Original Research Article

Molecular or clinical diagnosis of RTT syndrome?

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Abstract

Objectives: Children with delayed developmental milestones and behavioral abnormalities are generally misdiagnosed. So we aim to develop molecular diagnosis of RTT. **Method:** Karyotyping of the cases has been carried out. We standardized PCR protocol and sequencing of MECP2 coding region (Exon 2, 3 and 4).Then studied 23 cases of clinically diagnosed RTT. **Result:** Clinically diagnosed 23 RTT cases showed normal karyotype. Based on clinical findings, classical RTT cases were 10 (43.48%) and 13 (56.52%) were atypical RTT. We looked for common mutations mentioned by International Rett Syndrome Foundation in 8 out of 10 classical RTT cases and found 1 case (12.5 %) positive for R306C mutation. Rest 7 cases (87.5%) were negative for common RTT mutations we studied. **Conclusion:** To support and confirm the clinical diagnosis, molecular diagnosis has to be established in case of Rett Syndrome for accurate and better management. This study will assists to calculate RTT incidence in India.
Key Word: RTT syndrome.

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INTRODUCTION

Rett syndrome (RTT OMIM#312750) is an X- linked dominant neurological disorder of development.[1] It affects mostly females 1:10,000 to 1:22000 and rarely males 1:1,00,000.[2] **C**lassic Rett syndrome, a progressive neuro developmental disorder primarily affecting girls, is characterized by apparently normal psychomotor development during the first six to 18 months of life, followed by a short period of developmental stagnation, then rapid regression in language and motor skills, followed by long-term stability. During the phase of rapid regression, repetitive, stereotypic hand movements replace purposeful hand use. Additional findings include fits of screaming and inconsolable crying, autistic features, panic-like attacks, bruxism, episodic apnea and/or hyperpnea, gait ataxia and apraxia, tremors, seizures, and acquired microcephaly. Atypical Rett syndrome is observed increasingly as MECP2 variants are identified in individuals previously diagnosed with: clinically suspected but molecularly unconfirmed Angelman syndrome; intellectual disability with spasticity or tremor; mild learning disability; or (rarely) autism. Jeffrey L. Neul *et al*[3] has revised and simplified the diagnostic criteria for RTT. The spectrum of phenotypes includes classic or typical, variant or atypical RTT and mild learning disabilities (females) or neonatal encephalopathy and nonsyndromic intellectual disability (males)[3]. Classic or typical RTT is diagnosed when there is postnatal deceleration of head occur, a period of regression followed by recovery or stabilization with all the main criteria and all the exclusion criteria are met. By contrast, atypical RTT defines those patients who meet a period of regression followed by recovery or stabilization, two of the main criteria and five of the 11 supportive criteria. Pathological mutations in the MECP2 gene, which encodes methyl-CPG binding protein-2, were first reported in RTT in 1999 by Amir *et al*[4]. The MECP2 gene contains four exons and encodes two major

functional domains: the methyl binding domain and the transcription repression domain.

Diagnosis: Clinical Diagnosis The spectrum of phenotypes in MECP2-related disorders includes the following: Classic Rett syndrome and Variant Rett syndrome. In 1988, well before the discovery of the genetic basis of Rett syndrome, clinical diagnostic criteria were developed. The following are limitations to clinical diagnosis of Rett syndrome using these criteria: Clinical diagnosis may be considered tentative until the affected individual reaches age two to five years, by which point she has likely gone through several stages of the disease. Atypical forms may be either milder or more severe than classic Rett syndrome: In the more severe variant, no period of grossly normal development occurs; and early manifestations include congenital hypotonia and infantile spasms. In the milder variant, girls have less dramatic regression and milder intellectual disability. Other children experience an even more gradual regression that begins after the third year, lose purposeful hand use, and develop seizures; however, they retain some speech and the ability to walk[5] The modified criteria put forward by Neul *et al* in 2010[3] has helped to categorize the patients into the Classic and atypical RTT and has helped in resolving the inconsistencies and ambiguities in diagnosing the condition.

MATERIAL AND METHODS

Our study consists of all 23 girl patients who were referred to our genetic center (MILS) to establish the molecular diagnosis of Rett syndrome. The age of the patients varied from 24 months to 40 months and the mean age was 30 months. We strictly employed the revised diagnostic criteria for Rett Syndrome and applied to all of the patient as given by Jeffrey L. Neul *et al.*[3]: Necessary permission was obtained from genetic center for publishing the data. The diagnosis is considered when there is postnatal deceleration of the head growth observed, however this is not mandatory.

Required criteria for typical or classic Rett syndrome

- A period of regression followed by recovery or stabilization
- All of the main criteria and all of the exclusion criteria
- Supportive criteria are not required,

Required for the atypical or variant Rett syndrome

- A period of regression followed by recovery or stabilization
- Two of the four main criteria
- Five of the 11 supportive criteria

Main criteria

- Partial or complete loss of acquired purposeful hand skills
- Partial or complete loss of acquired spoken language or language skill
- Gait abnormalities: impaired (dyspraxia) or absence of ability
- Stereotypic hand movements including hand wringing/squeezing, clapping/tapping, mouthing and washing /rubbing automatis

Exclusion criteria for typical Rett syndrome

1. Brain injury secondary to peri-or postnatal trauma, neurometabolic disease or severe infection that causes neurological problems
2. Grossly abnormal psychomotor development in the first six months of life, with early milestones not being met.

Supportive criteria for the atypical Rett syndrome (currently or at any time)

- Breathing disturbances when awake
- Bruxism when awake
- Impaired sleep pattern
- Abnormal muscle tone
- Peripheral vasomotor disturbances
- Scoliosis/kyphosis
- Growth retardation
- Small cold hands and feet
- Inappropriate laughing/screaming spells
- Diminished response to pain
- Intense eye communication - “eye pointing”

We carried out cytogenetic studies, karyotyping in all the patients to observe any chromosomal abnormalities. This is followed by the MECP2 gene mutational analysis by standardized PCR protocol.

RESULTS

Revised criteria given by Neul *et al.*[3] were used in present study to diagnose RETT Syndrome.

Table 1: distribution of no of classical and atypical RTT patients

Clinically Diagnosed RTT	Classic Rtt	Atypical Rtt	Karyotype
	10 (p<0.013)	13 (p<0.13)	Normal in all cases

Table 2: Phenotypic presentation of the typical and atypical RTT syndrome.

Criterion	Typical RTT (10)	%	Atypical RTT (13)	%
Deceleration of growth	8	80	11	84
A period of regression followed by stabilization	10	100	13	100
Complete or partial loss of acquired purposeful hand skills	10	100		
Loss of acquired spoken language or skill	10	100		
Gait abnormalities dyspraxia	2	20		
Gait ataxia	8	80		
Hand wringing/ squeezing	4	40		
Hand clapping/ tapping	3	30		
Hand mouthing or washing/ rubbing	3	30		
Breathing disturbances when awake	6	60		
Bruxism when awake	5	50		
Impaired sleep pattern	9	90		
Abnormal muscle tone	7	70		
Peripheral vasomotor disturbances	3	30		
Scoliosis/kyphosis				
Growth retardation				
Small cold hands and feet				
Inappropriate laughing/screaming spells				
Diminished response to pain				
Intense eye communication - “eye pointing”				
Seizure	9	90	12	92
Microcephaly				
Muscle wasting and hypotonia				
Stages 1,2,3,4				
Cognitive impairment				

Table 3: Gene mutation frequency

	No of classic RTT patient analysed for mutation		No of atypical RTT patient analysed for mutation	
	08		13	
MECP2 gene mutation (R306C mutation)	positive 01 (12.5%)	Negative 07 (87.5%)	positive Nil (p<0.009)	Negative 13 (100%)

DISCUSSION

While diagnosing the RTT, differential diagnosis has to be considered. We considered each of the criteria in all typical and atypical RTT. It can be seen from table 1 that all of the 23 patient had no karyotyping abnormality and out of 23 cases (after strict application of revised criteria), 10 (4.47%) were the classical RTT syndrome while other 13 (56.52%) belonged to atypical variety of RTT. The abnormality present in all cases of classical RTT syndrome were; A period of regression followed by stabilization, Complete or partial loss of acquired purposeful hand skills, Loss of acquired spoken language or skill. Impaired sleep pattern, seizure and gait ataxia were the next commonest abnormality. While none of the Classical case presented with; Scoliosis/kyphosis, Growth retardation, Small cold hands and feet, Inappropriate laughing/screaming spells, Diminished response to pain, Intense eye communication- “eye pointing”, Microcephaly, muscle wasting and hypotonia, Stages 1,2,3,4 and Cognitive impairment. While in atypical RTT period of regression was the criteria present in all the patients followed by seizure[12] and deceleration of growth[11] 8 out of 10 typical RTT were analyzed for common mutations in MECP2 gene. We found 1 case positive (12.5 % positive) for the R306C mutation (table).

Amir *et al*[4] found that mutations in MECP2 is in the range of 95 to 97% with the typical RTT. The difference in the positive cases in the Amir *et al* and our study might be attributed to the methodologies for different mutations, small sample size and can be of various other mutations. Amir *et al* stated that even with the best methodologies, 3-5 % of patient who meets the strict criteria of typical RTT do not have an identified mutations in MECP2. It had been emphasized that mutations in gene is not required to make the diagnosis of typical RTT[4]. Amir *et al*[4]quoted that in case of atypical RTT, 50 to 70% show the mutations in MECP2. Compared to our study where no atypical RTT showed any mutational changes. Microdeletion studies in the group of atypical Rett Syndrome or classical Rett syndrome are helpful in establishing diagnosis but we have not employed.

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