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IL-1B POLYMORPHISMS AND ITS INTERACTION WITH NRG-1 ON THE RISK OF INTELLECTUAL DISABILITY

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ABSTRACT:

Trophic factors and cytokines are involved in neural development and regulate neurotransmission. In particular, interleukin-1β (IL-1β) and neuregulin-1 (NRG-1)have been recognized to play a key role in activity-dependent maturation and plasticity of excitatory synaptic structure and function. Genetic functional dysregulations of these molecules could impair neuronal processes influencing cognitive development and may have a wide range of neurological consequences. Here we studied the impact ofIL-1β rs16944(-511 T>C), rs1143634(+3962 C>T), NRG-1rs6994992, NRG-1rs35753505 and ErbB4 rs7598440 polymorphisms on the risk of cognitive impairment in children affected by mild/moderate intellectual disability (ID).

Our findings indicate that both the IL-1β variants are associated to ID. Moreover, analysis of gene-gene interaction suggests that there is an interaction between IL-1β and NRG-1 in ID risk. Measuring IL-1β levels we evidenced higher IL-1β serum concentration in ID patients than in controls.

Introduction

Intellectual disability (ID) (current term for mental retardation) is a complex neurodevelopmental disorder characterized by significant limitations in both intellectual functioning and adaptive behaviour. ID can be the result of a number of known unknown genetic, neurophysiological or environmental causes, trauma or combination thereof. Genetics plays an important role in ID aetiology; mutation, deletion or reorganization of the genes that encode proteins can indeed influence brain development or cognitive functioning.

In central nervous system (CNS) cytokines take part in brain functions involved in molecular and cellular mechanisms mediating complex cognitive processes [16], playing a key role in synaptic plasticity, long-term potentiation (LTP), neurogenesis and memory consolidation [19]. Cytokine production is under genetic control. Different individual levels of interleukins can be linked to gene polymorphisms within the coding, intron, or promoter regions. Epidemiological evidence show that the onset of cognitive decline [21] and dementia [7] are characterized by increased peripheral cytokine levels. Furthermore, some genetic studies support associations between pro-inflammatory cytokine gene variations and cognitive impairment [1] or decline in an elderly population [24]. Among cytokines, IL-1β is one of the most prominent candidates for these genetic studies. The association between the IL-1β SNPs and cognitive impairment has been studied mainly in memory performance, working memory, attention/processing speed or motor function of adults with conflicting results recently reviewed by Stacey [18]. In addition, specific IL1-related gene variants have been associated with increased serum levels [13]. The NRG-1 is part of the growth factor family and influences neuro-developmental processes such as myelination, synapse formation, neuronal migration. NRG-1 and its receptor ErbB4, as regulator of N-methyl-d-aspartic acid (NMDA) and GABA receptors, have been associated with schizophrenia [10]. Importantly, memory impairment is a feature of schizophrenia as patients experience deficits in cognition, executive function, and memory processes [17]. In addition, NRG-1 also appears to be involved in both short-term and long-term plasticity suggesting that it plays a role in cognitive processes [23]. In 2013 Kukshal [11] reported the association between two SNPs of NGR-1(rs35753505 and rs6994992) and cognitive functions in Indian population confirming prior reports in Caucasian samples [23] and in rodent models [15]. The interaction between cytokines and growth factors has been largely demonstrated and in many physiological functions they

modulate each other [9]. Here we extend a previous study [1] with the aim of determining if there is an association of IL-1β and NRG-1 SNPs and the susceptibility to ID. To do this, we considered a children population with a diagnosis of mild/moderate ID without comorbidity or neurological impairment.

Material and Methods

Patients and diagnostic assessment

A case control study was conducted. Following the study approval by the local Ethics Committee (0102550), 45 ID patients (25 males and 20 females), at age between 3-18 years were enclosed in this study. All patients were Italians, Caucasians and have been enrolled at the Child Development and Neuropsychiatric Clinic of the S. Salvatore Hospital, L'Aquila, Italy. Based on age, the Wechsler Primary and Preschool Scale of Intelligence (WPPSI-R) and Wechsler Intelligence Scale for children (WISC-III) were used to study intellectual functioning in verbal and performance cognitive domains and the child's general intellectual ability. Vineland Adaptive Behaviour Scales were used to measure personal and social skills required for everyday living. The Child Behavior CheckList (CBCL) was used to detect emotional and behavioral problems in children and adolescents and the Conners' Continuous Performance Test (C-CPT), which measures and evaluates a child's attention span and ability to maintain focus on a task, was used to support the conclusions. According to the criteria of Diagnostic and Statistical Manual of Mental Disorders- IV Edition (DSM-IV) that recognizes four degrees of ID severity, the children included in this study had an IQ score comprised between 50 and 70 that reflect a mild to moderate level of intellectual impairment. Exclusion criteria for ID patients were genetic conditions (Down syndrome, fragile X syndrome, and phenylketonuria). Environmental conditions that interfere with the growth and development of the brain such as prenatal (severe maternal malnutrition, alcohol and drug abuse), during birth (hypoxia, extreme prematurity) and after birth (severe head injury, malnutrition of the child, severe emotional neglect or abuse) factors were included.

Controls

Thirty-one healthy controls matched with the patients for age, gender and ethnicity were considered. A written informed consent was obtained from all participants.

DNA extraction

Genomic DNA was extracted from whole blood according to the manufacturer's protocol (QIAamp DNA blood MiniKit, Qiagen, Courtaboeuf, France) and kept at −20°C until use. DNA concentration and purity was determined using a spectrophotometer (Beckman Instruments, Inc. Fullerton, CA. 92834-3100).

Genotyping

IL-1β rs16944 and rs1143634 gene polymorphisms were investigated in patients and controls using a polymerase chain reaction (PCR)-based method (Pel-Freez Cytokine Genotyping Kit) as previously described [1]. The SNPs rs6994992 and rs35753505 of NRG-1 gene and rs7598440 of the ErbB4 were amplified by PCR using the primers shown in Table 1. Amplifications were carried out on 50 ng of genomic DNA per 50 µl reaction. PCR products were identified on 1.5% agarose gel and then purified by a PCR clean-up reagent (EXOSAP). Sequence reactions were performed using the BigDye Terminator Chemistry v 1.1 (Applied Biosystems, Foster City, CA), processed on an Applied Biosystems 3130 Genetic Analyzer and then purified. Typing was obtained on the basis of alignment of the processed sequences with the sequences retrieved from the Genbank Database.

IL1-β measurements

Serum samples from patients and healthy controls were separated from venous blood and stored at −80°C until cytokine analysis. IL1-β levels were evaluated by enzyme-linked immuno sorbent assay (ELISA) using commercially available kits (Boster Biological Technology) and performed in accordance with the manufacturer's protocol. The sensitivity of the assays was< 0.15pg/ml. Samples were run in duplicate and the mean value was used for further statistical analysis.

Statistical Analysis

Person's chi-square test and Fisher's exact test were used to evaluate genotype and allele frequencies among cases and controls. The odds ratio (OR) was calculated to assess the relative risk conferred by a particular allele and genotype. Comparison of serum IL-1β level between cases and control group was done with one-way analysis of variance (ANOVA). Statistical significance was set at $p < 0.05$. Calculations were performed using the SPSS/Win software (Version 12.0, SPSS Inc., Chicago, IL).

Results

The genotype and allele frequencies of the IL-1β rs16944 and IL-1β rs1143634polymorphisms in ID patients and control subjects are shown in Figs.1 and 2. Our findings indicate that both the IL-1β variants are associated to ID. We found that subjects carrying the rs16944 CC genotype had an increased risk to develop ID (p=0.02; OR: 3.29) compared to those with the rs16944 TT genotype (p=0.0003; OR:0.57). We also found that C allele was more frequent in patients than in controls (p=0.0007 OR:3.29) and on the contrary, T allele was more frequent in controls than in patients (p=0.0007 OR:0.30). The analysis of the IL-1β rs1143634 revealed a positive association with ID for the CC genotype and a negative one for the TT genotype (p=0.005; OR: 4.15 and p=0.01; OR:0.05). A significant difference was also observed in the frequency of C and T alleles of rs1143634 in patients and controls (p=0.0006 for C allele, OR:4.21 and p=0.0006 for T allele, OR:0.24). Considering together the two SNPs of IL-1β we noted that CC CC combination was associated with an increased risk to develop ID (p=0.003 OR:8.33) (Fig.3). Examining the genotype and allele frequencies of the NRG-1 rs6994992, NRG-1rs35753505 and ErbB4 rs7598440 polymorphisms, we did not observe statistical significant differences between patients and controls.

Analysis of gene-gene interaction revealed some combinations associated with ID. Given the complexity of the statistical analysis, only associations with p<0.05 (Fisher-test) were taken into account. The first significant combination, founded only in the group of patients, was between the CC genotype of IL-1β rs1143634 with the CC genotype of NRG-1 rs6994992 (p=0.01 OR:1.81). Instead, the combination CT of IL-1β rs1143634 with TT of NRG-1 rs6994992 was found only in controls (p=0.009 OR:0.6), Fig.4. The last significant combination, founded only in patients, was CC of IL-1β rs1143634 with CC of NRG-1 rs6994992 and TT of NRG-1rs35753505 (p=0.004 OR: 2.02), Fig.4.

From the study of the IL-1β serum concentration we detected a significantly higher level of IL-1β in patients with ID than in control subjects (p=0.005), Fig.5.

Discussion

Severe ID is quite sporadic, whereas more often milder forms can occur and spread out in family and population. For the present investigation we recruited only a children population whose clinical diagnosis was mild to moderate ID with exclusion of other co-morbidity. Studying 2 SNPs located in the IL-1β gene (rs16944 and rs1143634) we demonstrated a positive association between the both CC genotype and ID and a negative one with TT genotype. These results were confirmed through at allelic level. In fact, we found that the presence in both SNPs of C allele was significantly associated with ID. On the contrary, T-allele was significantly more frequent in control children. The involvement of inflammatory cytokines, especially IL-1, IL-6, and tumour necrosis factor (TNF-a) in the normal physiological regulation of hippocampal-dependent memory has been widely analysed [22] and it has been demonstrated that the influence of IL-1β on memory processes includes both detrimental and beneficial effects [2]. The key role of

pro-inflammatory cytokines and specifically of IL-1β in neuronal functionality seems to be thinly regulated in a dose-effect manner. Many research conducted mainly in rodent models, underlined that both peripherally and centrally altered levels of IL-1β might be responsible for hippocampal-dependent memory impairments [3]. This is important because peripheral cytokines are able to affect the brain both blood-borne and neural routes [5] and can lead to de novo production of pro-inflammatory brain cytokines [20]. The IL-1β gene displays many SNPs both in promoter and coding regions, which have been associated with IL-1β production. The rs16944 andrs1143634 SNPs are respectively a promoter polymorphism and a silent polymorphism in exon 5 of IL-1β and are able to influence circulating protein expression [6]. There are several evidences of association between the rs1143634 C allele and higher serum IL-1β concentrations [12]. In our study, we show a significantly higher IL-1β serum level in ID than in controls. In addition, patients with CC genotype and C allele of both SNPs had higher IL-1β expression than controls even if not statistically significant (p = 0.06) (data not shown). NRG-1 is a growth factor with multiple functions in the embryonic and postnatal brain and it is involved in a variety of physiological processes, including migration of embryonic interneurons, maturation and survival of oligodendrocytes [8] and modulation of synaptic plasticity [14]; therefore it may influence cognitive function. The analysis of the frequencies showed no association between ID and the two SNPs of NRG-1 nor with the SNP of its receptor ErbB4 but, logistic regression analysis demonstrated IL-1β and NRG-1 genes interaction. More specifically, our results provide evidence that IL-1β rs1143634 and its interaction with both NRG-1 SNPs affects the risk when CC genotype of IL-1β SNP combines with CC and TT NRG-1 carriers. On the contrary, CT TT combination of IL-1β rs1143634 and NRG-1 rs6994992 appeared to be protective as it is present only in controls. Here we reported data from patients that are apparently non syndromic ID, as mentioned in exclusion criteria.

Conclusion

The impact of IL-1β SNPs and the IL-1β/NRG-1 interaction have not been previously described in the ID risk. These results could be a start point for further investigations and can provide information to develop future projects on this topic and/or to planning strategies that identify ID early. All this with the aim of improve access to management interventions and specialized care for ID.

Conflict of interest statement.

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethics Statement

All studies have been approved by the appropriate ethics committee (n. 1931/ 17.11.2011) and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Table 1. The primer sequences used for amplification of NRG-1 and ErbB4 genes.

NRG-1 rs6994992	5'-AGTAGGATTGGATGTTTG-3'
	5'-TTAGCAGCATAGTTGGGCAG-3'
NRG-1 rs35753505	5'-GCATTAGAACTAGAACTTGCGTGA-3'
	5'-TGGGAACTCTCCATCTCTTTC-3'
ErbB4 rs7598440	5'-CTCAGCAGAGGCATCAAC-3'
	5'-CTGGACACTCATGGGAAG-3'

SNP	Genotype	ID Patients n(%)	Controls n(%)	p-value (Fisher Test)	OR (95% CI)
IL-1β rs16944	CC	24 (53.4)	8 (25.8)	0.02	3,29 (1.21-8.89)
	CT	21 (46.6)	15 (48.4)	ns	-
	TT	0	8 (25.8)	0.0003	0.57 (0.19-1.69)
IL-1β rs1143634	CC	36 (78.3)	13 (46.4)	0.005	4,15 (1.50-11.5)
	CT	10 (21.7)	11 (39.3)	ns	-
	TT	0	4 (14.3)	0.01	0.05 (0-1.039)

SNP: single nucleotide polymorphism; OR: odds ratio
p-value calculated using Fisher's exact test.

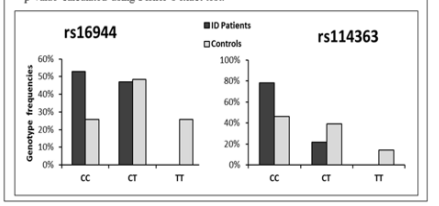

Fig. 1. Upper: Table reports the genotype association analysis of the 2 SNPs of IL-1β. Below: Graphs represent genotype percentages of each IL-1β SNP.

SNP	Allele	ID Patients n(%)	Controls n(%)	P value	OR (95% CI)
IL-1β rs16944	C	69 (76.6)	31 (50.0)	0.0007	3.29 (1.64-6.60)
	T	21 (23.4)	31 (50.0)	0.0007	0.30 (0.15-0.61)
IL-1β rs1143634	C	82 (89.1)	37 (66.0)	0.0006	4.21 (1.78-9.94)
	T	10 (10.9)	19 (34.0)	0.0006	0.24 (0.10-0.56)

SNP single nucleotide polymorphism
p-value calculated using Fisher's exact test

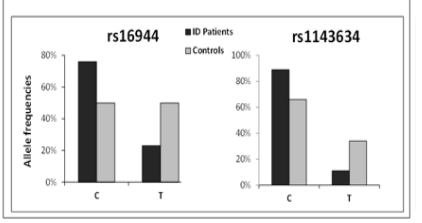

Fig. 2. Upper: the table accounts for the allele association analysis of the 2 SNPs of IL-1β. Below: Graphs represent allele percentages of each IL-1β SNP.

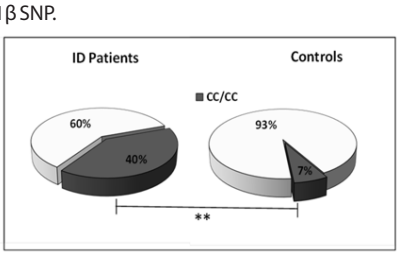

Fig. 3. Pie chart indicating the percentage of CC CC haplotype of two IL-1β SNPs. ** p = 0,003 calculated using Fisher's exact test. OR:8,33; CI 95%: 1,97-44,16.

IL-1β rs1143634	NRG-1 rs6994992	NRG-1 rs35753505	Patients n(%)	Controls n(%)	p-value (Fisher Test)	OR (95% CI)
CC	CC	-	9 (22.5)	0	0.01	1.81 (1.44-2.29)
CT	TT	-	(0)	4 (15.4)	0.009	0 (0-1.17)
CC	CC	TT	9 (22.5)	0	0.004	2.02 (1.63-2.52)

p-value calculated using Fisher's exact test

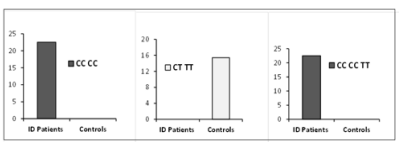

Fig. 4. Upper: The allele association analysis of the 2 SNPs of IL-1β is shown on the table. Below: Gene-gene interactions analysis of IL-1β and NrG-1. Each graph represents a genetic combination of the 3 SNPs. Combination CC CC and CC CC TT were found only in ID patients. Combination CTTT was found only in controls.

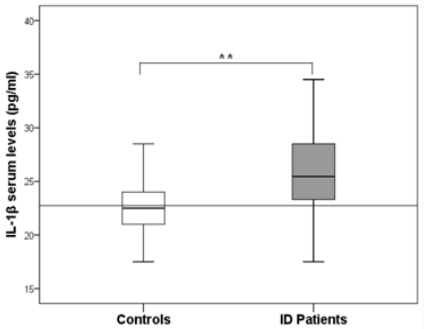

Fig. 5. Boxplot represents the serum levels of IL-1β protein. Cut-off value (horizontal line=22.75) was determined by receiver operating characteristic (ROC) curve. Differences were calculates with one-way ANOVA; **p=0,005.

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