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Val16Ala-SOD2 polymorphism modulates hypothalamic-pituitary-adrenal axis molecules and BDNF levels in healthy adults under no psychological stress

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ABSTRACT. Chronic psychological stress alters the hypothalamic–pituitary–adrenal axis (HPA-axis), triggering chronic oxidative-inflammatory states that are associated with physical and psychiatric conditions. However, it is not clear if basal oxidative-inflammatory states triggered by genetic variation affect the HPA-axis by altering cortisol, adrenocorticotropic hormone (ACTH) and dehydroepiandrosterone sulfate (DHEA-S) levels. Humans have a single nucleotide polymorphism (SNP) found in manganese-dependent superoxide dismutase (Val16Ala-SOD2, rs4880), which has two alleles (V and A) which affect the basal efficacy of SOD2 antioxidant enzyme in the mitochondria. The VV-genotype, which

presents low SOD2-efficacy, has been associated with chronic inflammatory states, as well as higher risk of depression and self-reported psychological stress. Therefore, basal oxidative imbalance could have some influence on modulation of HPA-axis physiology. We tested this hypothesis comparing morning blood levels of cortisol, ACTH and DHEA-S and other biochemical markers in 90 healthy adult university students previously genotyped for the SOD2-SNP (30 volunteers for each genotype, 26.5 ± 8.7 years old). Only volunteers who self-reported no perception of psychological stress were included in the study. The VV group had higher morning cortisol and ACTH, and lower DHEA-S and brain-derived neurotrophic factor (BDNF) than A-allele subjects. These results indicate some influence of S-imbalance on modulation of this molecule. Therefore, we suggest that genetically controlled pro-oxidative and inflammatory states could modulate physiological markers for stress and neurogenesis.

Key words: Oxidative stress; Antioxidant enzyme; Psychiatric diseases; Adrenocorticotropic hormone; Dehydroepiandrosterone sulphate

INTRODUCTION

Evidence from epidemiological and experimental studies have suggested that chronic stress of psychosocial origin causes alterations in the hypothalamic-pituitary-adrenal (HPA) axis triggering pro-inflammatory and pro-oxidative states (Black et al., 2017). Consequently, the oxidative-inflammatory processes associated with psychological stress would constitute a risk for the development of somatic, psychiatric and other chronic non-transmissible diseases (Russell and Lightman, 2019). Previous evidence suggested that the association between psychological stress and chronic inflammation involves some cellular imbalances that increase the level of oxidative molecules and reduce the autophagy of cellular metabolic wastes producing dangerous and pro-inflammatory molecules (Feldman et al., 2015; Straub and Cutolo, 2016). However, an open question is whether intrinsic basal oxidative states could interfere in the modulation of HPA-molecules that are altered under psychological stress states, such as cortisol, adrenocorticotropic hormone (ACTH) and dehydroepiandrosterone sulfate (DHEA-S).

A genetic superoxide-hydrogen peroxide (S-HP) imbalance has been associated with a human single nucleotide polymorphism (SNP) found in the manganese-dependent superoxide dismutase (Val16Ala-SOD2, rs4880) gene. The SOD2-SNP has two alleles (A-Alanine and V – Valine) and produces three genotypes (VV, AA and AV), which change the basal efficacy of SOD2 antioxidant enzyme in the mitochondria. The homozygous genotypes have an S-HP imbalance that has been associated with risk for several non-transmissible chronic diseases (Bresciani et al., 2013 and 2015). Some evidence indicates that superoxide dismutases have an important role in vascular function and disease (Miao et al., 2009; Fukai et al., 2011), and low-efficiency of SOD2-enzyme could be a risk factor. This presumption is corroborated by epidemiological investigations suggesting that VV-carriers, who have higher basal S-levels than A-allele carriers, have a higher risk to develop

hypercholesterolemia (Duarte et al., 2010) and resistance to statin treatment (Duarte et al., 2016), obesity (Montano et al., 2009; Becer and Çırakoğlu, 2015), several diabetes *mellitus* type 2 complications (Huang et al., 2017; Yahya et al., 2019) and some cardiovascular diseases (Gottlieb et al., 2005; Fujimoto et al., 2010; Chen et al., 2012; Pascotini et al., 2015; Souiden et al., 2016; Flores et al., 2017). Moreover, there are some studies that suggest potential association between VV-genotype and mood disorders (Gałecki et al., 2010; Wigner et al., 2018; Jung et al., 2020).

On the other hand, AA-genotype has been associated with risk of DNA damage (Taufer et al., 2005), higher risk of developing sepsis states (Paludo et al., 2013; Majolo et al., 2015) and some cancer types, since the individual presents high basal HP-levels (Berto et al., 2015; Kang 2015; Li et al., 2016; Wang et al., 2018). A possible association between Val16Ala-SOD2-SNP and non-transmissible chronic diseases seems to involve a differential inflammatory response associated with homozygous VV and AA-genotypes: in the presence of a pro-inflammatory factor, VV-carriers have a high susceptibility to maintain low-grade chronic inflammatory states, whereas AA-carriers have a higher faster and shorter inflammatory response than V-allele carriers (Barbisan et al., 2017).

Therefore, one can infer that basal S-HP imbalance produced by Val16Ala-SOD2-SNP has some influence on modulation of HPA-axis's physiology. Considering that HPA-axis alterations have been consistently associated with depression and other mood disorders (Juruena et al., 2018), we hypothesized that oxidative imbalance related to Val16Ala-SOD2 SNP could influence some HPA-axis molecules, such as ACTH and DHEA-S. In order to test this hypothesis, we compared the morning blood levels of these molecules in non-stressful and mental healthy adults.

MATERIAL AND METHODS

General study design and ethics

The present investigation is a part of a broader research project developed by Biogenomics Laboratory (Federal University of Santa Maria, Brazil) that studies the role of S-HP imbalance triggered by Val16Ala-SOD2 SNP on the risk of developing non-transmissible chronic diseases prevalent in elderly population and its potential pharmacogenetic, nutrigenetic and toxicogenetic effects. This has been also evaluated by *in vitro* genetic and /or pharmacological S-P imbalance protocols (Barbisan et al., 2014, Berto et al., 2015; Azzolin et al., 2016; Schott et al., 2017; Barbisan et al., 2018). Therefore, participants of this study were selected from an initial databank of 180 free-living community subjects (26.5 years old, minimum = 18; maximum = 40 years old). All subjects were previously Val16Ala-SOD2 SNP genotyped using isolated DNA samples and by standard polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) techniques, a procedure similar to thatg used by Barbisan et al., 2014. Similar to the study previously performed by Jung et al (2020), volunteers were asked if they felt psychologically stressed. Only those who did not have any self-perceived psychological stress were include in the study.

From this sample, we selected a group to participate in the HPA-axis analysis; all of them were students at the Federal University of Santa Maria (Brazil) ($\leq$40 years old). All subjects lived in the same region of Brazil (Rio Grande do Sul), were non-smokers, non-

obese, didn't have any previous, psychiatric or not, non-transmissible chronic diseases, especially obesity, hypercholesterolemia and diabetes *mellitus* type 2, identified by a structured interview that included evaluation of occurrence of daily medicine intake of psychotropics, dietary supplements and other drugs that could affect the HPA-axis modulation. Moreover, we excluded volunteers that self-reported living a chronically stressful time in their lives in the last six months. Therefore, 30 subjects of each genotype (n = 90) were selected to compose the sample investigated here. As most subjects included in the protocol were undergraduate and graduate students blood samples were not taken during periods of school exams, which could influence the modulation of the HPA axis.

All the study participants provided their written informed consent, and this protocol was approved by the Human Ethics Committee of the Federal University of Santa Maria (CAE nº 23081.015838/2011-10). The study described here was also carried out in accordance to The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Laboratory analysis

The HPA-axis markers were quantified by a commercial clinical analysis laboratory (Labimed Company) located in Santa Maria-RS, Brazil. The pharmacists who performed the biochemical analyses did not know the genotype of the volunteers participating. In order to guarantee that subjects present similar physiological profile some biochemical variables were also evaluated and compared among samples including the quantification of: glucose, lipids (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides) determined by spectrophotometry assays, and high-sensitivity C reactive protein (hs-CRP) quantified by nephelometry (Dade Behring, Newark, DE, EUA) (Freitas et al., 2018). Inflammatory status was also evaluated by cell-free circulating DNA on plasma, quantified by PicoGreen assay (Quant-iT PicoGreen®, Invitrogen, Eugene, OR, USA) using a microplate reader (SpectraMax M2e, Molecular Devices, Austria) with fluorescence quantified at an excitation of 485 nm and an emission of 520 nm. The cfDNA levels indicate leukocytes apoptosis, event very common in infections and inflammatory states (Frank et al., 2016). The concentration of glucose, lipid prolife and hs-CRP are presented as mmoL and cfDNA as pg/mL.

Considering that Pascotini et al. (2018) described the association between lower levels of Brain Derived Neutrophic Factor (BDNF) and VV-SOD2 genotype, this marker was also quantified in subjects studied here. BDNF is a neurotrophin of crucial relevance in the central nervous system to neural plasticity and network formation that presents inverse correlation with cortisol levels (Barfield and Gourley, 2018). BDNF levels were determined by an enzyme immunoassay (ELISA) using a commercial kit from eBIOSCIENCE (E-EL-H0010, San Diego, USA) according to the manufacturer's instructions. The detection range of the kit is 31.25-2000 pg/mL.

The levels of cortisol, ACTH and DHEA were also analyzed through enzyme immunoassay (ELISA) for determination of markers in human serum and/or plasma

using a commercial kit according to the manufacturer's instructions (Immuno Biological Laboratories, cortisol = RE52611, ATCH = RE53081, and DHEA = IB79342) (Freitas et al., 2018). Cortisol is the main glucocorticoid hormone in human beings and presents a circadian fluctuation achieving the highest level in the morning. Cortisol secretion by adrenal cortex is regulated by a negative feedback mechanism via Corticotropin releasing hormone (CRH) in the hypothalamic region and the ACTH in the pituitary gland. However, its secretion is also affected by different stress situations. Therefore, in the present study, free cortisol, ACTH and DHEA-S levels were measured from blood samples collected from 8am - 9am by venipuncture while the subjects were fasting. The blood aliquots were centrifuged at 4°C at 500 × g for 15 min, sample aliquots were frozen at -80°C until their analysis. In the commercial kit used here analytical sensitivity (limit of detection) was BDNF= 18.75 pg/mL; cortisol = 0.005 µg/dL; ACTH= 1 pg/mL and DHEA-S = 0 0.002 µg/mL. We provided intra-assay and inter-assay coefficients of variation as follow: BDNF (5.39% and 4.21%), cortisol (5.9% and 13%); ACTH (7.6% and 7.1%) and DHEA-S (6.8% and 12.2%).

The general immunoassay quantification of BDNF and HPA-axis markers was performed as follow: 50 µL of each standard, control and sample was pipetted into respective wells of microtiter plate. Further, 100 µL enzyme conjugate was added into each well, the plate was covered with adhesive foil, shaken carefully and incubate for 2 h at room temperature (RT) on an orbital shaker (400-600 rpm). After adhesive foil removal and removal and disposal of the incubation solution, the plate was washed 4 x with 250 µL of diluted Wash Buffer. Each time, the excess solution was removed by tapping the inverted plate on a paper towel. TMB substrate solution (100 µL) was added to each well and incubated for 30 min. Finally, 100 µL of the stop solution was added to each well and the optical density was determined within 30 min using a microplate reader set to 450 nm. The concentrations of BDNF and ACTH were presented as pg/mL, cortisol as µg/mL and DHEA-S as ng/mL.

Statistical Analyses

Data analysis was performed with SPSS (version 22.0.1; SPSS Inc., Chicago, IL). Quantitative variables without normal distribution determined by Kolmogorov-Smirnoff test were log-transformed before comparison among SOD2-genotypes. These variables were compared among genotypes by analysis of variance followed by Bonferroni *post hoc* test or by Student *t test*, when dose-allele effect was analyzed. Categoric variables were compared by chi-square test or Fisher Exact test. General Pearson correlation between HPA-axis markers were also performed. The alpha value considered was set at 0.05, and all P values were two-tailed. Multivariate logistic regression analysis (Backward Wald) was performed in order to determine some sex, age and education level influence on association between Val16Ala-SOD2 SNP and HPA-axis and other biochemical markers evaluated here. All statistical analysis with $P \leq 0.05$ were considered significant.

RESULTS

Among the 90 subjects included in the study, 43% were male and 57% female. Most subjects had >12 years of school education (74%). Table 1 presents some biochemical characteristics baselines with total sample independent of Val16Ala-SOD2 SNP. The levels of metabolic variables, such as glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides, as well as hs-CRP were similar among subjects carrying different SOD2 genotypes. However, BDNF levels were dose-allele influenced (P = 0.001), with higher values found in AA-subjects (30.29 ± 5.64 pg/mL), intermediary values in AV-subjects (22.02 ± 3.69 pg/mL) and lower values in VV-subjects (14.20 ± 2.54 pg/mL).

Table 1. Baseline biochemical characteristics of healthy adults not under psycho-emotional stress enrolled in the study.

Variables	Subjects (n= 90)
BMI (Kg/m^2)	24.9 ± 3.4
Glucose (mmol/L)	4.16 ± 0.07
Total cholesterol (mmoL)	3.76 ± 0.07
LDL-cholesterol (mmoL)	1.86 ± 0.04
HDL-cholesterol (mmoL)	1.31 ± 0.05
Tryglicerides (mmoL)	1.03 ± 0.07
hs-CRP (mg/L)	0.22 ± 0.03
BDNF (pg/mL)	22.26 ± 7.57
Cortisol (ng/mL)	11.48 ± 4.61
ACTH (pg/mL)	6.77 ± 3.32
DHEA-S (ng/mL)	5.86 ± 2.07

BMI – body mass index; LDL– low density lipoproteins; HDL- high density lipoproteins; hs-CRP = High-sensitivity c-reactive protein; BDNF= Brain-derived neurotrophic factor; ACTH = Adrenocorticotropic hormone; DHEA-S = dehydroepiandrosterone sulfate

The SNP also affected significantly (P = 0.0001) the levels of three HPA-axis markers analyzed here (Figure 1). VV-subjects presented higher levels of cortisol and ACTH and lower levels of DHEA-S than AA-subjects. On the other hand, heterozygotes had intermediary values of these markers, when compared to homozygous subjects. However, additional multivariate analysis did not find influence of sex and age in these results.

As expected, a significant and positive correlation between cortisol and ACTH morning-blood levels was found in the subjects analyzed in this investigation (r^2 = 0.745, P < 0.001). On the other hand, cortisol presented a significant negative correlation with DHEA-S levels (r^2 = - 0.566, P < 0.001). ACTH was also negatively correlated with DHEA-S levels (r^2 = - 0.519, P < 0.001).

Potential correlation between BDNF and HPA-axis values was evaluated, and main results are presented in the Figure 2. Negative correlation between BDNF and cortisol and BDNF and ACTH levels was observed in all subjects analyzed here. A significant positive correlation between BDNF and DHEA morning-blood levels was also observed in the young healthy subjects investigated here. However, Val16Ala-SOD2 SNP did not influence the correlation between BDNF and HPA-axis markers.

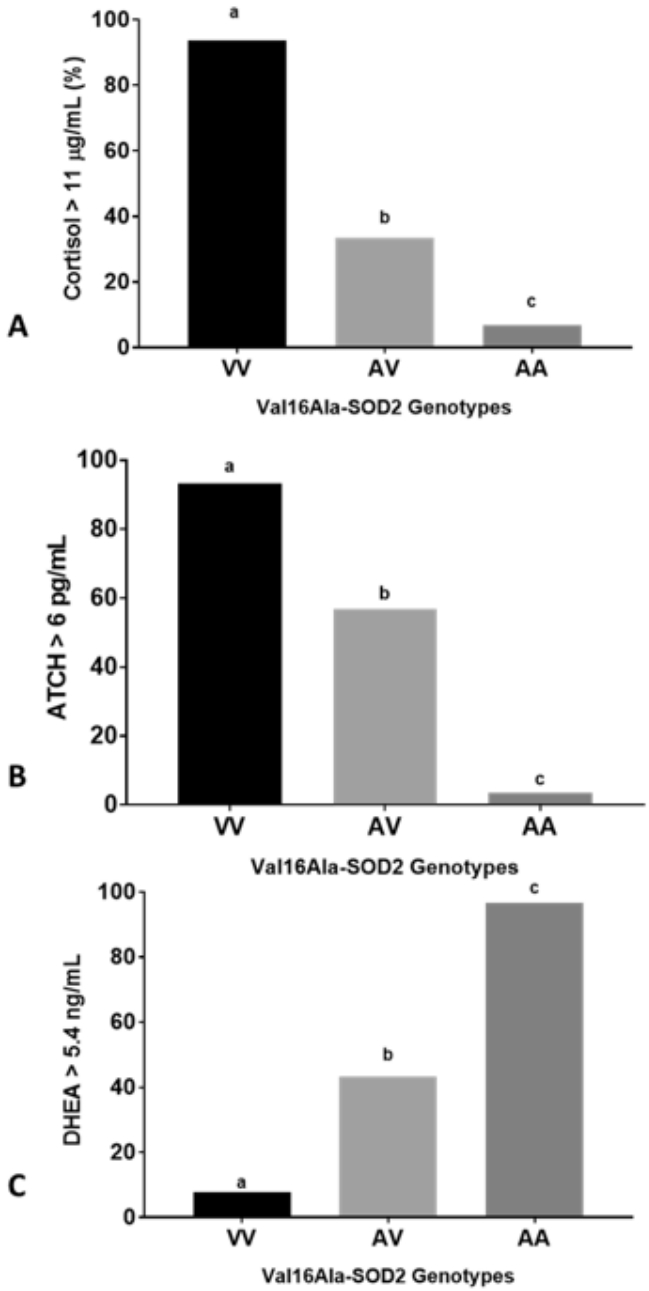

Figure 1. Comparison of fasting morning-blood cortisol, adrenocorticotropic hormone (ACTH) and dehydroepiandrosterone sulfate (DHEA-S) levels among healthy adult subjects carrying different Val16Ala-SOD2 genotypes (Valine-Valine, VV; Alanine-Alanine, AA and Alanine-Valine, AV) by One-way analysis of variance followed by Bonferroni *post hoc* test. Different letters indicate significant differences among genotypes at $P \leq 0.01$.

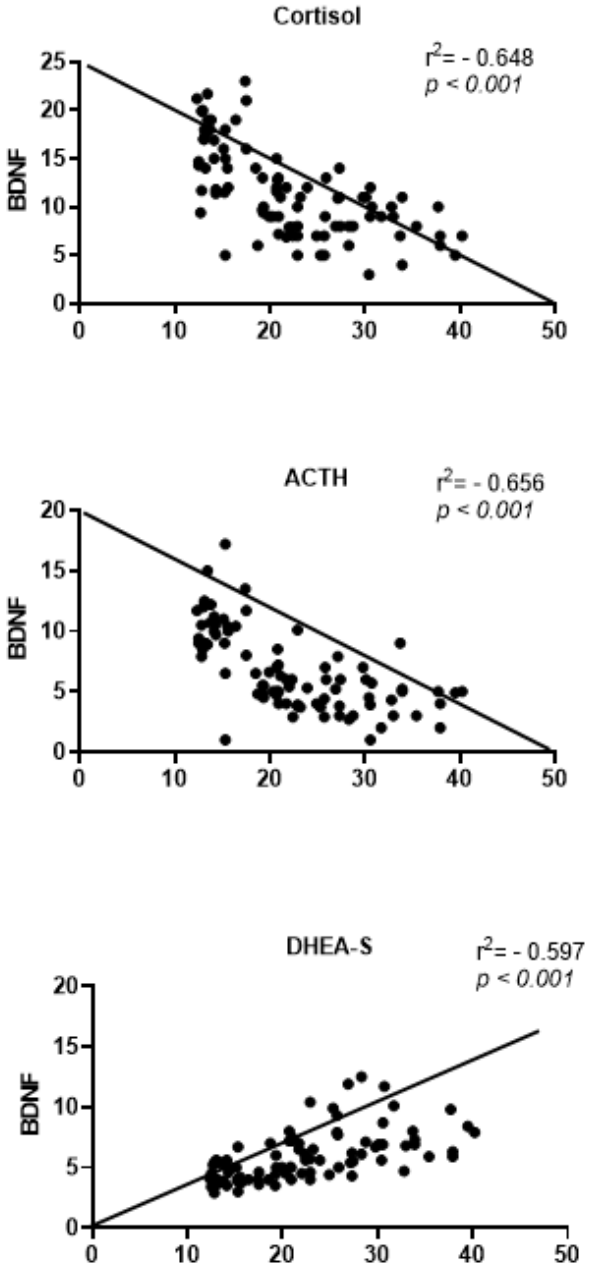

Figure 2. General Pearson correlation between Brain-derived neurotrophic factor (BDNF) and hypothalamic-pituitary-adrenal (HPA) axis molecules (independent of Val16Ala-SOD2 genotypes) in fasting morning blood in healthy adult subjects. Correlation (r^2) and significance (P) are shown in each graph.

DISCUSSION

We evaluated if intrinsic basal S-HP imbalance caused by a genetic variation found in the SOD2 gene could differentially modulate HPA-axis molecules. The VV-subjects that have higher S-basal levels than A-allele subjects presented higher levels of stress-related molecules (cortisol and ACTH) and lower levels of DHEA-S. However, the correlation between these molecules was not affected by SOD2 SNP. Moreover, VV-subjects presented lower morning-blood BDNF levels than others indicating some influence of S-imbalance on modulation of this molecule. From these results, we consider relevant to discuss three main points: potential influence of S-HP imbalance on HPA-axis modulation; potential influence of S-HP imbalance on BDNF levels and finally methodological constrains that could present some influence on results described here.

It is well accepted that psychological stress in life is unavoidable. However, persistent psychological stress can lead to the development of anxiety, depression, and cognitive impairment, and contribute to a higher risk for chronic non-transmissible diseases (Adam et al., 2017). In fact, chronic psychological stress can result in sympathetically mediated negative effects that alter UTC mitochondrial function, increasing the levels of ROS molecules (Picard et al., 2015). Moreover, there are many evidences that suggest that neurodegenerative and neuropsychiatric disorders are closely related to excess of oxidative stress that alters SNC functions (Wadhwa et al., 2018).

However, studies investigating whether basal states of oxidative imbalance could affect HPA axis modulation are still incipient. The present study sought to contribute to the elucidation of this question through the analysis of the basal levels of cortisol, ACTH and DHEA-S in individuals carrying different Val16Ala-SOD2 genotypes. The potential effect of VV-genotype on HPA-axis modulation could be plausible based in the increase of S-levels into mitochondria. In order to understand this association is necessary to comment some aspects related to S-basal imbalance triggered by Val16Ala-SOD2 SNP, that is summarized in the Figure 3. The SOD2-enzyme is synthetized from a nuclear gene located in chromosome 6 at the 25.3 position.

From SOD-2 mRNA, an inactive SOD2-protein is initially produced, which contains a peptide sequence named mitochondrial target sequence (MTS) that is crucial for the entry of the enzyme into the mitochondria. By the time SOD2 enters the mitochondria the MST sequence is lost, and the enzyme becomes active. S-anion is the primary ROS generated by mitochondrial electron transport chain (ETC) (Figure 3A).

The passage of electrons through ETC generates energy; and ultimately these electrons reduce molecular oxygen in to water. This process involves a series of redox carriers known as Complex I (NADH: ubiquinone oxidoreductase), complex II (succinate dehydrogenase), complex III (ubiquinol-cytochrome c reductase) and complex IV (cytochrome c oxidase). The exergonic process of electron transport through complex I–IV will be the last step related to adenosine triphosphate (ATP) generation. However, this process is not 100% efficient occurring the exit of some electrons from the ETC before they get reduced to water at Complex IV. This exit is named “electron leak”, that is the major causative factor for production of mitochondrial S-anion, mainly in Complexes I and IV. Therefore, SOD2-enzyme has a crucial role in the mitochondria avoiding detrimental S-anion action, that can react with nitric oxide (NO) producing peroxynitrite and other ROS molecules (Sutton et al., 2003; Murphy 2009; Rich and Marechal 2010).

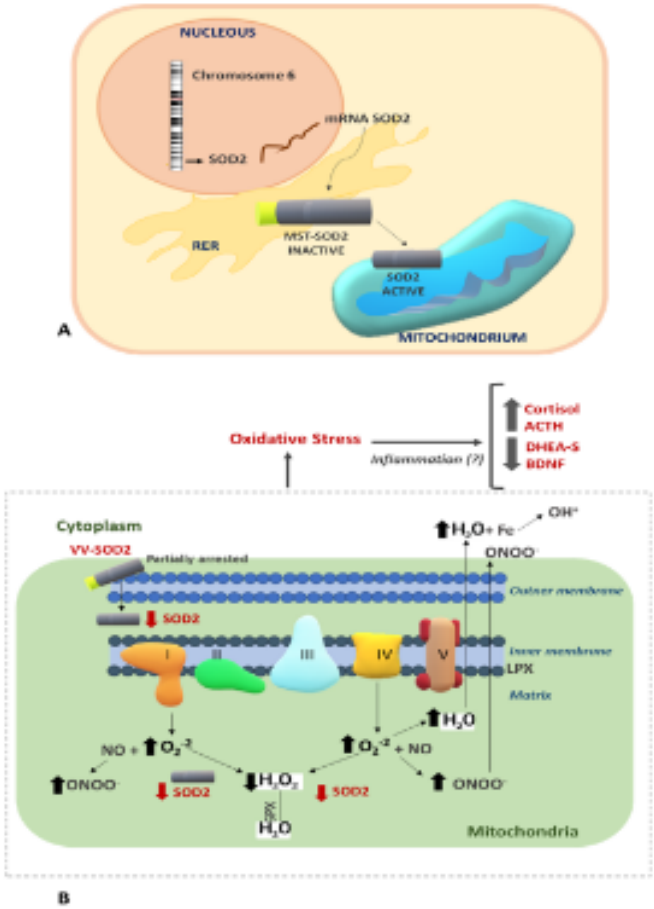

Figure 3. General scheme of superoxide dismutase manganese dependent (SOD2) metabolism and the potential Val16Ala-SOD2 SNP effect on superoxide-hydrogen peroxide (S-HP) imbalance related to VV-genotype on production of reactive oxygen and nitrosative molecules. (A) SOD2-gene is located in chromosome 6. Initially a SOD2-inactive protein is synthetized that has a peptide sequence named "mitochondrial sequence target, MTS" (represented in yellow) that triggers the protein into the mitochondria. In this organelle, MTS is cleaved and SOD2 becomes an active enzyme catalyzing S-anions that are constantly produced by mitochondrial electron transport chain; (B) V-allele produces a beta-sheet SOD2-protein that is partially arrested in outer mitochondrial membrane. A lower production of SOD2-active enzyme is not completely efficient to catalyze S-anion in H_2O_2. Basal S-imbalance contributes to generates other oxidative and nitrosative molecules increasing the risk of oxidative stress and chronic low-grade inflammatory states. ACTH - adrenocorticotropic hormone; DHEA-S = dehydroepiandrosterone sulfate (DHEA-S); BDNF = Brain-derived neurotrophic fator.

The Val16Ala-SOD2 SNP interferes in the SOD2-enzyme efficiency because it induces alterations in the conformation of SOD2-inactive protein. Whereas V-allele produce a beta-sheet SOD2-inactive protein, A-allele produce an alpha-helix SOD2-inactive protein. The VV-SOD2 conformation induces a partial arrest of inactive protein in outer mitochondrial membrane. In consequence, VV-mitochondria has lower levels of SOD2-enzyme than A-allele mitochondria determining low-efficiency in the catalysis of S-anion in HP-molecules (Figure 3B) (Sutton et al., 2003). Previous studies have suggested that VV-imbalance could contributes to establishment of chronic low-grade inflammation processes

that are observed in several non-transmissible chronic diseases, such as hypercholesterolemia, diabetes *mellitus* type 2 and cardiovascular morbidities (Bresciani et al., 2015). Moreover, some *in vitro* and *in vivo* protocols have suggested potential pharmacogenetic effects of Val16Ala-SOD2 SNP (Costa et al., 2012; Duarte et al., 2016).

Considering potential SOD2-SNP on mental health, previous studies have suggested that VV-genotype could increase the risk of depression, which is a psychiatric condition closely associated with psychological stress (Gałecki et al., 2010; Wigner et al., 2018; Jung et al., 2020). However, although the SOD2-polymorphism affected morning-blood levels of cortisol, ACTH and DHEA, the correlation between these molecules was altered indicating that the S-imbalance induced by VV-genotype does not act on the feedback mechanism of HPA-axis.

Another relevant result described here is in respect to the association between SOD2-SNP and BDNF levels since VV-subjects presented lower levels of this important neurotrophic molecule, confirming a previous report performed by Pascotini et al. (2018). A complementary analysis was performed to determine potential influence of SOD2 SNP on correlation between BDNF and HPA-axis molecules. In fact, expression and secretion of neurotrophines, such as BDNF are modulated by neuronal activity that is directly influenced by light and dark periods. As BDNF is also influenced by circadian period, some studies (Begliuomini et al., 2008; Pluchino et al., 2009) showed that BDNF and cortisol levels are significantly higher in the morning than at night in healthy men subjects. Experiments in rats and mice also demonstrated the involvement and relevance of BDNF in basal HPA-axis regulation (Naert et al., 2015).

However, elevated levels of cortisol due to stressful experiences have deleterious effects on neuroplasticity (McEwen et al., 2008). Previous studies described an inverse correlation between cortisol and BDNF levels in some psychiatric conditions including schizophrenia (Issa et al., 2010), female suicide attempters (Ambrus et al., 2016) and depression (Malhi et al., 2018). Elevated cortisol levels during a lifetime is a predictor of human hippocampal atrophy related to cognitive deficits found in depressive patients (Lupien et al., 1998). On the other hand, genetic studies involving a Val66Met SNP in the BDNF gene have described an association with this SNP on cortisol responses to stress in women (Jiang et al., 2017). Some experimental studies also suggested that reduced DHEA and BDNF levels could be involved in the pathophysiology and pharmacotherapy of childhood depression (Malkesman et al., 2009). In other studies, there are some evidences showing lower cortisol and BDNF levels in child and adolescent victims of sexual abuse (Şimşek et al., 2015).

Thus, it seems that the modulatory association of BDNF and the HPA-axis could be altered in the presence of some physiological and neuropsychiatric states and by genetic influence, as is the case of the polymorphism in the previously mentioned BDNF gene. However, results described in this study also appear to show that the basal oxidative state would influence the modulation of BDNF and HPA-axis.

Some investigations, such as performed by Mansur et al. (2016) also indicate that BDNF has a positive correlation with SOD activity, acting as a regulatory molecule on SOD2 gene expression of circulating angiogenic cells (He et al., 2012). Therefore, the higher susceptibility of VV- subjects presenting high levels of cortisol and ACTH and reduced levels of BDNF is in agreement with previous studies published in the literature, such as conducted by Bouvier et al., 2017. These authors observed that intense social defeat

stress produced vulnerability to depression in rats, and that depression was a consequence of a persistent oxidative stress state and lower BDNF-levels. They also indicated that treatments with antioxidants were able to reverse this vulnerability.

In another experimental investigation performed by Freitas et al. (2018), high intensity interval training (HIIT) induced elevation of hippocampal BDNF levels, and reduction of oxidative stress by elevation of SOD enzyme in young adult, male Wistar rats. The experiment also showed decreasing proinflammatory cytokines (IL-1β, IL-6 and TNF-α) in these rats. This also demonstrates the close modulation of psychological stress via HPA-axis and BDNF alterations, oxidative stress and inflammatory states.

Previous studies have suggested that VV-SOD2 genotype is associated with high basal S-levels, chronic inflammatory states including elevated levels of pro-inflammatory cytokines (Montano et al., 2012; Barbisan et al., 2017) and risk of some cardiometabolic morbidities and depression (Gałecki et al., 2010; Flores et al., 2017). In these terms, the main novelty of the results described here lies in the fact that independent of psychological chronic stress occurrence, individuals carrying the VV genotype have a "pro-stress condition" potentially determined by higher levels of cortisol and ACTH and lower levels of DHEA-S and BDNF. Perhaps this "pro-stress condition" could explain an elevated risk of VV-subjects to develop depression. How much preventive actions could minimize this potential susceptibility to the existent stress in VV individuals, through a diet rich in antioxidants and moderate physical activity, is a question to be investigated better in the future.

Finally, it is important to comment some methodological constraints of our study. A consistent number of studies investigate HPA axis modulation through analysis of cortisol, ACTH and DHEA-S levels of saliva or blood samples collected at different times of the day. However, in these cases it is quite difficult to control if the subjects of the research ended up being exposed to some level of acute stress, which could mask the results. Actually, it is not easy to establish association studies involving genetic of oxidative metabolism, since there are several potential intervenient factors that could act as attenuators or enhancers of oxidative stress associated to psychological stress and psychiatric disorders. For this reason, we opted to investigate the morning levels of cortisol, ACTH and DHEA-S in a high and controlled sample of healthy subjects previously SOD2-genotyped.

CONCLUSIONS

In summary, we suggest that HP imbalance determined by Val16ala-SOD2 SNP modulates physiological markers for stress and neurogenesis. Further experimental *in vitro* and *in vivo* investigations could help confirm these findings. Based on our observations and review of the literature, this is a new finding that could have clinical impact.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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