RESEARCH PAPER

Molecular Testing of *MECP2* Gene in Rett Syndrome Phenotypes in Indian Girls

MEENAKSHI LALLAR[1], ARCHANA RAI[1], PRIYANKA SRIVASTAVA[1], KAUSIK MANDAL[1], NEERJA GUPTA[2], MADHULIKA KABRA[2] AND SHUBHA R PHADKE[1]

From Department of [1]Medical Genetics, SGPGI, Lucknow, Uttar Pradesh; and [2]Genetics Division, Department of Pediatrics, AIIMS, New Delhi; India.

Correspondence to: Dr Shubha Phadke, Department of Medical Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow 226 014, Uttar Pradesh, India. shubharaophadke@gmail.com

Received: April 13, 2017; Initial review: June 20, 2017; Accepted: January 30, 2018.

Objective: To assess yield of *MECP2* gene sequence variations analysis and large deletions in suspected cases of Rett syndrome.

Design: Descriptive study.

Setting: Tertiary-care medical genetics center.

Patients: Girls with neuroregression, postnatal microcephaly and signs and symptoms suggestive of classical and atypical Rett syndrome were classified into two groups. Group I consisted of girls with Classical and atypical Rett syndrome on basis on the Revised Rett Syndrome diagnostic criteria, 2010. Group II included girls with neuroregression and postnatal microcephaly and other Rett like features but not fulfilling the above criteria.

Procedure: Sanger sequencing of coding regions and large deletional analysis of *MECP2* gene.

Outcome measure: Identification of mutation in *MECP2* gene.

Result: Mutation in *MECP2* gene was identified in 74% (14/19) in group I and none (0/17) in group II. The mutation detection rate was 93% (13/14) in group I classical Rett syndrome girls (2 with large deletions identified with Multiplex ligation dependent probe amplification) and 20% (1/5) in group I atypical Rett syndrome girls. One novel *MECP2* sequence variation was identified in group I classical Rett syndrome.

Conclusion: The yield of the mutation detection in *MECP2* is higher in classical Rett syndrome. In girls with some Rett like features, but not fulfilling revised Rett syndrome diagnostic criteria, mutation testing for *MECP2* gene has a low yield.

Keywords: *MLPA, Mutation testing, Sanger sequencing.*

Published online: February 09, 2018. PII:S097475591600115

Rett syndrome (OMIM No. 312750) is caused by heterozygous mutations in the *X*-linked gene, *MECP2*, which encodes methyl-CpG binding protein 2 (*MECP2*). It has a prevalence of around 1 in 10,000 female births [1]. More than 1000 different mutations have been reported in the *MECP2* gene [2]. It has been classified into Typical Rett syndrome (classic form) and Atypical Rett syndrome [3]. Classic Rett syndrome is characterized by an approximately 6-18 month period of overtly normal development which precedes a period of regression. This period of regression is followed by recovery or stabilization.

There have been a few case reports from India identifying isolated sequence variants and deletions in *MECP2* gene [4,5]. In a large Indian study, the common and novel sequence variations in *MECP2* gene in Rett syndrome patients were identified, but large deletions in *MECP2* gene were not studied [6]. In this study, we present the sequencing variations and deletional forms of *MECP2* gene identified in Rett syndrome patients, classified into classical and atypical forms using the using the Rett syndrome revised diagnostic criteria and nomenclature, 2010 [3].

METHODS

This cross-sectional study included girls with a clinical suspicion of Rett syndrome. Ethical clearance was obtained from Institutional Ethics committee of SGPGI and AIIMS. Informed written consent was taken from parents/guardians of all participants. All the girls included in the study had history of neuroregression (prior to five years of life) and postnatal microcephaly with normal MRI findings. The girls were divided into two groups. Group I included girls with a clinical diagnosis of classical or atypical Rett syndrome based on the Rett syndrome revised diagnostic criteria and nomenclature, 2010 [3]. Group II included all girls who did not fulfil all the criteria listed in the revised diagnostic

criteria and nomenclature, 2010 but had neuroregression, postnatal microcephaly (with normal MRI) and other features suggestive of Rett syndrome were observed like autistic features, poor social interaction and eye contact, isolated play, inappropriate laughter, stereotypical movements, delayed speech etc. In both the groups, Sanger sequencing of *MECP2* gene (two, three and four exons) was performed followed by Multiplex ligation dependent probe amplification (MLPA) (P0245- covering *MECP2* exon 1 upstream and exons 3 and 4) in *MECP2* mutation negative cases to look for partial and whole gene deletions of *MECP2* gene. The Sanger sequencing was done using five sets of primers, one each for exon 2 and 3 and three overlapping fragments for exon 4; exon 1 was not sequenced [7].

RESULTS

A total of 36 girls, with postnatal microcephaly (with normal MRI) and history of neuroregression, prior to five years of age, were studied.

Group I consisted of 19 girls – 14 classical Rett syndrome and five atypical Rett syndrome girls, and Group II included 17 girls. These patients were tested as some features suggestive of Rett syndrome (other than neuroregression and postnatal microcephaly) were observed. The mean age at diagnosis in the three groups was 4 year, 3 year and 4.5 years, respectively. All the cases were of sporadic occurrence. The age of girls ranged from 1.6 to 10 years. The mean age of neuroregression was significantly higher in group II (3 years) as compared to group I (1.2 years).

The Sanger sequencing of *MECP2* gene in group I identified sequence variations in 11 out of 14 classical Rett syndrome girls (78%) and one out of five atypical Rett syndrome girls (20%). In group I, MLPA detected large exonic deletions in two classical Rett syndrome girls (14%) (***Web Fig. 1***). The total yield of molecular testing (Sanger sequencing and MLPA was 92.8% (n=13) in classical Rett syndrome girls and 20% (n=1) in atypical Rett syndrome girls. The total yield of molecular testing in group I was around 74%. Molecular testing did not identify any *MECP2* sequence variation or copy number change in group II (17 girls). Additional molecular testing were done and revealed pathogenic copy number variations in two out of 17 group II girls (11%).

Out of the 12 sequence variations identified in *MECP2* gene, 11 were known pathogenic mutations and one was a novel mutation (***Table* I**). The novel missense mutation- c.427G>A was identified in group I, in classical Rett syndrome girl. The missense variant was predicted as likely pathogenic by mutation prediction softwares (Mutation taster, SIFT (Sorting Intolerant From Tolerant) and Polyphen 2).

TABLE I MUTATIONS IDENTIFIED IN *MECP2* GENE

Mutation in MECP2 gene	*Type of mutation*	*Exon*
*c.427G>A	Missense	4
c.502C>T	Nonsense	4
c.473C>T	Missense	4
c.916C>T	Missense	4
c.905C>G	Missense	4
c.763C>T	Nonsense	4
c.799A>T	Missense	4
c.1153_1190del 38	Homozygous deletion	4
Deletion of exon 1 and 3	Large deletion	
Deletion of exon 3 and 4	Large deletion	

**Novel mutation.*

A known deletion was identified, c.1153_1190del 38 in group I classical Rett syndrome girl in the homozygous form. The finding was confirmed by repeat testing. Apart from being a homozygous deletion; it can be a heterozygous deletion with probable allele drop out or selective amplification of shorter fragment. Uniparental disomy was ruled out by SNP microarray. Karyotype was normal 46, XX. Whole exonic or whole deletion of MECP2 was ruled out by MLPA (P0245).

DISCUSSION

In the present study, *MECP2* gene mutations (sequence variations and larger deletions) were identified in 93% classical Rett syndrome patients and 20% atypical Rett syndrome patients in group I patients. In group II patients no *MECP2* mutation was identified. This study illustrates the usefulness of the Rett syndrome revised diagnostic criteria as a tool for clinical categorisation of Rett syndrome. According to previous studies, mutations in *MECP2* gene can be found in 65-96% of individuals with classical Rett syndrome. The yield for *MECP2* mutations is much lesser with atypical Rett phenotype ranging from 30-50% [8-12]. In an Indian study, 30% (27/90) patients with Rett phenotype, had a mutation in *MECP2*. The lower yield in this study could be due to use of DSM IV criteria for inclusion of cases in the study. In addition, the study did not look for large deletions in *MECP2* [6]. Another, limitation of our study and previous studies is that exon 1 has not been sequenced, rarely it may have disease causing mutations in it [7]. Exon 1 could not be sequenced in our study, despite several efforts, due to high GC content.

The novel mutation identified in this study most likely leads to loss of function of the MBD domain of *MECP2*

WHAT IS ALREADY KNOWN?

- *MECP2* mutations (sequence variations and deletions) are more frequently found in classical than atypical Rett syndrome.

WHAT THIS STUDY ADDS?

- *MECP2* mutations should only be evaluated in classical or atypical Rett syndrome patients if they fulfill the Revised Rett syndrome diagnostic criteria, 2010.

protein, which is essential to bind MECP2 to methylated DNA and hence, this might affect transcriptional regulation. Interestingly, all the sequence variations in *MECP2* gene were located in the exon 4 and most were C>T transitions. No genotype phenotype correlation was observed as severity of clinical manifestations was same – being classical Rett phenotype in patients with different mutations: missense, and small and large deletions. The general restriction of Rett syndrome to females and the very low recurrence rate appears to be the result of a large proportion of these mutations arising *via* spontaneous deamination of 5-methylcytosine to thymine in the heavily methylated male germ cells [13].

Large exonic deletions are found in approximately 8-10% of Rett syndrome (including both classical and atypical forms) [14,15]. As compared to previous studies which looked at deletions of *MECP2* gene with specific probe set (*e.g.* P015), we used the MLPA-P0245 probe set. Though it is commonly used and very useful, it is not specific for *MECP2* gene. This probes set, with a limited number of probes for each specific chromosome region, is meant to designed routinely to detect the common deletion/duplication involving different chromosomes, in a cost effective way. We hereby put forward the importance of using the commonly used probe set P0245 for detection of large exonic deletions of *MECP2* gene.

In Group I cases where molecular testing of *MECP2* did no show mutation other genes like *CDKL5* and *FOXG*, can be tested [16,17]. In group II patients with no *MECP2* mutations, other causes of neuroregression and postnatal microcephaly should be sought for. In two of these 17 patients, cytogenetic microarray detected pathogenic copy number variations.

All the cases in the given study were sporadic. The *MECP2* sequencing of mothers of girls with mutations in *MECP2* was not done. Rett syndrome is of sporadic occurrence in 99 % of cases. In the remaining one percent there is either gonadal mosaicism in mother or there is highly skewed *X*-chromosome inactivation leading to no phenotype of Rett syndrome in the mother [18]. The risk of recurrence of Rett syndrome in next pregnancy is low, but it cannot be totally excluded as germline mosaicism has been documented in Rett syndrome. Hence, prenatal diagnosis can be provided in the next pregnancy.

As per our study, revised clinical diagnostic criteria are useful guide for clinical and molecular diagnosis of Rett syndrome. *MECP2* mutations are more likely to be identified in classical than atypical Rett syndrome. Around 10% mutations in Rett syndrome patients are large exonic deletions of *MECP2* gene and hence, MLPA should be done when mutation is not identified by Sanger sequencing of *MECP2* gene. MLPA with the commonly used probe set, P0245, is found to be effective in detection of large deletions in girls with classical Rett syndrome.

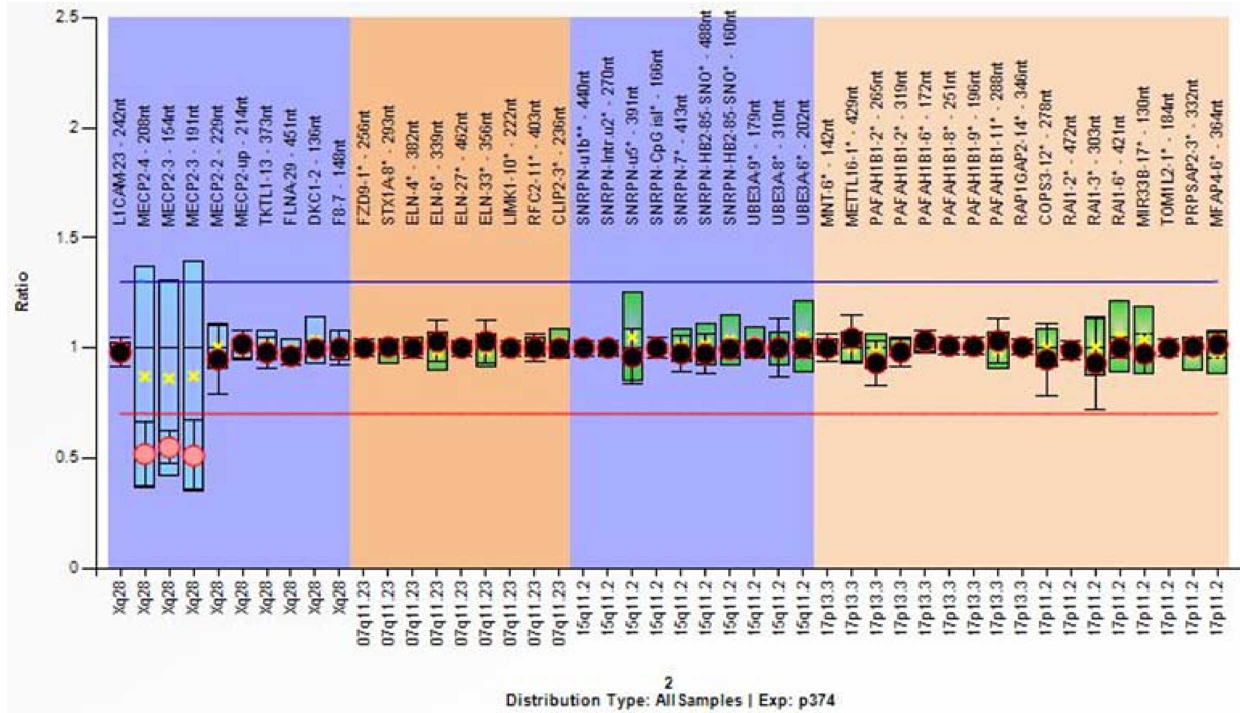
Acknowledgements: Naveen Sankhyan, Pediatric Neurology, PGIMER, Chandigarh; Rekha Gupta, Medical Genetics, MGMCH, Jaipur: Viswanathan V, Pediatric Neurologist, Kanchi Kamakoti CHILDS Trust and Apollo Children's Hospitals, Chennai; and Leena Deshpande, Dr Yewale Hospital, Mumbai.
Contributors: ML,KM,SRP: conception and design of the study, the acquisition of data, and the analysis and interpretation; AR, PS: conducting mutation analysis; KM, SRP, NG,MK: drafting and critical review.
Funding: Indian Council of Medical Research (63/8/2010/ BMS); *Competing interests:* None stated.

REFERENCES

1. Lyst MJ, Bird A. Rett syndrome: a complex disorder with simple roots. Nat Rev Genet. 2015;16:261-75.
2. Christodoulou J, Grimm A, Maher T, Bennetts B. RettBASE: The IRSA *MECP2* Variation Database - A new mutation database in evolution. Hum Mutat. 2003; 21:466-72.
3. Neul JL, Kaufmann WE, Glaze DG, Christodoulou J, Clarke AJ, Bahi-Buisson N, *et al*. Rett syndrome: Revised diagnostic criteria and nomenclature. Ann Neurol. 2010;68:944-50.
4. Khajuria R, Gupta N, Sapra S, Gulati S, Ghosh M, Kalra V, *et al*. Novel non-identical MECP2 mutations in Rett syndrome family: a rare presentation. Brain Dev. 2012; 34:28-31.
5. Khajuria R, Gupta N, Sapra S, Gulati S, Ghosh M, Kalra V, *et al*. A novel MECP2 change in an Indian boy with variant Rett phenotype and congenital blindness: Implications for

genetic counseling and prenatal diagnosis. J Child Neurol. 2011;26:209-13.

6. Das DK, Raha S, Sanghavi D, Maitra A, Udani V. Spectrum of MECP2 gene mutations in a cohort of Indian patients with Rett syndrome: report of two novel mutations. Gene. 2013;515:78-83.
7. Saunders CJ, Minassian BE, Chow EW, Zhao W, Vincent JB. Novel exon 1 mutations in MECP2 implicate isoform MeCP2_e1 in classical Rett syndrome. Am J Med Genet A. 2009;149A:1019-23.
8. Percy AK, Lane JB, Childers J, Skinner S, Annese F, Barrish J, *et al*. Rett syndrome: North American database. J Child Neurol. 2007;22:1338-41.
9. Bienvenu T, Carrié A, de Roux N, Vinet MC, Jonveaux P, Couvert P, *et al*. MECP2 mutations account for most cases of typical forms of Rett syndrome. Hum Mol Genet. 2000;9:1377-84.
10. Laurvick CL, de Klerk N, Bower C, Christodoulou J, Ravine D, Ellaway C, *et al*. Rett syndrome in Australia: a review of the epidemiology. J Pediatr. 2006; 148:347-52.
11. Olson HE, Tambunan D, LaCoursiere C, Goldenberg M, Pinsky R, Martin E, *et al*. Mutations in epilepsy and intellectual disability genes in patients with features of Rett syndrome. Am J Med Genet A. 2015;167:2017-25.
12. Miltenberger-Miltenyi G, Laccone F. Mutations and polymorphisms in the human methyl CpG-binding protein MECP2. Hum Mut. 2003;22:107-15.
13. Francke U. Mechanisms of disease: neurogenetics of MeCP2 deficiency. Nat Clin Pract Neurol. 2006;2:212-21.
14. Hardwick SA, Reuter K, Williamson SL, Vasudevan V, Donald J, Slater K, *et al*. Delineation of large deletions of the MECP2 gene in Rett syndrome patients, including a familial case with a male proband. Eur J Hum Genet. 2007;15:1218-29.
15. Pan H, Li MR, Nelson P, Bao XH, Wu XR, Yu S. Large deletions of the MECP2 gene in Chinese patients with classical Rett syndrome. Clin Genet. 2006;70: 418-19.
16. Weaving LS, Christodoulou J, Williamson SL, Friend KL, McKenzie OL, Archer H, *et al*. Mutations of CDKL5 cause a severe neurodevelopmental disorder with infantile spasms and mental retardation. Am J Hum Genet. 2004;75:1079-93.
17. Ariani F, Hayek G, Rondinella D, Artuso R, Mencarelli MA, Spanhol-Rosseto A, *et al*. FOXG1 is responsible for the congenital variant of Rett syndrome. Am J Hum Genet. 2008;83:89-93.
18. Ishii T, Makita Y, Ogawa A, Amamiya S, Yamamoto M, Miyamoto A, *et al*. The role of different X-inactivation pattern on the variable clinical phenotype with Rett syndrome. Brain Dev. 2001;23:S161-4.



***Web Fig.* 1** *MLPA P0245 results showing exon 3 and 4 deletion in MECP2 gene.*