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ORIGINAL ARTICLE

GENETIC ANALYSIS OF FAMILIES HAVING AUTOSOMAL RECESSIVE INTELLECTUAL DISABILITY

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ABSTRACT

Background: Intellectual disability (ID) is a neuro-developmental defect that is manifested by development delay and learning disability. Such defects may be caused due to chromosomal disorders (trisomy 18 or Down syndrome) or single gene mutation. Its worldwide prevalence is estimated to be 1-3%. The genetic etiology of non-syndromic ID is poorly understood. To date, more than 100 loci have been reported to be associated with non-syndromic ID. The objective of this study was to identify the causative genes for three intellectual disable (ID) families from Kohat, Pakistan.

Materials and Methods: This cross-sectional study was conducted in the Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan from March 2014 to August 2015. The inclusion criteria set for the families was consanguineous relation and more than two patients per family (including cousins). All the patients were tested individually in friendly atmosphere using IQ test to scale the ID on the basis of performance. Thereafter, blood samples were taken by aseptic method and DNA was extracted for the purpose of doing genetic analysis. In genetic analysis, exome sequencing was performed to find the pathogenic variants. Subsequently. Sanger sequencing was also done to see the segregation of pathogenic variants.

Results: Genetic analysis found mutation in AP4B1 in Family 1, in WDR62 in Family 2, while Family 3 was unremarkable.

Conclusions: The study involved genetic analysis of three consanguineous families and found mutation in AP4B1 in Family 1, in WDR62 in Family 2, while Family 3 was unremarkable. The present research will help in devising molecular diagnostic technics for pre-marital and pre-conception testing.

KEY WORDS: Intellectual disability; DNA; Genotype; Exomes; Exome sequencing; Gene clusters; Chromosome; Mutation; Chromosomal disorders; Molecular diagnostic technics.

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INTRODUCTION

Intellectual disability (ID) is caused by genetic defects which are not inherited. These disabilities can be caused by mutations in genetic development. Its main characteristics are impaired intellectual abilities of the patients expressed throughout their growing phases subsidizing to their whole stages of brain power like cognition, speaking and neuronal functions. ID sometimes presents itself in infancy with decreased muscular tone, visual contact and impaired motor activities along with delayed developmental milestone.[1]

Autosomal recessive intellectual disabilities (ARID) contribute to about 10% of cases in an outbred population. In consanguineous families, the risk for ARID is higher in magnitude than outbred population by 2-3 times.

Intellectual disability is categorized from mild to profound depending upon the severity of cognitive impairment on the basis of their IQ test score. Mild or slight intellectual disability have IQ score between 51 and 70, in case of moderate intellectual disability IQ score is between 36 and 50 and in case of severe intellectual disability the IQ score is between 20 and

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35, while score less than 20 is labeled as profound intellectual disability.[2,3]

Clinical examination of ID children is done to assess shortages in their adapted performance and a child's efficiency in achieving the principles of maturing, learning, self-individuality or community accountability and particularly learning presentation that is predictable of the patient's age level and social norms. Mild ID patients can live almost normal life with a little support. Moderate ID patients require an instructor. 10% of the ID patents require little to no assistance. Severe ID patients have significant abnormalities, they may understand dialogue but they have less aptitude to express themselves. These patients require assisted living and nursing and need full observation and care. Profound ID patients need full time provision and assistance in their life activities. This group of ID patients totally depend on family members for nursing care in all aspects of daily life and have tremendously incomplete aptitude to communicate with someone as well as this category of patients have more than two bodily developmental limitations.[4]

On the basis of associated abnormalities, intelligent disability can be divided in to two groups.

Non-syndromic intellectual disability (NSID) has isolated features of ID without showing any-other associated consistent handicaps. Syndromic intellectual disability (SID) is there when ID is combined with other physical and behavioral abnormalities.[5]

The objective of this study was to identify the causative genes for three intellectual disable (ID) families from Kohat, Pakistan.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan from March 2014 to August 2015. The study included three ID families from district Kohat, Khyber Pakhtunkhwa, Pakistan.

Inclusion Criteria: The inclusion criteria were set on the following parameters.

1. Those ID families were included which had two or more than two congenital ID patients, with the history of intellectual disability before the age of 18 years.
2. Only those families were included in which the affected patients were brothers and sisters and their parents were maternal or paternal cousins.

Exclusion Criteria: The exclusion criteria were set on the following parameters.

1. Those ID families whose blood samples were already taken by someone else for research purpose.
2. Those families which were unwilling for an informed consent or extraction of blood samples.
3. Those patients who were suffering from some sort of generalized active infection.

All the patients were tested individually in friendly atmosphere using IQ test (Wechsler Intelligence Scale for Children (WISC) or Stanford-Binet or Wechsler Adult Intelligence Scale- Third Edition (WAIS--III) to scale the ID on the basis of performance.[6] During this study, we also interviewed the parents and relatives of the ID patients. Some tasks were also assigned to the control group (normal members of the same families) to check their motor skills and reflexes. All the intellectual disable patients were examined thoroughly. Five ml blood samples were collected from ID patients, their normal siblings and parents. These blood samples were processed in laboratory for DNA extraction and later whole exome sequencing. After that, whole exome sequencing was done to find the pathogenic variants. The data was analyzed by CATCH (Cas9-Assisted Targeting of Chromosome Segments). CATCH enables one-step targeted cloning of large gene clusters.[7] Sanger Sequencing was carried out to see the segregation of disease associated variant.

Exome Sequencing: The exome was captured using the SureSelect Human All Exons IV2 (1 patient), IV3 (1 patient), V4 (2 patients) and V5 (2 patients) reagents (Agilent Technologies Inc., Santa Clara, CA, USA). Sequencing was done in HiSeq™ 2000 Sequencing System (Illumina® Inc., San Diego, CA, USA). Individually exome library was indexed, divided into two equivalent halves, and sequenced in two different lanes. The raw results were analyzed using a customized pipeline that utilizes published algorithms in a sequential manner (Burrows-Wheeler Aligner-BWA) for mapping the reads, SAM tools for detection of variants, Pindel for the detection of Indels, and ANNOVAR (Wang Genomics Lab, Philadelphia, PA, USA). The entire coding sequence corresponding to the human RefSeq (NCBI Reference Sequence Database) coding genes was used as the reference for the calculation of coverage and reads on target. All procedures were done using the manufacturer's recommended protocols without modifications.

RESULTS

In families' pedigrees/ trees, roman numbers (I, II, III) are used for the designation of generations, while English numbers (1, 2, 3) are used to show the individual numbers within each generation. The circles and boxes represent female and male gender, respectively. The blacked colored boxes/ circles show affected members (ID patients) of the family. A slash line over the boxes/ circles show the dead members of the family, and double marriage lines between parents show a consanguineous relationship. All the individuals which are marked with arrow and P symbol were checked for segregation

after exome sequencing with special numbers i.e. 10494, 10495 etc.

A. Genetic Outcomes of Family 1:

All the ID patients in the Family 1 had the same phenotype characteristics, i.e. intellectual disability with spastic paraplegia & short stature. Mutations of AP4B1 and WDR62 were co-segregated with disease phenotype.

These mutations are known to cause intellectual disability. (Figure 1)

Segregation analysis: The AP4B1 mutation is the only mutation segregating with the disease phenotype. Normal sibling V:3 was carrier while V:1 was normal for disease variant.

All the mutant genes are shown as:

AP4B1exon5:c.968dupC:p.S323fs **(M1)**

HCRTexon2:c.47_49del:p.16_17del **(M2)**

GHDCexon3:c.17_19del:p.6_7del **(M3)**

The mutation analysis in this family found a frameshift insertion of cytosine nucleotide [c.968dupC (p.S323fs)] in the fifth exon of AP4B1 gene. The mutation was segregating with the disease phenotype and showed that normal sibling V:3 was carrier while V:1 was wild-type for disease variant. (Figure 2)

B. Genetic Outcomes of Family 2:

All the patients in Family 2 have the same phenotypic characteristics of ID. The associated abnormalities in these patients include intellectual disability with spastic paraplegia & short stature. All the individuals which are marked with arrow and P symbols were checked for segregation after exome sequencing. (Figure 3)

Segregation analysis: All putative mutations show co-segregation with disease phenotype. V:7 was normal while V:3 was carrier for disease genotype.

All the mutant genes are shown as:

KCNK6, chr19.C314T:p.T105 **(M1)**

EML2, chr19.T1810A:p.C604S **(M2)**

WDR62, chr19.exon11:c.G1531C:p.D511H **(M3)**

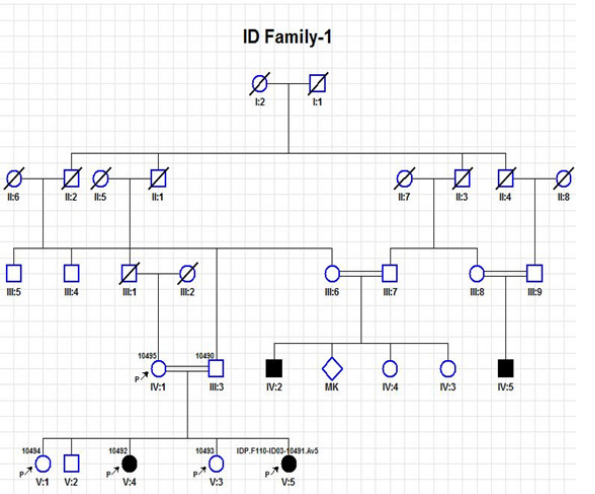
In this family individual IV:1, IV:2, V:3 are shown in blue color as heterogenecity, the black color V7 declare the normal individual while the red color M1/M2 demonstrates mutation.

Sequence alignment in this family determined a missense mutation [G1531C (p.D511H)] in the eleventh exon of WDR62 gene.

In this family, individual IV:1, IV:2, V:3 are shown in blue color as heterogenecity, the white color V7 declare the normal individual while the red color denotes mutation as shown in above Family tree 2 and as in chromatogram report. (Figure 4)

C. Genetic Outcomes of Family 3

In the ID Family 3 both of the patients had the same phenotype characteristics i.e. intellectual disability with epilepsy. There were no other associated abnormalities like spastic paraplegia or short stature. In this family no putative mutation was found and segregation analysis was not done. (Figure 5)



+/+	M1/M1	M1/+	M1/M1
+/+	+/+	+/+	+/+
+/+	+/+	+/+	+/+

Figure 1: Segregation Analysis of ID Family 1

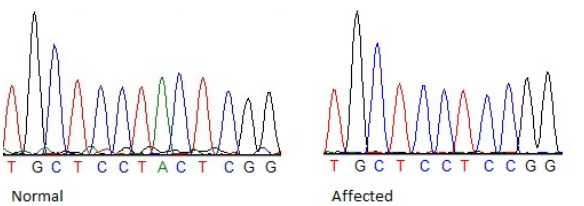
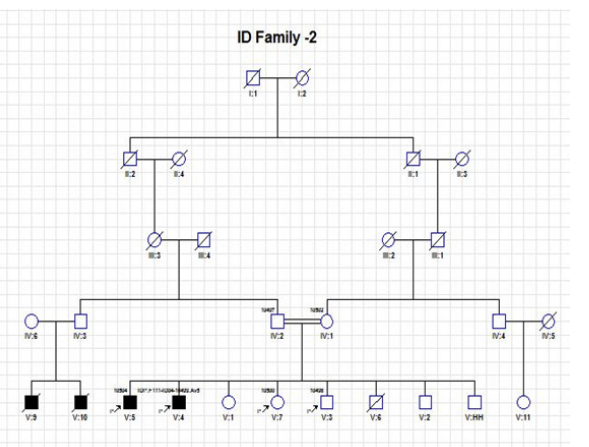


Figure 2: Chromatogram Report of ID Family 1



M1/M1	M1/M1	+/+	M1/+
M2/M2	M2/M2	+/+	M2/+
M3/M3	M3/M3	+/+	M3/+

Figure 3: Segregation Analysis of ID Family 2

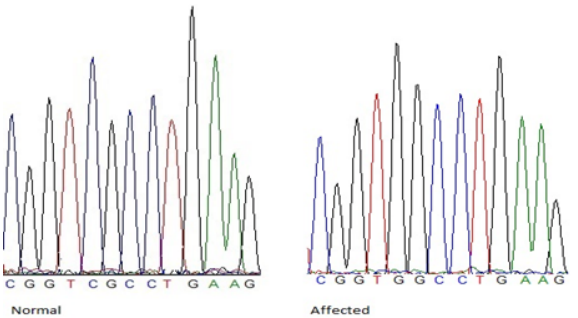


Figure 4: Chromatogram Report of ID Family 2

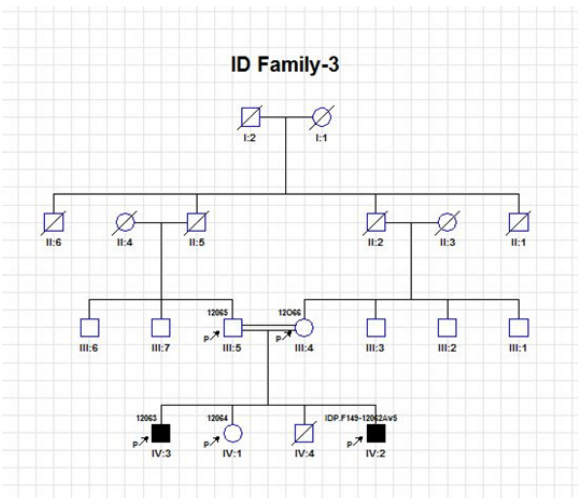


Figure 5: Intellectual disability (ID) Family 3

DISCUSSION

The causes and clinical presentations of ID patients are heterogeneous. Genetic basis of ID is believed to be present in 25-50% of cases, even though this number rises proportionally with severity. One fourth of ID patients with non-syndromic intellectual disability have an autosomal recessive manner of inheritance. Consanguineous families are significant in defining autosomal recessive bases of the disease.[8]

Modell and Darr in 2002 described that recessively inherited congenital disorders are more common in consanguineous families.[9] The off springs of consanguineous persons have an increased probability of rare recessive disorder causing variants (alleles) being inherited from both paternal and maternal lineages. The prevalence of consanguineous families is high in Pakistan. In ID disorder, although families are consanguineous but the number of ID patients in a pedigree is not large because patients with ID do not reproduce. Due to this reason more work is needed to illustrate intellectual disability in Pakistani population.[10]

By linkage analysis followed by candidate gene sequencing of a consanguineous Israeli Arab family with autosomal recessive mental retardation and spasticity, a homozygous truncating mutation in the AP4B1 gene was identified. The authors concluded that AP4-complex-mediated vesicular trafficking plays a crucial role in brain development and function.[11,12]

In 2 sibs, born of consanguineous Arab parents, with SPG47, identified a homozygous truncating mutation in the AP4B1 gene. The mutation was found by exome sequencing of the candidate region on chromosome 1p13-p12 identified by linkage analysis and also reported the phenotypic similarities to the patients.[13] The AP4B1 gene encodes a subunit of the heterotetrameric adaptor protein (AP) complex, a component of intracellular transport of proteins that is thought to have a unique role in neurons. AP4 is composed of 2 large chains, beta-4 (AP4B1) and epsilon-4, a medium chain, mu-4, and a small chain, sigma-4.

This study was designed to characterize the variant genes responsible for intellectual disability in three ID families of district Kohat of Khyber Pakhtunkhwa province of Pakistan, affected with non-syndromic autosomal recessive intellectual disability (NSARID). The exome sequencing studies of the families with ID have many benefits. It helps in localizing the disease causing regions on genome and positional cloning of the localized segments.

Thus it is helpful to control the disease transmission in next generations by genetic counseling and knowing the carrier status of the normal members in the affected families. The exome sequencing also helps in establishing genotype-phenotype correlations.

AP4B1 (adaptor-related protein complex 4, beta 1 subunit)

This gene encodes a subunit of a heterotetrameric adapter-like complex 4 that is involved in targeting proteins from the trans-Golgi network to the endosomal-lysosomal system (protein transporter activity). AP4-complex-mediated trafficking plays a crucial role in brain development and functioning. Mutations in this gene are associated with cerebral palsy, spastic quadriplegic type 5 (CPSQ5) disorder.

Role of WDR62 (WD Repeat Domain 62)

Only WDR62 is known to play a role in cerebral cortical development, neuronal proliferation and migration. Mutations in this gene have been associated with microcephaly, cortical malformations, and mental retardation.

Comparative symptoms of patients are explained with both the families with symptoms of above genes.

CONCLUSION

The present study was conducted on three consanguineous families for the determination of the responsible genes for intellectual disability. Exome sequencing revealed putative mutations in AP4B1 in Family 1 and in WDR62 in Family 2. In Family 3, we could not locate any putative mutation. These mutations were found to cause intellectual disability

and additional phenotypes. The study would help in devising the tests for determining the carrier status of persons before marriage. The study would also be helpful in preconception counseling. It can educate the people in making positive decisions about the future of their children.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	JK, MJ
Acquisition, Analysis or Interpretation of Data:	JK, MJ, SU, KM, UU, SA
Manuscript Writing & Approval:	JK, MJ, SU, KM, UU, SA

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.