the absence of alpha- and gamma-synucleins. *J Mol Neurosci* 2005;25:157–164. [PubMed: 15784963]
- Perez RG, Waymire JC, Lin E, Liu JJ, Guo F, Zigmond MJ. A role for alpha-synuclein in the regulation of dopamine biosynthesis. *J Neurosci* 2002;22:3090–3099. [PubMed: 11943812]
- Self DW, Nestler EJ. Molecular mechanisms of drug reinforcement and addiction. *Annual review of neuroscience* 1995;18:463–495.
- Shibasaki Y, Baillie DA, St Clair D, Brookes AJ. High-resolution mapping of SNCA encoding alpha-synuclein, the non-A beta component of Alzheimer's disease amyloid precursor, to human chromosome 4q21.3-->q22 by fluorescence in situ hybridization. *Cytogenetics and cell genetics* 1995;71:54–55. [PubMed: 7606927]
- Sidhu A, Wersinger C, Vernier P. alpha-Synuclein regulation of the dopaminergic transporter: a possible role in the pathogenesis of Parkinson's disease. *FEBS letters* 2004;565:1–5. [PubMed: 15135042]
- Spillantini MG, Divane A, Goedert M. Assignment of human alpha-synuclein (SNCA) and beta-synuclein (SNCB) genes to chromosomes 4q21 and 5q35. *Genomics* 1995;27:379–381. [PubMed: 7558013]
- Spitzer RL, First MB. Classification of psychiatric disorders. *Jama* 2005;294:1898–1899. [PubMed: 16234493]author reply 1899–1900
- Uhl GR, Liu QR, Walther D, Hess J, Naiman D. Polysubstance abuse-vulnerability genes: genome scans for association, using 1,004 subjects and 1,494 single-nucleotide polymorphisms. *American journal of human genetic* 2001;69:1290–1300.
- Volkow ND, Wang GJ, Fowler JS, Logan J, Gatley SJ, Wong C, Hitzemann R, Pappas NR. Reinforcing effects of psychostimulants in humans are associated with increases in brain dopamine and occupancy of D(2) receptors. *The Journal of pharmacology and experimental therapeutics* 1999;291:409–415. [PubMed: 10490931]
- Walker SJ, Grant KA. Peripheral blood alpha-synuclein mRNA levels are elevated in cynomolgus monkeys that chronically self-administer ethanol. *Alcohol (Fayetteville, NY)* 2006;38:1–4.
- Yavich L, Tanila H, Vepsäläinen S, Jakala P. Role of alpha-synuclein in presynaptic dopamine recruitment. *J Neurosci* 2004;24:11165–11170. [PubMed: 15590933]

Figure 1.

Measures of alpha synuclein in serum samples from recently abstinent cocaine abusers. **A.** Median and quartiles (box plots) as well as 10th and 90th percentiles (whiskers) of the concentration of alpha synuclein in cocaine dependent (N = 38) and aged-matched drug-free control subjects (N = 14). Alpha synuclein serum concentrations were significantly increased in the cocaine abusers compared with those of the aged-matched drug-free control group (*t*-test, $p < 0.0001$). Thick lines represent the mean value. Correlation between alpha synuclein serum protein concentrations and self-reported ratings for craving intensity and the pattern of use in cocaine dependent patients (N = 38). A significant positive correlation was demonstrated between alpha synuclein serum concentrations and **B)** intensity of desire to use (Pearson $r =$

0.60, $p < 0.0001$), and **C**) days of use in month prior to treatment (Pearson $r = 0.56$, $p < 0.0005$). Similar significant results were obtained for the frequency rating of cocaine craving as described in the Results section.

Table 1**Demographic Characteristics**

Age (years); mean (S.D.)	36.8 (9.86)
Male (%)	86.5
Race/ethnic group (%)	
White	83.8
Hispanic	16.2
Years of Education; mean (S.D.)	14.1 (1.96)
Years of Cocaine Use; mean (S.D.)	12.7 (7.1)
Days of Use in Last Month; mean (S.D.)	9.0 (9.9)
ASI Composite Scores; mean (S.D.)	
Medical	0.17 (0.31)
Educational	0.32 (0.29)
Alcohol	0.20 (0.25)
Drug	0.23 (0.09)
Legal	0.12 (0.19)
Family	0.41 (0.27)
Psychiatric	0.36 (0.23)
Current Alcohol Dependence %	16.2
Current Co-morbid Axis I Disorder %	21.6
Current Axis II Disorder %	35.1