Potential Biomarkers for Intellectual Disability: A Gypsy Family Study

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Abstract

Intellectual disability (ID) is frequently due to the synergic action of environmental and genetic factors. Here we describe the particular case of three Gipsy Italian siblings, fifteen, twelve and eleven years old, with ID. Genetic analysis stated that the parents are not consanguineous, and no human leukocyte antigen (HLA) alleles related to autism and/or other neurological disorders are present in the 3 ID patients. Instead, a positive association of ID with brain derived neurotrophic factor (BDNF) (Val66Met and C270T), IL6 (-174) and interleukin 1 receptor antagonist (IL1RA) mspa 11100 polymorphisms was demonstrated in these three ID patients. Moreover, serum levels of interleukin 1beta (IL1B), interleukin 6 (IL6) were significantly different between the three patients and controls.

Keywords: Intellectual disability; Polymorphism; Cytokine; HLA; Neurotrophic factor

Introduction

Several genes are involved in ID etiology but the mechanisms by which these candidates regulate cognitive function remain poorly understood [1,2]. Cytokines play a central role in CNS functions and, some of them such as IL-1beta and IL-6 have been associated with cognitive decline and dementia [3,4] and are released during neuronal activity, playing a key role in regulating the strength of synaptic [5,6].

Peripheral expression of cytokines can influence both in pathological and physiological conditions, the hippocampus-related memory and synaptic plasticity [7-9] and there is growing evidence that single nucleotide polymorphisms (SNPs) regulate their expression [10,11]. Several polymorphisms of BDNF gene have been described and in our previous study a positive association between ID and two BDNF SNPs was demonstrated (Val66Met and C270T) [12]. Current research increasingly also demonstrates a role of the HLA proteins in the functional interactions of neural cells, in CNS development [13], and even in neurological disorders [14]. The phenylketonuria (PKU), a defect in the hepatic enzyme phenylalanine hydroxylase (PAH), is an inherited disorder that induces delayed mental and social skills. A higher incidence of PKU was demonstrated in Gypsies than in other populations [15], and two missense mutations have been identified in the PAH gene of an Italian PKU patient [16].

Herein, we report the particular case of three Gipsy ID siblings in which functional cytokine gene polymorphisms, serum levels of cytokines, HLA class I and II allele polymorphisms, BDNF gene polymorphisms and two PAH missense mutations have been studied.

Case Report

Three children belonging to a Gipsy family living in the area of the coast of the Adriatic Sea were enrolled at the Department of Neuropsychiatric Disorders of the Civil Hospital of L’Aquila, Italy (Figure 1A).

Patient 1

The first patient is a female born in 1999 without complications after a normal pregnancy. From birth, her psychomotor development was delayed, and included feeding problems. She was able to walk at the age of 18 months. At 5 years old she spoke only a few words and started intensive linguistic training. A severe intellectual disability was diagnosed (IQ 35). She is described as an easily irascible although quite sociable child.

Patient 2

The other female, born in 2002 after an uneventful pregnancy, developed critical anaemia after the birth and received blood transfusions. She began walking at 15 months old and a delay in language development and in the control of the sphincters was reported. Severe intellectual disability (IQ 34) was noticed early on. Some spread electric anomalies, particularly in the frontal lobe area, were observed by her electroencephalogram. She is shy and unable to integrate with peers.

Patient 3

The younger affected brother was born in 2003 by caesarean section. The same psychomotor development pattern seen in his affected sisters was present. His speech was delayed and he started to speak at 3 years old. A mild intellectual disability was diagnosed (IQ 59). He is short-tempered and does not socialize easily.

According to Diagnostic and Statistical Manual of Mental Disorders- IV Edition (DSM-IV) criteria, to access ID diagnosis Wechsler Intelligence Scale for Children-III (WISC-III), a standardized test of intellectual aptitude for children between ages 6 and 16 years, and the Vineland Adaptive Behaviour Scales (VABS) were administrated. The WISC-III subscales are used to generate four composite scores: verbal comprehension, perceptual reasoning, working memory, and processing speed. The VABS is a widely used tool for assessing an individual’s ability to care for one’s self personally and socially. The Child Behaviour CheckList (CBCL), a parent-report
questionnaire to detect emotional and behavioural 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, were used to further support conclusions and to evaluate general intellectual development (Figure 1B).

Controls for cytokines and BDNF polymorphisms pertain to a larger case-control group studied previously [12]. Nine healthy children age and sex matched with the siblings were selected as controls for cytokine levels (Figure 2B).

Venous blood was drawn from three siblings and their parents. Genomic DNA was extracted using QIAGen’s DNA blood Midi Kit. Cytokine gene polymorphisms were investigated in patients and controls using the Pel-Freez Cytokine Genotyping Kit [17].

All 3 ID Gipsy siblings presented the specific IL-6 (-174) G/C and the IL-1RA mspa1100 T/T genotypes both associated with an increased risk to develop ID [17] and the IL6 GG/CA predisposing apototype [17].

HLA class I (A, B, and Cw) and class II (DRB1) gene polymorphisms was conducted at high resolution by sequence-based typing (SBT) assay using commercial class I and class II sequencing kits (Abbott Molecular AlleleSEQR HLA-SBT). Sequencing was carried out on a 3130 Genetic Analyzer (Applied Biosystems). Assign SBT v 3.5+ software (Conexio Genomics, Applecross, Australia) used the allele assignment to detect the heterozygous positions within each electropherogram and assess the typing on the basis of an alignment of the processed sequence with an updated HLA sequence library. (B) Measurements of cytokine levels were carried out using the Multi-Analyte Profiler ELISArray Kit (Qiagen) according to the manufacturer’s instructions. Three fourteen and three twelve years old females and three eleven years old males were selected as controls. Results are reported as absorbances at 450 nm. Statistical analyses were performed using the statistical package for the social sciences (SPSS) version 11.0 (SPSS, Tokyo, Japan). A Mann-Whitney test for non-parametric data was used to compare medians. *p=0.011 for IL6; *p=0.035 for IL1β.

Discussion
In this case report we show a set of genetic characteristics associated with ID in sibs that have not shown evident hereditary problems linked to cognitive impairment. Moreover, the anamnestic investigations did not highlight the relevant problems in the history of the family.
The involvement of pro-inflammatory cytokines, has been amply demonstrated in neurological conditions [20-22] since they alter the activity of the central nervous system and neuroendocrine, increase the release of neurotransmitters, induce the activation of immediate early genes in the brain and change the basic behaviours [23], as well as the functions of learning and memory [24,25]. In the three ID Gypsy patients, we found the entire predisposing genetic cytokine profile previously observed in a larger case-control study [17] and a significant higher level of IL1β and IL6 in the three ID patients.

Confirming previous reports [12,26], we observed the positive association between both Val/ Met and C270T BDNF SNPs and ID. In addition, the children not showed any HLA class I (A2) and class II (DR4) alleles related to autism and/or to cognitive impairment [27,28]. On the contrary the HLA investigations allowed us to trace the Gipsy origin of the family and to exclude the parental consanguinity.

Last but not least, considering the gipsy origin of the father, we investigated two most prevalent mutations of PAH associated to PKU, showing the normal homozygous C/C genotype for both mutations in all the three sibs.

The case reported here summarizes results previously published by us and by others [12-26] and demonstrates the possible clinical relevance of immune and neurotrophic factor polymorphisms in cognitive impairment. This knowledge could represent a useful diagnostic tool to clinicians and could help to assess the risk to a person carrying a particular allele or haplotype of developing a neurological disease and thus contribute to a better understanding of its pathogenesis.

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