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Association of the leukemia inhibitory factor gene polymorphism rs929271 with idiopathic mild intellectual disability

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Aim. To investigate the possible association of *LIF* gene polymorphism rs929271 with mild intellectual disability (ID). **Methods.** The group of patients with mild (IQ score between 50 and 70) idiopathic intellectual disability consisted of 64 individuals including 40 (62.5 %) males and 24 (47.5 %) females. The control group consisted of 238 healthy volunteers from different regions of Ukraine. Polymorphic variants of *LIF* gene rs929271 were detected using PCR followed by *Hinf1* RFLP analysis. **Results.** The data concerning *LIF* genotypes and allelic variants distribution in ID patients and control group were obtained. Statistical analysis shows significant differences at rs929271 for both genotype and allele frequency when comparing ID cases and controls ($p = 0.01$ and 0.02, respectively). **Conclusions.** Our results suggest that *LIF* gene polymorphism rs929271 is associated with idiopathic mild intellectual disability. Therefore, we propose LIF as a new marker of genetic susceptibility for intellectual disability.

Keywords: *LIF* gene, intellectual disability, polymorphism, population.

Introduction

Intellectual disability is a neurodevelopmental disorder, affecting about 3 % of the population, and is associated with a series of social and medical handicaps [1]. The causes of intellectual disability vary with the severity of the condition: moderate-to-severe intellectual disability (IQ less than 50) is much more likely to be due to a single pathological cause (genetic or environmental) whereas mild ID (defined as an IQ score between 50 and 70) is rather due to the complex condition in origin [2].

Leukemia inhibitory factor (LIF) is a member of the neuropoietic family of neurotrophins and was found to regulate the neuronal phenotype and coordinates astrocyte, oligodendrocyte, microglia, and inflammatory cell responses [3–5]. Furthermore, LIF is shown to act as a survival factor for neurons and oligodendrocytes [6, 7].

Upon binding to the heterodimeric glycoprotein 130 (gp130)/LIF receptor (LIFR) complex, LIF activates several major intracellular signaling pathways including ERK/MAPK signaling [8, 9]. It was discovered that ERK/MAPK pathway is important for normal cognitive development and is required for certain types of synaptic plasticity [10, 11]. It was shown that the mutations in genes coding for ERK/MAPK pathway proteins and regulators such as *SYNGAP1* and *RPS6KA3* cause non-syndromic intellectual disability (NS-ID) and autism spectrum disorders [12–16].

The leukemia inhibitory factor gene is located on chromosome 22q12.1-q12.2 [17]. The T to G transversion rs929271 is located in the 3 primed untrans-

lated region of the *LIF* gene. This polymorphism is suggested to reduce mRNA stability and finally may have an effect on the amount of secreted LIF [18]. Previous reports have demonstrated that the *LIF* gene variant (rs929271) may produce susceptibility to vascular dementia, hebephrenic schizophrenia and deterioration of working memory function [19, 20].

Aim of this study is to evaluate the possible association of the *LIF* gene polymorphism rs929271 with mild intellectual disability.

Materials and Methods

DNA-samples were extracted from peripheral blood leucocytes of unrelated volunteers from different regions of Ukraine and ID patients by the standard phenol-chloroform method. Informed consents were obtained from all the individuals participating in our study.

The group of patients with mild (IQ score between 50 and 70) idiopathic intellectual disability consisted of 64 individuals including 40 (62.5 %) males and 24 (47.5 %) females, where previous extensive genetic investigations have revealed no abnormalities. All patients underwent physical and neurological examination (test used for IQ: WISC III, WISC-R, WISC) and standard G-banding karyotype analysis. DNA tests to determine Fragile X status (*FRAXA, FRAXE, FRAXF* loci) and Prader Willi/Angelman syndromes (PW/AS) were performed to rule out the known genetic causes of ID prior to further investigation. Array-CGH analysis (400K resolution) revealed no any pathological rearrangements in all patients.

The control group consisted of 238 individuals including 128 (53.8 %) males and 110 (46.2 %) females. This group may be considered representative for the estimation of DNA polymorphism frequency in autosomal genes [21, 22].

The presence of *LIF* polymorphism rs929271 was examined by PCR-RFLP (restriction fragment length polymorphism) analysis. Specific oligonucleotides, designed and synthesized in accordance to corresponding sequences of *LIF* gene, were used as primers: forward: 5'-GGGGACACAGAAACAAG GACAGGG -3' and reverse: 5'-AAGGGTCGGAT CTGAGAGAATGGG-3'. Primers were designed using a web-based PRIMER 3.0 program (http://workbench.sdsc.edu). We used the «BLAST» program at http://www.ncbi.nlm.nih. gov/blast to check for the specificity of the primers. Hypothetical RFLP results

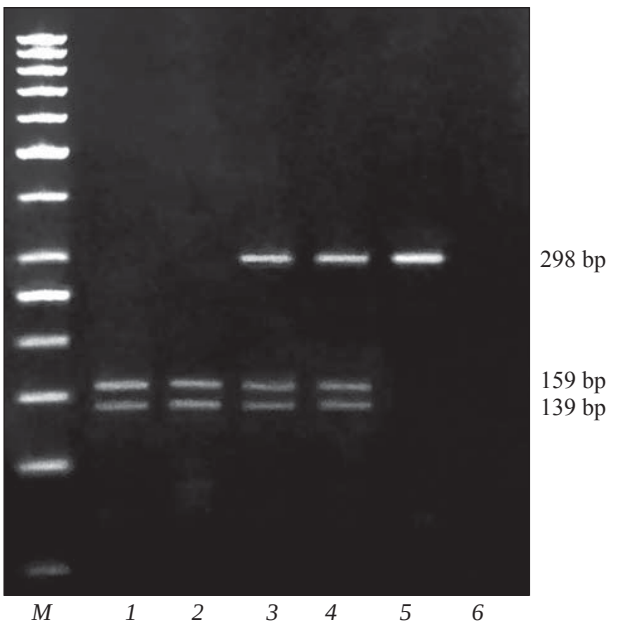

Fig. 1. RFLP analysis of rs929271 *LIF* gene variant (2 % agarose gel electrophoresis): *M* – molecular mass marker (Ladder 50 bp); *1, 2*, – individuals with homozygous genotype TT; *3*, *4* – individuals with heterozygous genotype TG; *5* – individual with homozygous genotype GG, *6* – negative control

Table 1. **Distribution of genotypes and allele variants in investigated groups**

	Control group, *n* = 238	ID patients, *n* =64
Genotype, *n* (%)		
TT	107 (45)	18 (28.1)
TG	106 (44.5)	35 (54.7)
GG	25 (10.5)	11 (17.2)
TG+GG	131(55)	46(71.9)*
Allele, *n* (frequency)		
T	320 (0.672)	71 (0.555)
G	156 (0.328)	57 (0.445)*

Note. N – number of individuals; * statistically reliable difference (P < 0,05).

were tested using NEB cutter V2.0 (http://tools.neb.com/NEBcutter2).

The PCR amplification was performed in a final volume of 25 µl containing 1 × PCR buffer, 1.5 mM MgCl2, 200 µM of each dNTP, 1 µM of each primer, 0.2 units of *Taq*-DNA polymerase and 200 ng of the DNA template. The cycling conditions were as follows: initial denaturation at 95 °C for 5 min, 30 cycles consisting of denaturation at 94 °C for 30 s, annealing at 63 °C for 30 s, extension at 72 °C for 30 s and a final elongation step at 72 °C for 3 min. The amplified fragments were digested with *HinfI*. Digestion was performed in 15 µl reaction volume containing 1 X reaction buffer, 0.5 units of the restriction enzyme and 10 µl of purified PCR product, incubated at 37 °C overnight and analyzed in 2 % standard agarose electrophoresis.

The results were statistically assessed using OpenEpi software and Fisher's 2 by 2 exact test, as well as odd ratio (OR) calculation; $p < 0.05$ was considered to be statistically significant test [23].

Results and Discussion

The designed primers successfully amplified the corresponding fragment (298 bp) of the *LIF* gene. The T to G transition in rs929271 variant removes a restriction site for endonuclease *HinfI*. Thereby three different patterns could be observed for the rs929271 variant after the restriction digestion: a 298 bp band (for rs929271 T/T); a 298 bp, a 159 bp and a 139 bp bands (for rs929271 T/G); a 159 bp and a 139 bp bands (rs929271 G/G) (Fig. 1).

Based on the RFLP analysis of the rs929271 variant, the individuals were classified into three groups: TT, TG and GG. Genotypes and allele frequencies of the rs929271 polymorphism are presented in Table 1. The observed genotype distributions showed no deviations from Hardy-Weinberg expectations in general population of Ukraine and in ID patients group.

It was determined that total frequency of hetero- and homozygous carriers of the *LIF* gene rs929271 minor allele, in this case that of guanine (G), is reliably higher ($p = 0.01$) in the ID patients group (71.9 %) compared to the control group (55 %). The minor G-allele occurred less frequently in the control group – 0.328 than in the ID patients group – 0.445 ($p = 0.02$). It was shown that the risk of mild ID development increased for both hetero- and homozygous carriers of minor rs929271 G-allele and the odd ratio was 2.09 (95 % CI: 1.14–3.81).

Our results suggest that the *LIF* gene polymorphism rs929271 is associated with idiopathic mild intellectual disability. These data can be explained by a decrease of LIF levels in the *LIF* gene rs929271 minor allele G carriers that in turn, may affect the regulation of neurogenesis and neuroprotection at least via LIF/ERK/MAPK signaling.

Therefore, we propose *LIF* as a new marker of genetic susceptibility for intellectual disability. Future investigations are necessary to explain the molecular mechanisms of the *LIF* involvement in ID pathogenesis.

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Асоціація поліморфізму rs929271 гена лейкемія-інгібуючого фактора з ідіопатичною легкою інтелектуальною недієздатністю

Мета. Дослідити можливу асоціацію поліморфізму rs929271 гена *LIF* з легкою інтелектуальною недієздатністю (ІН). **Методи.** Група пацієнтів з легкою (IQ між 50 і 70) ідіопатичною інтелектуальною недієздатністю складалася з 64 індивідів, включаючи 40 (62,5 %) чоловіків і 24 (47,5 %) жінки. Контрольна група складалася з 238 здорових добровольців з різних регіонів України. Поліморфні варіанти rs929271 гена *LIF* виявляли за допомогою ПЛР з подальшим *Hinf1* ПДРФ аналізом. **Результати.** Були отримані дані про розподіл генотипів і алельних варіантів гена *LIF* в групі пацієнтів з ІН і в контрольній групі. Статистичний аналіз показує значимі відмінності по rs929271 як для частот генотипів, так і алельних варіантів при порівнянні досліджуваної та контрольної груп (p = 0,01 і 0,02, відповідно). **Висновки.** Наші результати показують, що поліморфізм rs929271гена *LIF* асоційований з легкою ідіопатичною інтелектуальною недієздатністю. Тому ми пропонуємо *LIF* в якості нового маркера генетичної схильності до інтелектуальної недієздатності.

Ключові слова: ген *LIF*, інтелектуальна недієздатність, поліморфізм, популяція.

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Ассоциация полиморфизма rs929271 гена лейкемия-ингибирующего фактора с идиопатической легкой интеллектуальной недееспособностью

Цель. Исследовать возможную ассоциацию полиморфизма rs929271 гена *LIF* с легкой интеллектуальной недееспособностью (ИН). **Методы.** Группа пациентов с легкой (IQ между 50 и 70) идиопатической интеллектуальной недееспособностью состояла из 64 индивидов, включая 40 (62,5 %) мужчин и 24 (47,5 %) женщины. Контрольная группа состояла из 238 здоровых добровольцев из разных регионов Украины. Полиморфные варианты rs929271 гена *LIF* выявляли посредством ПЦР с последующим *Hinf1* ПДРФ анализом. **Результаты.** Были получены данные о распределении генотипов и аллельных вариантов гена *LIF* в группе пациентов с ИН и в контрольной группе. Статистический анализ показывает значимые различия по rs929271 как для частот генотипов, так и аллелей при сравнении исследуемой и контрольной групп (p = 0,01 и 0,02, соответственно). **Выводы.** Наши результаты показывают, что полиморфизм rs929271гена *LIF* ассоциирован с легкой идиопатической интеллектуальной недееспособностью. Поэтому мы предлагаем *LIF* в качестве нового маркера генетической предрасположенности к интеллектуальной недееспособности.

Ключевые слова: ген *LIF*, интеллектуальная недееспособность, полиморфизм, популяция.

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