Detection of in rs876657372 (delACGT) in PRSS12 Gene, Risk Factors and Associated Congenital Abnormalities in Non-Syndromic Intellectual Disability-Case Control Study.

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Research article

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Abstract

Background

PRSS12 gene was the first gene to be identified as a cause of a non-syndromic autosomal recessive form of intellectual disability (ID). A 4-base pair deletion (delACGT; rs876657372) in the PRSS12 gene has been detected in two different families diagnosed with autosomal recessive non-syndromic intellectual disability (NS-ID). Here we aimed to find out if rs876657372 is associated with NS-ID in Sudanese and the possible risk factors that could be linked the disability.

Methods

The study included 60 individuals; 30 were diagnosed with NS-ID and 30 healthy controls. The IQ level, parent’s degree of consanguinity, family history of ID, exposure to X-ray, bacterial or viral infection, smoking, and taking of medication during pregnancy were all analyzed as possible risk factors. We also investigated the possible associated congenital abnormality. Restriction fragment length polymorphism method (RFLP) was performed using primers that include the 4 base pair deletion site.

Results

Male to female ratio of 3:2 with a mean age was 15 years for both study groups. The degree of intellectual disability (ID) varies between patients group, however 60% had a moderate ID. Furthermore, 57% of the patients had consanguineous parents. Assessment of risk factors among the patient group showed mothers of the patients were more exposed to risk factors than control group. Patients’ families had history of ID more than the controls; this could indicate the possibility of autosomal recessive ID. Furthermore, Psychomotor delay appeared to be common among the patients group. The SNP analysis revealed that only two patients were heterozygous delACGT; both patients were with moderate ID. None of the control group had the mutation.

Conclusion

Causes of NS-ID could be correlated to certain environmental and hereditary factors. DelACGT is correlated to NS-ID mainly in patients with moderate ID.

Name of the registry

Al-Neelain University Institutional Review Board

Trial registration number

NU-IRB-18-5-5-22

Date of registration

22/05/2018
**Background**

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 [1, 2].

The causes of Intellectual Disability (ID) are diverse and include environmental factors, teratogens, chromosomal anomalies, and metabolic diseases impairing neuronal function [3]. Non-genetic factors include maternal viral infections [4], child exposure to ionizing radiation during the gestational period [5], 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 [7] or idiopathic. Nearly a quarter of individuals with NS-ID follow an autosomal recessive mode of inheritance [8].

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 [9]. In a review by Kaufman et al [9] 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, MBDS, MECP2, NLGN4, OPN1, PAK3, PQBP1, PRSS12, PTCHD1, RPS6KA3, SHANK2, SHROOM4, SLC6A8, STXBP1, SYNGAP1, SYT, TSPAN7, TRAPPC9, TUSC3, UPF3B, ZNF41, ZNF674, ZNF711, ZNF81 [9]. 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 [10].

In humans, the PRSS12 gene is responsible for coordinating various physiological functions, including digestion, immune response, blood coagulation, and reproduction [10]. PRSS12 plays a role in neuronal plasticity and may subserve structural reorganizations associated with ID [11]. 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 excitatory synapses [12]. The loss of motopsin function causes nonsyndromic mental retardation in humans and impairs long-term memory formation in Drosophila [13].

There is a relation between families who has children with ID and mutation is PRSS12 [14]. A 4-base pair deletion (delACGT, rs876657372) in PRSS12 gene was associated with NS-ARID in an Algerian family [8, 15] following autosomal recessive mode of inheritance [8].

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.

**Methods**

This is a case-control study included 60 individuals; of whom 30 were diagnosed with NS-ID and 30 healthy controls. 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 (supplementary I).

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/) to generate a 571 bp amplicon (F: 5´GTGACCAGAGTAAGGGGA´3, R: 5´ GTTCCCTGAACCAAGACAGACA`GAGACAAGAGACAGA´3). 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 (0.5 µg/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. 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 1). Unfortunately the parents of the control group were reluctant to give information about grade of consanguinity. 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 1
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 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%)          |

We excluded the possibility of genetic factors that might be the cause if ID and all study participants had normal karyotypes. The exposure of the mothers to certain possible non genetic risk factors was also measured. And only 4 mother (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 3).
Family history of intellectual disability (ID) was examined in both case and control groups and we found that 6 (20%) from the patients had a family history of ID and 2 (6%) from the control had a previous family history of ID (Table 4).

Further clinical features that convey ID were also measured. Fifteen (50%) patients showed additional clinical feature of which 6 (20%) have a psycho-motor problems; 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 (Table 5). All controls were normal and didn't have abnormal congenital abnormalities.
Table 5  
Associated symptoms with ID in study groups

| Clinical presentations | Number of case group | Control group |
|------------------------|----------------------|---------------|
| Psycho-motor problems  | 6                    | 0             |
| 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            |

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. None of the control group had the mutation (Table 6).

Table 6  
The results of SNP and its genotypes in the study groups Mutation analysis result.

| Genotype   | Patients NO(%) | Controls NO (%) |
|------------|----------------|-----------------|
| Wild type  | AGCT           | 28/30           | 30/30          |
| Mutant type| AGCT del       | 2/30            | 0/30           |

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 the age of 18 [16].

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; however, psycho-motor delay constituted the majority of the accompanied congenital abnormality. Similar findings were reported on a study by Chentouf et al., [17], who showed association between epilepsy, ID and consanguinity indicating the possibility of escorted
disability. Here we illustrate and for the first time psycho-motor problems as additional feature in children diagnosed with NS-ID.

Furthermore, parents who are second-degree relatives are more common. This degree of consanguineous marriage is frequent among Sudanese [18]. Many studies had confirmed the association between consanguinity and ID [17, 19, 20, 21 and 22] indicating the possibility of hereditary. The high frequency of family history of ID among patients group confirmed the theory of possibly hereditary factors.

The heterozygous delACGT (rs876657372) in PRSS12; was detected in two of the patients but none of the control samples. Interestingly those two patients were diagnosed moderate ID only without congenital malformations and one of them was autistic. This is different from Molinari et al [8] who showed that the SNP was associated with patients diagnosed with severe ID.

**Conclusion**

Consanguinity and family history are risks to ID. Non-genetic environmental factors were probably not the cause of ID in this study. Psychomotor delay is an expected congenital abnormality accompanies NS-ID. In this study; delACGT (rs876657372) in PRSS12 is linked to ID specifically moderate ID.

**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

**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 parents. A written and signed informed consent to participate and scientific publication in the study was obtained from the parents of all study participants under the age of 16 years old.
Furthermore all study participants who were more than 16 years also obtained a signed consent form of participation and scientific publication.

**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**

EE: conducted the laboratory work and contributed in manuscript writing.

ME: Is the supervisor, the principle investigator of rare diseases biobank in Faculty of Medical laboratory Sciences and the writer of the manuscript.

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