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ORIGINAL ARTICLE

Association of Spastic Paraplegia and Short Stature in Patients of Autosomal Recessive Intellectual Disability

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ABSTRACT

Background: Intellectual Disability "ID" is a genetic disorder, which lead to arrested or incomplete development of the brain. It is the limitation of cognitive skills impairment and decline ability of a person in learning process. ID is the most common health problem in worldwide. These patients have decline intellectual functions and at least limitation in their two or more adoptive skills such as reading, writing abilities, social interactions, Behavioral habits, self-care, communication ability etc. The time period for the diagnosis of ID is the onset of disease before the eighteen years of age. Spastic Paraplegia and short stature is a general terminology using for a group of an uncommon inherited diseases that cause stiffness and weakness in the lower limbs muscles. Gradually its symptoms get worse with the passing of time. It's also called as familial spastic paraparesis or Strümpell-Lorrain syndrome. SP is classified clinically as "complicated" (syndromic) or "uncomplicated" (nonsyndromic) Spastic Paraplegia.

Methods: This study was started in March 2014 to Aug 2015. The criteria for the selection of families were consanguineous families with two or more than two ID patients. The patients were examined, interviewed one by one in friendly atmosphere. Then the blood samples were taken by aseptic method. Blood samples were processed in laboratory. DNA extraction and PCR was done. After that Exome sequencing was used to find the pathogenic variants. The data was analyzed by CATCH. Sanger Sequencing was applied to see the segregation.

Results: In ID-family1 the variant of AP4B1 was segregated with the disease phenotype. These ID patients have short stature and Spastic Paraplegia. Mutation in AP4B1 is known to cause intellectual disability. In ID-family2 the variants of WDR62, EML2 and KCNK6 were co-segregated with disease phenotype. But only mutations in WDR62 are known to cause intellectual disability. ID family2 also identified as short stature. In ID-family3 Exome sequencing data reveal no putative variants.

Conclusion: The present study was conducted in three consanguineous families for the determination of the responsible genes for intellectual disability. Exome sequencing revealed putative mutations in AP4B1 and WDR62 in two out of three families. In third family we could not locate any putative mutation.

Keywords: Intellectual disability ID, Autosomal recessive disorders, Autosomal recessive nonsyndromic ID, Behavioral Abnormality, Segregation, Exome sequencing, Spastic Paraplegia, Short stature.

INTRODUCTION

Intellectual disability or (ID) is define as a patient has some limitations in their cognitive and social skills, that include communication skills, social and self-care skills. ID is the most common health problem in worldwide. These patients have decline intellectual functions and at least limitation in their two or more than two adoptive skills such as reading, writing abilities, social interactions, Behavioral habits, self-care, communication ability etc. Intellectual functioning (such as judgment, learning and problem solving,). Adaptive functions such as (daily life activities such as communication in society and independent living). Symptoms of ID appearing usually before the age of 18 years. Sometimes ID presents itself in early infancy with decline muscular tone, short stature, visual contact and motor activities, Spastic Paraplegia, along with delayed neuro developmental milestones. ID can be classified in four major groups on the basis of ID patient IQ test score ie mild, moderate, severe, profound. Slight or Mild ID have IQ score in between 50 and 70, in case of moderate ID have IQ score in between 35 and 50 and in case of severe ID the IQ score is in between 20 and 35, while score less than 20 is labeled as profound intellectual disability. Clinical examination of ID children is done to assess shortages in their adapted performance that meaningfully bound a child's efficiency in achieving with the principles of maturing, education, self-individuality or community accountability and particularly learning presentation that is predictable of the patient's age level and social gathering. Mild ID patients can live almost normal life with a little support. While moderate ID patients are able to care of their own selves, but still require an instructor. Many of these ID patients can live independently, but some still need support. This category of ID patients is counting for about 10% of the ID patients. Severe ID patients have significant interruptions in growth, they can understand dialogue but they have less aptitude to express themselves. These patients can't achieve all own-care events self-sufficiently and require full observation and care. Profound ID patients have noteworthy developing postponements in all aspect of life, they are suffered by noticeable bodily and hereditary irregularities because of which they require adjacent administration and these patients also require complete and proper assistant to support in their own-care events. More severe patients group which is called profound ID need full time provision and maintenance 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. Spastic Paraplegia and short stature is a general terminology using for a group of an uncommon inherited diseases that cause stiffness and weakness in the lower limbs muscles. Known gene for Spastic Paraplegia is the SPG4 gene (spastin protein) are responsible for about 40% of Autosomal dominant Spastic Paraplegia cases. The commonest causes of Spastic Paraplegia and short stature are familial (genetic) short stature and delayed (constitutional) growth, which are considered as normal, nonpathologic variants of growth. This study was done in highly consanguineous families to identify causative genes of intellectual disability (ID).in the above three ID families 65% of the ID patients were as Spastic Paraplegia and with presentation of short stature.

MATERIALS AND METHODS

This study was started in March 2014 to Aug 2015 at KPK. ID patients were related from 03 ID families under the following criteria.

Inclusion Criteria:

1 Those ID families were included which had 2 or more than 2 congenital ID patients, with the history of intellectual disability either from their birth or before the age of 18 years of age.

2 Only those families were included in which the affected patients were real brothers and sisters.

3 The parents of all the selected patients were close cousins, either from maternal side or from paternal side of relation.

Exclusion Criteria:

1 Those ID families' whose blood samples had already been taken by someone else for research purpose.

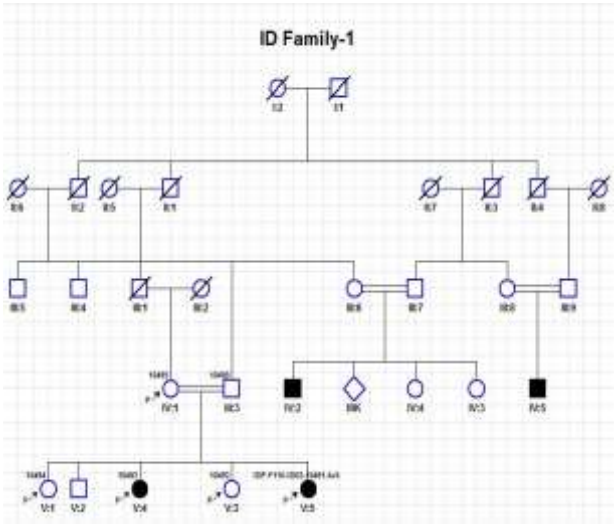
2 Those patients whose onset of disease was after the age of 18 years.

3 Those families which were unwilling for an informed consent or extraction of blood samples.

4 Those patients who were suffering from some sort of active infection.

All the patients were interviewed one by one in friendly atmosphere. Blood samples were taken by aseptic method. All the ID patients were physically examined thoroughly. Blood samples were sent to laboratory. DNA extraction and PCR was done. After that Exome sequencing was used to find out the pathogenic variants. The data was analyzed by CATCH. Sanger Sequencing was applied to see the segregation. The sampling technique used was non probability consecutive sampling technique. From each ID patients, their parents and their normal sibling's 5cc blood samples were collected.

RESULTS



Family-1: Intellectual Disability

In all the three families' pedigrees /trees roman numbers I, II, III, are used for the identification of the number of generations. While English numbers 1, 2, 3, are used to show the numbers of members in the family. The circular boxes are for female sex and the quadrangle box for male sex in each family. The blacked colored boxes are for the infected members (ID patients) of the family. The slash line on the boxes show the dead members of the family. While the double lines between parents show the relation of consanguinity. 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. All the ID patients in the family 1 had the same phenotype characteristics, i.e. Intellectual disability with spastic paraplegia & short stature.

Segregation Analysis of ID Family-1: All the ID patients in this family have the same phenotype characteristics, ie Intellectual disability with spastic paraplegia & short stature.

Table 1: Sequencing Result of ID Family-1

	ID_F-1_10491		
Variants		%	dbSNP 135
synonymous SNV	10075	50.10%	98.06%
nonsynonymous SNV	8914	44.32%	96.72%
stopgain SNV	57	0.28%	85.96%
stoploss SNV	17	0.08%	100.00%
wrong annotation in DB	483	2.40%	83.64%
frameshift deletion	113	0.56%	10.62%
frameshift insertion	80	0.40%	18.75%
frameshift substitution	0	0.00%	0.00%
nonframeshift deletion	175	0.87%	16.57%
nonframeshift insertion	191	0.95%	53.93%
nonframeshift substitution	6	0.03%	0.00%
	20111		95.13%
splicing (± 10bp)	1042	4.93%	83.97%
	21153		

Table 2: Exome Sequencing Results of Total Reads and Coverage

	ID_F-1_10491
Total Reads	148'273'800
Rmdup	138'829'740
On target	81'575'934
Coverage	
1	98.90%
8	90.14%
10	86.93%
20	73.24%
30	64.93%
40	59.71%
50	55.92%

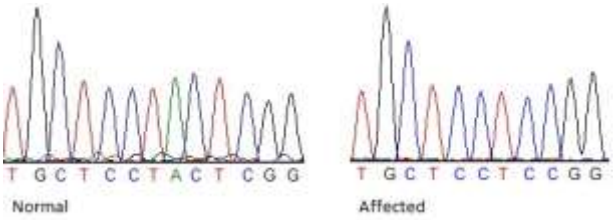
Segregation analysis: The AP4B1mutation was the only mutation segregating with the disease phenotype. Normal sibling V:3 was carrier while V:1 was normal for disease variant.

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

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

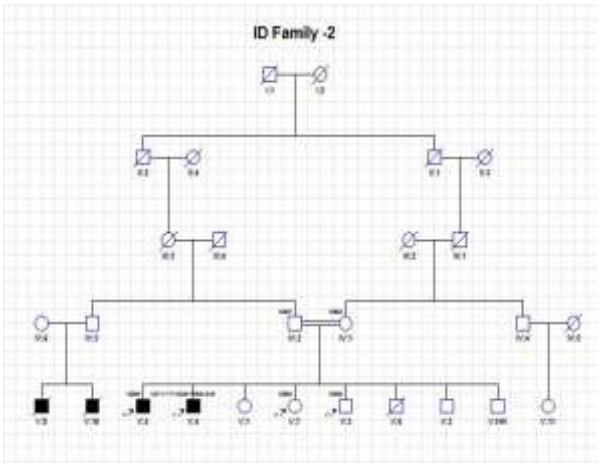
Table 3: Variants Prioritization: Putative Variants Analyzed by CATCH

Chr	Gene	ExonicFunc	AAChange	Obs	Ref	Zyg	QS	Cov	INFO	esp6500siv	HGMD_2012_1_ALL.or phrset.regional
chr1	AP4B1	frameshift insertion	exon5:c.968dupC:p.S323fs	G	-	het	4681	74667	PNDEL;END=114442675;DP=746;SVTYPE=D;AF1=0.124665	Score=679	ID_ spastic paraplegia & short stature|664delC|AP4B1
chr17	HCRT	nonframeshift deletion	exon2:c.47_49del:p.16_17del	-	GCA	het	600	1086	PNDEL;END=40336503;DP=1086;SVTYPE=D;AF1=0.009208	Score=6207	Narcolepsy_ early onset|47T>G
chr17	GHDC	nonframeshift deletion	exon3:c.17_19del:p.6_7del	-	GCA	het	300	36162	PNDEL;END=40345503;DP=361;SVTYPE=D;AF1=0.013850		



Chromatogram Report of ID Family-1

In the family ID-2 all the patients have the same phenotype characteristics as of ID family-1. The associated abnormalities in all these four patients are the same; all these patients have Intellectual disability with spastic paraplegia & short stature. All the individuals which are marked with arrow and P symbol were checked for segregation after exome sequencing.



Family-2 Intellectual Disability

Table 4: Sequencing Result of ID Family-2 Showing the Different Quality of Mutations

	IDP_F111_10499		
Variants		%	dbSNP 135
synonymous SNV	10897	49.78%	97.77%
nonsynonymous SNV	9734	44.47%	96.49%
stopgain SNV	75	0.34%	81.33%
stoploss SNV	14	0.06%	100.00%
wrong annotation in DB	515	2.35%	84.47%
frameshift deletion	123	0.56%	12.20%
frameshift insertion	88	0.40%	23.86%
frameshift substitution	2	0.01%	0.00%
nonframeshift deletion	206	0.94%	17.96%
nonframeshift insertion	232	1.06%	47.84%
nonframeshift substitution	3	0.01%	0.00%
	21889		94.75%
splicing (± 10bp)	1296	5.59%	80.40%

Table 5: ID Family-2: Exome Sequencing Results of Total Reads and Coverage

	IDP_F111_10499
Total Reads	126'716'220
Rmdup	118'357'041
On target	69'648'155
Coverage	
1	99.30%
8	97.49%
10	96.98%
20	93.98%
30	90.62%
40	87.00%
50	83.07%

Table 6: Variants Prioritization: Putative Variants Analyzed Manually

Chr	Func	Gene	AAChange	Zyg	QS	Cov	mce46way	snp137	ljb2_sift	ljb2_pp2hvar	ljb2_mt	ljh_gerp++	HGMD
chr19	exonic	KCNK6	exon1:c.C314T:p.T105I	hom	222	110	Score=509;lod=156	rs201520164	0	0.978	1	2.83	
chr19	exonic	EML2	exon18:c.T1810A:p.C604S	hom	222	192	Score=568;lod=272		0.002	0.991	0.999	4.17	
chr19	exonic	WDR62	exon11:c.G1531C:p.D511H	hom	222	59	Score=612;lod=408		0	0.991	0.988	5.36	Microcephaly 1531G>A 511D>N

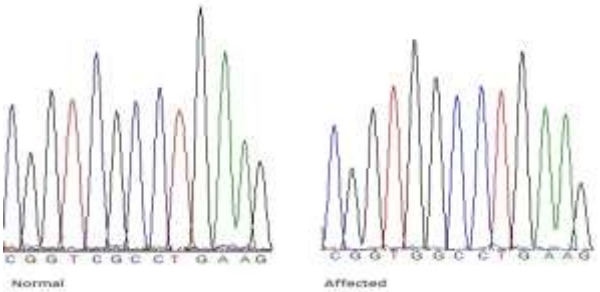
Segregation Analysis of ID Family-2

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

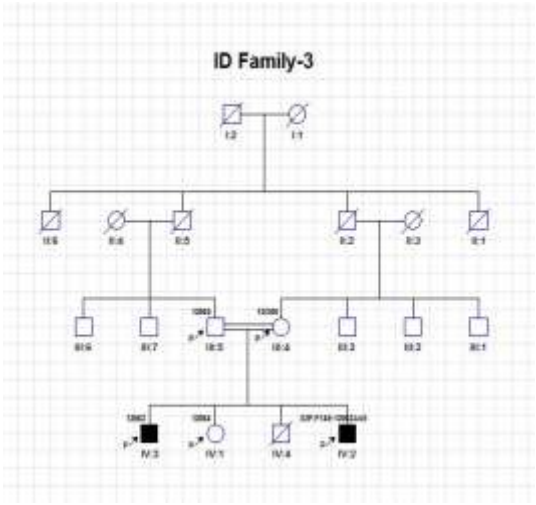
KCNK6, chr19.C314T:p.T105 (M1)

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

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



Chromatogram Report of ID Family-2



Family-3 Intellectual Disability

In the ID family 3 both of the patients had the same phenotype characteristics intellectual disability with epilepsy. There were no other associated abnormalities like spastic paraplegia or short stature. In this family no putative mutation was found there for segregation analysis was not done.

DISCUSSION

The clinical presentation and causes of ID patients is heterogeneous. According to Genetic basis study of ID is believed to be present in 25–50% of cases, even this number may rises proportionally with disease severity. One fourth of ID patients with nonsyndromic intellectual disability (NSID) have an Autosomal recessive manner of inheritance. Consanguineous families are very important in defining Autosomal recessive bases of ID disorder. In 2002 Modell and Darr described that recessively inherited congenital disorders are more common in consanguineous families .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. Consanguineous families' ratio is high in Pakistan which have high prevalence of congenital disorders.

In ID disorder till now only four loci have been identified where as several are reported in Pakistani population for ARNSID; these are MRT13/TRAPPC9 on chromosome 8, MRT14 on chromosome 2, MRT15 & MRT16 on Chromosome 9. Due to

this reason more work is needed to illustrate intellectual disability in Pakistani population.

A study on a consanguineous Israeli Arab family with Autosomal recessive mental retardation and spasticity was reported in which a homozygous truncating mutation in the AP4B1 gene was identified. The authors of concluded that AP4-complex-mediated vesicular trafficking plays a crucial role in brain development and function. 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.

This study was conducted to find out the variant genes responsible for intellectual disability in KPK Pakistani families affected with ARNSID. 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.

This will helpful to control and prevent 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.

CONCLUSION

Autosomal recessive forms of ID disorder (ARID) contribute about 10% of cases in an out bred population. In consanguineous families, the risk for ARID is a higher magnitude and the total risk for ID is about 2 to 3 times higher than for children of parents who are not related. In literature review study the undiagnosed causes of ID, 15–20% are suspected to be ARID.

The present study was done on three consanguineous families for the determination of the responsible genes for Intellectual Disability. Exome sequencing revealed putative mutations in AP4B1 and WDR62 in two out of three families. In third family we could not locate any putative mutation.

Mutations of AP4B1, WDR62, EML2 and KCNK6 were co-segregated with disease phenotype. These mutations were found to cause intellectual disability with spastic paraplegia & short stature.

Wide range of testing offers should be provided to find out the carrier status before marriage and also preconception counseling should be provided, this can educate the people in making positive decisions since we know that consanguineous marriages are favored in a substantial number of people in this region

Further advancement is required in this research to identify genes for possible gene editing.

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