

A new form of autosomal recessive hypophosphatemic rickets is associated with an inactivation mutation in the ENPP1 gene

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Introduction: Human disorders of phosphate (Pi) handling and hypophosphatemic rickets have been shown to result from mutations in PHEX, FGF23 and DMP1, presenting as X-linked recessive, autosomal dominant and autosomal recessive patterns, respectively.

Patients/ Methods: We have characterized an enlarged consanguineous Bedouin family displaying autosomal recessive Hypophosphatemic rickets. Two patients presented with short stature and bowing legs. A third patient has normal stature and was apparently healthy except for delayed healing of post traumatic fracture of his left tibia. The structure and size of the family were suitable for positional cloning of the causing gene through linkage study. After excluding linkage to the three above mentioned known genes, we carried out a genome wide search for the chromosomal region containing the mutated gene.

Results: We identified linkage to the chromosomal locus 6q23. The linkage interval of 7.39Mb contains 70 genes including the ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) gene, a known player in phosphate metabolism. The cDNA coding region for ENPP1 was directly sequenced and a novel mutation Y901S that presented homozygously in all patients, and segregated as expected in the healthy family members was found. The possibility that this variation represents polymorphism, was excluded by testing 236 control Bedouins of the same geographic region. Moreover, The tyrosine in position 901 resides in the nuclease-like domain, and is strictly conserved in ENPP1. ENPP1 generates inorganic pyrophosphate that is an essential physiologic inhibitor of calcification. The function of the mutation was tested by transfection of a pSVT7 expression vector harboring the mutated full-length ENPP1 coding sequence into cells and measuring the NPP activity in comparison to the activity in cells transfected with the normal sequence vector. We found that the mutation reduced NPP activity by 96% to 4% of residual activity. ENPP1 is a glycosylated ecto-enzyme, with its amino terminus in the cytoplasm and extracellular catalytic domains. We found that the cell membrane localization of the mutated protein is comparable to the wild type enzyme

Conclusions: ENPP1 generates inorganic pyrophosphate (PPi), an essential physiologic inhibitor of calcification, and previously described inactivating mutations in this gene were shown to cause aberrant ectopic calcification disorders, whereas no aberrant calcifications were present in our patients. Our surprising result suggests a different pathway involved in the generation of ARHR and possible additional functions for ENPP1.

The magic of replacement therapy – treating severe hypoparathyroidism with continuous infusion of PTH 1-34

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Introduction: Hypoparathyroidism is usually treated with low-phosphate diet, calcium supplements, and vitamin D analogs. However, metabolic control is not always achieved, and treatment may lead to nephrocalcinosis and renal insufficiency. Twice-daily PTH 1-34 injection was suggested as an alternative treatment. We describe an 18 year old female treated successfully with PTH 1-34 administered continuously via a pump.

Patients/ Methods: The patient has glycogen storage disease type III, severe hypoparathyroidism, and celiac disease. Extensive molecular studies were performed in order to achieve a specific diagnosis.

Results: The patient was diagnosed with glycogen storage disease type III at age 1 year. She presented at age 5 years with hypoparathyroidism, and was treated with calcium and magnesium supplements and alfacalcidol. She was reportedly clinically stable with that regimen until 2007, albeit severe hyperphosphatemia persisted. She was first admitted to our hospital on 3/2007 and had two prolonged hospitalizations with severe hypocalcemia, severe hyperphosphatemia, and hypomagnesemia and was dependent on intravenous calcium and magnesium to prevent tetany. CT scan revealed multiple brain calcifications. EEG showed general epileptic activity. At the first hospitalization treatment with PTH 1-34 at 20micgX2/day was started. Significant improvement was initially observed, but after one year treatment was no longer effective and the patient became again dependent on intravenous calcium and magnesium. We initiated treatment with continuous infusion of PTH 1-34 via an insulin pump. Within 24 hours we observed a dramatic increase in serum Ca and Mg and a decrease in serum PO₄. Urinary calcium excretion decreased. EEG normalized. After one year of therapy, the patient continues to have normal Ca, PO₄ and Mg levels, and is very happy with the treatment, reporting better sleep, better academic performance, and an improvement in quality of life. The current PTH 1-34 dose is 18micg/24h. Recently she was found to have celiac disease. Molecular diagnostic studies: Sequencing for the paracellin gene (PCLN1=CLDN 16) revealed normal coding exons. Sequencing for the CaSR revealed compound heterozygosity A986S / R990G. The patient's mother and brother both carry the A986S polymorphism, while the patient's father carries the R990G mutation. All 3 are normocalcemic. HLA typing found the patient to be positive for DQB1*03 – compatible with celiac disease. A genome-wide homozygosity screen is pending.

Conclusions: This patient presented a complex diagnostic and therapeutic challenge. We do not know if her three diseases are related and how they affect each other's clinical course. This case demonstrates the biochemical advantages of PTH replacement therapy, including the avoidance of side effects such as nephrocalcinosis and renal failure.

The impact of increase in effective osteoporosis drugs availability on the incidence of osteoporotic fractures in the Negev population

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Introduction: Osteoporosis is characterized by decreased bone mass and distortion of the skeletal microarchitecture, resulting in increased vulnerability for fractures following mild trauma. Osteoporosis is more common among postmenopausal women, however, men are also affected. Previous surveys carried-out in our area in the pre-effective drug era (late '1970th) and on the verge of the widespread introduction of effective anti-osteoporosis drugs (about 2000), indicated a marked increase in the incidence of hip fractures in the population at risk of women and men age 50 and older, which was mainly attributed to an increase in the fracture incidence in the very old (75 years and older) and to aging of the population. The present study was aimed to evaluate the impact of widespread availability of anti-osteoporosis drugs on the incidence of hip fractures in the same population.

Patients/ Methods: The study included women and men 50 years and older with radiographic evidence of a new fracture caused by low impact trauma. Only residents of the Negev were included. Incidence rates of hip fractures were calculated based on population data obtained from the official Central Bureau of Statistics. Data on osteoporosis drug use in the period of 6 month prior and 6 month following the fracture event were restricted to 75% of the patients who belonged to the Clalit health Services and were extracted from the hospital charts and the Ofek computerized database.

Results: Compared with previous surveys conducted close to the inclusion of effective anti-osteoporosis drugs in the health basket of the State of Israel and immediately after, we observed a persistent increase in the rate of use of effective anti-osteoporosis drugs (bisphosphonates, raloxifene, calcitonin, teriparatide) as well as of all anti-osteoporosis drugs (including calcium and vitamin D preparations) during the 6 months period preceding the fracture event. Anti-osteoporosis drug use was consistently higher in the period following fracture event, but the rate did not increase from that observed immediately after the introduction of effective anti-osteoporosis drugs into the health basket. The absolute rate of use of anti-osteoporosis drugs remained relatively low: use of all treatments before the fracture event was 31% in women and 14% in men, which changed after the fracture event to 53% and 19%, respectively. Use of effective anti-osteoporosis drugs treatments before the fracture was 18% in women and 9% in men, which changed after the fracture event to 27% and 4.5%, respectively. A statistically significant 45% decrease in the incidence of proximal-hip fractures in women 75 years and older was observed in the current survey compared to the year 2000 survey [from 1,900/100,000 (95% CI 1,537–2,356) to 1,044/100,000 (95% CI 1,253–870)]. In men 75 years and older, a 37% decrease in the incidence of hip fractures was observed [from 1,053/100,000 (95% CI 710–1,505) to 649/100,000 (95% CI 482–857)], which did not reach statistical significance.

Conclusions: These results draw a direct line between an increase in the use of anti-osteoporosis preparations and reduction in the incidence of proximal hip fractures in the Negev population in the 7-9 years period since the inclusion of effective anti-osteoporosis drugs in the health basket . This is underlined by the more pronounced decline in hip fractures observed in women, among whom the use of anti-osteoporosis drugs was far more widespread than in men, implicating causality between use of anti-osteoporosis drugs and the decrease in fracture incidence. Despite the increase in the anti-osteoporosis drugs availability, the majority of the population at risk, including et extra-risk, who experienced fracture events, still does not receive them, suggesting that osteoporotic fracture incidence could be decreased even further by filling of the treatment gaps through encouragement of the use of osteoporosis drugs by patients at risk, both women and men.

Post fracture osteoporosis treatment program, is it efficient?

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Introduction: Fragility fractures resulting from low trauma events such as a fall from standing height are common in the elderly. Up to 50% of women and 30% of men will experience an osteoporotic fracture during their lifetime. Patients with fragility fractures have a fivefold increase in risk for further osteoporotic fractures. Orthopedic care usually does not include initiation of fracture prevention treatment.

Patients/ Methods: Fractures Prevention Program (FPP) was initiated in Rambam Health Care Campus in March 2009. All patients (pts) with fragility fractures were referred from the Department of Orthopedic Surgery to the Bone and Mineral Metabolism Unit for fracture prevention treatment.

Results: 347 pts, aged 46-107 (74.5 ± 12.7), 93 (27%) men and 254 (73%) women were hospitalized with fractures in 2009. During ten months period, 228 hip fractures, 23 vertebral fractures and 98 fractures at other sites (proximal and distal humerus, olecranon, wrist, distal femur and femoral shaft, tibia, fibula, and ankle) were diagnosed, 65 (18.7%) patient had previous fragility fractures. Prior to hospitalization only 56 (22%) women have received a fracture prevention treatment: 51 (19.7%) were treated with oral bisphosphonates (48 – Alendronate, 3 - Risedronate), 4 (1.6%) – with SERM (Raloxifen), 1 (0.4%) – with Recombinant PTH (Forteo). None of the men was treated before hospital admission. 25OHD serum level prior to hospitalization was available for 83 (24%) patients. 25OHD level was 60.2 ± 23.7 (14.3-113) nmol/L, 26 (31%) patients had vitamin D deficiency (25OHD ≤ 50 nmol/L), 7 (8.4%) patients had severe vitamin D deficiency (25OHD ≤ 25 nmol/L). 82 (23.6%) pts, 8 (8.6%) men and 74 (29%) women, adhered to the FPP clinic visits, including 30 (53.6%) women previously treated in the community. 47 (20.6%) program participants had femoral neck fractures and 35 (29.4%) – fractures at other locations. 265 (76.4%) pts stayed out of the FPP: 26 (7.5%) women continued their community initiated treatment, 1 (0.3%) man was started on therapy by his family physician, 44 (12.7%) patients refused to join the FPP and remain untreated, 27 (7.8%) – were lost for follow-up, 157 (45.2%) – were unable to reach the clinic and remained untreated in the community, 10 (2.9%) patients died. 109 (32.2%) pts are currently treated for osteoporosis, including 27 in the community: 55 (16.3%) pts receive alendronate, 19 (5.6%) – risedronate, 2 (0.6%) – raloxifen, 10 (3%) – zoledronate, 10 (3%) - teriparatide, 13 (3.8%) – calcium and vitamin D only to ensure vitamin D replenishment prior to bisphosphonate treatment. 229 (67.8%) patients remain untreated.

Conclusions: Despite high prevalence of osteoporosis in the elderly, most patients remain untreated even after fragility fractures. Hospital based FPP increased by 10% the rate of post fracture treatment. Male patients and patients with hip fractures are more likely to stay untreated after hospitalization. Taking into account post-fracture disability and low compliance, a structured post fracture hospital initiated program with intravenous zoledronate might lead to better results and is worth to be tested.

Osteoprotegerin as an independent marker of subclinical atherosclerosis in osteoporotic postmenopausal women

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Introduction: Osteoprotegerin (OPG) appears to represent the molecular link between bone resorption and vascular calcification, and may help to explain the high prevalence of atherosclerosis and osteoporosis in postmenopausal women. We investigated a possible association between serum OPG levels and arterial stiffness in postmenopausal women with osteoporosis.

Patients/ Methods: 70 postmenopausal women with osteoporosis and cardiovascular risk factors but without coronary artery disease were evaluated for metabolic, inflammatory parameters and serum OPG levels. Pulse wave velocity (PWV) and augmentation index (AIx) were performed as a simple noninvasive recording of the two artery sites pressure waveform using SphygmoCor (version 7.1, AtCor Medical, Sydney, Australia).

Results: Serum OPG levels were significantly, positively associated with AIx ($r=0.39$, $p=0.003$) and with PWV ($r=0.81$, $p<0.0001$). No association between OPG levels and hemodynamic variables or measures of glucose metabolism was observed. Among inflammatory markers, OPG was significantly, positively associated with fibrinogen ($r=0.323$, $p=0.015$). In a multiple linear regression analysis, OPG was independent predictor of PWV (standardized beta=0.75, $p<0.0001$) and AIx (standardized beta=0.41, $p=0.01$).

Conclusions: Serum OPG is potentially an independent predictor of early vascular adverse changes in osteoporotic postmenopausal women.

Bone turnover markers in the follow-up of bisphosphonate treated osteoporotic patients

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Introduction: Tools for assessment of long term effects of bisphosphonates (BP) in osteoporotic patients are needed, especially, due to the occasional clinical reports of severely suppressed bone turnover and atypical skeletal fragility, as well as presumably increased risk of osteonecrosis of jaw in patients with carboxy terminal telopeptide (CTX) plasma level <0.2 ng/ml. Drug holiday is a common practice after long term BP use. Data about the rate of bone turnover recovery after long term BP treatment is scarce.

Patients/ Methods: 300 patients(pts) aged 68.56±8.14 (mean±SD) years were evaluated after 4-10 years of alendronate therapy. Two bone turnover markers (BTM): aminoterminal propeptide of type I collagen (P1NP) and carboxy terminal telopeptide (CTX) were assessed in pts' fasting morning plasma samples during long term BP treatment.

Results: Plasma levels (mean±SD, ng/ml) after 4 years of treatment were 7.7±25.32 and 0.2± 0.09, after 5 years 11.23±26.02 and 0.13±0.23, after 6 years 10.08±22.32 and 0.12±0.19 after 7 years 8.09±23.67 and 0.11±0.21, after 8 years 6.94±22.49 and 0.006±0.18, after 9 years 6.94±22.49 and 0.006±0.18, after 10 years 12.17±27.17 and 0.1±0.21 for P1NP and CTX respectively. There was no significant change in the levels of both bone turnover markers over the duration of therapy. Normal premenopausal range, a target range for BP treated pts, for CTX was 0.025-0.573 ng/ml and for P1NP - 15.13-58.59 ng/ml, as reported by the manufacturer (Roche). One (0.3 %) pt had CTX values below premenopausal range, 156 (51.9 %) had CTX levels <0.2 ng/ml, 26 (8.75%) pts had P1NP levels below premenopausal in all treatment duration groups. None of the pts reported abnormal healing after oral cavity procedures. Forty five pts that were assessed during BP treatment, stopped treatment for drug holidays. From these 30 were evaluated 6 months after treatment discontinuation: 9 (20 %) demonstrated a significant rise in P1NP (increase by ≥ 10 ng/ml) and in CTX (> 0.2 ng/ml), in 2 pts (4.4%) only P1NP value increased and in 5 (11.1%) only CTX. 31 pts were evaluated 1 year after treatment discontinuation: in 5 (11.1%) increase in both markers was observed, in 1 (2.2%) a rise in P1NP and in another (2.2%) a rise in CTX only was observed.

Conclusions: Most pts on long term bisphosphonate treatment had bone turnover markers levels within normal premenopausal range irrespective of treatment duration. Suppressed bone turnover was observed in a small subset of patients. After treatment discontinuation only one fifth of the pts demonstrated increase in bone turnover.

Sirtuin 1 (sirt1) is a regulator of marrow Adipogenesis

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Introduction: Bone loss is an inevitable consequence of aging. Age-associated bone loss results from insufficient osteoblasts attributed to a shift of multipotential mesenchymal stem cell progenitors towards the adipocyte lineage at the expense of the osteoblast lineage. Sirtuin 1 (SIRT1) a NAD⁺-dependent deacetylase was found to be a key regulator of life span in lower species and in multiple metabolic pathways and age-associated diseases in mammals. First identified for its role in chromatin remodeling and gene silencing, it was then discovered to be the mediator of the life extending effect of calorie restriction. Importantly, SIRT1 was found to repress the transcription of the nuclear receptor peroxisome proliferator-activated receptor γ (PPAR γ), a master regulator of adipocyte differentiation and its transcriptional activity (Picard et al. Nature 2004). By recruitment to the PPAR γ promoter together with the corepressors NCoR and SMRT SIRT1 represses PPAR γ gene expression. Since osteoblasts and adipocytes originate from a common progenitor, we sought to test the hypothesis that SIRT1 is involved in the marrow mesenchymal stem cell fate to differentiate into an osteoblast or adipocyte.

Patients/ Methods: Studies were performed in the murine embryonic mesenchymal stem cell line C3H10T1/2 and in primary bone marrow mesenchymal cell cultures obtained from 12 week-old female SIRT1^{+/-} mice and their WT counterparts (general SIRT1 ablation in inbred 129 sv mice is lethal). SIRT1 over-expression in C3HT101/2 cells was modified through retroviral infection with pBABE-SIRT1 with puromycin selection. Adipogenesis was induced with insulin, dexamethasone, indomethacin IBMX and rosiglitazone. Adipogenesis was assessed 14 days post induction by Oil-Red-O staining. RNA was extracted and gene expression was determined by Real Time PCR. Protein expression was evaluated by western blotting.

Results: Following induction of adipogenic differentiation Sirt1^{+/-} -derived bone marrow mesenchymal stem cell cultures exhibited increased adipocyte formation, corresponding with decreased osteoblast formation, as determined by Oil-Red-O staining. There was a five fold increase in PPAR γ gene expression and a 3 fold increase in CEBP α gene expression 48 hours post adipogenesis induction in SIRT1-derived cultures as compared to WT-derived cultures. A reciprocal finding was demonstrated in SIRT1-overexpressing C3HT101/2 cells with reduced adipogenesis compared to control cells.

Conclusions: These results indicate that SIRT1 plays a role in the bone marrow mesenchymal progenitor cell fate to differentiate into an osteoblast or an adipocyte possibly via its influence on PPAR γ . Our findings suggest that SIRT1 is involved in the pathogenesis of osteoporosis.