ORIGINAL RESEARCH

Skeletal Abnormalities and VDR1 Gene Polymorphisms in Mucopolysaccharidosis Patients

Camelia Alkhzouz[1,2]
Georgiana Cabau[2]
Cecilia Lazea [ID][2,3]
Carmen Asavoaie [ID][3]
Simona Bucerzan[1,2]
Andreea Manuela Mirea [ID][2]
Marius Farcas [ID][2]
Maria Miclaus Jnr[2]
Radu Popp[2]
Diana Miclea[1,2]

[1]Department of Medical Genetics, Clinical Emergency Hospital for Children, Cluj-Napoca, Romania; [2]"Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; [3]First Pediatric Clinic, Clinical Emergency Hospital for Children, Cluj-Napoca, Romania

Introduction: Articular and bone damage, which is so disabling in Mucopolysaccharidosis (MPS), requires attention as to the explanatory bias of the pathogenetic mechanisms identified to date. The vitamin D receptor (VDR) has been investigated in many studies in correlation with bone metabolism, osteoporosis, and the impaired bone mineral density associated with certain polymorphisms of the VDR gene.

Aim: This study aims to observe whether there is an association between clinical features, phospho-calcium metabolism parameters and the VDR gene polymorphisms in patients with MPS.

Patients and Method: We evaluated six patients with MPS type I, 20 patients with MPS type II, two patients with MPS types IIIA and IIIB and three patients with MPS type IVB. In these patients, phospho-calcium metabolism, markers of bone formation, bone radiographs and bone densitometry were evaluated, as were four polymorphisms of the VDR gene (ApaI, BsmI, FokI and TaqI).

Results: There was a deficiency in 25 hydroxy vitamin D in MPS type I patients at the final evaluation and in MPS type II patients, both at ERT initiation and at the last evaluation. The analysed polymorphisms were not associated with modified calcium-phosphor levels, but some differences were observed regarding the level of 25 OH vitamin D. Thus, in the case of AA polymorphism, all patients have a 25 OH vitamin D deficiency, and one patient with the AA genotype and three with Aa have a 25 OH vitamin D deficiency and secondary hyperparathyroidism due to this deficiency (four patients), all of them having the Bb phenotype.

Conclusion: In MPS patients, vitamin D deficiency is observed, as it is in some patients with secondary hyperparathyroidism, which indicates vitamin D supplementation to protect bone metabolism. There are no obvious correlations between VDR polymorphism and bone metabolism in MPS patients.

Keywords: mucopolysaccharidosis, vitamin D receptor, ApaI, BsmI, FokI, TaqI

Introduction

Mucopolysaccharidoses (MPS) are characterised by the malfunctioning of lysosomal enzymes involved in the degradation of mucopolysaccharides (glycosaminoglycans or GAGs), which accumulate in various tissues, affecting cell function and the extracellular matrix. Depending on the particular deficient lysosomal enzyme involved, there are eleven MPS types (MPS I, II, IIIA, B, C, IVA, IVB, V, VI, VII, VIII and IX). Characteristic clinical features are represented by musculoskeletal, cardiovascular, CNS, ophthalmic, auditory, and pulmonary involvement. The skeletal features developed progressively in these pathologies, due to GAGs

Correspondence: Diana Miclea
Department of Medical Genetics, Clinical Emergency Hospital for Children, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania
Email diana.miclea@umfcluj.ro

accumulation in joints and connective tissues, are very limiting regarding the mobility and activity of these patients, entailing a significant decrease in the quality of life.[1,2] Bone damage is classically referred to as multiplex dysostosis, which includes vertebral abnormalities, deformity and shortening of long bones, macrocephaly, hips dysplasia, coxa valgum, genu valgum, claw hands, thorax involvement, facial dysmorphism, odontoid hypoplasia, and osteopenia. Joint and skeletal damage, so disabling in MPS, requires attention regarding the explanatory bias of the pathogenetic mechanisms known at present.

Going beyond the classical conception of MPS pathogenesis as being due to accumulation of undegraded GAGs, the influences exerted by these molecules, as biological response modifiers, can be much more diverse, including influences on different signalling pathways (STAT, BMP4, FGFR3), on cytokines (IL2, IL6), on oxidative stress and mitochondrial dysfunction, on cathepsin activity, on autophagy, on abnormal intracellular transport and on other substrate pathways.[3–7] On the other hand, MPS bone remodelling is influenced by cathepsin K activity, a lysosomal enzyme highly expressed in osteoclasts and leading to osteopenia and osteoporosis.[5,8–11] Osteoporosis and osteopenia observed in MPS patients, as modifications in the extracellular matrix, should have consequences in bone phospho-calcium metabolism.

Bone mineral density and vitamin D levels were assessed and proportionally correlated in MPS patients, but there is no clear pathogenetic mechanism known so far for vitamin D metabolism in correlation with MPS, other than insufficient oral intake.[12–15] It is known that the MPS therapies employed to date do not have significant efficacy in reversing the conjunctive tissue changes.[16,17] Conversely, the peculiarities of phosphocalcic metabolism due to genetic changes concomitant with those responsible for MPS may influence bone metabolism in MPS patients, but there have been no clear data until now.[3]

A genetic change often correlated with bone metabolism involves the vitamin D receptor (VDR), through which vitamin D mediates its biological effects. VDR is a member of the nuclear hormone receptor superfamily, has a size of 75 kb, comprises 11 exons, gives rise to a protein with 427 amniotic acids, and regulates gene transcriptions with an effect on calcium and skeletal metabolism.[18–20] Vitamin D (1.25 dihydroxy vitamin D) binds to the receptor (VDR) and thus influences intestinal calcium absorption as well as osteoblastic differentiation, thus leading to proper bone mineralisation and remodelling. VDR abnormalities could alter mineral metabolism, as well as other pathways such as cancer or immune response.[21,22]

The VDR gene has been investigated in many studies in correlation with bone metabolism, initially in relation to Type 2A-dependent vitamin D rickets, in which there is a clear cause-effect correlation, and subsequently in relation to growth, osteoporosis and impaired bone mineral density, in the case of certain polymorphisms of the VDR gene.[23] There are no data about the involvement of VDR changes as aggravating factors for bone disease in MPS patients.

The aim of the study is to observe whether there is an association between clinical features, phospho-calcium metabolism parameters, and genetic polymorphisms of the VDR gene in patients with MPS.

Table 1 Auxological Characterisation in MPS Patients

	Height (SD) at Diagnosis Average (Min, Max)	Height (SD) 1Yr After ERT Average (Min, Max)	Height (SD) Last Assessment Average (Min, Max)	BMI (SD) at Diagnosis Average (Min, Max)	BMI (SD) 1Yr After ERT Average (Min, Max)	BMI (SD) Last Assessment Average (Min, Max)
All patients	−0.40 (−6.63, 5.2)	NA	−2.75 (−7.9, 1.76)	0.73 (−2, 3.3)	NA	0.70 (−2, 3)
Patients MPS type I	−2.25 (−6.63, 2.04)	NA	−4.80 (−7.90, 0.20)	0.32 (−2, 1.8)	NA	1.55 (0.3, 1.8)
Patients MPS type II	0.33 (−4.06, 5.2)	−0.31 (−5.26, 4.05)	−1.52 (−5.89, 1.76)	0.96 (−2, 3.3)	1.30 (−2, 3)	0.70 (−2, 3)
p*	0.03	NA	0.02	0.29	NA	0.25

Note: *p obtained compared patients with MPS I and MPS II.
Abbreviations: ERT, enzyme replacement therapy; SD, standard deviation; BMI, body mass index; NA, data not available.

350

Pharmacogenomics and Personalized Medicine 2021:14

Patients and Method

We evaluated six patients with MPS type I, 20 patients with MPS type II, two patients with MPS types IIIA and IIIB, and three patients with MPS type IVB, who presented at the Emergency Clinical Hospital for Children, Cluj-Napoca, between 1st January 2009 and 31st December 2019.

These patients were clinically evaluated, noting the clinical picture (auxological parameters, goniometry, and clinical signs). There were also investigations by different specialities to evaluate the other features of this disease: neurological and psychological assessments, ENT examinations (Ear, Nose and Throat), audiograms, ophthalmological examinations, and cardiological evaluations. The specific diagnosis was carried out by performing enzymatic and molecular tests.

The following evaluations were conducted at diagnosis (before enzyme replacement therapy, ERT), one year after ERT (for MPS I and MPS II) and at the final evaluation (between 3 and 8 years of treatment): phospho-calcium metabolism (phosphorus, calcium, PTH, 25 OH vitamin D, alkaline phosphatases), markers of bone formation (osteocalcin), bone radiographs and bone densitometry. Four

polymorphisms of the *VDR* gene were evaluated (rs7975232-ApaI, rs1544410-BsmI, rs2228570-FokI and rs731236-TaqI).

Study approval was obtained from the hospital ethics committee, with informed consent being obtained for each patient from the parents or legal guardians.

Genetic Testing

Genomic DNA was extracted for all subjects from 300 μL of blood, using a commercial kit (Wizard Genomic DNA Purification kit, Promega, Cambridge, MA, USA).

The polymorphisms rs7975232-ApaI, rs1544410-BsmI, rs2228570-FokI and rs731236-TaqI were evaluated, using the adapted protocol of Kaya et al.[24] The VDR gene region carrying the polymorphic restriction sites ApaI, BsmI, TaqI was amplified by PCR, using the upstream primer in exon 8 (5′-CAACCAAGACTACAAGTACCGCGTCAGTGA-3′) and the downstream primer in exon 9 (5′-CACTTCGAGCACAAGGGGCGTTAGC-3′). For the FokI polymorphic restriction site, a primer pair spanning exon 2 of the VDR gene was used for amplification (5′-AGCTGGCCCTGGCACTGACTCTGCTCT-3′ and 5′-ATGGAAACACCTTGCTTCTTCTCCCTC-3′). The PCR

Table 2 Biological Assessment in MPS Patients

	Moment of Analysis	Calcemia (mg/dl) Average (Min-Max) (NR 8.8–10.3)	Phosphoremia (mg/mL) Average (Min-Max) (NR 3–5.4)	25-Hydroxy Vitamin D (μg/L) Average (Min-Max) (NR ≥30)	Alkaline Phosphatase (UI/l) Average (Min-Max) (NR 90–315)	Parathormone (pg/mL) Average (Min-Max) (NR 8.5–88)	Osteocalcin (ng/mL) Average (Min-Max) (NR 6.6–65.4)
All patients	Last assessment	9.55 (8.84–10.24)	4.46 (2.59–5.67)	23.04 (9.48–42.2)	187.07 (45–469)	47.02 (8.67–114.3)	78 (19.3–132.3)
Patients MPS type I	Before ERT	9.70 (8.7–10.13)	5.00 (4.1–5.7)	30.02 (24.5–33.6)	260 (212–336)	NA	NA
	Last assessment	9.72 (9.22–10.16)	4.30 (2.59–5.35)	28.44 (24.5–36.1)	147 (55–306)	38.32 (21.4–60.4)	57.20 (19.3–95.1)
Patients MPS type II	Before ERT	9.68 (8.58–10.53)	4.70 (3.3–5.8)	17.40 (3.5–25.8)	264 (143–430)	40.5 (20.4–71.5)	NA
	Last assessment	9.43 (8.84–10.24)	4.52 (3.8–5.67)	22.15 (9.85–42.2)	191.2 (45–469)	50.02 (8.67–114.3)	85.03 (58.97–132.3)
p *	Before ERT	0.936	0.25	0.03	0.91	NA	NA
	Last assessment	0.76	0.03	0.16	0.95	0.20	0.11

Note: *p obtained compared patients with MPS I and MPS II.
Abbreviations: NA, data not available; NR, normal range.

reaction was performed using Thermo Scientific PCR Master Mix containing Taq DNA polymerase 0.05U/μL, reaction buffer, 4 mM MgCl2, 0.4 mM of each dNTP (dATP, dCTP, dGTP and dTTP), UltraPure BSA (Invitrogen™), forward primer (0.1–1.0 μM), reverse primer (0.1–1.0 μM) and template DNA. The PCR conditions were 3 minutes at 94.5°C for initial denaturation, followed by 35 cycles of denaturation of 1 minute at 94.5°C, annealing 1 minute at 61°C and 2 minutes at 72°C for extension, with a final extension at 72°C for 7 minutes.

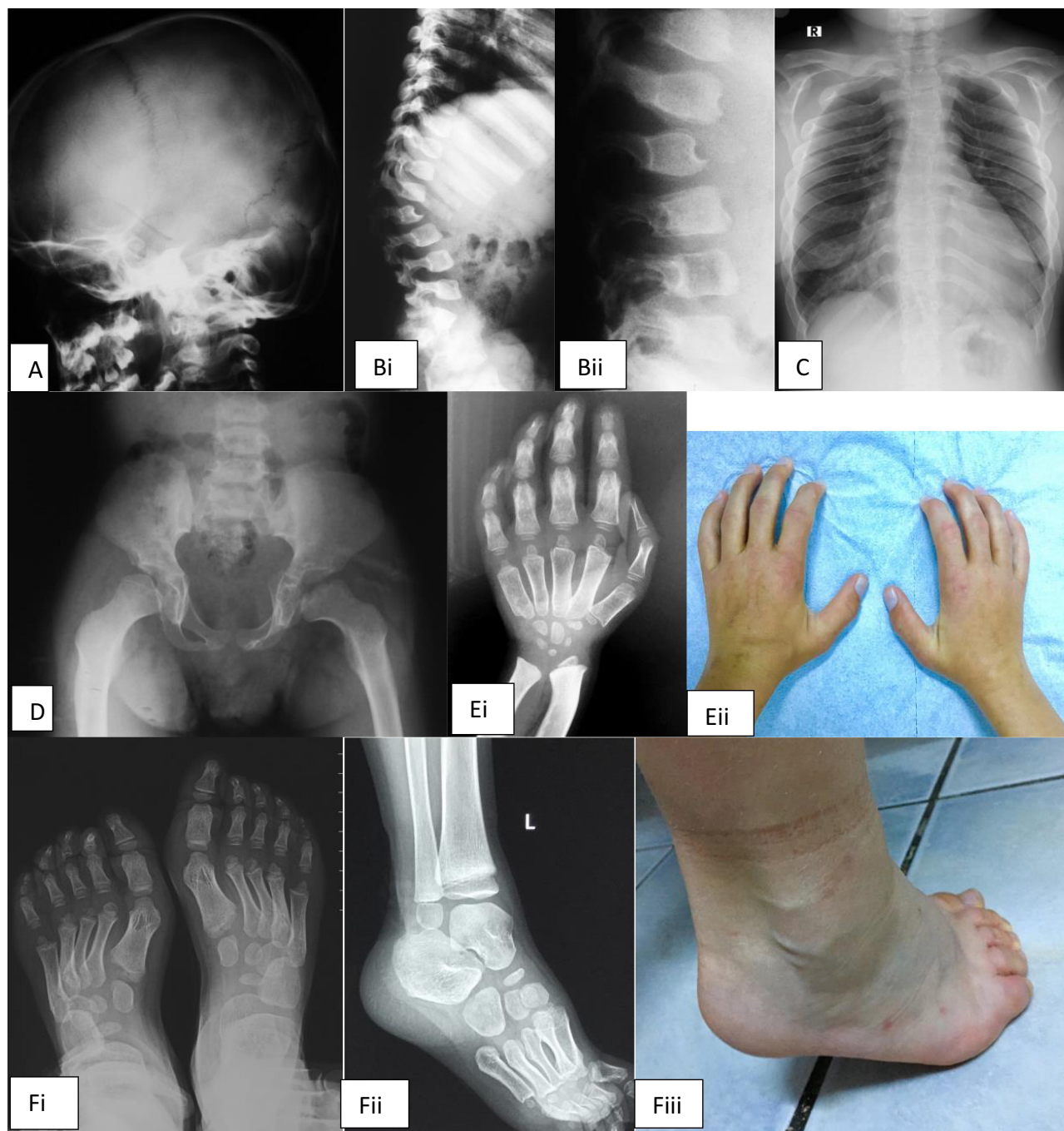The amplified PCR products were digested by FastDigest FokI (10U/uL) (Thermo Scientific) restriction endonuclease

Figure 1 Radiological abnormalities seen in our patients with MPS type II. (**A**) Macrocephaly in 6 years old MPS II patient; (**B**) **i,ii**. Dorsolumbar kyphosis and a hypoplastic transitional vertebra with anterior beaking; (**C**) Short, thick clavicles, paddle-shaped ribs, scoliosis; (**D**) Round and flared iliac wings, an underdeveloped acetabulum and signs of hip dysplasia; (**E**) **i,ii**. Thick and hypoplastic distal radius and ulna creating a V-shaped deformity (Madelung deformity); small/underdeveloped carpal bones; bullet-shaped phalanges; (**F**) **i,ii,iii**. Small and hypoplastic tarsal bones and calcaneovalgus deformity.

for 15 minutes at 37°C, followed by enzyme inactivation at 65°C for 5 minutes, FastDigest TaqI (10U/uL) (Thermo Scientific) 15 minutes at 65°C, ApaI (10U/uL) and BsmI (10U/uL) for 16 hours at 37°C, followed by inactivation for 20 minutes at 65°C. With the enzymes ApaI, BsmI, TaqI and FokI, the respective genotypes were defined as A, B, T, and F (indicating the absence of the restriction site) or a, b, t, and f (indicating the presence of the restriction site). The digestion products were visualised under UV light using a gel electrophoresis visualising system.

Results

Six patients with MPS type I, 20 patients with MPS type II, two patients with MPS types IIIA and IIIB and three patients with MPS type IVB were analysed. Among the patients with MPS type I were three females and three males. All patients with MPS type II were males, considering the X-linked recessive transmission of this disease. The mean age of the patients at the time of ERT initiation was 9.08 years for those with MPS type I and 7 years for those with MPS type II.

The auxological characterisation of these patients can be observed in Table 1. A higher height deficit is observed at the ERT initiation in patients with MPS type I (mean −2.25SD), compared to MPS type II (mean 0.33SD) (p = 0.03). At the final evaluation, under ERT, there is a similar height growth and a higher height deficit for MPS type I (−4.80SD), compared to MPS type II patients (−1.52SD) (p = 0.02). A body mass index (BMI) within normal limits is observed for all patients with MPS.

Concerning the biological analysis, in Table 2 the main findings are described. For the group with MPS types I and II, data were available from the initiation of therapy to the present (between 3 and 8 years after ERT initiation). There was a deficiency in 25-Hydroxy vitamin D in MPS type I patients at the last evaluation and, in MPS type II, both at ERT initiation and at the final evaluation. Concerning 25-Hydroxy vitamin D levels, a difference was observed between MPS type I and MPS type II patients at the ERT initiation (p = 0.03) and a slight difference was also noticeable at the last evaluation (without reaching a statistically significant threshold).

There was a slightly higher level of osteocalcin in patients with MPS II than in those with MPS I, indicating a slightly more intense osteogenic process in patients with the former type, without reaching a threshold of statistical significance. The DEXA examination did not reveal osteoporosis or osteopenia in the examined patients. All patients with MPS types IIIA, IIIB and IVB also presented low levels of vitamin D (<30 μg/L), one patient with MPS IIIB and two patients with MPS type IV presenting a deficiency of 25-Hydroxy vitamin D (<20μg/L).

The radiological skeletal findings in these MPS patients are illustrated in Figure 1. All I, II and IV MPS patients in our study group presented musculoskeletal abnormalities on imaging studies with varying degrees of severity. Dysostosis multiplex is a term that includes several radiological features typically encountered in MPS patients. Some of the most common findings associated with dysostosis multiplex in our patients are presented in Table 3.

The genotypes observed in our study are described in Table 4. Compared to the total GnomAD frequency, similar frequencies of these genotypes are reported. Correlations between phospho-calcium metabolism and ApaI polymorphisms are shown in Table 5.

Table 3 Radiological Findings Associated with Dysostosis Multiplex in MPS Patients

Skeletal	Anomalies
Skull	Macrocephaly J shaped sella turcica Calvarial thickening of bone cortex Facial anomalies
Spine	Vertebral anomalies (platyspondily, odontoid hypoplasia, transitional dorso-lumbar hypoplastic vertebra) Thoracolumbar kyphosis Scoliosis Spinal cord stenosis and cord compression
Thorax	Clavicle anomalies Paddle-shaped ribs
Pelvis	Hip dysplasia Underdeveloped/flattened acetabulum Coxa valga Hypoplasia of the inferior part of the iliac bones Round iliac wings
Hands and feet	V-shaped deformity of distal ulna and radius Hypoplastic carpal and tarsal bones Pointed metacarpals and metatarsals Bullet-shaped phalanges
Knees	Genu varum

Table 4 Genotypes and Alleles Frequencies for the Variants Studied

Genotype*	ApAI (A/C) (rs7975232)			BsmI (C/T) (rs1544410)			FokI VDR (T/C) rs2228570			TaqI (T/C) (rs731236)		
	AA	**Aa**	**aa**	**BB**	**Bb**	**bb**	**FF**	**Ff**	**ff**	**TT**	**Tt**	**tt**
MPS type I (n=6)	3 (50%)	3 (50%)	0	1 (17%)	2 (33%)	3 (50%)	0	6 (100%)	0	2 (33%)	4 (67%)	0
MPS type II (n=20)	4 (27%)	11 (72%)	0	4 (24%)	11 (64%)	2 (12%)	7 (35%)	11 (55%)	2 (10%)	5 (28%)	9 (50%)	4 (22%)
MPZ type III (n=2)	0	2 (100%)	0	1 (50%)	0	1 (50%)	0	2 (100%)	0	1 (50%)	1 (50%)	0
MPZ type IV (n=3)	0	3 (100%)	0	0	2 (67%)	1 (33%)	0	2 (67%)	1 (33%)	1 (33%)	2 (67%)	0
Genotypes frequencies GnomAD	31%	49%	20%	43%	45%	12%	13%	45%	42%	44%	44%	12%
Alleles Frequencies	**A**	**a**	**Total Alleles**	**B**	**b**	**Total Alleles**	**F**	**f**	**Total Alleles**	**T**	**t**	**Total Alleles**
MPS type I	9 (75%)	3 (25%)	12 (100%)	4 (33%)	8 (67%)	12 (100%)	6 (50%)	6 (50%)	12 (100%)	8 (67%)	4 (33%)	12 (100%)
MPS type II	19 (63%)	11 (37%)	30 (100%)	19 (56%)	15 (44%)	34 (100%)	25 (63%)	15 (37%)	40 (100%)	19 (53%)	17 (47%)	36 (100%)
MPS type III	2 (50%)	2 (50%)	4 (100%)	2 (50%)	2 (50%)	4 (100%)	2 (50%)	2 (50%)	4 (100%)	3 (75%)	1 (25%)	4 (100%)
MPS type IV	3 (50%)	3 (50%)	6 (100%)	2 (33%)	4 (67%)	6 (100%)	2 (33%)	4 (67%)	6 (100%)	4 (67%)	2 (33%)	6 (100%)
Total Alleles MPS type I–IV	33 (63%)	19 (37%)	52 (100%)	27 (48%)	29 (52%)	56 (100%)	35 (56%)	27 (44%)	62 (100%)	34 (59%)	24 (41%)	58 (100%)
Alleles frequencies GnomAD	78,693	63,629	142,322	92,946	50,164	143,160	50,164	92,946	143,110	94,346	48,454	142,800
	55%	45%	100%	65%	35%	100%	35%	65%	100%	67%	33%	100%

Note: *Not for all the analysed patients was observed a result after genetic testing.

In patients with MPS type II, a lower level of 25-Hydroxy vitamin D is observed in patients with homozygous phenotype AA, compared to those with heterozygous phenotype Aa ($p = 0.04$). An MPS type II patient with genotype AA has calcium at the upper limit of normal (10.24mg/dl), low phosphoremia (4.24 mg/dl) and 25-Hydroxy vitamin D deficiency (15.1ug/l). Also, in MPS type II patients with AA genotype, a deficiency level for vitamin D is observed in four of them (14–18ug/l), one showing secondary hyperparathyroidism due to vitamin D deficiency. Vitamin D deficiency is observed in four patients with the Aa genotype, three of them presenting secondary hyperparathyroidism from vitamin D deficiency. The four patients with vitamin D deficiency and secondary hyperparathyroidism have the heterozygous Bb (Bsm1) genotype. In patients with MPS type I, no significant changes were observed between the various ApaI genotypes and changes in phospho-calcium homeostasis. There is also a higher level of osteocalcin in MPS type II patients with the Aa genotype than in those with AA, indicating a lower level of bone synthesis in the latter.

Table 5 Correlations Between Phospho-Calcium Metabolism and ApaI Polymorphisms in Patients with MPS Type II at Last Evaluation

Genotypes in Patients with MPS Type II*	AA	Aa	P (AA,Aa)
Height (SD) average	−0.23	−1.89	0.86
Calcium (mg/dl) average (NR 8.8–10.3)	9.46	9.53	0.92
Phosphore (mg/mL) average (NR 3–5.4)	4.32	4.65	0.47
25-Hydroxy vitamin D (µg/L) average (NR ≥30)	16.32	24.70	0.04
Alkaline Phosphatase(UI/l) average (NR 90–315)	150	212.78	0.15
Parathormone (pg/mL), average (NR 8.5–88)	52.15	49.82	0.92
Osteocalcin (ng/mL) (NR 6.6–65.4)	59.59	91.34	0.03

Note: *The genotype aa was not observed in patients with MPS type II.
Abbreviation: NR, normal range.

All patients with MPS type II and the BB phenotype have a vitamin D deficiency. There is a statistically significant difference between the 25OH vitamin D level and the BB and Bb polymorphisms for Bsm1 in patients with MPS type 2, patients with BB having a lower level. All patients with the BB genotype are deficient in vitamin D 25OH (<20). The four patients with MPS type II with secondary hyperparathyroidism have a heterozygous Bb genotype. It is also observed that the PTH level is lower in MPS type II patients with the BB phenotype than in those with the bb phenotype (see Table 6).

No statistically significant changes were observed between FokI polymorphisms and phospho-calcium homeostasis in patients with MPS types II (see Table 7).

Except for an association of the TT genotype with a lower level of serum phosphorus in MPS type II, no significant changes of TaqI polymorphisms with phosphocalcic homeostasis in MPS types II are observed (refer to Table 8).

Discussion

In this study, characteristics of growth and phospho-calcium metabolism in patients with MPS have been described, as well as associations of this metabolism with genetic polymorphisms of the vitamin D receptor gene. In both MPS type I and II, a deficit of 25-hydroxy vitamin D was observed. The analysed polymorphisms were not associated with modified calcium-phosphor levels, but some differences regarding the level of 25-Hydroxy vitamin D were noted. Thus, in the case of AA polymorphism, all patients have a 25-Hydroxy vitamin D deficiency, one of them presenting secondary hyperparathyroidism. Also, three patients with the Aa have a 25-Hydroxy vitamin D deficiency and secondary hyperparathyroidism from this deficiency. All patients with secondary paratiroidism presented Bb phenotype.

The frequency of these *VDR* polymorphisms among patients with MPS is quite similar to that of the general population, as observed in the GnomAD database. Until now, there have been no data on these *VDR* polymorphisms in patients with MPS.

It is known that pathogenic variants of the *VDR* gene lead to vitamin D-dependent rickets type 2A. There are more subtle variations of the *VDR* sequence, as these polymorphisms, which are more common in the population, and while their effect is often not well known, they can influence many biological processes. These include decreased bone mineral density and osteoporosis, tall stature, hyperparathyroidism, osteoarthritis, autoimmune diseases (psoriasis, Graves' disease, type 1 diabetes, multiple sclerosis), or infectious diseases such as tuberculosis.

Table 6 Correlations Between Phospho-Calcium Metabolism and BsmI Polymorphisms in Patients with MPS Type II at Last Evaluation

Genotypes in Patients with MPS Type II	BB	Bb	bb	P (BB, Bb)	P (BB,bb)	P (Bb,bb)
Height (SD)	0.63	−2.67	−0.54	0.09	0.38	0.80
Calcium (mg/dl) (NR 8.8–10.3)	9.55	9.46	9.64	0.69	0.74	0.20
Phosphore (mg/mL) (NR 3–5.4)	4.86	4.41	4.77	0.09	0.84	0.09
25-Hydroxy vitamin D (µg/L) (NR ≥30)	15.12	26.57	18.91	0.05	0.35	0.22
Alkaline Phosphatase (UI/l) (NR 90–315)	177.25	164.6	306	0.74	0.08	0.01
Parathormone (pg/mL) (NR 8.5–88)	32.75	57.44	57.93	0.27	0.004	0.98
Osteocalcin (ng/mL) (NR 6.6–65.4)	64.55	89.60	88.78	0.11	0.20	0.96

Abbreviation: NR, normal range.

Table 7 Correlations Between Phospho-Calcium Metabolism and FokI Polymorphisms in Patients with MPS Type II at Last Evaluation

Genotypes in Patients with MPS Type II	FF	Ff	ff	P (FF,Ff)	P (FF,ff)	P (Ff,ff)
Height (SD)	−1.58	−1.30	−1.24	0.66	NA	NA
Calcium (mg/dl) (NR 8.8–10.3)	9.51	9.54	9.46	0.82	NA	NA
Phosphore (mg/mL) (NR 3–5.4)	4.42	4.7	4.88	0.30	NA	NA
25-Hydroxy vitamin D (μg/L) (NR ≥30)	21.56	21.41	18	0.97	NA	NA
Alkaline Phosphatase (UI/l) (NR 90–315)	202.28	211.8	45	0.83	NA	NA
Parathormone (pg/mL) (NR 8.5–88)	47.08	53.9	41	0.69	NA	NA
Osteocalcin (ng/mL) (NR 6.6–65.4)	77.04	85.177	83	0.59	NA	NA

Abbreviation: NR, normal range.

Polymorphisms of the *VDR* gene have been studied in different diseases, but the most important associations were found in osteoporosis studies. Also, amongst all the genes studied in osteoporosis, the *VDR* gene polymorphism was the most relevant. ApaI polymorphism was associated with protection against osteoporosis;[23,25] however, there are other studies that find some detrimental effects or no effect at all on osteoporosis.[26–28] BsmI polymorphism was associated with an increased risk of osteoporosis,[23] but other authors have not found any association.[28,29]

FokI and TaqI were significantly associated with the development of osteoporosis.[23,25,28] Fok1 is located in exon 2 of the gene and leads to the formation of a new start codon, resulting in a shorter VDR product that interacts more efficiently with the transcription factor TFIIB.[30] BsmI and ApaI are located in intron 8 of the *VDR* gene and influence mRNA stability by altering a splice site or altering intronic regulatory elements.[30] TaqI polymorphism is located in exon 8 and represents a synonymous substitution, not influencing the VDR protein but being involved in the regulation of mRNA stability.[30] Other

authors have observed that, if *VDR* polymorphisms are not clearly associated with bone health, a haplotype of these polymorphisms, such as Bat or BAt, could be more relevant in association with bone mineral density.[31,32]

The variability of skeletal involvement in MPS patients could also be influenced by epistatic gene interactions or gene-environment interactions. Other factors which exacerbate bone mineralisation abnormalities in MPS could be secondary to immobilisation, poor nutritional status, vitamin D deficiencies, and little sun exposure.[33]

Among the findings arising from this study is the lack of data regarding associated factors that could exacerbate skeletal disease in MPS patients. *VDR* polymorphisms have not been evaluated until now in MPS patients. Phosphocalcic metabolism in MPS patients has not often been evaluated in the literature,[33] so data regarding these factors is needed. Vitamin D deficiency was also observed by Zuber et al in MPS patients, and our study reinforces the idea that these patients need vitamin D supplementation to protect a particular bone metabolism.[31,33,34]

Table 8 Correlations Between Phospho-Calcium Metabolism and TaqI Polymorphisms in Patients with MPS Type II at Last Evaluation

MPS Type II						
	TT	Tt	tt	p(TT,Tt)	p(TT,tt)	p(Tt,tt)
Height (SD)	−1.59	−0.93	−1.60	0.55	0.43	0.30
Calcium (mg/dl) (NR 8.8–10.3)	9.51	9.56	9.50	0.84	0.87	0.75
Phosphore (mg/mL) (NR 3–5.4)	4.82	4.26	4.75	0.02	0.79	0.11
25-Hydroxy vitamin D (μg/L) (NR ≥30)	23.72	19.05	22.42	0.36	0.79	0.62
Alkaline Phosphatase (UI/l) (NR 90–315)	250.83	182.37	157.46	0.13	0.07	0.48
Parathormone (pg/mL) (NR 8.5–88)	0.15	0.77	0.08	0.79	0.46	0.46
Osteocalcin (ng/mL) (NR 6.6–65.4)	0.83	0.35	0.37	0.24	0.56	0.72

Abbreviation: NR, normal range.

The limitations of this study are the low number of MPS patients. However, the low prevalence of this disorder in the general population makes it very difficult to recruit a higher number of patients: these patients represent all the patients treated with ERT in our country. Another limitation is the retrospective nature of the study, which has resulted in an absence of data for some patients in relation to skeletal metabolism, such as bone mineral density.

Conclusions

1. Vitamin D deficiency is observed in MPS type I and type II patients and in some patients associated with secondary hyperparathyroidism, indicating supplementation with vitamin D in order to protect the bone metabolism.

2. There are no obvious correlations between *VDR* polymorphism and bone metabolism in patients with MPS types I and II.

Details of Ethics Approval

The research study was approved by the Ethics Committee of Clinical Emergency Hospital for Children, Cluj-Napoca, Romania.

Patient Consent Statement

The written, informed consent was obtained from participants or their guardians prior to the genetic analysis. All genetic procedures were done in accordance with the ethical standards on human experimentation, of the hospital committee, and with the Helsinki Declaration of 1975, as revised in 2000. Approval from the Institutional Committee for Care and Use of Laboratory Animals. This article does not contain any studies with animal subjects performed by any of the authors.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Disclosure

Dr. Camelia Alkhzouz, Dr. Carmen Asavoaie and Dr. Diana Miclea report personal fees from Speaker in conference organized by Takeda Pharmaceuticals Company, Romania, "News on integrated care and management of patients with Hunter's syndrome", Cluj Napoca, 08 November 2019. The authors report no other conflicts of interest in this work.

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