Review Article

Rett syndrome: Methyl-CpG binding protein 2 transformation and phenotypic-genotypic characteristics

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Abstract

Methyl-CpG-binding protein 2 (MECP2) mutation has been coupled to Rett syndrome; X-linked neurological disorder, leading to steady slowing down of neurodevelopment that exclusively affects girls worldwide. As this gene is known for its specific localization and careful binding to methylated Cytosine residues in genomic DNA and is expected to intercede biological function of transcriptional oppression and chromatin concentration too. But a finest delivery of properly folded and tailored MECP2 protein is required for its part in directive gene transcription. The detailed research work done worldwide shows that MECP2 function is not only vital for normal brain development and neuronal pledge but is eventually requisite for establishment and upholding of normal neuronal occupation. The percentage of MECP2 mutation in classic Rett patients is up to 80% however this transformation spectrum ranges from severe to mild with congenital encephalopathy, mental retardation and various neurological side effects. Altered MECP2 shows relevance to phenotypic manifestation but the skewed X inactivation appears to influence its seriousness in a far more extensive sense. The patients with identical MECP2 mutation may show very different phenotypic features and strictness because of variation in x-inactivation prototype among individuals. This study is done to review the availability of diagnosis and treatment at molecular level. In this review the clinical spectrum and molecular findings in females with MECP2 dysfunctions are also reviewed with special reference to evaluate the sufficient available criteria for molecular diagnosis and treatment across the globe.

Keywords: Genotype; MECP2 mutation; Phenotype; Rett syndrome

Introduction

Rett is an X-linked dominant neurodevelopmental syndrome, cause severe intellectual disability predominantly among girls. Hereditary changes of MECP2 known as methyl-CpG binding protein 2, is prime reason of Rett. Neurological disorders are associated with varied medical manifestations inclusive of harshness of

diseases, age, prognosis and cure. Due to the heterogeneity and sundry classes of neurological disorder, some are brought about by single locus while for other's disease disposition is heterogeneous in nature resulting from interaction between genetic as well as environmental factors. According to researchers [1, 2] genomic variations play a significant role in disease vulnerability and

treatment options [3]. In this regard, some of molecular methods are under exploration for adjustment of mutant and freak genes and their regulation [4, 5].

Among neurodevelopmental syndrome, Mental Retardation (MR) is base of most serious handicap children as well as young ones. Mental retardation (MR) from modest to severe that is less than 50 is likely to influence 0.3-0.5% of inhabitants and frequency increases to 1-1.5% if mild MR is considered, but approximations swing extensively among pandemic studies. Genetic causes are tremendously heterogeneous include noticeable chromosomal anomalies and monogenic diseases [2]. In the Online Mendelian database more than 1000 entries with 282 genes recorded with retardation but 25-40% of severe cases remain unexplained and studies show that numbers of instances in all likelihood involve combinations of multigenic and surrounding elements [6-9].

MECP2 structure and functional units

Basically MECP2 is the most expressly DNA-binding protein positioned in chromosome Xq28 on distal portion of long arm and tied in 5-methyl-cytosine, e.g. Figure

1 [7, 10-12]. It is a founder member protein that binds to methyl CpG domain, consist of single polypeptide that compose of CpG proteins and a transcription repression domain. It is likewise proficient of binding to 5-methyle cytosine residues to distinct correspondence methylated CpG dinucleotides both in naked DNA as well as within chromatin in mammalian genome [13]. It is comprises of four exons in which exon 3 is the biggest one, spans 1084 base pairs and encoding 486 amino acids, holds two functional domains; 84 aa Methyl CpG-restricting Domain and 104 aminoacids Transcription repression domain. Basically MECP2 encodes two key isoforms MECP2-E1 and E2. Exon 2 is joined with MECP2-E1 and initiate translation, ensuing in a diverse N-terminal end at exon 1. The two isoforms are identical in both domains [14]. Initially the MBD ties to cytosine deposits residues symmetrically to CpG dinucleotides situated at promoter site and later on facilitate transcriptional silencing after the completion of DNA methylation [15]. The TRD interacts with histone deacetylase and SIN3A and interaction causes deacetylation of core histones follow-on transcriptional repression.

Figure 1. Here is the graphical representation of the chromosome contain MECP2 gene [7]

Risk factors

Hereditary and non-hereditary components contribute similarly and equally for MECP2 mutation. Non-genetic factors incorporate nutritional and environmental factors, poisonous exposure at some stage in pregnancy and some of socio-economic factors for example; level of income, own family situations and cultural surroundings ought to be a contributory role in prevalence of neuro developmental illnesses. The phenotypic articulation of Rett is X-inactivation pattern which is an ordinary and random process in females because one X chromosome is turned off. Females have equal number of paternal as well as maternal active X chromosome and in rare cases show nonrandom or skewed inactivation (XCI). This pattern can significantly impact phenotypic characteristics, bringing about a varied degree of severity such as a normal MECP2 gene to an extreme abnormal atypical Rett syndrome whenever altered gene is active [16].

A choicest degree of efficiently folded and tailor-made MECP2 is mandatory for its role in instruction of transcription and either slight or a lot may result with pathogenic penalty [17]. Consequently disturbance of the supply in nucleus via defective co/ post-translational N-terminal modification leads to sooner degradation and can cause deregulations of many other genes, ultimately leads to Rett phenotype [14].

Causes

A genetic change of MECP2 gene causes Rett [18]. MECP2 related disorder is from rising class of genetic diseases and over 200 known mutations in Rett are because of defects in epigenetic regulation. However the mutations in a particular gene aren't definitive for the diagnosis of Rett but 95% of diagnosed individuals have been observed with mutations inside the gene encoding transcriptional regulator [19]. The excessive recurrence of cytosine to thymidine

transitions shows that the deamination of methylated cytosines is not an unusual purpose of Rett whilst spontaneous deamination is likely to make a contribution to the high frequency of methyl-cytosine transitions to thymidine. Number of elements has been observed to accelerate deamination, such as, cytosine protonation because of unusual base pair formation or modification [20].

Almost 80% of MECP2 mutation present in exon 3 [21]. To give bit of knowledge about mutated MECP2 locus [22] looked into 48 girls with classical sporadic Rett, seven families with conceivable familial genetic changes and five sporadic with mild phenotypic characters, however no longer diagnostic for Rett. They used Long Distance PCR and Long-read Direct Sequencing techniques to sequence the coding regions. In all instances of recognized mutations, forty-four out of fifty-five had been classical sporadic or familial Rett sufferers and just 20% cases were non-demonstrative highlights. Additionally they found considerably few symptoms in cases hauling missense mutations as compare to those with nonsense and frameshift (truncating) mutations [23].

Sixty-five to 80 percent of Rett sufferers have mutations in exon 3 or 4 in related gene. In spite of enormous number of changes distinguished, eight very commonplaces i.e. R106w, R133c, T158m, R168x, R255x, R270x, R294x and R306c represent dominant part of transformation in girls [16, 24].

In this era of molecular diagnosis the exact mechanism with the aid of which MECP2 dysfunction causes Rett Syndrome is obscure; however mounting proof indicates that neuronal fitness and improvement rely upon precise law of MECP2 expression [25]. Examination of brain uncovers profound microencephaly, densely packed neurons, neuronal dystrophy in cerebral cortex and reduction in dendritic arborization [18].

Accordingly heterozygous mutant girls show mosaic pattern for MECP2 deficiency with huge variety of phenotype while guys progressively demonstrate severe phenotypes and rarely live beyond 2 years. So by the age of 4-6 weeks, progressive physical deterioration starts and individual typically die at ~10-12 weeks. In addition, neurons of Rett people seem to demonstrate a decrease in dendritic maturity. Furthermore, dendritic arborization and dendritic back bone density is decreased in several brain regions as well [26-28].

Generally cerebrum size can be reduced by as much as 34%. Young Rett sufferers have expanded thickness of GABA receptor inside the caudate that reduces with age such as ionotropic glutamate receptor i.e. AMPA and NMDA are uniquely decreased in basal ganglia in patients of more than eight years old [19, 29]. Ubiquitously expressed MECP2 mutation, leads to various phenotypes and its incidence ratio in males is 1-2% only and usually leads to lethality [30].

Basically Rett syndrome is evolved in four typical tiers. First degree is stagnation period with onset between 6-18 months and lasts weeks to months and is characterized by formative capture without relapse. Second stage begins among 1-4 years old and is described by way motor and language skills. Stage 3 is pseudostationary can also takes place for a considerable length of time where patients may improve communication but motor function get worsen. In stage four, loss of ambulation is observed however there is no abrupt progress from one to other stage [31, 32].

Globally lot of research work is done on genetic causes of Rett such as [33] presented mutation heterogeneity in Salvic population in 2007 and identified 78.16% classic Rett patients. The transversion 323>A in exon 3 prompts substitution of Lysine to Histidine at function 108, lies within MBD and affect methyl specific binding [34]. At position 302

the 904>T change causes substitution of proline with serine ought to meddle with get together of transcription silencing complex, cancelling connection with Sin3A co-repressor and histone deacetylase recruitment. The frameshift mutations in exon three that are 189-190delGA deletion with threonine at position 63 and another missense mutation of 17bp deletion 816-832del17 in TRD makes stop codon. A mixed cancellation /reversal change 1063-1236del; 1189-1231inv 43 became additionally affirmed by cloning of influenced vicinity of methyl binding proteins. Majority of missense changes have been positioned in MBD while nonsense situated from end part of MBD to TRD and cancellations were generally found for most part in C-terminal place. The eight most regular changes i.e. 316C >T, 397 >T, 473C >T, 502 >T, 763C >T, 808C >T, 880C >T, 916C >T representing for greater than half of Rett located at CpG [35].

Prevalence

Rett syndrome initially was perceived as a neural disease in 1966 by Austrian pediatrician Andreas Rett and after the dynamic work on this disease it was recognized as genetic disorder in 1982 worldwide, e.g. Table 1 and most common reasons of mental retardation among women [25, 36, 62]. Rett syndrome (RS) common features are normal psychomotor advancements until first six months of life accompanied via cognitive impairment, lack of satisfactory and gross motor skills, lack of communicative abilities and acquired microcephaly. Individuals with Rett ordinarily reach into middle age and they will continue to exist even longer [28, 37]. This neurodevelopmental disorder had an expected prevalence of 1 in 10,000-15,000 girls beginning with maximum cases being sporadic [38, 39].

Because of the high proportion of consanguinity in Asian countries the

prevalence of neurodevelopmental disorder is three folds higher in underdeveloped countries as compare to Western countries [40]. Consanguinity is an important aspect to determine the birth defects including intellectual disability with relatively high prevalence of autosomal recessive forms. Consanguineous marriage is defined as a

relationship between two persons having common ancestor [41]. According to a report 2011 10.4% of all marriages crop up in family worldwide while its fraction is much higher in Arab and central Asian population. In Pakistan its rate is >60% with 17-38% as first-cousin marriages [42].

Table 1. Across the world Rett prevalence from 1982 to 2006 [62]

Country	Study Year	Classification of Rett syndrome cases	Prevalence per 10,000 females	Prevalence 95% CI	Age range
Southwest Sweden	1982	Classical just	0.65	0.25-1.05*	6-17
West Scotland	1982	Classical just	0.69	0.41-0.99*	3-15
Southwest Sweden	1982	Classical just	0.84	0.38-1.30*	6-17
Switzerland	1967-1982	Classical just	0.41	0.25-0.56*	4-19
Northern Italy	1973-1983	Classical just	22.03	7.2-51.3*	4-12
Tokyo, Japan	1988	Classical, atypical	0.50	0.32-0.68	6- 14
North Dakota, USA	1989	Classical just	0. 53	0. 06-0. 96	0- 18
Northern Tuscany	1978- 1990	Classical, atypical	2. 1	0. 04-4. 16	3- 14
Furkui prefecture	1993	Classical just	0. 22	0.05-1. 13*	6- 14
Estonia	1993	Classical just	0. 66	0.24-1. 07*	0- 15
Australia	1994	Classical, Atypical	0. 67	0.55-0. 79	5- 18
Norway	1995	Classical, atypical	2. 17	1. 87-2. 59*	3- 19
Texas, USA	1990	Classical, atypical	0. 58	0. 36-0. 53	2-18
France	2004	Mutative positive	0. 58	0. 51-0.66	4-15
Australia	2004	Classical, atypical	0. 88	0. 83- 93	5-18
Hong Kong West	2006	Classical just	0. 57	0. 15-0. 98	≤ 35

*Confidence intervals were not presented in Australia in 2004 but data was calculated given in article

Diagnosis

The diagnosis in MR requires multidisciplinary exertion yet investigation begins with parental record that mainly includes tetragenic exposure such as alcohol, cigarette and drugs [43]. Maternal illnesses together with myotonic dystrophy, pregnancy history, amniotic liquid sum and fetal utero hypotonia are also taken into account. Family ancestry provides important insights for MR, such as miscarriage provide evidence for X-connected impediments as well as

psychological signs may be considered in patient diagnosis. American College of Medical Genetics recommendations are presented for screening of 20-29- metabolic disorder in newborn. Physical examination highlights mainly center around assessment of dysmorphic neurological abnormalities and behavioral phenotype, for example, hand-wringing of Rett patients. Magnetic Resonance Imaging (MRI) indicates cranial shape variations such as microcephaly, macrocephaly and neurological

manifestation. Molecular testing is also a useful diagnostic tool for prenatal analysis for family. Variability in diagnosis is based on differing populace based investigation, age of patient and the time of study [44-46].

Modern mutation detection assays analysis show 97% of individuals with typical Rett. So a consortium was established in 2006 by experts from 13 different countries to address the confusion regarding diagnosis of Rett by the collaboration of International Rett Syndrome Association (IRSA). At present it is supported by International Rett Syndrome Foundation, an emerged foundation established by collaboration of IRSA and RSRF Rett Syndrome Research Fund [47]. As per IRSA, main criteria of primary Rett are incomplete and complete loss of hand aptitude, verbally expressed language and impaired gait abnormalities. Exclusion criteria for a typical Rett: neurometabolic disease due to severe infection, hideously strange psychomotor progress in first 6 months of life, precariousness in breathing, bruxism when conscious, impaired rest patterns, irregular muscle tone, fringe vasomotor unsettling influences, scoliosis/kypnosis, development hindrance and undersized cold hands and feet, ill-advised giggling/ spells, decreased reaction to torment, and extreme eye dispatch [48].

In 2005 team of scientists, exhibited the characteristic testing and learning on genotypic-phenotypic relationship of recognized transformation with extraordinary weight on their importance to far reaching screening programs in presymptomatic people. According to them testing measures should be readily valid, technically straightforward and consistent with high detection speed, the most minimal conceivable recurrence of lowest possible frequency of false-negative and false-positive outcomes [39].

Pre-birth screening is structured at distinguishing pregnancies at expanded

dangers of inborn or chromosomal variation from a norm utilizing a noninvasive methodology, for example, investigation by biochemical markers in maternal serum and ultrasound finding to spot fetuses with transformation. However the invasive diagnostic procedure includes; check the level of alpha-fetoprotein to find out the risks of neural cylinder and imperfections of ventral wall, pregnancy related plasma protein A (PAPP-A) β -human chorionic gonadotrophin joined with extended nuchal translucency on ultrasonography. It is also essential that all prenatal diagnosis must be gone before by non-directive hereditary advising and educated assent or informed consent.

An invasive prenatal diagnostic procedure (DNA-based mutation analyses) should offer to the parents of an infant with Rett syndrome and an identified MECP2 change that has an estimated approximately 1% recurrence rate. A thorough pedigree and careful history to identify the family members at risk are also vital.

Treatment

Henceforth, this field does not have a live human model that give endless supply of neurons so investigation could be executed in controlled circumstances. In addition, hardened tissues portions are of constrained use for neural system. Brain imaging does not allow studying subtleties of short hardware in cerebrum. Indeed, even creature models frequently do not reiterate complex human ailments. In 2012, team of scientists [49] created human neurons from somatic cells by induced pluripotent stem cell (iPSC) technology. They reprogrammed substantial cells into pluripotent position by over exploitation of explicit genes. Isogenic pluripotent cells (iPSC) demonstrated striking and comprehension of multifaceted illnesses with heritable and sporadic setting. On account of the high fraction of consanguinity in Pakistani population,

Homozygosity mapping is a perfect system to distinguish mutated genes responsible for Non Syndromic Autosomal Recessive Intellectual Disability (NS-ARID). Ultimately, this would be helpful to screen transporters so as to diminish quantity of influenced babies in a family [50].

The system used to display Rett disorder, utilizing mutated female patients with X-connected influenced genes as the age of isogenic cell lines had the advantage of x-inactivation in iPSC clones that hold freak just as wild-type. However, challenges are greater where mutations are unknown because of sporadic cases [51, 52]. In their investigation, they got neurons from Rett iPSC conveying four assorted MECP2 changes with a few adjustments in contrast to five non-affected people, for example, reduced soma measure, and altered dentritic spine mass and compact neurotransmitters.

Besides, to demonstrate causality, these phenotypes were endorsed against MECP2 in increase and loss of limit preliminaries utilizing wild type MECP2 cDNA and particular shRNA. Most importantly, these cellular defects were approved via self sufficient groups, revealing the qualities and reproducibility of the framework and in like manner prepared to save the deformities in the quantity of glutamatergic synapses [53] by two competitor drugs Insulin-like Growth Factor1 and gentamicin. The former one is reviewed as possibility for pharmaceutical treatment of Rett and potential issues of persistent clinical fundamentals while gentamicin was additionally accustomed to ensure neurons having a nonsense MECP2 alteration by raising its protein level [54-56]. The iPSC system offers some of confinements; cells in culture represent research artifacts that may miss or overestimate the important signaling information, mask possible cellular phenotypes or can create artificial ones. To overcome the other challenges of the descent

of the relevant neuronal subtypes, unambiguous reporter genes need to be used with further optimization setup [57, 58].

In 2013, Chapleau and colleagues reviewed specific aspects of treatment plan based on clinical trials with already accepted US-FDA approved medication. Nutritional issues were a highlighted in the treatment of Rett as 25% of patients feed by gastrostomy to meet high metabolic demands of body but was a difficult decision for caregivers. Gastrointestinal issues including constipation and gastroesophageal reflex could be treated by polyethylene glycol 3350 or milk of magnesia. Moreover esophageal irritation and acid secretion prevented by use of H2 receptor blockers or protein pump inhibitors. The use of antiepileptic drug lamotrigine had been shown to be profoundly successful in looking after seizures. Another approved drug for its affects on motor dysfunction was the opiate antagonist naltrexone. The aminoacids derivate L-carnitine was found to be useful in rest support and to improve correspondence capacity. Folate supplementation was proposed in Rett for a number of reasons because of its capacity to methylate segments of DNA. But results and findings of the study presented essential considerations for future clinical trials such as disease rigorousness, age and mutation type that would offer great impact on data analysis [28, 59].

Discussion

The detailed review of the research work done worldwide shows that MECP2 function is not vital for normal brain development and neuronal vow although is required for institution and continuance of normal neuronal function. Some definite aspect of neural maturation for which MECP2 is required remain to be determined [60].

It is important to take note of that because of verity in X-inactivation design in females with same MECP2 mutation may has diverse phenotypic highlights and severity and

believed that these modifications are influenced probands acquired from non-penetrant guardian are very conceivable for MECP2. Among variations distinguished either because of rehash extends bringing about length changes of polyanaline, missense mutations or regions just upstream of the start codon along these lines possibly influencing the capacity just as the articulation of neurological issue [61].

This is not amazing as run of the mill patients can be either pretty much seriously influenced than classical patients. In this way, it is trusted that medicinal region would probably get familiar with the trial of counseling, development and valuable assessment of kids distinguished by presymptomatic screening if a quicker and less expensive test winds up available [39].

The currently used diagnostic tests will have to be improved and new psycho-diagnostic tests should be created to calibrate the distinctive parts of cognitive processes in patients with mental retardation linked with X chromosome. The genotypic-phenotypic studies at present will offer point by point physical neurological examination and used to incorporate extra knowledge about possible mechanisms involve in cognition, learning behavior and brain development. Moreover this study would highlight dimensions to be improved among molecular techniques and tools such as, biochemical analysis, neuropsychological and behavioral testing, joined with Electroencephalography, Computerized Tomography or Magnetic Resonance Imaging and Single-photo Emission Computed Tomography Imaging of brain [30].

A definitive likely profit by unfavorable le finding and treatment is a standpoint amongst the most basic thought range from sparing the lives of influenced infants to improve their prognosis, excellence of life and neurodevelopment. Furthermore, the screening must be cost compelling, helpful

and extensively available from general wellbeing perspective. Based on these particular standards criteria for testing would be fulfilled some but not from all aspects of child testing. The ordinaries of Rett disorder is of around 10,000-15,000 females makes it a sufficiently noteworthy problem to justify open fixation worldwide [39].

Conclusion

In human neuronal maintenance and maturation is disturbed by a kind of alteration in methyl-CpG binding protein 2 shortly known as MECP2 and leads to Rett syndrome. This MECP2 gene mediates transcriptional repression and chromatin condensation because of the methylated Cytosine residue binding to genomic DNA. Since yet, there are no obvious treatments available. So universal newborn or prenatal screening is a big challenge in this modern era, until then secondary genetic testing and other warning signs based on behavior should be preferred substitutive approaches for this purpose. Though preclinical research on creature models of Rett syndrome makes the presymptomatic testing worthwhile but prenatal diagnosis in females with a family record is noticeable. Great care need to be exercised in this diagnostic and counseling domain as it raises sensitive ethical issues. A comparative view on the research work has done worldwide highlights its importance as a serious genetic disease. That is why; to devise targeted therapeutic strategies for Rett patients is ultimate goal and a step closer to complete the whole picture. All in all consanguineous carrier screening would create opportunities to discover disease causing mutations, thus providing huge benefit to families, communities and to public health.

Authors' contributions

Conceived and designed the experiments: T Hussain, Performed the experiments: MF Nasir, Analyzed the data: A Saeed & T Hussain, Contributed materials/ analysis/

tools: A Saeed, MF Nasir, Wrote the paper:
A Saeed, MF Nasir & T Hussain.

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