

Review article: Breaking new ground with Rett syndrome

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Introduction

Awareness of Rett syndrome (RS) among clinicians in English-speaking countries was prompted by a publication by Hagberg *et al.* (1983) – almost two decades after the condition was described by Andreas Rett in Vienna (Rett 1966). Much was therefore already known about its clinical and pathological characteristics before the discovery by Amir *et al.* (1999) that the syndrome results from mutations in the *MECP2* gene, located at Xq28. This discovery has led to a further rapid increase in understanding of the pathogenesis of the disorder. It is now apparent that mutations within the coding region of *MECP2* are responsible for more than three quarters of cases with classic (typical) features of RS and close to half of those with atypical features, including some males (Shahbazian & Zoghbi 2002). Mice have been developed with equivalent mutations on the corresponding gene *Mecp2* (Guy *et al.* 2001; Chen *et al.* 2001) and there are new insights into the disturbed cellular processes responsible for this severe neurodevelopmental disorder, to the extent that testable hypotheses about therapeutic interventions are emerging.

Clinical aspects

The clinical criteria for diagnosis of the RS have been progressively refined by experience (Hagberg *et al.* 1983; Kerr & Stephenson 1986; Diagnostic Criteria Working Group 1988; Kerr *et al.* 2001a) and the advent of genetic diagnostic testing has strengthened confidence in their specificity. Linking information from patient surveys and molecular genetic testing indicates a wide range in severity while the profile of disability is remarkably consistent (Kerr *et al.* 2001a,b; Kerr & Witt Engerstrom 2001a). The acute period of regression which usually occurs in the second year of life led to the initial assumption that the child was functioning normally beforehand. However, a series of studies have established that indicators of delayed development are often present from birth (Kerr 1987, 1995; Naidu 1997; Leonard & Bower 1998, Kerr & Witt Engerstrom 2001a; Burford *et al.* 2003). The newborn looks normal but is usually placid with disturbances of muscle tone, posture and movement. Although there is initial developmental progress, fine manipulative skills commonly fail to appear and language development seldom progresses beyond single words. The characteristic regression phase with decline in skills may be absent in the mildest cases or may be so early as to be overlooked in the most severe cases (Kerr *et al.* 2001b). By 5 years, the condition has usually stabilized and brain growth continues (Percy 1992; Kerr 1995; Hagberg

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et al. 2000) with some learning possible (Kerr & Witt Engerstrom 2001a). Following the phase of regression, each of the cardinal features of RS may be mild or severe, including intellectual disability, brainstem cardio-respiratory dysregulation, disturbances of muscle tone, limb contractures, scoliosis and reduction in linear and brain growth. Epilepsy may be severe or absent.

Survival into adult life is common and in the UK the death rate is 1.2% per annum with 50% of deaths occurring in severely malnourished people (Kerr *et al.* 1997). Twenty per cent of deaths are sudden and unexpected in otherwise robust people, with evidence mounting that disturbed cardio-respiratory regulation plays a significant role in these premature deaths (Kerr *et al.* 1997; Julu *et al.* 2001). The same regulatory disturbance, possibly within the brainstem, may also account for early deaths in the severely affected males (Kerr *et al.* 2001b).

Before the causative link between *MECP2* mutations and RS was established, the prevalence of classic RS among females was estimated to be at least 1 in 10,000, lower among males (Kerr 1992, Hagberg & Hagberg 1997). The increased ascertainment of cases and the wider clinical spectrum associated with *MECP2* gene mutations will lead to adjustment of this estimate (Hagberg & Gillberg 1993).

Current mutation testing for Rett syndrome

It is becoming apparent that the sensitivity of laboratory testing for RD can and must be improved beyond its current level. Although no mutation is identified in the *MECP2* coding region in up to a quarter of cases of classic RS, genetic laboratories worldwide have been experiencing a substantial demand for *MECP2* screening simply because of the clinical usefulness of a detected mutation, which confirms the clinical diagnosis and eliminates the need for further diagnostic evaluation. Unfortunately, a wildtype result is much less useful, especially as 'false negative' results may further delay diagnostic confirmation. In this context, there is mounting evidence that there are other molecular lesions which produce phenotypes that sometimes overlap with RS, most notably Angelman syndrome (AS). Provision of a negative test result can be disappointing and unsettling for parents of children with a long-standing clinical diagnosis of RS, as well as to

their doctors. It is important therefore that the over-all performance of RD diagnostic testing, especially test sensitivity, is improved.

While the current mutation screening methods being applied to *MECP2* in diagnostic laboratories worldwide are focused on detecting all heterozygous nucleotide variants in the coding region of the gene, it is becoming apparent that these are not the only disease-causing lesions within *MECP2* and that testing for RD can be improved further, although there are no data yet available that reveal to what extent test sensitivity can be improved. The range of published mutations has increased to include deletions that extend beyond the genomic positions of the commonly used polymerase chain reaction primers (Cheadle *et al.* 2000; Bourdon *et al.* 2001a). Mutations in mosaic form have been reported in both males and females (Clayton-Smith *et al.* 2000; Armstrong *et al.* 2001; Bourdon *et al.* 2001b; Topcu *et al.* 2002). Finally, comparison of the human *MECP2* genomic sequence with the mouse equivalent reveals strikingly high levels of evolutionary conservation in large non-coding tracts of gene. Review of the primer locations used by groups reporting *MECP2* mutations reveals that none have been routinely assessing these conserved tracts. It is possible that inclusion of these conserved regions in routine diagnostic screening will contribute to an improvement in test sensitivity for RD, although the challenge in this work will be to demonstrate unequivocally whether or not a non-coding variant is pathogenic. Finally, there is mounting evidence for the involvement of at least one other gene. However, clearly mutations in genes other than *MECP2*, if found, would be responsible for only a minority of cases. That other genes may be involved in the aetiology of RD is not surprising now, especially in view of the range of proteins that contribute to the transcription repression process mediated by MeCP2 (Shahbazian & Zoghbi 2002).

A wider phenotypic range

Beyond the RS phenotype, other presentations reported to be associated with *MECP2* pathology now include cases previously diagnosed with mutation negative AS, autistic disorder, mild intellectual impairment and non-progressive tremor also normally functioning carrier females identified from family investigation. Among males, *MECP2*-linked

disorders range from a fatal neonatal disorder, the severest RD manifestation (Kerr *et al.* 2001b), through to non-specific mental retardation (Hammer *et al.* 2002). Milder *MECP2* mutations have also been identified in families with X-linked intellectual disability associated with spasticity and movement disorder. Early onset psychosis, both childhood onset schizophrenia and X-linked recessive manic depressive psychosis, have been reported among those with a recurrent missense mutation (A140V) located within the methyl-binding domain (Klauck *et al.* 2002). Our experience with the British cohort (1143 cases) suggests that the RS phenotype, mild or severe, is associated with different *MECP2* mutations to those reported in non-RS cases (e.g. Klauck *et al.* 2002). The broad range of phenotypes associated with *MECP2* gene dysfunction, together with increased ascertainment of cases owing to gene testing indicates that *MECP2* mutations may contribute proportionately much more than is currently appreciated to profound combined intellectual and physical disability, especially in females.

Advances in genetic knowledge

At the molecular level, *MECP2* is involved in the regulation of many other genes. The protein product, MeCP2, is one of five methyl-CpG-binding proteins, four of which influence gene expression through transcriptional repression (Hendrich *et al.* 2001; Nan & Bird 2001; Wade 2001). These proteins, which recognize methylated DNA, recruit corepressor complexes containing histone deacetylase (HDAC) to effect gene silencing (Bird & Wolffe 1999). MeCP2 binds to methylated chromatin (Nan *et al.* 1997) at a highly conserved methyl-binding domain, allowing the transcription repression domain to activate the repression mechanism that acts through pathways mediated by several corepressors, including mSin3A (Jones *et al.* 1998; Nan *et al.* 1998; Kokura *et al.* 2001). MeCP2 has the unique ability to bind a single symmetrical pair of methyl-CpG dinucleotides (Lewis *et al.* 1992) and the intracellular distribution of MeCP2 parallels that of 5-methyl cytosine (Nan *et al.* 1996) which is located in all chromosomal regions, particularly those with high levels in transposon-derived repeats, in the inactive X chromosome and at imprinted loci (Lander *et al.* 2001).

Insights into features common to Rett syndrome and other clinical entities

In the light of recent discoveries, it is now possible to consider whether there is a molecular basis for the clinical features that RS shares with AS autism, a-thalassaemia mental retardation syndrome and aspects of Prader-Willi syndrome (PWS). The major clinical consequences of impaired MeCP2 function are believed to arise from loss of HDAC activity, with resulting persistence of acetylated histones H3 and H4, (Wan *et al.* 2001; El-Osta *et al.* 2002). The consequence of this is excessive expression of many genes, including those that are imprinted (Drewell *et al.* 2002). The molecular basis of AS involves an imprinted locus on chromosome 15 (15q11-q13) and it seems possible that downstream effects of impaired HDAC activity in this imprinted region may contribute to some clinical features of RS. A recent report of a male with a missense *MECP2* mutation, who has clinical features reminiscent of PWS (Kleefstra *et al.* 2002) is of interest, as PWS also occurs as a result of imprinting disturbances within the 15q11-q13 locus. Could it be that a disturbance of expression of paternally derived genes in the 15q11-q13 region is responsible for the occurrence of obesity in some people with RS? Interestingly, some of the *Mecp2*-null mice (Guy *et al.* 2001) developed an obvious increase in deposited fat, which was dependent on the genetic background. This suggests that modifier genes may mediate the effects of *Mecp2*, at least on body weight.

Neurophysiological consequences of *MECP2* mutations

Although *MECP2* is active in cells throughout the body, the clinical effects of mutations are most apparent in neuronal tissue, and particularly in the function of mature neurones (Chen *et al.* 2001). Brain volume is moderately reduced with neurones that are smaller and more densely packed than usual. Dendritic territories are reduced in some pyramidal neurones in the frontal, motor, temporal and hippocampal cortex (Armstrong 2002). There is a reduction in cerebrospinal fluid (CSF) brain-derived neurotrophic hormone (Riikonen 2001). There is a selective reduction in immunostaining for microtubule fibrillary protein MAP2 throughout the neocortex. This agent contributes to the early development and

mature function of neurones (Kaufmann 2001). Glutamate receptor staining is greatly increased in the brain, in early childhood only (Blue *et al.* 1999). There are reports of elevation of CSF glutamate (Lappalainen & Rikkonen 1996) and reduced cortical cholinergic innervation (Wenk & Hauss-Wegrznjak 1999). There is a reduction in substance P immunoreactive staining (Deguchi *et al.* 2000) and a huge increase in serotonin receptor binding in the brainstem (Armstrong 2002). Although it is still uncertain how and when the disease process starts, these findings and the early clinical signs suggest onset before birth (Nomura *et al.* 1997; Guy *et al.* 2001; Kerr & Witt Engerstrom 2001b). Mice with *Mecp2* mutations have a similar pattern of disease manifestation with an initial period of developmental progress followed by regression (Chen *et al.* 2001; Guy *et al.* 2001). However, in the mouse, the early developmental progress continues until after the first litter has been delivered. MeCP2 expression is greater in mature than in immature mammalian neurones or in glial cells and this has prompted the suggestion that the normal developmental increase in MeCP2 expression may act to protect the function of mature neurones by repressing genes that are important during brain-stem development but which become deleterious as neurones mature (Shahbazian *et al.* 2002).

The role of X inactivation

Clinical-molecular correlations which have emerged since the discovery of *MECP2* mutations are yielding further insights on the range of severity associated with *MECP2* mutations in RS. With *MECP2* located on the X chromosome, it is not surprising that X chromosome inactivation (XCI) plays a key role. Classic RS arising from a heterozygous *MECP2* mutation is, in fact, an attenuated phenotype, which reflects functional mosaicism at the cellular level. When a girl has inherited a *MECP2* mutation, cells express either an impaired MeCP2 protein from the mutant gene or a fully functional protein from the wildtype *MECP2* allele, the choice having been imposed by the biological processes initiating XCI in early foetal life. The fatal neonatal RD in males demonstrates the full clinical effect of total loss of MeCP2 protein function when the mutation is present in a hemizygous state (Kerr *et al.* 2001b). Skewed XCI with gross asymmetry in the proportions of wildtype

and mutant allele inactivation may thus result in a more severe or milder form of the disease, depending on whether the wildtype or mutant allele has been preferentially inactivated (Kerr *et al.* 2001b; Hoffbuhr *et al.* 2002). People with relatively mild RS in whom *MECP2* analysis and XCI studies have been completed are of particular interest. Among those with the milder R133C mutation, there has been no evidence to suggest that the mild phenotype is owing to skewed XCI. By contrast, marked skewing of XCI is present among those milder cases with the more severe T158M mutation (Hoffbuhr *et al.* 2001; Nielsen *et al.* 2001; Zappella *et al.* 2001), presumably with preferential inactivation of the mutant allele.

Issues of counselling

The question of recurrence of RD is a key issue for families. The empirically based recurrence estimate before *MECP2* gene testing became available was less than 1 in 300 (Killian 1986) and the emerging molecular data are in accord with that earlier empirically based recurrence risk estimate.

The accumulation of published reports over the past 3 years of screening for mutations in parents of girls with a *MECP2* mutation confirms that the great majority of *MECP2* mutations causing RS arise *de novo*, and are not present in DNA extracted from maternal blood. Consistent with this is the observation that approximately 90% of RS-causing mutations in *MECP2* occur within the paternally inherited gene (Cummings 1986; Girard *et al.* 2001; Trappe *et al.* 2001). It is noteworthy that in the few families in which RD has recurred, most transmitting females have been asymptomatic (Amir *et al.* 1999, 2000; Wan *et al.* 1999; Bienvenu *et al.* 2000; Villard *et al.* 2000; Hoffbuhr *et al.* 2001; Ishii *et al.* 2001; Zappella *et al.* 2001). A small number of these mothers with more than one affected child testing have mosaicism, demonstrated by testing DNA from a peripheral blood sample (Amir *et al.* 1999; Villard *et al.* 2000). Among those few transmitting females found to harbour an inherited germline mutation, inactivation studies have revealed skewing of XCI, which has protected them from the deleterious effects of the mutation (Wan *et al.* 1999; Villard *et al.* 2000).

Despite a recurrence risk of well under 1%, genetic diagnostic laboratories offering *MECP2* gene testing are experiencing a steady demand for testing blood

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samples from mothers and sisters of people with RS in whom a mutation has been identified. This is understandable because of the clinical severity of the disorder and the high risk of recurrence in a few cases. It is important, however, while offering testing for anxious members of the family, to ensure that families and their advisers are aware of the low overall recurrence risk.

Discussion

The genetic contribution to certain key clinical observations in RD deserves careful investigation and we suggest that this may lead to real therapeutic advance.

Through its highly selective effects, the RD throws light on the genetic programmes for brain development. During the early months of life, the child with RD displays developmental progress simultaneous with developmental failures, suggesting that different neural functions vary in their dependence on *MECP2*. At this time of life, the normal cortex is subject to major changes with huge synaptic development and programmed removal of many neurones, permitting fuller development of the infrastructure for cortical control. The brain in RS seems peculiarly ill-equipped to sustain this development.

Developmental demands may pick out the infrastructures which are most dependent on a responsive energy supply and we question whether the RD affected neurones can support the additional metabolic stress of normal major infancy developments. Sudden deaths reported to the British Survey suggest that in spite of their long survival, people with RD are poorly equipped to survive particular kinds of stress (Julu *et al.* 2001).

The distinctive rhythmic stereotyped movements suggest escape of underlying oscillatory motor rhythms from higher controls (Wright *et al.* 2003). A better understanding of the processes and mechanisms underlying such clinical observations may provide leads in the search for ways to treat or ameliorate RD and other disorders of brain development.

Particular skills and capabilities are predictably retained, even in the most severely affected people with RD and one outstanding feature is the universal enjoyment of music. Families spontaneously report that each person demonstrates distinct musical preferences which change over time. In music therapy, personal interactions can be seen to develop over a

series of sessions with active choices being made (Kerr 1987; Merker *et al.* 2001; Elefant 2002). Recent brain imaging indicates that the reception of music normally activates a prefrontal 'nexus' area linking emotional, cognitive and motor processing (Janata *et al.* 2002). The neuronal infrastructures required for the mind to accept and respond to music may be intact in RD constituting a valuable resource when other avenues are closed to the individual, a means by which the therapist may access the plastic capacities of the central nervous system and facilitate learning.

It is clear that the *MECP2* gene has an important role in many aspects of the development and mature function of neurones and their connectivity and that a range of different mutations leads to a number of neurological presentations in addition to the RS. Authors reporting such cases should provide full clinical descriptions in addition to any diagnostic labels to facilitate the investigation of the pathogenetic links (Kerr *et al.* 2001a).

Although the pathogenesis of RS is still only partially understood, enough is known to hypothesize about novel therapeutic interventions. An affected girl with a *MECP2* mutation has an accompanying normal *MECP2* gene in every cell – as has been so vividly demonstrated by those few healthy mothers who have a germline mutation and favourable skewing of XCI. Will it ever be possible to override the foetal programming of XCI and induce selective *MECP2* gene expression from the wildtype allele only? It is an alluring possibility although much more will need to be learned about the biology of XCI in humans before this can be attempted.

A promising start has been made to pharmacological intervention in RS by modifying some aspects of the abnormal respiratory rhythm (Julu *et al.* 2001) and trials are proceeding aimed at compensating for the neurotransmitter abnormalities (Percy 2002). However, until the results of such studies are known, it is advisable to limit routine medication to anticonvulsants for undoubted epilepsy and therapeutic doses of supportive vitamins.

Conclusion

There are many remaining unanswered questions about RD and about the role of *MECP2* in the devel-

opment and function of the normal brain and in other disorders now associated with *MECP2* mutations. We suggest that the answers to these will throw light on many presently unknown aspects of brain development and mature function. The Rett disorder is indeed a remarkable paradigm for understanding the development of the brain.

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