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Molecular Analysis of Hotspot Regions of *ARX* and *MECP2* Genes in Intellectual Disability and Cornelia De Lange Syndrome

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ABSTRACT Intellectual disability is a generalized developmental disorder. The present paper is focused on mutational screening of *ARX* and *MECP2* genes in syndromic (Cornelia De Lange Syndrome cases (CdLS)) and non-syndromic intellectual disability. Mutational analysis was carried out by PCR followed by conformation sensitive gel electrophoresis and sequencing. In CdLS cases two polymorphisms were obtained in *ARX* gene where one of these was probably deleterious polymorphism. In NSID cases total five polymorphisms were obtained in *ARX* gene, where one was probably deleterious polymorphism. The *MECP2* gene showed presence of one polymorphism which was known deleterious variant. Except one all sequence variants found in this paper were novel.

INTRODUCTION

Mental retardation (MR) also known as intellectual disability (ID) is a common neurological disorder that affects one percent of the population and is characterized by significant limitation in one or more adaptive behavior as well as in intellectual functioning (Maulik et al. 2011). ID can be divided into syndromic and non-syndromic forms. Syndromic ID (SID) can be defined by clinical, metabolic or biological features (Iqbal and van Bokhoven 2014). Commonly known syndromic forms of ID include Down syndrome, Fragile X syndrome, Glycerol kinase deficiency syndrome, West syndrome, Partington syndrome, Aarskog-Scott syndrome, Proud syndrome, Rett syndrome, Epilepsy, and Cornelia De Lange syndrome (Ropers and Hamel 2005).

Cornelia De Lange syndrome (CdLS) is one of the syndromes associated with ID which has been studied in the present work. CdLS is a multisystem developmental disorder. Physical abnormalities in CdLS patient include dysmorphic facial features, hirsutism, limb abnormalities, growth retardation, gastro esophageal dysfunction, mental retardation, and cardiac, genitourinary, as well as ophthalmologic anomalies. The reported prevalence of CdLS is 1 case per 10 000 people (Ansari et al. 2014). *NIPBL* gene mutation is the cause of the disease in sixty percent of CdLS cases. Other two genes that contribute to CdLS include *SMC1A* and *SMC3* which account for minority of cases (Mannini et al. 2013).

Non-syndromic intellectual disability (NSID) is characterized by cognitive impairment as a major manifestation and the term was introduced by Kerr et al. in 1991 (Renieri et al. 2005). Genetic changes are associated with twenty five to fifty percent of ID cases. In recent years, it has been observed that mutations in single gene are responsible for non-syndromic mental retardation. De novo mutations are mainly responsible for severe ID (Hamdan et al. 2014; Vissers et al. 2016). Different autosomal genes responsible for NSID includes *MBD5, MRD2, CRBN, CDH15, SYNGAP1, STXBP1, SHANK3* and *GRIK2* gene (Kaufman et al. 2010). Different studies on ID have shown higher ratio of affected males over females, and it has been assumed that more cases of male ID may occur due to X-linked genes (Philips et al. 2014). The prevalence of X-linked intellectual disability (XLID) is 1:1000 per birth. Approximately eighty percent of gene mutations

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responsible for NSID are present on X chromosome. It has been observed that genes responsible for XLID are important for rapid development of cognitive abilities during human growth. Mutations in X-linked genes *ATRX, ARX, MECP2, JARID1C* and *SCL6A8* are common in NSID. Various types of mutations have been reported for NSID including insertions, deletions, missense, frame-shift, truncating, microduplication and splice site mutation (Kaufman et al. 2010).

The *ARX* and *MECP2* genes are most frequently mutated in ID (Kaufman et al. 2010; Das and Mukhopadhyay 2012). The Aristaless-related homeobox (*ARX*) gene is a paired class of homeobox gene which plays an important role in embryonic brain development. The known frequent mutations in *ARX* gene in NSID include c.428_451dup (24 bp) and c.333_334ins (GCG) 7. (Abedini et al. 2012) The other frequently reported mutation in *ARX* gene in NSID is c.333_334ins (GCG) 1 (Gecz et al. 2006; Poirier et al. 2006; Abedini et al. 2012).

MECP2 (Methyl-CpG-binding protein 2) gene is present on chromosome Xq28. *MECP2* protein binds to DNA methylated at CpG sites through a methyl-CpG-binding domain (MBD) and suppresses the transcription of downstream genes by recruiting co repressor complexes through a transcriptional repression domain. *MECP2* mutations are commonly found in Rett syndrome. Other than Rett syndrome, a wide spectrum of phenotypes has currently been reported to be associated with *MECP2* mutations in females which include non-specific X- linked mental retardation (Bianciardi et al. 2016; Grasshoff et al. 2011). A140V in *MECP2* gene is the most frequent mutation causing severe ID (Orrico et al. 2000; Couvert et al. 2001; Rujirabanjerd et al. 2007).

As the detection rate of CdLS is low and there may be the possibility of involvement of other genes than *NIPBL, SMC1* and *SMC3*, researchers have considered CdLS as syndromic condition of ID for this paper and screened the genes implicated in neurological development. Authors are already working for last five years on mutational analysis of CdLS (Bajaj et al. 2013; Bajaj et al. 2014; Bajaj et al. 2016). Study of X-linked genes *ARX* and *MECP2* in CdLS was a totally different approach than the study of well known genes involved in causing CdLS.

Objectives

This research was conducted to observe the mutations with respect to *ARX* and *MECP2* genes linked to syndromic and non-syndromic mental retardation patients. This research also investigates the relationship between phenotype and genotype.

METHODOLOGY

Patients

Total eleven patients were selected for this study. Amongst these five were CdLS (SID) patients which included three males and two females from five different families. Remaining six were NSID patients including four males and two females. These were selected from six different families. The study also included six normal healthy controls having no previous history of ID. The criteria for selection of patients were the IQ level and phenotypic features along with previous clinical records. Patients having IQ less than 70 and no specific phenotypic features related to syndromic ID were selected as NSID and patients showing phenotypic characters of CdLS were selected as SID for this study. Consent forms were obtained from parents of both CdLS and NSID patients. 5ml blood was collected from patients, parents and control samples. Detail family history was collected. Project was approved by institutional ethical committee. All used chemicals were of molecular biology grade.

Mutational Analysis of *ARX* and *MECP2* Genes

DNA Isolation

DNA was extracted from leukocytes by using QIAgen kit in author's research laboratory. DNA quality and quantity was checked on agarose gel and by nanodrop.

Mutational screening of exon 2, exon 4 of *ARX* gene and Exon 3 of *MECP2* gene was performed using PCR amplification followed by conformation sensitive gel electrophoresis (CSGE) for identification of heteroduplex formation.

Amplification of ARX Gene

Amplification was done by using Eppendorf thermal cycler. Amplification of *ARX* gene was

performed using different primers as given in Table 1 (Abedini et al. 2012). Exon 2 of the *ARX* gene was amplified in four different overlapping fragments using the primers 2B, 2BB, 2C and 2X. To amplify fragments 2B and 2BB, 25-µl PCR reactions were carried out in 1X buffer (Banglore Genei), 200 µM of each dNTP, 1.5 mM MgCl2, 100 pmol of each specific primer, 2.5 µl of dimethyl sulfoxide, 200 ng DNA and 2 U Taq polymerase (Merck). Reaction conditions included initial denaturation at 95°C for 7 min followed by 14 cycles (denaturation at 95°C for 45 sec, annealing at the specific annealing temperature for 30 sec, elongation at 72°C for 1 min) followed by 16 cycles (denaturation at 94°C for 45 sec, annealing at the specific annealing temperature for 15 sec, elongation at 72°C for 15 sec) followed by a final extension at 72°C for 7 min. Fragment 2C and 2X of exon 2 and fragment 4A and 4B of exon 4 of *ARX* gene were amplified using previously reported PCR conditions (Poirier et al. 2006).

Amplification of MECP2 Gene

To amplify fragment 3A and 3B of exon 3 of *MECP2* gene, the 25-µl PCR reactions were performed in 1X buffer (Banglore Genei), 200 µM of each dNTP, 1.5 mM $MgCl_2$, 100 pmol of each specific primer (Table 1), 200 ng of DNA and 2 U of Taq polymerase (Bangalore Genei). After initial denaturation at 95°C for 5 min, 35 cycles (denaturation at 95°C for 30s, annealing at the specific annealing temperature for 30 sec and elongation at 72°C for 1 min), followed by a final extension at 72°C for 7 min were performed (Bienvenu et al. 2000).

Analysis of Amplified Products

Amplified fragments were analyzed by agarose gel electrophoresis to check quality and quantity of amplicons.

Polyacrylamide Gel Electrophoresis (PAGE) and Conformation Sensitive Gel Electrophoresis (CSGE)

Size variation screening was done using eight percent polyacrylamide gel electrophoresis. CSGE was performed using standard protocol to check the presence of heteroduplexes and thus the sequence variation. Equal amounts of wild type amplified DNA was mixed with amplified patients DNA, denatured at 95°C for 5 min and then annealed at 68°C for 30-60 min. From this mixture 20 µl DNA along with 4 µl 10X loading dye were loaded on CSGE gel. After running the samples on CSGE, the staining was done using ethidium bromide staining and silver staining method (Ganguly et al. 1993).

Sequencing

The amplicons showing size variations or presence of heteroduplexes on CSGE or PAGE were purified using PCR purification kit (thermoscientific) and sent to Genomebio Pvt Ltd, Pune and Eurofins Pvt Ltd, Bangalore for sequencing. Sequencing was done using Sangers dideoxy method on ABI 3730 DNA analyzer.

Sequence Analysis

Sequences were studied using DNA baser software to identify the variants. NCBI blast was carried out to obtain the relative position of sequence variants. Effects of observed variants were studied using mutationtatser software (http://www.mutationtaster.org). Different models, 'silent' (non-synonymous or intronic) alterations (without_aae model), substitution/insertion/deletion of a single amino acid (simple_aae model) or complex changes of the amino acid sequence (complex_aae model) of this software

Table 1: Primers and annealing temperature used for PCR amplification

Gene	*Forward primer*	*Reverse primer*	*Product size (bp)*	*Annealing temperature (°C)*
ARX 2B	CCCCTCCGCCGCCACCGCCAAC	TCCTCCTCGTCGTCCTCGGTGCCGGT	313	75
ARX 2BB	CTGATAGCTCTCCCTTGCCC	CGTTCTCGCGGTACGACTT	354	60
ARX 2C	TGGCCGGCGGCCCGGGCAGC	CAGCTCCTCCTTGGGTGACA	266	66
ARX 2X	GCAGTGGCCACAGAGGGCG	CGCGACCACCCTACGCGCAT	255	60
ARX 4A	ACCCACCCGCTCAGCCCCTA	AGACAGACAGACTTCCGAGGCTGC	261	66
MECP2 3A	TGTGTCTTTCTGTTTGTCCC	GATTTGGGCTTCTTAGGTGG	182	58
MECP2 3B	CCTCCCGGCGAGAGCAGAAA	TGACCTGGGTGGATGTGGTG	240	60

were considered. Variants having probability value close to one were considered.

RESULTS

Total five CdLS patients and six NSID patients were studied. Phenotype of CdLS cases was mental retardation, hypertrichosis, microcephaly, synophrys, small hands and feet. There was no family history for disease. NSID patients showed mild to moderate ID. All families of CdLS and NSID patients along with controls were screened for mutations in exon 2 and 4 of *ARX* gene and exon 3 of *MECP2* gene. These exons were selected as hotspot region because mutations have been already reported in these exons. The presence of amplified products was confirmed by agarose gel electrophoresis. Figure 1 shows the amplicons of DNA using different primers.

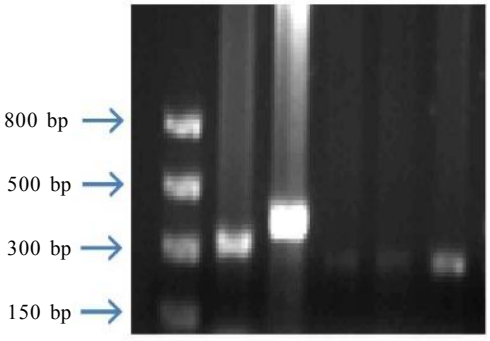

Fig. 1. Amplicons on agarose gel (Well 1- Ladder, Well 2 to 6 – Amplicons using primer *ARX* 2B, *ARX* 2BB, *ARX* 2C, *ARX* 2X, *MECP2* 3a)

Analysis of *ARX* Gene in CdLS Patients

Five families of CdLS were studied. Amplicons showing presence of heteroduplex on CSGE are shown in Figure 2 (well no. 3,4,5). Respective amplicons were purified and sequenced. Out of five patients only one patient showed two variations. Exon 2 of *ARX* gene showed variations c.509G>C and c.651G>T in patient 3 (female) (Table 2). First polymorphism led to change of amino acid S170T while second polymorphism was silent. Figure 3a and Figure 3b shows the electropherogram of control c.509 G and c.509G>C variation respectively. The results were confirmed by NCBI BLAST.

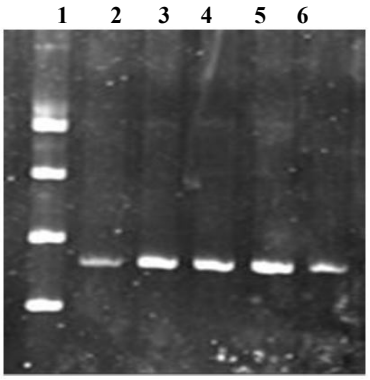

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Fig. 2. CSGE of amplified products (Well 1- Ladder, Well 2- control, Well 3 to 6 - samples)

Table 2: Observed polymorphism in exon 2 of *ARX* gene in CdLS patients

Sequence variations	*Effect of mutation*	*Number of patients*
G>C at c.509 (S170T)	Splice site change	1
G>T at c.651	No effect	1

Analysis of *ARX* Gene in NSID Patients

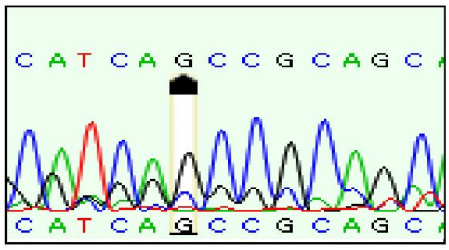
Out of six studied patients three showed polymorphism in exon 2. Two polymorphisms were observed in patient 1 (male) in exon 2, c.782A>T variation is shown in electropherogram given in Figure 4b (electropherogram for control c.782A is shown in Fig. 4a) , it led to amino acid change K261M and it was also seen in patient 3, while other polymorphism c.843A>C was silent. Patient 2 (male) showed c.331G>A variation that led to change of amino acid A111T. Patient 3 (female) showed two variations, the variation c.772G>C is shown in electropherogram in Figure 5b (electropherogram for c.772G control is shown in Fig. 5a). This led to amino acid change A258P. The other silent variation c.774G>T was observed in exon 2 (Table 3).

Analysis of *MECP2* Gene in CdLS Patients

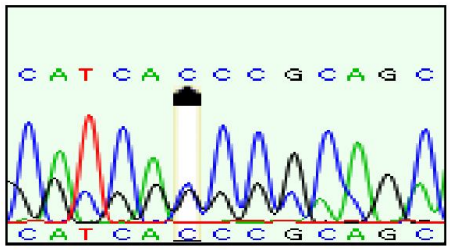
No nucleotide base change or variation was found in *MECP2* gene in studied five CdLS cases.

Analysis of *MECP2* Gene in NSID Patients

Polymorphism was observed in only one patient out of six studied families in exon 3. Poly-



3a



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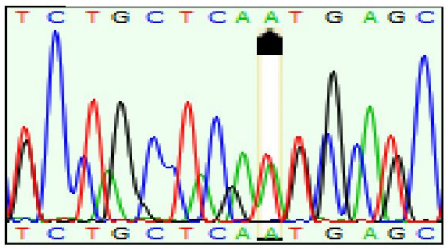
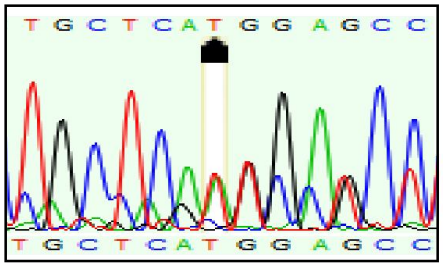


4a



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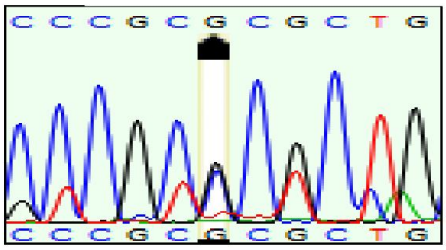
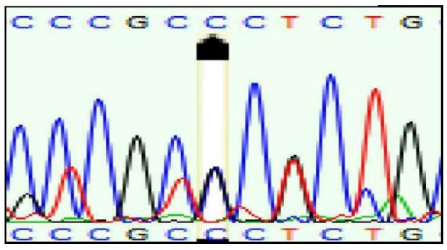


5a



5b

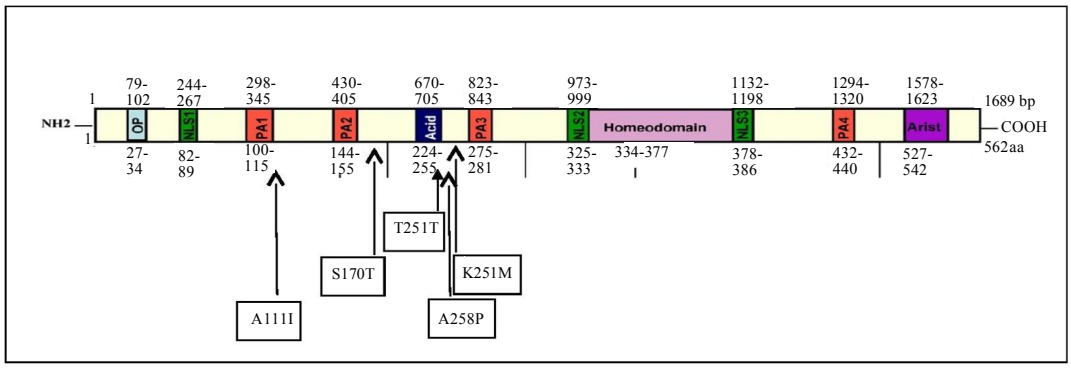


Figure 3 to 5 shows DNA sequencing electropherogram of control and patients
Figure 3: (a) Control c.509 G (b) Polymorphism c. 509 G>C
Figure 4: (a) Control c. 782 A (b) Polymorphism c. 782 A>T
Figure 5: (a) Control c. 772 G (b) Polymorphism c.772 G>C
Figure 6: Position of observed variants on, ARX protein domain

Table 3: Observed polymorphism in *ARX* and *MECP2* gene in NSID patients

Gene	*Sequence variations*	*Effect of mutation*	*Number of patients*
ARX	G>A at c.331(A111T)	Splice site change	1
ARX	G>C at c.772(A258P)	Splice site change	1
ARX	A>T at c.782(K261M)	Splice site change	2
ARX	G>T at c.774	No effect	1
ARX	A>C at c.843	No effect	1
MECP2	G>T at c.609	No effect	1

morphism c.609G>T was observed in patient 2 (male). It does not lead to any amino acid change (Table 3).

DISCUSSION

For last several years, research is being carried out on X-chromosome as there are higher affected males of ID than females (Lehrke 1972, 1974).

Present paper is focused on analysis of mutations in *ARX* and *MECP2* genes in CdLS and NSID patients. Since both conditions have mental retardation as a common feature, researchers wanted to check and compare molecular studies on both the genes in CdLS as well as non-syndromic cases.

Mutations in *ARX* and *MECP2* gene have not been reported in CdLS patients to date. In the present paper for the first time researchers have done mutational analysis in *ARX* and *MECP2* genes in CDLS patients.

In the present paper two polymorphisms were found in exon 2 of *ARX* gene in CdLS patient of third family (Table 2). First observed polymorphism G>C was at c.509 which is probably deleterious (as per mutationtaster). Screening of this polymorphism in family suggested that polymorphism was inherited from mother. This variation led to alteration of codon AGC to ACC. As this codon was mutated in the splice site it caused increase in donor site and showed significant score. It may result into loss of homeobox, alanine and proline rich region and OAR fragment that are downstream of altered splice site of *ARX* protein as per the analysis done by mutationtaster software (Fig. 6) and may play important role in neuronal development of patient. As the amino acid was found to be conserved the substitution S170T may affect protein function. Second polymorphism G>T at c.651 was a silent polymorphism. Since these polymorphisms were not seen in control DNA these may be considered as a disease associated polymorphism. Since these findings are not reported earlier these are novel polymorphisms.

When compared genotype of patients with phenotype, researchers have observed little variation in patient 3. Two polymorphisms were seen in case of patient 3. Phenotypes of patient 3 includes hypertelorism, long palpebral fissure, b/l proximally found thumb, small hands and feet, hypertrichosis. One characteristic phenotype change, b/l proximally found thumb was not found in other patients. Other phenotypic expressions were similar in all six studied CdLS cases.

The sequence variations found in this paper have not been reported earlier. However the reported variations in exon 2 of *ARX* gene in NSID include c.428_451 dup24 (Poirier et al. 2006; Fullston et al. 2011; Abedini et al. 2012), c.333_334 (GCG) 7 (Poirier et al. 2006), c.431_454del (24 bp) (Grønskov et al. 2004), c.448_456del, c.429_452del (Bienvenu et al. 2002) c.441_464dup, c.441_455del (Marques et al. 2015).

Five different polymorphisms were observed in exon 2 of *ARX* gene in NSID patient (Table 3). First variation G>A at c.331 was observed in patient 2. Patient was 19 years old male having moderate ID. This polymorphism led to alteration of codon from GCG to ACG. This resulted into increased donor splice site. It may cause loss of homeobox, alanine, proline rich region and OAR domain of *ARX* gene as these domains are located at downstream of mutated splice site (As per mutationtatser). This may affect the neuronal development in embryonic stages.

Second polymorphism found was c.772 G>C in *ARX* gene in third patient. Because of this polymorphism change in codon from GCG to CCG was observed, that affected the splice site. Both donor and acceptor splice site get increased and the significant score was obtained as per mutation taster. It may result into loss of protein domains downstream of this site. Amino acid change A258P was observed. But it's a non-conserved amino acid.

Polymorphism A>T was observed at c.782 in *ARX* gene in both first and third patient. Change in codon from AAG to ATG was observed due to this polymorphism. This variation caused increase in donor as well as accepter region of splice site (significant score as per mutationtaster). There may be a loss of homeobox, alanine, proline rich region and OAR domain of *ARX* protein. Amino acid change observed was K261M. The amino acid is non-conserved.

Two silent polymorphisms were found in patient number 3 and 1 respectively in *ARX* gene. c.774G>T was observed in third patient that led to splice site change. A>C polymorphism was observed at c.843 in 1st patient. As per mutationtaster this is probably deleterious variation. This variation resulted into change in splice site. Both changes lead to the loss of *ARX* protein domains that are present downstream of the altered splice site. Since all five polymorphisms are absent in control DNA these can be considered disease associated. Since these five findings are not reported earlier these are novel polymorphisms.

The reported variations in *MECP2* are different from the ones which were obtained in this paper. Most of the known mutations in *MECP2* gene in NSID includes A140V (Orrico et al. 2000; Couvert et al. 2001; Rujirabanjerd et al. 2007), P225L (Ylisaukko-Oja et al. 2005), A201V (Kaufman et al. 2010), p.Gly185Val and p.Arg167Trp (Bianciardi et al. 2016) in exon 4.

In the present paper mutational analysis of *MECP2* gene in NSID showed one variant c.609G>T in exon 3 in patient 2. It was not associated with any amino acid change or splice site change. As per the mutationtaster this is known to be deleterious (known disease mutation at this position HGMD CI067907). Exon 3 of *MECP2* gene did not show any other variations. This variation has not been reported earlier for NSID and is a new finding. All the observed variations that resulted into splice site change are shown in Figure 6.

Researchers tried to correlate genotype with the phenotype. Patient 1 and 3 showed moderate ID and IQ 35-49. Two variations found in both the patients resulted in changed splice site and led to loss of homeobox, alanine, proline rich region and OAR domain of *ARX* protein. Two other variants in patient 1 were silent polymorphisms. Patient 2 had Severe ID and IQ – 20 and genotypic variation found resulted into splice site change.

CONCLUSION

The present paper is focused on exploring involvement of *ARX* and *MECP2* gene variants in CdLS and NSID. One probably deleterious polymorphism and one silent polymorphism in *ARX* gene in CdLS cases was observed in this paper. This paper also report four polymorphisms and one probably deleterious polymorphism in *ARX* gene and one known deleterious polymorphism in *MECP2* gene in NSID. This is a different approach of studying CdLS than the routine protocol. Researchers conclude that the novel sequence variants have been obtained in *ARX* and *MECP2* genes in NSID and CdLS patients. The paper also adds new molecular finding to the existing data about *ARX* and *MECP2* genes in NSID which were found in Indian patients (Maharashtra Region).

RECOMMENDATIONS

As the detection rate of CdLS is low one can focus studies on *ARX* gene in CdLS along with other known genes. Patients having sequence variations showed lower IQ (moderate to severe ID) whereas patients having no sequence variation had little higher IQ (mild ID).

FUTURE STUDIES

The entire *ARX* and *MECP2* genes may be screened for sequence variations in SID and NSID patients. In future exome sequencing or array CGH may help to pin point the mutations in NSID patients.

LIMITATIONS

PCR is a laborious and time consuming technique for mutational analysis of entire gene length as well as larger genome regions. Next generation sequencing technique can help in screening genome of these patients.

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REFERENCES

Abedini S, Kahrizi K, Behjati F, Banihashemi S, Ghasemi Firoozabadi S, Najmabadi H 2012. Mutational screening of *ARX* gene in Iranian families with X-linked intellectual disability. *Arch Iran Med,* 15(6): 361-365.

Ansari M, Poke G, Ferry Q, Williamson K, Aldridge R et al. 2014. Genetic heterogeneity in Cornelia de Lange syndrome (CdLS) and CdLS-like phenotypes with observed and predicted levels of mosaicism. *J Med Genet,* 51(10): 659-668.

Bajaj S, Gambhir P, Ranade S 2013. Nucleotide sequence analysis of *NIPBL* gene in Indian Cornelia de Lange syndrome cases. *Indian J Hum Genet,* 19(1): 9-13.

Bajaj S, Gambhir P, Ranade S 2014. Short tandem repeats in CdLS - causing genes: Distribution and comparison. *J Genet,* 93(3): 104-107.

Bajaj S, Nampoothiri S, Yesodharan D, Gambhir P, Ranade S 2016. First report on complete *NIPBL* gene deletion in Indian case of Cornelia de Lange syndrome. *International Journal of Human Genetics,* 16(1, 2): 61-69.

Bianciardi L, Fichera M, Failla P, Di Marco C, Grozeva D et al. 2016. *MECP2* missense mutations outside the canonical MBD and TRD domains in males with intellectual disability. *J Hum Genet,* 61(2): 95-101.

Bienvenu T, Carrié A, de Roux N, Vinet MC, Jonveaux P et al. 2000. *MECP2* mutations account for most cases of typical forms of Rett syndrome. *Hum Mol Genet,* 9(9): 1377-1384.

Bienvenu T, Poirier K, Friocourt G, Bahi N, Beaumont D et al. 2002. *ARX*, a novel Prd-class-homeobox gene highly expressed in the telencephalon, is mutated in X-linked mental retardation. *Hum Mol Genet,* 11(8): 981-991.

Couvert P, Bienvenu T, Aquaviva C, Poirier K, Moraine C et al. 2001. *MECP2* is highly mutated in X-linked mental retardation. *Hum Mol Genet,* 10(9): 941-946.

Das A, Mukhopadhyay K 2012. Molecular genetics of intellectual disability with special emphasis on the idiopathic type. *International Journal of Genetics,* 4(3): 99-110.

Fullston T, Finnis M, Hackett A, Hodgson B, Brueton L et al. 2011. Screening and cell-based assessment of mutations in the Aristaless-related homeobox (*ARX*) gene. *Clin Genet,* 80(6): 510-522.

Ganguly A, Rock MJ, Prockop DJ 1993. Conformation-sensitive gel electrophoresis for rapid detection of single-base differences in double-stranded PCR products and DNA fragments: Evidence for solvent-induced bends in DNA heteroduplexes. *Proc Natl Acad Sci USA,* 90(21): 10325-10329.

Gecz J, Cloosterman D, Partington M 2006. *ARX*: A gene for all seasons. *Current Opinion in Genetics and Development,* 16(3): 308-316.

Grasshoff U, Bonin M, Goehring I, Ekici A, Dufke A et al. 2011. De novo *MECP2* duplication in two females with random X-inactivation and moderate mental retardation. *Eur J Hum Genet,* 19(5): 507-512.

Grønskov K, Hjalgrim H, Nielsen IM, Brøndum-Nielsen K 2004. Screening of the *ARX* gene in 682 retarded males. *Eur J Hum Genet,* 12(9): 701-705.

Hamdan FF, Srour M, Capo-Chichi JM, Daoud H, Nassif C et al. 2014. De novo mutations in moderate or severe intellectual disability. *PLoS Genet,* 10(10): 1-9.

Iqbal Z, van Bokhoven H 2014. Identifying genes responsible for intellectual disability in consanguineous families. *Hum Hered,* 77(1-4): 150-160.

Kaufman L, Ayub M, Vincent JB 2010. The genetic basis of non-syndromic intellectual disability: A review. *J Neurodevelop Disord,* 2(4): 182-209.

Lehrke R 1972. Theory of X-linkage of major intellectual traits. *Am J Ment Defic,* 76(6): 626-631.

Lehrke RG 1974. X-linked mental retardation and verbal disability. *Birth Defects Orig Artic Ser,* 10: 1-100.

Mannini L, Cucco F, Quarantotti V, Krantz ID, Musio A 2013. Mutation spectrum and genotype-phenotype correlation in Cornelia de Lange syndrome. *Hum Mutat,* 34(12): 1589-1596.

Marques I, Sá MJ, Soares G, Mota Mdo C, Pinheiro C et al. 2015. Unraveling the pathogenesis of *ARX* polyalanine tract variants using a clinical and molecular interfacing approach. *Mol Genet Genomic Med,* 3(3): 203-214.

Maulik PK, Mascarenhas MN, Mathers CD, Dua T, Saxena S 2011. Prevalence of intellectual disability: A meta-analysis of population-based studies. *Res Dev Disabil,* 32(2): 419-436.

Orrico A, Lam C, Galli L, Dotti MT, Hayek G et al. 2000. *MECP2* mutation in male patients with non-specific X-linked mental retardation. *FEBS Letters,* 481(3): 285-288.

Philips AK, Sirén A, Avela K, Somer M, Peippo M et al. 2014. X-exome sequencing in Finnish families with intellectual disability four novel mutations and two novel syndromic phenotypes. *Orphanet J Rare Dis,* 11: 1-13.

Poirier K, Lacombe D, Gilbert-Dussardier B, Raynaud M, Desportes V et al. 2006. Screening of *ARX* in mental retardation families: Consequences for the strategy of molecular diagnosis. *Neurogenetics,* 7(1): 39-46.

Renieri A, Pescucci C, Longo I, Ariani F, Mari F, Meloni I 2005. Non-syndromic X-linked mental retardation: From a molecular to a clinical point of view. *Journal of Cellular Physiology,* 204(1): 8-20.

Ropers H, Hamel B 2005. X-linked mental retardation. *Nature Reviews Genetics,* 6(1): 46-57.

Rujirabanjerd S, Tongsippunyoo K, Sripo T, Limprasert P 2007. Mutation screening of the Aristaless-related homeobox (*ARX*) gene in Thai pediatric patients with delayed development: First report from Thailand. *European Journal of Medical Genetics,* 50(5): 346-354.

Vissers LE, Gilissen C, Veltman JA 2016. Genetic studies in intellectual disability and related disorders. *Nat Rev Genet,* 17(1): 9-18.

Ylisaukko-Oja T, Rehnström K, Vanhala R, Kempas E, von Koskull H et al. 2005. *MECP2* mutation analysis in patients with mental retardation. *Am J Med Genet A,* 132A(2): 121-124.

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