Bone-Related RTT

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Rett syndrome (RTT) is a monogenic neurodevelopmental disorder primarily caused by mutations in X-linked MECP2 gene, encoding for methyl-CpG binding protein 2 (MeCP2), a multifaceted modulator of gene expression and chromatin organization.

MeCP2 bone mineral density bone metabolism osteoblast WNT pathway

1. Introduction: Rett Syndrome

Rett syndrome (RTT; OMIM ID 312750) is a devastating neurodevelopmental disorder, which mainly affects female subjects with a prevalence rate of approximately 1:10,000 live births [1][2][3][4][5]. RTT represents the second most common origin of intellectual disability in females [6]. In its classic form, after a period of normal development and growth for 6–18 months, developmental stagnation and regression of acquired psychomotor skills occur (e.g., loss of purposeful hand skills and replacement with incessant stereotypic movements), followed by motor impairment, severe mental retardation, seizures, ataxia, and autistic features [6].

The primary causes of RTT in approximately 95% of patients are de novo mutations at the X-linked MECP2 gene, which encodes for methyl-CpG binding protein 2 (MeCP2), a genome-wide epigenetic modulator responsible for activating/repressing gene transcription, modifying chromatin compaction, and regulating RNA and miRNA processing [7][8]. MeCP2 is widely expressed throughout the body [9][10][11][12][13][14], with abundant levels in the central nervous system, in particular in neurons, but also in microglia, astrocytes, and oligodendrocytes [15][16][17][18]. Moreover, the X-chromosome inactivation, a known mechanism of gene dosage compensation, leads cells and tissues of RTT patients to show a mosaic pattern for MECP2; hence, the ratio between the wild-type and mutated versions of MeCP2 protein can in part be responsible for the severity of the disorder phenotype [19][20][21][22]. Therefore, although RTT is considered a monogenic neurologic disorder, it presents a highly complex nature, which reflects the wide range of both cellular/systemic alterations and co-morbidities, indicating the importance of MeCP2 expression even outside the central nervous system.

RTT is now considered a multisystem pathology, and common co-morbidities include periodic breathing disorder, various sleep disturbances, abnormal pubertal development, electrocardiograms with prolonged cardiac QT interval, numerous gastrointestinal disorders, osteopenia, and scoliosis [23][24][25][26][27][28]. Surgery for correction of scoliosis is widely performed on girls with RTT. Moreover, several efforts are oriented towards the study of bone mass, bone health, and fracture occurrence in RTT [29][30][31], although the molecular mechanisms underlying the altered bone status in RTT patients are not well clarified yet.

2. Clinical Aspects of Altered Bone and Mineral Metabolism in RTT

Twenty years after Naidu and colleagues evaluated 70 RTT female patients between 2 and 34 years of age for different clinical parameters and progressive scoliosis was detected in 25 of these patients, in particular in non-ambulatory girls, and this clinical feature was not associated with the age [32]. In addition, Keret et al. found accelerated progression of scoliosis with mainly thoracolumbar curve patterns in 8 of 10 RTT patients [33]. In addition, phenotype–genotype correlations revealed a clear association between more severe MECP2 mutations (i.e., R106W, R168X, R255X, R270X, and large deletions) and the prevalence and progression of scoliosis [34][35]. For severe scoliosis with a curve greater than 40–45°, surgical intervention is recommended to prevent further progression of the spinal curvature and maintain functional capabilities [34].

The work analyzed 20 cases of RTT patients with ages comprised between 2–20 years by comparing them to 25 age-matched healthy girls and 11 girls with cerebral palsy. Using dual-energy X-ray absorptiometry, which is considered a more precise measure of bone health [36], the authors identified deficient bone mineralization in RTT compared to the other two groups. Moreover, in RTT, there was no increase in mineralization with weight and age as observed in the other populations examined. The authors concluded that, although there was an adequate dietary intake of calories, calcium, and vitamin D, osteopenia found in RTT patients could predispose them to fractures and early osteoporosis [37].

Anterior iliac crest bone biopsies were obtained from three RTT patients. Quantitative bone histomorphometry indicated a decreased bone volume accompanied by low bone formation rates in two patients. The authors assumed that the slow rate of bone formation decreases bone volume in RTT patients by interfering with the development and accumulation of bone mineral mass [38]. In addition, two other independent studies supported the finding of a decreased bone formation in RTT with an impaired bone development rather than increased bone resorption, particularly evident in the younger patients [29][30].

in 1995 also demonstrated dysmorphogenetic defects in a sample of 17 RTT patients [39]. The results showed the prevalence of metatarsal shortness in 65% of cases and metacarpal shortness in 57%, using radiological evaluation; these anomalies were more common in older patients. Furthermore, there was also evidence for reduced bone density in the hands in 86% of the evaluated patients [39]. The same authors, in a larger and more representative cohort of 100 RTT patients from the Australian RTT Database, confirmed their previous results, showing a distinctive RTT profile characterized by short second metacarpal and short distal phalanx of the thumb.

At the same time, different groups focused their attention on the possible contributing factors that can predispose to bone demineralization in RTT patients (Table 1). One of the first studies in this direction was performed in 1999 by comparing hand radiographs of 101 RTT patients from the Australian RTT Database with controls matched for age, sex, and laterality [40]. Mean Z-score values for cortical thickness and percentage cortical area were significantly lower in RTT than in control children, indicating a decrease in bone mass. Moreover, an association of

these bone abnormalities with increasing age and the use of anticonvulsant medication was also highlighted in this study [40].

A few years later, in 2001, another study found a similar relationship between anticonvulsant therapy and the reduction in bone mass and bone quality in RTT patients [41]. Eighty-two RTT patients and 82 controls were compared for bone mineral density (BMD) by dual X-ray absorptiometry and other ultrasonographic parameters. In addition, among RTT patients, those treated with anticonvulsants known to affect bone metabolism or those who were non-ambulatory showed more prominent defects of bone mass and bone quality parameters, suggesting that ambulatory status, in primis, and anticonvulsant therapy could play a significant role in the altered bone status of RTT [41]. In this context, the observed association between the use of antiepileptic drugs, such as lamotrigine and carbamazepine, which are chronic inducers of hepatic CYP450 enzymes, and severe vitamin D deficiency in RTT patients, especially in those receiving antiepileptic polytherapy [42], may find in the interference with vitamin D metabolism a partial explanation for impaired bone health in RTT.

However, additional factors other than anticonvulsants/vitamin D interaction can affect bone homeostasis in RTT. Indeed, in 2010, a cross-sectional observational study on 49 RTT patients found low bone mass that was unrelated to anticonvulsant usage or scoliosis and only marginally associated with clinical severity and ambulation [43]. Between the baseline and follow-up scans, there was a significant decline over time in bone mineral content, bone mineral density, bone area, and lean tissue mass Z-scores for all outcome measures. For most bone measurements, multiple regression analysis showed a correlation with the overall reduced mobility skills observed over time [44].

Similarly, a 10-year longitudinal study on 58 RTT girls highlighted the relationship between the extent of ambulatory disability and the deterioration of bone status [45]. In fact, levels of biochemical and quantitative ultrasound parameters at phalanges were significantly lower in non-ambulatory than in ambulatory RTT, although they showed a similar worsening of bone status during the 10-year follow-up [45].

In 2006, an interesting work conducted by Motil et al. tried to understand the cause of RTT osteopenia by evaluating several factors related to calcium metabolism, including dietary intake, intestinal absorption, and renal excretion [46]. In the presence of adequate dietary intake of calcium, the authors did not find a defect in intestinal calcium absorption. Rather, they observed an increase in fractional calcium absorption in RTT patients, probably a compensatory mechanism in an attempt to meet their metabolic needs for bone mineralization. Moreover, analysis of urinary calcium excretion was consistent with mild and subclinical hypercalciuria, a phenomenon interpreted as a response to impaired bone mineralization in the RTT girls [46].

In a subsequent work, the same authors expanded the number and age range of RTT patients for investigating their bone status in relationship to the type of MECP2 mutation [47]. In a cohort of 50 RTT girls, the total body bone mineral content and bone mineral density were significantly lower in older than in younger patients. In parallel, a Danish study on 61 RTT patients did not detect associations between several bone parameters obtained by dual-energy X-ray absorptiometry and type of MECP2 mutation or pattern of X chromosome inactivation. On the other

hand, low bone mass, low bone density, and small bones in RTT were most affected by mobility status, epilepsy diagnosis, and treatment with antiepileptic drugs [48].

On the other hand, in a more recent study on 232 RTT patients, a clear genotype–phenotype correlation between MECP2 mutation and bone status has been recognized (Table 1) Values of bone mineral density at the femoral neck and at the total hip were significantly lower in RTT patients with the most severe mutations (i.e., R106T, R168X, R255X, and R270X) compared to subjects with less severe mutations (i.e., R133C, R294X, R306C, and T158 M). In line with these findings, a previous study in an Australian RTT cohort showed that R168X and T158M mutations are strong predictors for low bone mineral density and content [44]. Similarly, in a RTT cohort consisting of 49 girls, the lowest values of bone mineral density and content were observed in patients with T158M or R270X mutations [43].

3. Therapeutic Approaches for Bone-Related Issues in RTT

In addition to the neurological symptoms, bone structural abnormalities and related complications, such as osteoporosis, fractures, and scoliosis, play a significant role in RTT pathophysiology and gravely impair the quality of life of patients as well as families. Therefore, management of bone status in these patients requires special attention, and, in this regard, a group of international experts on RTT has recently developed clinical guidelines for both the assessment and management of bone health in the affected children [25]. Regarding the therapeutic strategies suggested to improve bone health and reduce the frequency of fractures, the guidelines encouraged increasing the level of physical activity and recommended ensuring adequate calcium and vitamin D intake through their supplementation [25]. Therefore, panel members warned about the need for follow-up and monitoring after one year from the start of treatment to verify the improvement in bone density and, thus, to evaluate whether the pharmacological therapy could continue [25].

Indeed, before 2016, only two studies evaluated the treatment with bisphosphonates in RTT subjects [49][50]. In 2013, a case study reported data related to the significant improvement in bone heath status in RTT, but the evaluation was limited to a single patient treated for 3 years with pamidronate (aminohydroxypropylidene diphosphonate disodium) [50]. Due to severe osteoporosis, the child suffered six fractures in less than 3 years before the treatment with the bisphosphonate. However, in the 3 years posttreatment, the use of pamidronate reduced the number of fracture episodes to zero with a 45% improvement in bone mass density values (i.e., from a Z-score of −3.8 to −1.3).

In this case, an 18-year-old RTT patient, who had a history of recurrent low-trauma fractures, was treated daily for 8 months with teriparatide, a recombinant fragment of human parathyroid hormone able to stimulate bone formation. It is worth noting that, in this case, the combined mechanisms of action of teriparatide and neridronate, i.e., stimulation of bone formation and inhibition of bone resorption, respectively, may have produced an additive effect in ameliorating bone health in the RTT child. Interestingly, in another case report published one year later, daily subcutaneous teriparatide treatment for 21 months in a 28-year-old RTT patient led to several improvements in bone status, including an increase in bone formation marker osteocalcin associated with a corresponding

increase in bone mineral density at both lumbar spine and femoral neck [51]. In addition, trabecular microarchitecture (i.e., trabecular density, trabecular bone volume, trabecular number and thickness) was also significantly improved.

In addition to the case reports described above, more recently, two other studies conducted an evaluation of the safety and efficacy of bisphosphonate use in larger cohorts of RTT patients [52][53]. In the first retrospective study, a cohort of 20 RTT children, who received pamidronate therapy for 2 years (specifically, on two consecutive days every 3 months), was analyzed for determination of bone mineral density, incidence of fractures, and other biochemical markers [52]. The treatment was well tolerated and associated with a recovery of different bone-related parameters: decrease in the incidence of fractures; increase in spine bone mineral density Z-score; reduction in urinary calcium/creatinine ratio; improvement in BMI and mobility skills [52].

Absence of severe adverse effects and efficacy of a parenteral third-generation bisphosphonate, i.e., zoledronic acid, have been confirmed in another retrospective evaluation on 19 patients affected by cerebral palsy and 8 RTT children [53]. Some benefits of zoledronic acid over pamidronate include the shorter infusion time and the fact that it can be administered as a single annual injection. In the RTT group, one year after injection of zoledronic acid, there was a significant improvement in both lumbar spine and femoral bone mineral density, along with a reduction in the incidence of fractures and pain. The most frequent adverse effects included flu-like symptoms and hypocalcemia, especially in younger patients [53].

The treatment mainly influenced the structural properties of trabecular bone, increasing bone volume fraction, trabecular number, connectivity density in all groups of mice (i.e., wild-type and Mecp2 mutants, both males and females). Of note, zoledronic acid treatment decreased bone remodeling parameters. Collectively, the observations of this study supported the previously mentioned clinical reports, confirming the efficacy of bisphosphonate treatment in sustaining bone health in RTT patients, as indicated by the clear improvement in trabecular bone architecture in Mecp2 mutant mice. On the other hand, the decline in bone remodeling indices raises doubts about the possible negative impact of zoledronic acid on other aspects of bone physiology.

In addition to the above-mentioned pharmacological treatments, there are other non-pharmacological interventions commonly indicated to cope with the well-known risk factors associated with poor bone health in RTT patients, including limited mobility and nutritional deficits [45]. Therefore, adequate dietary calcium intake and serum levels of bone markers, such as calcium and 25-hydroxyvitamin D, are usually monitored in RTT patients and, in case of deficiencies, vitamin D and calcium supplementations are used, as also recommended in the 2016 guidelines for assessment and management of bone health in RTT [25][45][54][53]. Furthermore, physical therapy is another essential intervention for the management of bone health in this disorder [25][55][56]. In this regard, a systematic review of the literature identified nine different approaches to physical therapy used in RTT that demonstrated to improve the quality of life in these patients, mostly helping to maintain autonomy [57].

Finally, it is worth mentioning the results of a study evaluating the effectiveness of low magnitude mechanical stimulation (LMMS) in improving bone mineral density in RTT patients [58]. Several evidences demonstrated the

ability of mechanical stimuli to modulate bone formation and remodeling. Accordingly, a significant improvement in spine bone mineral density was observed in nine RTT patients enrolled in a 12-month crossover pilot study designed as a 6-month intervention period with LMMS and a 6-month period without [58]. Based on these preliminary data, LMMS could be considered as an additional feasible intervention to associate with previous reported therapeutic approaches, although further evaluation on larger cohorts of RTT patients is needed.

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