Gene 576 (2016) 577–580

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Short communication

BEND3 is involved in the human-specific repression of calreticulin: Implication for the evolution of higher brain functions in human

A. Aghajanirefah [a], L.N. Nguyen [a], M. Ohadi [b,*]

[a] Department of Molecular Biology, Faculty of Science and Medicine, Radboud Institute for Molecular Life Sciences, Radboud University Nijmegen, Nijmegen, The Netherlands
[b] Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Received 2 September 2015
Received in revised form 1 October 2015
Accepted 15 October 2015
Available online 19 October 2015

Keywords:
Calreticulin
BEND3
Nuclear factor I
DNA pulldown
Human-specific

ABSTRACT

Recent emerging evidence indicates that changes in gene expression levels are linked to human evolution. We have previously reported a human-specific nucleotide in the promoter sequence of the calreticulin (CALR) gene at position −220C, which is the site of action of valproic acid. Reversion of this nucleotide to the ancestral A-allele has been detected in patients with degrees of deficit in higher brain cognitive functions. This mutation has since been reported in the 1000 genomes database at an approximate frequency of <0.0004 in humans (rs138452745). In the study reported here, we present update on the status of rs138452745 across evolution, based on the Ensembl and NCBI databases. The DNA pulldown assay was also used to identify the proteins binding to the C- and A-alleles, using two cell lines, SK-N-BE and HeLa. Consistent with our previous findings, the C-allele is human-specific, and the A-allele is the rule across all other species (N = 38). This nucleotide resides in a block of 12-nucleotides that is strictly conserved across evolution. The DNA pulldown experiments revealed that in both SK-N-BE and HeLa cells, the transcription repressor BEN domain containing 3 (BEND3) binds to the human-specific C-allele, whereas the nuclear factor I (NFI) family members, NF1A, B, C, and X, specifically bind to the ancestral A-allele. This binding pattern is consistent with a previously reported decreased promoter activity of the C-allele vs. the A-allele. We propose that there is a link between binding of BEND3 to the CALR rs138452745 C-allele and removal of NFI binding site from this nucleotide, and the evolution of human-specific higher brain functions. To our knowledge, CALR rs138452745 is the first instance of enormous nucleotide conservation across evolution, except in the human species.

1. Introduction

Aberrant expression of the genes related to the chaperone/immune system is linked with the pathophysiology of major psychiatric disorders (Sperner-Unterweger and Fuchs, 2015; Bozidis et al., 2014; Kazeminasab et al., 2012; Arion et al., 2007; Matigian et al., 2007; Iwamoto and Kato, 2006; Kakiuchi et al., 2003). There is emerging evidence linking calreticulin (CALR) to psychiatric disorders. One of the most widely-used mood stabilizers, valproic acid (VPA), exerts its therapeutic effect partially through altering CALR gene expression (Kim et al., 2005; Corson et al., 2004; Bown et al., 2002). CALR is a $Ca^{+}2$-dependent chaperone that interacts with 4-aminobutyrate type A (GABAA) receptor associated protein, which also interacts with integrins, indicating the involvement of CALR in synaptogenesis (Mohrluder et al., 2007). CALR also regulates the proper folding and differential trafficking of glutamate receptors (Jaskolski et al., 2005). Convergence of the glutamate and GABA-ergic pathways is a proposed mechanism for the pathophysiology of schizophrenia (Sodhi et al., 2011; Harrison and Weinberger, 2005). CALR is emerging as an immune-relevant gene, involved in both the adaptive and innate branches of the immune system (Liu et al., 2013; Raghavan et al., 2013).

We have previously reported low frequency functional mutations in the promoter sequence of CALR that are present in cases with major psychiatric disorders in humans (Ohadi et al., 2012; Esmaeilzadeh-Gharehdaghi et al., 2011; Farokhashtiani et al., 2011; Olad Nabi et al., 2010; Nunes et al., 2008; Aghajani et al., 2006). One of those mutations, −220C > A, is unique in two respects: Firstly, human specificity of the −220C nucleotide, and secondly, reversion of the nucleotide to the ancestral A-allele, in a spectrum of major psychiatric disorders, with degrees of deficit in human-specific higher-order cognitive functions (Ohadi et al., 2012; Farokhashtiani et al., 2011). We have also shown that this mutation is functional, and results in increase in gene expression (Esmaeilzadeh-Gharehdaghi et al., 2011). Of note, the site of action of VPA encompasses nucleotide −220 (Ohadi et al., 2012). The above properties support a possible link between the human CALR nucleotide −220 and the evolution of human-unique brain processes

Abbreviations: BEND3, BEN domain containing 3; CALR, Calreticulin; NFI, Nuclear factor I; VPA, Valproic acid.

* Corresponding author at: Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran.
E-mail address: ohadi.mina@yahoo.com (M. Ohadi).

http://dx.doi.org/10.1016/j.gene.2015.10.040
0378-1119/

that are widely impaired in major psychiatric disorders, such as language, judgment, and conceptual thinking. Indeed, the -220A mutation is the first reported instance of a cognition-deficit mutation, which reverses a human gene promoter to the ancestral type. Since the publication of the −220A mutation (Farokhashtiani et al., 2011), we have located information in the 1000 genomes database (www.1000genomes.org), on two individuals heterozygous for the −220C > A alleles (rs138452745) in populations of European and African origin, respectively. The phenotype of those two individuals is unknown at this time. However, this data supports our observation that the A-allele is present at very low frequency in humans (Farokhashtiani et al., 2011)]..

In the study reported here, we identify proteins binding to the rs138452745 human-specific C- vs. the ancestral A-allele, using the DNA pulldown assay. We also present update on the evolutionary status of rs138452745 across evolution.

2. Materials and methods

2.1. Bioinformatics update

Homology analysis across species for the CALR promoter sequence was performed by retrieving sequences from Ensembl (http://www.ensembl.org/index.html) and NCBI (http://www.ncbi.nlm.nih.gov), and comparing them using the EBI ClustalW2 (http://www.ebi.ac.uk/Tools/clustalw2/).

2.2. DNA pulldowns

DNA pulldowns, dimethyl labeling, mass spectrometry, and data analysis were performed as previously described (Hubner et. al., 2015), with minor modifications. Briefly, biotin tagged oligonucleotides (of the −220A and −220C allele) were immobilized to streptavidin beads. SK-N-BE and HeLa nuclear extract was then co-incubated with the beads, together with poly-DIDC and -DADT (to decrease the number of non specific interactions). After incubation, the beads were washed several times to remove non specifically bound proteins, followed by reduction of the proteins with TCEP, alkylation with MMTS, and overnight digestion with Trypsin/LysC. The protein digest was then dimethyl labelled. The pulldown experiment was performed in duplicate, where in the 'forward' experiment the pulldown of the −220C oligonucleotide was labelled with CD_2O (heavy) and the pulldown of the −220A oligonucleotide with CH_2O (light). In the 'reverse' experiment, the labels were swapped between the two pulldowns.

Significance of outliers was determined using intensity based outlier statistics (significance B) as described previously (Cox and Mann, 2008). Interactors were statistically significant if their significance B values were <0.01.

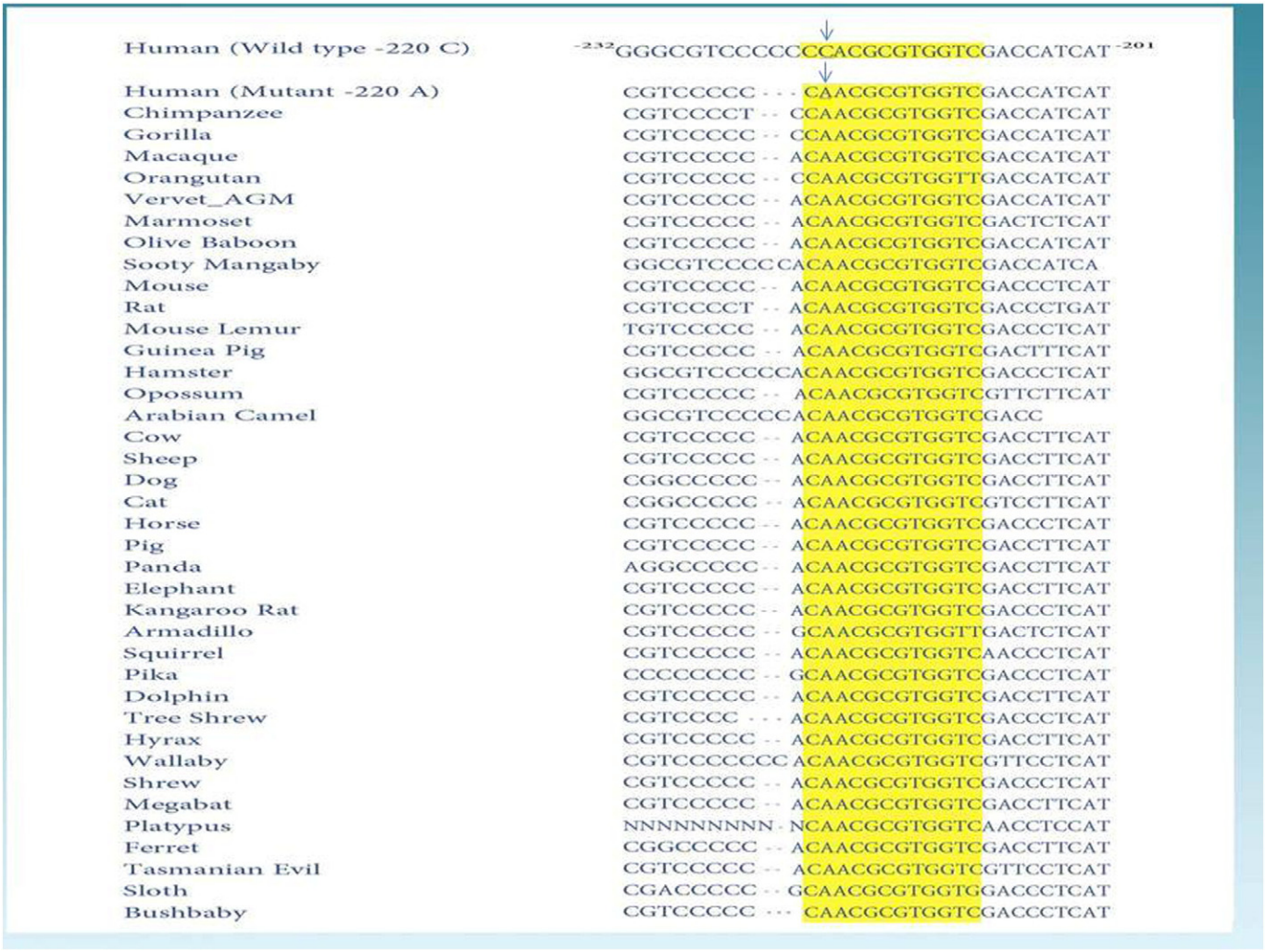

Fig. 1. The CALR promoter conserved block (highlighted in yellow) encompassing nucleotide −220 (rs138452745) (shown by arrows) across evolution. The C-allele is human-specific, and the A-allele is the rule across all other species (N = 38). The A-allele has been detected in patients with degrees of cognition deficit.