ORIGINAL PAPERS / ARTICLES ORIGINAUX

DETECTION OF IN RS876657372 (DELACGT) IN PRSS12 GENE, RISK FACTORS AND ASSOCIATED CONGENITAL ABNORMALITIES IN NON-SYNDROMIC INTELLECTUAL DISABILITY-CASE CONTROL STUDY.

DETECTION DE RS876657372 (DELACGT) DANS LE GENE PRSS12, DES FACTEURS DE RISQUE ET DES ANOMALIES CONGENITALES ASSOCIEES DANS LES DEFICIENCES INTELLECTUELLES NON SYNDROMIQUES : UNE ETUDE CAS-TEMOIN.

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Keywords: *PRSS12 gene, non-syndromic intellectual disability, DelACGT, psychomotor delay, rs876657372*

ABSTRACT

Background:

Certain mutations in the *PRSS12* gene were linked to non-syndromic autosomal recessive form of intellectual disability.

Objective:

Here we wanted to find out whether delACGT (rs876657372) is associated with non-syndromic intellectual disability (NS-ID) in Sudanese and what possible risk factors might cause the disability.

Methods:

The study included 30 patients with NS-ID and 30 healthy controls. The intelligence quotient (IQ) level, the degree of consanguinity of the parents, the family history of intellectual disability (ID), exposure to X-rays, bacterial or viral infections, smoking and the use of medication during pregnancy were all measured. We also examined the possible associated congenital anomaly.

Results:

The ratio of men to women was 3:2 with an average age of 15 years for both study groups. The degree of ID varied from moderate to severe in the patient group. In addition, 57% of the patients had consanguineous parents. Furthermore, patients' mothers were more exposed to the risk factors than the control group. The patients' families had a history of ID more than the control group. In addition, psychomotor delay seemed to be a common congenital abnormality in the patient group. Single Nucleotide Polymorphism (SNP) analysis revealed that only two patients were heterozygous delACGT. None in the control group exhibited the mutation.

Conclusion:

None of the risk factors tested were associated with NS-ID. The heterozygous DelACGT was not associated with NS-ID. We speculate that the causes of NS-ID may be correlated with complex environmental and hereditary factors.

RESUME

Contexte

Certaines mutations du gène *PRSS12* ont été associées à une forme autosomique récessive de déficience intellectuelle non syndromique.

Objectif

Nous avons cherché à déterminer si le gène delACGT (rs876657372) était associé à une déficience intellectuelle non syndromique (NS-ID) chez les Soudanais et quels facteurs de risque pourraient être à l'origine de cette déficience.

Méthode

L 'étude a inclus 30 patients diagnostiqués avec une déficience intellectuelle non syndromique et 30 témoins sains. Le QI, le degré de consanguinité des parents, les antécédents familiaux de déficience intellectuelle, l'exposition aux rayons X, les infections bactériennes ou virales, le tabagisme et l'utilisation de médicaments pendant la grossesse ont tous été mesurés. Nous avons également examiné la possible anomalie congénitale associée.

Résultats

Le ratio hommes / femmes était de 3/2 avec un âge moyen de 15 ans pour les deux groupes d'étude. Le degré de déficience intellectuelle (DI) variait de modéré à sévère dans le groupe de patients. De plus, 57% des patients avaient des parents consanguins et les mères des patients étaient plus exposées aux facteurs de risque que le groupe témoin. Les familles des patients avaient plus d'antécédents de DI que le groupe témoin, et le retard psychomoteur semblait être une anomalie congénitale courante dans le groupe de patients. L'analyse SNP (Single Nucleotide Polymorphism) a révélé que seuls deux patients étaient des delACGT hétérozygotes. Aucun dans le groupe témoin n'a présenté la mutation.

Conclusion

Aucun des facteurs de risque testés n'était associé aux déficiences intellectuelles non syndromiques. La delACGT hétérozygote n'était pas associée à la déficience intellectuelle non syndromique. Nous supposons que les causes de déficience intellectuelle non syndromique pourraient être corrélées à des facteurs environnementaux et héréditaires complexes.

INTRODUCTION

Non-Syndromic Intellectual Disability (NS-ID) is a disorder defined by the presence of incomplete or arrested mental development and deterioration of concrete functions at each stage of development (14,1).

The causes of Intellectual Disability (ID) are diverse and include environmental factors, teratogens, chromosomal anomalies, and metabolic diseases impairing neuronal function (17). Non-genetic factors include maternal viral infections (6), child exposure to ionizing radiation during the gestational period (21), and sometimes people links taking certain medication during pregnancy to child developmental malformations. Thus, the etiologies of ID are heterogeneous and unfortunately, in about more than half of the cases the cause of ID is elusive (4) or idiopathic. Nearly a quarter of individuals with NS- ID follow an autosomal recessive mode of inheritance (20).

Over the past years, different single genes were linked to NS-ID. Many of these genes may also cause Syndromic Intellectual Disability (S-ID), autism, or other neurodevelopmental phenotypes (15). In a review by Kaufman et al (15) examples of these genes were mentioned as; ACSL4, AFF2/FMR2, AGTR2, AP1S2, ARHGEF6, ARX, ATRX, BRWD3, CASK, CC2D1A, CDH15, CRBN, DLG3, DOCK8, FGD1, FTSJ1, GDI1, GRIK2, HUWE1, IL1RAPL1, JARID1C (KDM5C), KIRREL3, MAGT1, MBD5, MECP2, NLGN4, OPHN1, PAK3, PQBP1, PRSS12, PTCHD1, RPS6KA3, SHANK2, SHROOM4, SLC6A8, STXBP1, SYNGAP1, SYP,

TSPAN7, TRAPPC9, TUSC3, UPF3B, ZNF41, ZNF674, ZNF711, ZNF81 (15). One of the identified genes linked to non-syndromic autosomal recessive intellectual disability (NS-ARID) is *PRSS12* (MIM: 606709), also known as Neurotrypsin and Motospin (12).

In humans, the *PRSS12* gene is responsible for coordinating various physiological functions, including digestion, immune response, blood coagulation, and reproduction (12). *PRSS12* plays a role in neuronal plasticity and may subserve structural reorganizations associated with ID (7). PRSS12 protein is secreted from neuronal cells and is localized to the synaptic cleft. Studies in mice show that this protein cleaves a protein, agrin, which is important for the formation and maintenance of exitatory synapses (11). The loss of motopsin function causes nonsyndromic mental retardation in humans and impairs long-term memory formation in Drosophila (19).

There is a relation between families who has children with ID and mutation is *PRSS12* (3). A 4-base pair deletion (delACGT, rs876657372) in *PRSS12* gene was associated with NS-ARID in an Algerian family (20,16) following autosomal recessive mode of inheritance (20).

In this study we aimed to examine delACGT, rs876657372 in a group of Sudanese diagnosed with NS-ID and further analyze the exposure to possible risks factors.

METHODOLOGY

This is a case-control study included 60 individuals of whom 30 were diagnosed with NS-ID, and 30 healthy controls. All study participants showed a normal karyotype, thus we excluded syndromes of ID that are due to chromosomal abnormalities. A structured questionnaire which include demographic data, intelligence quotient (IQ) degree, the parent grade of consanguinity, and the previous medical history of ID in the family. There were also questions about the history of bacterial or viral infection during pregnancy, X-ray exposure, smoking, and uptake of any medication at the time of pregnancy.

The variant (https://www.ncbi.nlm.nih.gov/variation/view) located in 118,313,332 – 118,313,336, allele delACGT, transcript (c.1355_1358del), NM_003619.4, p.Asp452fs.

Blood samples were collected from all study participants and DNA extraction was done by using Qiagen extraction kits from blood. The part of the *PRSS12* gene which contains the rs876657372 variant was designed using primer3 web tool (http://primer3.ut.ee/) (Table 1) to generate a 571 bp amplicon. The PCR reaction was achieved according to the manufacturer (Maxtime PCR premix kit i-startaq). PCR was carried out in a Thermocycler (techne TC412., UK) and included an initial denaturation at 94°C for 2 min followed by 34 cycles of denaturation at 94°C for 30 seconds, primer annealing at 58°C for a 20-second extension at 72°C for 40 second and a final extension at 72°C for 5 min. The electrophoresis of PCR products was performed in 1.5 % (w/v) of agarose gel containing ethidium bromide (0.5µg/ml) and photographed using a gel documentation system (syn gen-Germany). The restriction enzyme was selected using (NEBcutter V2.0) tool, New England BioLabs (http://nc2.neb.com/NEBcutter2/) specific to the position of the polymorphism. Consequently, the 571 base pair product of the *PRSS12* gene was digested with (Zral) restriction enzyme (New England BioLabs) according to the manufacturer's instructions to generate fragments. Data analysis results were then expressed in frequencies.

RESULTS:

Case group were match with the control group by age and sex. Hence, the study included 18 males 12 females in both control and patients' groups with male to female ratio of 3: 2. Their ages ranged between 3-18 years old and mean age was 15 years. A professional medical doctor measured the degree of intelligence quotient **(**IQ); accordingly, 9 (30%) patients showed mild ID, 18 (60%) moderate and 3 (10%) severe ID (Table 2) and all control samples had a normal IQ (Table 2). The parents' grade of consanguinity among cases group showed that 11 (36.7%) were second grade relatives, 6 (20%) were third grade, 7 (23.3%) were not related and 6 (20%) refused to give information on grade of consanguinity (Table 3). Unfortunately, the parents of the control group were reluctant to give information about grade of consanguinity. Furthermore, 20% of the patients group have family history of ID and 80 % hasn't while only 6.7%) of the control group has family history of ID and 92.3% hasn't (p. value 0.12; OR 3.50 and 95% CI was 0.64 to 18.98) (Table 4). The exposure of the mothers to certain possible non-genetic risk factors was also measured. And only 4 mothers (13.3%) from the patients' group were exposed to risk factors during pregnancy. One (3.3 %) was a passive

smoker, one (3.3%) exposed to X-ray and two (6.7%) used medications that was later stopped by their doctors during pregnancy. However, they didn't provide information on what was these medications. None of the mothers were diagnosed with bacterial or viral infections and none has complication during pregnancy. On the other hand, and within the control parents, 3 (10%) were exposed to non-genetic risks, 2 (6.7%) were passive smokers and 1 (3.3%) complained from infection (Table 5).

Further clinical features that convoy ID were also measured. Fifteen (50%) patients showed additional clinical feature of which 6 (20%) have a psycho-motor problem, 2 (6.7%) have seizures, 2 have deafness, 2 (6.7%) have vision problem, one (3.3%) had epilepsy and 4 (13.3%) were later diagnosed with autism (p.value 1.0; OR 1.0 and 95% CI 0.0192 to 52.0394) (Table 6). All controls were normal and didn't have abnormal congenital abnormalities.

The SNP analysis revealed that two patients were heterozygous delACGT; one of them was diagnosed with NS-ID without other physical congenital abnormality, the other one was autistic and both patients were with moderate ID and none of the control group had the mutation (p value 0.15 OR 1.07 and 95% CI 0.97 to 1.17) (Table 7).

DISCUSSION:

In this study, females' ratio slightly exceeded their males' counterparts. According to our knowledge, ID affects males and females equally and the only difference is in syndromes linked to X-linked disorders which affects males more than females. Thus, our findings could be influenced by the availability of the samples. The age of the patients confirms that the disability is commonly diagnosed before age of 18 (18). Furthermore, parents who are second-degree relatives are more common. This degree of consanguineous marriage is frequent among Sudanese (8). Many studies had confirmed the association between consanguinity and ID (5,22,2,13,10) indicating the possibility of hereditary. However, in this study family history showed no association with ID.

Although certain environmental factors were previously associated with ID, here few mothers of both study groups were exposed almost equally to these factors. Thus, it is unlikely to say that these risk factors cause NS-ID.

All patients were diagnosed with NS-ID and psycho-motor delay constituted the majority of the accompanied congenital abnormality. Similar findings were reported on a study by Chentouf et al., (8), who showed association between epilepsy, ID and consanguinity indicating the possibility of escorted disability. Here we illustrate that, although psycho-motor problems is an additional feature in children diagnosed with NS-ID however there is no association between NS-ID and any addition congenital features.

The heterozygous delACGT (rs876657372) in *PRSS12*; was detected in two of the patients but none of the control samples with no statistical association. Furthermore; since the SNP follows autosomal recessive mode of inheritance, we cannot conclude it is the cause of NS-ID (20).

CONCLUSION:

The delACGT (rs876657372) in *PRSS12* was found in heterozygous form in few patients. Thus, the SNP was not associated with NS-ID in this study. The study rejected that consanguinity and family history roles in NS-ID. In addition, and contradicting other studies; additional congenital abnormalities are not associated with NS-ID.

List of abbreviations

ID : Intellectual Disability

NS-ID : None syndromic intellectual Disability

S-ID : Syndromic intellectual Disability

IQ : Intelligence quotient

NS-ARID : Non-syndromic autosomal recessive intellectual disability

SNP : Single Nucleotide Polymorphism

Declarations

Ethics approval and consent to participate

The study was approved by central institutional ethical board of Al-Neelain University (NU-IRB- 18- 5-5-22). Because all study participants were children, confidentiality of participant's information was explained to their guardians.

Consent for publication

Participant guardian has agreed to participate in the study by signing the consent form approved by the ethical board.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors contributions

Ebtihal Esmail conducted the laboratory work and contributed in Manuscript writing. Dr. Mona Ellaithi was the principle investigator and writer of the manuscript.

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Table 1: forward and reverse primers

F	**5´ GTGACCGAGGAGTAAGGGGA´3**
R	5´ GTTCCCTGAACCAAGAGACAGA´3

Table 2: severity of intellectual disability in cases group.

Degree of IQ level	Number of patients
Mild	9(30%)
Moderate	18 (60%)
Severe	3 (10%)
Total	30 (100%)

Table 3: Levels of consanguinity among cases group

Degree of consanguinity	Number of patients (%)
Second degree relatives	11 (36.7%)
Third degree relatives	6 (20%)
Not related	7 (23.3%)
Refused to give information	6 (20%)
Total	30 (100%)

Table 4: Family history of ID among all study participants.

Risk factor		Patients NO (%)	Controls NO (%)	P-value	OR 95%CI
Family History	Yes	6/30 (20%)	2/30(6.7%)	0.12	3.50
	NO	24/30 (80%)	28/30 (92.3%)		0.64 to 18.98

Table 5: Risk factor during pregnancy in study groups.

Risk	Number of exposed in case group (%)	Number of exposed in control group (%)
Smoking/ passive smoking	30 (3%)/1	30 (6.7%)/2
Exposure to X-ray	30 (3%)/1	0/30 (0%)
up take of medication	/30 (6.7%)2	0/30 (0%)
bacterial or viral infection	30 (0%)/0	30 (3.3%)/1
Complications during pregnancy	30 (0%)/0	30 (0%)/0

Table 6: Associated symptoms with ID In study groups.

Clinical presentations	Number of case group	Control group	p. value	OR 95% CI
Psycho-motor problems	6	0	1	1
				0.0192 to 52.0394
Seizers	2	0		
Deafness	2	0		
Vision problem	2	0		
Epilepsy	1	0		
Autism	4	0		
ID without other symptoms	13	30		
Total	30	30		

Table 7: The results of SNP and its genotypes in the study groups Mutation analysis result.

Genotype		Patients NO (%)	Controls NO (%)	P-value	OR 95%CI
Wild type	AGCT	28/30	30/30	0.15	1.07 0.97 to 1.17
Mutant type	AGCT del	2/30	0/30		

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