Brief Report

Alpha2C-adrenoceptor Del322-325 polymorphism and risk of psychiatric disorders: significant association with opiate abuse and dependence

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ABSTRACT

Objectives \textsuperscript{a}\textsuperscript{2}\textsubscript{C}-adrenoceptors (\textsuperscript{a}\textsuperscript{2}\textsubscript{C}-AR) are involved in behavioural responses relevant to psychiatric disorders and suicide completion. The genetic polymorphism \textsuperscript{a}\textsuperscript{2}\textsubscript{C}Del322-325-AR confers a loss-of-function phenotype. Functional human studies have associated \textsuperscript{a}\textsuperscript{2}\textsubscript{C}Del322-325-AR polymorphism with major depression pathophysiology. The aim of this study was to analyse, for the first time, the association of \textsuperscript{a}\textsuperscript{2}\textsubscript{C}Del322-325-AR polymorphism with suicide completion and with related psychiatric disorders: major depression, schizophrenia, opiate and alcohol abuse and dependence. Methods Post-mortem brain DNA was extracted (n = 516) and genotyping performed by HaeIII restriction endonuclease digestion of PCR products and DNA fragment analysis on capillary sequencer. Amplified products were sequenced to confirm the presence of the polymorphism. Results The frequency of \textsuperscript{a}\textsuperscript{2}\textsubscript{C}Del322-325-AR in suicide (9%, n = 236) and non-suicide victims (11%, n = 280) was similar. Genotype frequencies for the \textsuperscript{a}\textsuperscript{2}\textsubscript{C}Del322-325-AR polymorphism in depressed (15%, n = 39) and schizophrenic subjects (18%, n = 39) were higher than in controls (7%, n = 187), but these differences did not reach statistical significance (P = 0.125 and P = 0.063, respectively). A selective and significant association of \textsuperscript{a}\textsuperscript{2}\textsubscript{C}Del322-325-AR polymorphism with opiate abuse and dependence was found (23%, n = 35, P = 0.011). Conclusions Our results indicate that \textsuperscript{a}\textsuperscript{2}\textsubscript{C}Del322-325-AR may play a role in the pathophysiology of opiate abuse and dependence and raise the interest for larger genetic associative studies.

Introduction

Suicide is a major public health issue. According to the World Health Organisation, approximately 1 million people die by suicide in the world every year (Wasserman et al. 2012). It has been suggested that genetics strongly influences suicidal behaviour (Brent and Melhem 2008). However, the most important risk factors for suicide completion are psychiatric disorders such as depression, anxiety, substance abuse and personality disorders (Rihmer 2007), major depression being the most important (Lonnqvist et al. 1995). Thus, suicide completion would be the final outcome of the interaction between genetic, psychological and environmental factors. Numerous neurochemical studies have reported an increased \textsuperscript{a}\textsuperscript{2}\textsubscript{C}-adrenoceptor (\textsuperscript{a}\textsuperscript{2}\textsubscript{C}-AR) expression, density and activity in brains of depressed suicide victims (Meana et al. 1992; González-Maeso et al. 2002; Rivero et al. 2014), which, in line with \textsuperscript{a}\textsuperscript{2}\textsubscript{C}-AR hetero- and autoreceptor function, may well account for the proposed deficits in noradrenergic/sеротонergic transmission. Therefore, it seems plausible that variants in genes codifying for \textsuperscript{a}\textsuperscript{2}\textsubscript{C}-AR confer risk for suicide completion.

Both \textsuperscript{a}\textsuperscript{2}\textsubscript{A}-AR and \textsuperscript{a}\textsuperscript{2}\textsubscript{C}-AR localise in the central nervous system, the latter being most abundant in the basal ganglia and hippocampus (De Vos et al. 1992; Ordway et al. 1993; Grijalba et al. 1996). Genetic association studies between ADRA2A gene and suicidal behaviour (Sequeira et al. 2004; Martín-Guerrero et al. 2006; Perroud et al. 2009) or mood disorders (Ohara et al. 1998) have mostly reported negative results. However, animal studies with \textsuperscript{a}\textsuperscript{2}\textsubscript{C}-knockout (\textsuperscript{a}\textsuperscript{2}\textsubscript{C}-KO) and \textsuperscript{a}\textsuperscript{2}\textsubscript{C}-overexpressing (\textsuperscript{a}\textsuperscript{2}\textsubscript{C}-OE) mice have revealed the implication of \textsuperscript{a}\textsuperscript{2}\textsubscript{C}-AR in the regulation of...
aggression, startle reactivity and the responses to stress (Sallinen et al. 1998, 1999). These studies have shown α2C-OE mice to be prone to the development of behavioural despair.

Del322-325 polymorphism at ADRA2C gene is a common 12-base-pair (bp) deletion of nucleotides 964–975 resulting in the deletion of four amino acids (Gly-Ala-Gly-Pro) in the third intracellular loop of α2C-AR (Feng et al. 2001). The deletion of these four amino acids has been suggested to result in a dysfunctional α2C-AR (Small et al. 2000) and to be associated with increased levels of cathecolamines (Neumeister et al. 2005). Studies have related α2C-del322-325-AR polymorphism with the development of heart failure in African Americans (Small et al. 2002), increased risk of silent brain infarction (Kim et al. 2014) and enhanced pain perception (Kohli et al. 2010). Interestingly, α2C-Del322-325-AR has also been described to contribute to the pathophysiology of major depression (Neumeister et al. 2006).

The aim of the present study was to explore the association between the functional polymorphism α2C-Del322-325-AR and suicide. For this purpose, the study focussed on suicide completion as an extreme phenotype of suicidal behaviour. Therefore, genotyping was performed on suicide victims and on subjects who had died by means other than suicide, including natural and accidental deaths. Furthermore, association between α2C-Del322-325-AR and various psychiatric diagnoses (major depression, schizophrenia, opiate and alcohol abuse and dependence) was also analysed in the same subjects, by access to established ante-mortem diagnoses of psychiatric disorders.

**Materials and methods**

**Subjects and samples**

To study the association between α2C-Del322-325-AR polymorphism and suicide completion, genotyping of samples of suicide and non-suicide victims was performed. Human brain samples were obtained at legal autopsies performed in the Basque Institute of Legal Medicine (IVML, Bilbao, Spain). The study was carried out in accordance with IVML ethical standards for post-mortem brain studies and with the Declaration of Helsinki.

Prefrontal cortex samples from a total number of 516 subjects were collected, dissected and immediately frozen and stored at −70 °C. The subjects were initially categorised as suicide victims (n = 236) and non-suicide victims (n = 280), as determined by medical examination. The sample size of this study was estimated to have 80% power to detect a positive association for α2C-Del322-325-AR polymorphism with a ≥15% frequency in suicide victims.

Retrospective information about diagnosis and treatment was obtained from medical examiner’s information and ante-mortem medical records of general and psychiatric hospitals (DSM-IV or ICD-10 criteria) in all the suicide and non-suicide cases. The 516 individuals included in the study were ascribed to case and control groups. Control samples (n = 187) were obtained from subjects without evidence of psychiatric or substance use disorder and who died by a non-suicide mechanism. The 329 case subjects were ascribed to the following groups: 39 subjects with major depression, 39 schizophrenic subjects, 40 subjects with affective disorders (other than major depression), 35 subjects with other diagnoses (11 subjects with bipolar disorders, 10 with personality disorders, three with dysthymic disorders, eight with anxiety disorders, one with hypnotic and one with amphetamine and cannabis use disorder, one with eating disorder), 35 subjects with opiate abuse and dependence, 67 subjects with alcohol abuse and dependence and 74 non-diagnosed suicide victims. In order to cope with the haplotype diversity between different ethnic groups, this study was performed in an homogenous set of Caucasian subjects from European origin, known to have much less haplotype diversity than other racial groups (Small et al. 2004). Ethnicity was assessed according to medical examiner’s records. The demographic characteristics, psychiatric diagnoses and mechanisms of death are shown in Table 1.

**α2C-Del322-325-AR genotyping**

Genomic DNA was extracted from brain tissue under blind conditions using standard procedures (Sambrook et al. 2001). α2C-Del322-325-AR genotyping was performed by PCR-restriction fragment length polymorphism using the enzyme HaeIII. The presence of the polymorphism produces the loss of HaeIII restriction site (wild-type: 107, 98, 89, 48, 44 and 8 bp fragments versus α2C-Del322-325-AR positive: 134, 107, 89, 44 and 8 bp). PCR and digestion fragment bands were resolved by electrophoresis in a 3% agarose gel containing ethidium bromide, and visualised under ultraviolet light. The polymorphism was validated by DNA fragment analysis on a capillary sequencer (AbiPrism 3100 Avant Genetic Analyzer, Applied Biosystems) with GeneScan analysis software, using LIZ 500 internal standard for allele sizing (Applied Biosystems, Foster City CA, USA; Figure 1). Primer sequences and PCR conditions are described in Supplemental Table 1.
(available online). As genotyping control, four samples (two wild-types and two positive for the polymorphism) were analysed by direct sequencing using AbiPrism 3100 Genetic Analyzer system (Figure 2).

### Statistical analysis

The presence of at least one allele with the polymorphism (deletion) was considered as a positive event. A nominal value of \( \alpha = 0.05 \) was selected to assess the significance of the associations. Analyses were performed using the SPSS 14.0 programme (SPSS Inc., Chicago, IL, USA) and R 2.15.1 software (R Development Core Team 2013).

#### Table 1. Demographic characteristics and mechanisms of death of suicide and non-suicide victims ascribed to psychiatric disorder and control groups.

| Phenotype                              | Frequency | Adjusted \( P \) values |
|----------------------------------------|-----------|-------------------------|
| Suicide victims (\( n = 236 \))        |           |                         |
| Female/Male                            | 75/161    |                         |
| Age (years)                            | 54 ± 1    |                         |
| Post-mortem delay (hours)              | 27 ± 1    |                         |
| Non-suicide victims (\( n = 280 \))    |           |                         |
| Female/Male                            | 66/214    |                         |
| Age (years)                            | 47 ± 1    |                         |
| Post-mortem delay (hours)              | 29 ± 1    |                         |
| **Psychiatric disorder and control groups** |           |                         |
| Major depression (\( n = 39 \))        |           |                         |
| Female/Male                            | 29/10     |                         |
| Age (years)                            | 65 ± 2    |                         |
| Post-mortem delay (hours)              | 22 ± 2    |                         |
| Suicide/Non-suicide victims            | 36/3      |                         |
| Schizophrenia (\( n = 39 \))           |           |                         |
| Female/Male                            | 5/34      |                         |
| Age (years)                            | 43 ± 3    |                         |
| Post-mortem delay (hours)              | 24 ± 2    |                         |
| Suicide/Non-suicide victims            | 33/6      |                         |
| Other affective disorders (\( n = 40 \)) |           |                         |
| Female/Male                            | 11/29     |                         |
| Age (years)                            | 55 ± 3    |                         |
| Post-mortem delay (hours)              | 29 ± 3    |                         |
| Suicide/Non-suicide victims            | 37/3      |                         |
| Other diagnoses (\( n = 35 \))          |           |                         |
| Female/Male                            | 12/23     |                         |
| Age (years)                            | 48 ± 3    |                         |
| Post-mortem delay (hours)              | 26 ± 3    |                         |
| Suicide/Non-suicide victims            | 31/4      |                         |
| Opiate abuse and dependence (\( n = 35 \)) |           |                         |
| Female/Male                            | 3/32      |                         |
| Age (years)                            | 34 ± 1    |                         |
| Post-mortem delay (hours)              | 23 ± 2    |                         |
| Suicide/Non-suicide victims            | 6/29      |                         |
| Alcohol abuse and dependence (\( n = 67 \)) |           |                         |
| Female/Male                            | 9/58      |                         |
| Age (years)                            | 54 ± 1    |                         |
| Post-mortem delay (hours)              | 30 ± 3    |                         |
| Suicide/Non-suicide victims            | 19/48     |                         |
| Non-diagnosed suicide victims (\( n = 74 \)) |           |                         |
| Female/Male                            | 21/53     |                         |
| Age (years)                            | 60 ± 2    |                         |
| Post-mortem delay (hours)              | 28 ± 2    |                         |
| **Control (\( n = 187 \))**            |           |                         |
| Female/Male                            | 51/136    |                         |
| Age (years)                            | 46 ± 2    |                         |
| Post-mortem delay (hours)              | 31 ± 1    |                         |
| Violent deaths                         | 163       |                         |
| Non-violent deaths                     | 3         |                         |
| Natural deaths                         | 21        |                         |

#### Table 2. Frequency of the \( \alpha_{2C}\)Del322-325-AR polymorphism in suicide and non-suicide victims and in psychiatric disorder and control groups.

| Phenotype                              | Frequency | \( P \) values |
|----------------------------------------|-----------|----------------|
| Suicide victims                         | 236       | 0.661          |
| Non-suicide victims                     | 280       | 10.7           |
| Major depression                        | 39        | 0.125          |
| Schizophrenia                           | 39        | 0.063          |
| Other affective disorders               | 40        | 0.480          |
| Other diagnoses                         | 35        | 0.100          |
| Opiate abuse and dependence             | 35        | 0.011*         |
| Alcohol abuse and dependence            | 67        | 0.446          |
| Non-diagnosed suicide victims           | 74        | 0.411          |
| Control                                 | 187       | 7.5            |

Polymorphism frequencies were compared between suicide victims and non-suicide victims and between psychiatric disorder groups and control group. *\( P < 0.05 \) vs. controls (two-sided Fisher’s exact test). Adjusted \( P \) values were obtained from either binary or multinomial logistic regression tests. Sex and suicide completion were used as covariates and the analysis followed by Benjamini-Hochberg’s false discovery rate.

Deviation from Hardy–Weinberg equilibrium in the control population was performed by \( \chi^2 \)-test. The polymorphism frequency in cases and controls was compared by Fisher’s exact test.

The effect of sex in the comparison between suicide and non-suicide victims was assessed by binary logistic regression. In the analysis of polymorphism frequency among psychiatric disorder groups, the effect of confounding factors sex and suicide completion, as well as multiple testing of several phenotypes, were assessed by multinomial logistic regression test followed by false-discovery test (Benjamini and Hochberg 1995).

#### Results

The minor allele frequency (MAF) of \( \alpha_{2C}\)Del322-325-AR in each group is shown in Table 2. Except for one homozygous subject (male, 67 years, natural death, diagnose of alcohol abuse and dependence), all individuals with presence of \( \alpha_{2C}\)Del322-325-AR polymorphism were heterozygous. Comparison of \( \alpha_{2C}\)Del322-325-AR frequency in suicide (9%, \( n = 236 \)) and non-suicide victims (11%, \( n = 280 \)) did not reveal any association between the polymorphism and suicide completion (\( P = 0.661 \)).

Subsequently, samples were categorised according to established ante-mortem diagnosis of psychiatric disorders. MAF of \( \alpha_{2C}\)Del322-325-AR was found to be 7% in the control population (\( n = 187 \)). The genotype frequency for \( \alpha_{2C}\)Del322-325-AR polymorphism was higher in subjects with major depression (15%, \( n = 39 \)), schizophrenic subjects (18%, \( n = 39 \)) and in the group of subjects with a variety of psychiatric disorders (17%, \( n = 35 \)). However, these differences failed to reach statistical significance (\( P = 0.125 \), \( P = 0.063 \) and \( P = 0.100 \), respectively). However, the frequency in those subjects
with affective disorders other than major depression was not higher than in controls (2%, \( n = 40, P = 0.480 \)). Interestingly, two previously unreported mutations in the exonic region of \( \alpha_2C \) gene were identified in the major depression group (Figures 1 and 2).

Analysis of \( \alpha_2C \)-Del322-325-AR polymorphism in subjects with opiate abuse and dependence showed a significant higher frequency (23%, \( n = 35, P = 0.011 \)) with respect to control subjects. By contrast, the frequency of \( \alpha_2C \)-Del322-325-AR polymorphism in subjects with alcohol abuse and dependence was not different from control subjects (10%, \( n = 67, P = 0.446 \)), demonstrating a selective and significant association between the \( \alpha_2C \)-Del322-325-AR polymorphism and opiate abuse and dependence.

A multinomial logistic regression was performed to ascertain the effect of confounding variables sex and suicide completion on the association between the polymorphism and psychiatric disorders. The logistic regression model was statistically significant, \( \chi^2(21) = 572.47, P < 0.001 \). The model explained 69% (Nagelkerke \( R^2 \)) of the variance in psychiatric disorders and correctly classified 52% of cases. Adjusted \( P \) values (Table 2) confirmed that the presence of the polymorphism was associated with opiate abuse and dependence (\( P = 0.049 \)). Those with at least one allele of the \( \alpha_2C \)-Del322-325-AR polymorphism were 3.9 times (95% confidence interval: 1.4–10.3) more likely to suffer from opiate abuse or dependence.

**Discussion**

The present study does not find an association between \( \alpha_2C \)-Del322-325-AR polymorphism and suicide completion, in spite of the described alterations in \( \alpha_2C \)-AR status in brains of depressed suicide victims (Meana et al. 1992; Callado et al. 1998; González-Maeso et al. 2002; Rivero et al. 2014). These results are in accordance with the lack of genetic association observed in the study of \( \alpha_2C \)-AR gene and suicide completion (Martín-Guerrero et al. 2006). Interestingly, the present study shows the selective association of \( \alpha_2C \)-Del322-325-AR with opiate (23 vs. 7% in controls) but not with alcohol abuse and dependence (10%), providing evidence that altered \( \alpha_2C \)-AR coding sequence may play a role in the pathophysiology of opiate abuse and dependence. Further, this work replicates the \( \alpha_2C \)-Del322-325-AR MAF value previously observed in Caucasian populations (Small et al. 2004).

The exact mechanism through which \( \alpha_2C \)-Del322-325-AR would confer susceptibility to opiate abuse and dependence is not known. In the human brain, \( \alpha_2C \)-AR are mainly located in the hippocampus and in the basal ganglia with predominance in caudate (De Vos et al. 1992; Ordway et al. 1993; Grijalba et al. 1996). In this sense, the principal neuronal circuitry involved in drug addiction processes and reward mechanisms is the dopaminergic system of the ventral tegmental area (VTA) and its projections to the anterior limbic areas (Williams et al. 2001). The localisation of \( \alpha_2C \)-AR in basal ganglia may account for a possible implication of \( \alpha_2C \)-AR in the regulation of the dopaminergic system, which might be altered in those subjects carrying the \( \alpha_2C \)-Del322-325-AR polymorphism. Actually, \( \alpha_2C \)-AR can be recognised and activated by dopamine (Zhang et al. 1999), and they also inhibit dopamine release.

![Figure 1. \( \alpha_2C \)-Del322-325-AR polymorphism genotyping. (A) Electrophoretic image of the bands corresponding to the \( HaeIII \) digestion fragments (only bands between 89 and 134 bp are shown). Lane 1: wild-type sample. Lane 2: heterozygous sample for \( \alpha_2C \)-Del322-325-AR polymorphism. Lane 3: homozygous sample. Lane 4: heterozygous sample for \( \alpha_2C \)-Del322-325-AR with an additional 9-bp deletion. Lane 5: wild-type sample for \( \alpha_2C \)-Del322-325-AR with a base change from G to A at position 971. (B) Capillary electrophoresis fragment-length. (1) Wild-type sample; (2) heterozygous sample; (3) homozygous sample; (4) heterozygous sample for \( \alpha_2C \)-Del322-325-AR polymorphism with a 9-bp deletion; (5) wild-type sample for \( \alpha_2C \)-Del322-325-AR polymorphim with a base change from G to A at position 971.](image-url)
Research on genetically modified animals has shown that overexpression of α2C-AR is associated with enhanced adrenocortical responses to stress and with decreased startle reactivity, aggression, and activity in the forced swimming test, whereas α2C-AR KO animals show opposite effects (Sallinen et al. 1998, 1999). These findings suggest that altered α2C-AR expression and/or functionality may increase the vulnerability to stress-related psychiatric disorders, including substance use disorders (Piazza and Le Moal 1998). Interestingly, the present study failed to show any association of α2CDel322-325-AR polymorphism with alcohol abuse and dependence, emphasizing the selectivity of the genetic association with opiate abuse and dependence. Actually, although drugs of abuse share general mechanisms (development of a euphoric state, craving, tolerance and withdrawal mechanisms), the specific action of ethanol and opioids at VTA neurons differ (Brodie et al. 1990; Johnson and North 1992). In this line, a selective association between a repetition polymorphism outside the coding region of ADRA2C gene and pathological gambling has been described in
gamblers without substance abuse but not in gamblers with substance abuse (Comings et al. 2001).

Finally, the analgesic synergy between the noradrenergic and opioid system has been extensively studied (Chabot-Doré et al. 2015). Analgesic synergy, at least at the spinal level, seems to be mediated by $\alpha_{2C}$-AR (Fairbanks et al. 2002). The mechanism for this synergy seems complex, as $\alpha_{2C}$-AR located in the dorsal horn have been shown to inhibit opioid release (Chen et al. 2008). Thus, the presence of $\alpha_{2C}$Del322-325-AR might be related to altered pain perception. Concordant to this, higher pain scores in response to cold pressor test have been reported in subjects with $\alpha_{2C}$Del322-325-AR polymorphism (Kohli et al. 2010).

With regard to major depression, a genetically determined link between $\alpha_{2C}$-AR function and the response of brain regions critical for emotional processing had been suggested in $\alpha_{2C}$Del322-325-AR carriers with major depression (Neumeister et al. 2006). However, the present study has not found any significant association neither in the group of major depression nor in the group of other affective disorders joining the negative results obtained for ADRA2A gene and mood disorders (Ohara et al. 1998; Burcescu et al. 2006). Similarly, in the schizophrenia group a larger proportion of $\alpha_{2C}$Del322-325-AR polymorphism with respect to control subjects was present. However, this difference failed to reach statistical significance, in line with the negative results obtained in African-American schizophrenic patients (Feng et al. 2001). Previous studies have also failed to see any association between ADRA2C gene and clinical response to antipsychotic treatment (De Luca et al. 2005) or attention-deficit and hyperactivity disorder (Barr et al. 2001).

To our knowledge, this is the first report of an association between polymorphisms at the ADRA2C gene and opiate abuse and dependence. The present finding suggests a possible role of $\alpha_{2C}$Del322-325-AR in the motivational behaviour leading to opiate self-administration and in the mechanisms of reward activated after opiate drug intake. A limitation of the current study is the small sample size ascribed to each of the psychiatric disorders groups. Although, in the case of opiate abuse and dependence the effect and sample size achieved a power of 69%, the association needs replication with expanded population sizes. Furthermore, studies with genetically modified animals having $\alpha_{2C}$Del322-325-AR polymorphism would be an ideal tool to delineate the effect of this polymorphism in the mechanisms of opiate abuse and dependence.

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Disclosure statement

None to declare.

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