

REVIEW

Potential Biomolecules Capable of Assessing Risk of Osteoporotic Fracture: A Scoping Review

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Abstract Osteoporosis as a systemic chronic skeletal disease is characterized by low bone mineral density and increased risk to osteoporotic fractures. Osteoporosis is prevalent in the middle-aged and elderly population, especially in the postmenopausal women. With population aging, osteoporosis has become a world-wide serious public health problem. Early recognition of the high-risk population followed by timely and efficient intervention and/or treatment is important for preventing osteoporotic fractures. In light of the high heritability and complex pathogenesis of osteoporosis, comprehensive consideration of vital biological/biochemical factors is necessary for accurate risk evaluation of fractures. For this purpose, we review recent research progress on molecules which can be applied to assess risk for osteoporotic fractures. Future integrative analyses and systematic evaluation of these molecules may facilitate developing novel methodologies and/or test strategies, i.e., biochips, for early recognition of osteoporosis, hence contributing to preventing osteoporotic fractures.

OSTEOPOROSIS (OP) is a chronic skeletal disease characterized by decreased bone strength and susceptibility to osteoporotic fractures (OFs). OP is prevalent in

the middle-aged and elderly population, especially in the postmenopausal women. With population aging, OFs have become an increasingly serious public health problem. A report from World Health Organization (WHO) in 2015 noted that OFs have adverse health effects on individuals in both developed and developing countries.^[1]

The FRAX tool is commonly used to determine the individuals who are at risk of OFs.^[2] The National Osteoporosis Foundation performed an investigation in Chinese using the Chinese FRAX® model, a modified FRAX tool, and demonstrated an increase in 10-year risk to hip fracture and major OF with age.^[3] However,

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a cohort study carried out in Hong Kong showed that the FRAX method dramatically underestimated the fracture risk of Chinese population.^[4] Therefore, it is necessary to identify more factors used in combination with existing methods and more fine-tuned method applied for Chinese population for the purpose of precision prediction/medicine.

OFs are complex conditions influenced by interactions of genetic and environmental factors. A large number of genes have been identified to be associated with bone mineral density (BMD) and OFs in humans.^[5, 6] Meanwhile, some novel biomolecules have been determined to be involved in OFs.^[7] Therefore, it is reasonable for us to believe that targeted evaluation of a panel of biomolecules would become feasible in the near future, and facilitate developing biomolecules to effectively assess OFs risk.

For the above purpose and perspective, we searched PubMed with the terms: "osteoporotic fractures AND molecular", "osteoporotic fractures AND protein", "osteoporotic fractures AND RNA", "osteoporotic fractures AND metabolite", and "osteoporotic fractures AND lipid" to identify clinical epidemiologic researches on potential molecular biomarkers such as proteins/peptides, RNAs, lipids, etc. We detected that a number of proteins such as insulin-like growth factor (IGF) and periostin, as well as metabolites including nucleic acid, amino acid and lipid mediators, may serve as indicators to identify or to predict the risk of OFs.^[8]

POTENTIAL BIOMARKERS HAVING THE CAPACITY FOR PREDICTING OFS RISK

Protein and peptide molecules

Table 1 lists a dozen of predictive protein molecules associated with OFs, which have been identified by several prospective studies.^[9-22]

Thereinto serum insulin-like growth factor binding protein-1 (IGFBP-1),^[9] periostin,^[10, 11] adiponectin,^[12, 13] C-reactive protein (CRP),^[14] bone-specific alkaline phosphatase (BAP)^[15] and fibroblast growth factor 23 (FGF23)^[16, 17] were considered as contributing factors towards OFs risk increase. Lundin *et al.*^[9] discovered that IGFBP-1 level in serum was an independent risk factor associated with hip fracture (HF) [adjusted hazard ratio (HR)=1.46 per SD increase, 95%CI (1.08-1.99)] and major OFs [HR=1.33, 95%CI (1.05-1.69), $P=0.017$] in Sweden women. Besides, periostin is a secreted γ -carboxyglutamic acid-containing pro-

tein, which is mainly expressed in the periosteum of adults.^[23] Higher level of periostin in serum can increase fracture risk. For instance, Rousseau *et al.*^[10] discovered that the highest quartile of serum periostin was associated with an increased risk of fracture with an adjusted relative ratio (RR) of 1.88 in a prospective study [95%CI (1.1-3.2)] after eliminating the effects of age, fragility fracture, and hip BMD T-score. Periostin fragment resulting from cathepsin K digestion was an independent risk factor of OFs in females as well [HR=2.14, 95%CI(1.54-2.97)].^[11] In addition, Johansson *et al.*^[12] reported a link between an increased risk of OFs and enhanced serum adiponectin level [HR/SD=1.46, 95%CI (1.23-1.72)], such association remained significant even after adjusting the confounding effect of age, time, BMD total hip, general health, and previous fracture [HR/SD=1.30, 95%CI (1.09-1.55)]. In addition, the significant association between serum adiponectin level and incident of OFs in males was also observed [HR=1.47, 95%CI (1.23-1.77)].^[13]

Serum CRP level was regarded as a risk factor associated with any type of OFs [HR=1.06, 95%CI (1.02-1.11)], HFs [HR=1.09, 95%CI (1.02-1.17)] and vertebral fractures (VFs) [odds ratio (OR)=1.34, 95%CI (1.14-1.58)] in a prospective study of 6386 participants (59% female).^[14] However, clinical value and potential ability of CRP in diagnosing OFs need to be further validated, especially for a single patient. In addition, serum BAP level increase may elevate the likelihood of osteoporotic VFs of 65 postmenopausal women whose menstruation had ceased for less than 5 years [RR per SD=4.38, 95%CI (1.45-13.21)].^[15] In a study involving 457 postmenopausal women who stopped menstruating greater than 5 years, those in the upper tertile for BAP might be more prone to VFs [RR=1.39, 95%CI (1.12-1.74)].^[15] Furthermore, Mirza *et al.*^[16] considered FGF23 protein level in serum above 55.7 pg/ml to be a risk indicator related to HFs [HR=2.30, 95%CI (1.16-4.58)] as well as non-VFs [HR=1.63, 95%CI (1.01-2.63)], respectively. In addition, they found that the risk of developing non-vertebral OFs in subjects with the highest quartile of FGF23 level in serum (>57.4 pg/ml) increased by 63% compared to those with the lowest three quartiles. For men who suffered from renal insufficiency only (eGFR_{CrCys}< 60 ml/min·1.73 m²), the highest quartile of serum FGF23 level was also found to be a risk for hip and non-spine OFs with relative hazard (RH) of 2.02 [95%CI (1.07, 3.79)].^[17]

Besides, a couple of protective factors related to OFs, including sclerostin^[18] and endogenous secretory receptor for advanced glycation end products (esRAGE)-to-pentosidine (PEN) ratio,^[19] have been identified thus far. For example, Szulc *et al.*^[18] reported that French men with higher concentration of serum sclerostin had lower OFs risk [$HR=0.55$, 95% CI (0.31-0.96), $P<0.05$ adjusted for hip BMD]. In another study carried out in Japanese, negative correlations between esRAGE-to-PEN ratio and the incidence of OFs at femoral neck [FN, $HR=0.67$, 95% CI (0.45-0.98)] or lumbar spine (LS, $HR=0.64$, 95% CI (0.43-0.95)] were detected.^[19]

Moreover, several peptide molecules, such as bone resorption markers C terminal cross linking telopeptide of type I collagen (CTX) and bone formation markers—N-terminal propeptide of type I procollagen (PINP), have been delineated to have great predictive value for OFs. For example, early in 1996, serum CTX level above the upper limit of the premenopausal range has been found to be associated with an increased risk of HF [OR=2.2, 95% CI (1.3-3.6)].^[20] In 2002, Garnero *et al.*^[21] reported that both elevated urinary alpha-L/beta-L-CTX ratio and low femoral neck BMD (T score<-2.5) might result in greater risk of OFs in women [$RR=4.5$, 95% CI (2.0-10.1)]. Afterwards, Yoshimura *et al.*^[22] confirmed that serum beta-CTX ($HR=1.80$, $P<0.001$) as well as N-terminal cross-linking telopeptide of type I collagen (NTX, $HR=1.96$, $P<0.01$) were independent risk factors of spinal OFs in Japanese women, furthermore, serum PINP level of the OFs patients was significantly higher than that of the controls in both men ($HR=2.80$, $P<0.05$) and women ($HR=1.65$, $P<0.05$).

Metabolites

Some polar metabolites are involved in nucleic acid metabolism, amino acid metabolism and lipid metabolism, which are closely related to the imbalance between bone resorption and formation and may underlie the development of OFs.^[24] **Table 2** summarizes three categories of potential predictive metabolite molecules associated with OFs.

Nucleic acid metabolites

According to the EPIDOS cohort study including 7598 French female, free deoxypyridinoline secretion above the upper limit of the premenopausal range was associated with an increased HF risk [OR=1.9, 95% CI (1.1-3.2)].^[20] Additionally, high level of urinary deoxy-

pyridinoline significantly predicted 10-year OFs risk of the LS in women ($HR=1.40$, $P<0.05$) but not in men [$HR=1.53$, 95% CI (0.63-3.73)].^[22] However, the significant associations were only validated in epidemiological research, rather than in specific clinical treatment, so the potential predictive value needs to be further addressed.

Amino acid molecules

There existed positive links between plasma total homocysteine (tHcy) and OFs risk.^[25, 26] The adjusted HR (95% CI) for OFs in subjects with high (≥ 15.0 $\mu\text{mol/L}$) vs. low levels (<9.0 $\mu\text{mol/L}$) of tHcy was significant in women [$HR=2.42$, 95% CI (1.43-4.09)] but not in men [$HR=1.37$, 95% CI (0.63-2.98)].^[25] Whereas the incidence of OFs was associated with quartiles of homocysteine (Hcy) ($P=0.047$), and high risk of OFs was only observed in females aged 55 years and over [adjusted for age, BMI, and FN-BMD, $HR=1.82$, 95% CI (1.02-3.22)] from the Rotterdam Study during 7 years of follow-up.^[26]

Meanwhile, dimethylglycine (DMG) is a product of the choline oxidation pathway and generated from betaine during the folate-independent remethylation of Hcy to methionine. Lower level of plasma DMG was associated with an increased risk of HF [$HR=1.70$, 95% CI (1.28-2.26)], as compared to the highest tertile.^[27]

Lipid mediators

Osteoporosis or osteopenia often co-exists with lipid metabolism disorders.^[28] Studies suggested that peroxisome proliferator activated receptor gamma (PPAR γ) may play a key role in the pathogenesis of both osteoporosis and lipid metabolism disorder, and lipid metabolism disorders caused by bone marrow fat may be the main cause of bone marrow microcirculation disorders and osteopenia.^[29]

Sphingosine-1-phosphate (S1P) plays diverse roles in cell differentiation, apoptosis, proliferation, motility, bone metabolism, and the most prominent role seems to be the augmentation of bone resorption.^[30] It was reported that the plasma S1P level was significantly higher in the postmenopausal women with OFs than those without OFs (7.23 ± 0.79 $\mu\text{mol/L}$ vs. 5.02 ± 0.51 $\mu\text{mol/L}$; $P<0.001$).^[31] After adjustment for age and other confounders, the HR of OFs was 6.12 (95% CI : 4.92-7.66) per SD increase in plasma S1P level, and the women in the highest quartile of S1P levels had a significantly increased fracture risk

Table 1. Potential protein and peptide molecules in serum associated with OFs

Molecules	References	Sample size	Gender	Ethnicity	Age (yrs) (mean $\pm$ SD or range)	Follow-up (yrs)	Phenotype	Concentration	Effect size or difference	
									Male	Female
IGFBP-I	Lundin <i>et al.</i> ^[9]	351	Female	Sweden	72.8 $\pm$ 2.31	10	OF	NA	NA	HF: Adj. HR=1.46, 95%CI(1.08-1.99) per SD increase; MOF: Adj. HR=1.33, 95%CI(1.05-1.69, P=0.017)
Periostin	Rousseau <i>et al.</i> ^[10]	607 (women with incident fracture: 75 vs. women without incident fracture: 532)	Female	French	66.6 $\pm$ 8.4	7	VF	1249 $\pm$ 340 vs. 1162 $\pm$ 298 ng/ml	NA	RR=2.81, 95%CI(1.1-7.3), P=0.03; Adj. RR=1.88, 95%CI(1.1-3.2)
K-Postn	Bonnet <i>et al.</i> ^[11]	695 (fracture: 66 vs. no fracture: 629)	Female	Geneva	65.0 $\pm$ 1.5	(mean $\pm$ SD) 4.7 $\pm$ 1.9	Low-trauma clinical fracture	57.5 $\pm$ 36.6 vs. 42.5 $\pm$ 23.4 ng/ml	NA	HR=2.14, 95%CI(1.54-2.97), P<0.001
Adiponec-tin	Johansson <i>et al.</i> ^[12]	999 (fracture: 150 vs. no fracture: 849)	Male	Swedish	NA	(mean) 5.2	OF	14.2 $\pm$ 8.9 vs. 11.5 $\pm$ 5.8 μ g/ml	HR/SD=1.46, 95%CI (1.23-1.72)	NA
Adiponec-tin	Johansson <i>et al.</i> ^[13]	989 (fracture: 124 vs. no fracture: 865)	Male	Swedish	70-81	(mean) 5.3	OF	14.4 $\pm$ 9.3 vs. 11.5 $\pm$ 5.8 μ g/ml	Adj. for age and time since baseline HR=1.47, 95%CI(1.23-1.77) per SD; Adj. for FN BMD and age HR=1.40, 95%CI(1.16-1.68) per SD	NA
CRP	Oei <i>et al.</i> ^[14]	6386	59% female	Rotterdam	69.1 $\pm$ 8.9	(mean) 11.6	OF	NA	OF: HR=1.06, 95%CI(1.02-1.11) HF: HR=1.09, 95%CI(1.02-1.17) VF: OR=1.34, 95%CI(1.14-1.58)	YSM<5 years: RR=4.38, 95%CI(1.45-13.21); YSM $\geq$ 5 years: RR=1.39, 95%CI (1.12-1.74)
BAP	Tamaki <i>et al.</i> ^[15]	522	Female	Japanese	54.7 (YSM<5 years); 66.6 (YSM $\geq$ 5 years)	(median) 10	VF	NA	NA	NA
FGF23	Mirza <i>et al.</i> ^[16]	2868	Male	Swedish	75.4 $\pm$ 3.2	(median) 3.35	OF/VF	NA	OF: HR=1.20, 95%CI(1.03-1.40); VF: HR=1.33, 95%CI(1.02-1.75); Above 55.7 pg/ml: HF: HR=2.30, 95%CI(1.16-4.58); non-VF: HR=1.63, 95%CI(1.01-2.63)	NA
FGF23	Lane <i>et al.</i> ^[17]	1680 (*Q1: 346; *Q2: 346; *Q3: 345; *Q4: 347)	Male	Swedish	Q1:73.40 $\pm$ 5.8; Q2:73.63 $\pm$ 5.91; Q3:74.40 $\pm$ 6.0; Q4:73.70 $\pm$ 5.8	5.3	Non-spine OF	Q1-Q3: 4.2-22.4 pg/ml; vs. Q4: 22.4-111.1 pg/ml	RH=2.02, 95%CI(1.07-3.79) in men with eGFR _{Crois} <60 ml/min-1.73 m ²	NA

Continued Table 1. Potential protein and peptide molecules in serum associated with OFs

Molecules	References	Sample size	Gender	Ethnicity	Age (yrs) (mean $\pm$ SD or range)	Follow-up (yrs)	Phenotype	Concentration	Effect size or difference	
									Male	Female
Sclerostin	Szulec et al. ^[18]	710 (fracture: 75 vs. no fracture: 635)	Male	French	65 $\pm$ 7	10	OF	0.54 (0.47, 0.64) vs. 0.61 (0.49, 0.77) ng/ml	All fracture: HR=0.55, 95%CI(0.31-0.96) per SD, P<0.05	NA
PEN	Tamaki et al. ^[19]	1285 (fracture: 25 vs. no fracture: 1260)	Male	Japanese	73.1 $\pm$ 5.1	5	OF	0.057 vs. 0.049 μ g/ml	FN:HR=1.48, 95%CI(1.00-2.18); LS:HR=1.51, 95%CI(1.03-2.21)	NA
esRAGE-to-PEN ratio	Tamaki et al. ^[19]	1285 (fracture: 25 vs. no fracture: 1260)	Male	Japanese	73.1 $\pm$ 5.1	5	OF	(geometric mean $\pm$ SD) 3.6 $\pm$ 1.8 vs. 4.9 $\pm$ 0.9	FN: HR=0.67, 95%CI(0.45-0.98); LS: HR=0.64, 95%CI(0.43-0.95)	NA
CTX	Garnero et al. ^[20]	7598 (hip fracture: 109 vs. controls: 292)	Female	French	HF: 82.6 $\pm$ 4.6; Controls: 82.5 $\pm$ 4.5	(mean) 22 months	HF	305 $\pm$ 163 vs. 266 $\pm$ 150 μ g/mmol Cr	NA	OR=2.2, 95%CI(1.3-3.6)
CTX	Garnero et al. ^[21]	408 (fracture: 65 vs. controls: 343)	Female	French	Fracture: 67 $\pm$ 9; Controls: 64 $\pm$ 9	(median) 6.8	VF and peripheral fractures	22.8 $\pm$ 8.0 vs. 21.2 $\pm$ 8.5 μ mol/mmol Cr	NA	RR=4.5, 95%CI(2.0-10.1)
beta-CTX	Yoshimura et al. ^[22]	400 (male: 199 vs. female: 200)	Male/female =1/1	Japanese	Male: 59.4 $\pm$ 11.4; Female: 59.8 $\pm$ 11.6	10	Spinal OF	Male: 0.187 $\pm$ 0.121 ng/ml; Female: 0.234 $\pm$ 0.145 ng/ml	NS	HR=1.80, P<0.001
NTX	Yoshimura et al. ^[22]	400 (male: 199 vs. female: 200)	Male/female =1/1	Japanese	Male: 59.4 $\pm$ 11.4; Female: 59.8 $\pm$ 11.6	10	Spinal OF	Male: 13.6 $\pm$ 4.2 nmolBCE/L; Female: 15.2 $\pm$ 4.0 nmolBCE/L	NS	HR=1.96, P<0.01
PINP	Yoshimura et al. ^[22]	400 (male: 199 vs. female: 200)	Male/female =1/1	Japanese	Male: 59.4 $\pm$ 11.4; Female: 59.8 $\pm$ 11.6	10	Spinal OF	Male: 38.2 $\pm$ 19.5 ng/ml; Female: 50.1 $\pm$ 21.9 ng/ml	HR=2.80, P<0.05	HR=1.65, P<0.05

OF: osteoporotic fracture; IGFBP-I: insulin-like growth factor binding protein 1; K-Postn: periostin fragment resulting from cathepsin K digestion; CRP: C-reactive protein; BAP: bone-specific alkaline phosphatase; FGF23: fibroblast growth factor 23; PEN: pentosidine; esRAGE-to-PEN ratio: advanced glycation end products-to-pentosidine ratio; CTX: C-terminal cross-linking telopeptide of type I collagen; NTX: N-terminal cross-linking telopeptide of type I collagen; PINP: N-terminal propeptide of type I procollagen; NF: non-fracture; NA: not available; YSM: years since menopause. HF: hip fracture; VF: vertebral fracture; RH: relative hazards; HR: hazard ratio; FN: femoral neck; BMD: bone mineral density; eGFR_{Croys}: cystatin C and creatinine-based estimated glomerular filtration rate; LS: lumbar spine; NS: not significant; MOF: major osteoporotic fractures; RR: risk ratio; OR: odds ratio.

*Quartiles of FGF23 (pg/ml): Q1: 4.2-12.0, Q2: 12.1-16.6, Q3: 16.6-22.4, Q4: 22.4-111.1.

[$HR=9.89$, 95% CI (2.83-34.44)].^[31]

Ginsberg *et al.*^[32] revealed a significant association of vitamin D with hip BMD and risk of HF during follow-up for about 8.4-years. They found both higher 24, 25(OH)₂D and vitamin D metabolite ratio [VMR, i.e., the ratio of 24, 25(OH)₂D to 25 (OH)D] were associated with lower risk of HFs [$HR=0.73$, 95% CI (0.61-0.87) and 0.74, 95% CI (0.61-0.88) per SD increase, respectively]. Swanson *et al.*^[33] also reported that 25(OH)D and 1, 25(OH)₂D were protective against HFs. Specifically, the risk of HFs was higher in men with the lowest 1, 25(OH)₂D levels (8.70 to 51.60 pg/ml) after adjustment for baseline hip BMD [HR (95% CI)=1.99 (1.19-3.33)].^[34] In addition, the lowest quartile of 25(OH)D (3.13 to 20.90 ng/ml) also increased the risk of HFs in American men [$HR=2.01$, 95% CI (1.24-3.26)].^[33] However, there existed some inconsistent results regarding to these associations.^[35] To our knowledge, no prior study has evaluated whether 24, 25(OH)₂D concentration or VMR is more strongly associated with bone health than 25(OH)D alone. These results do not support the hypothesis that measures of 1, 25(OH)₂D could improve the ability to predict adverse skeletal outcomes when 25(OH)D measures are available. Still, there is long way before reaching the potential diagnostic role of 25(OH)D and 1, 25(OH)₂D in predicting risks to OFs. Epidemiological outcomes need to be further elaborated when applying to clinical trials.

To be noted, higher concentration of plasma polyunsaturated fatty acids (PUFAs) decreased the OFs risk in older adults.^[36] The highest tertile of plasma PUFAs was associated with decreased OFs risks [$HR=0.60$, 95% CI (0.41-0.89)] in men; as well as in women ($P_{trend}=0.06$).^[36] However, PUFAs are novel molecules related to OFs and only conducted in epidemiological research among population, and the further significant value in clinical usage to indicate OFs needs to be validated in single patient.

POTENTIAL MOLECULES THAT EXHIBIT DIAGNOSTIC VALUE FOR OFS

At present, a large number of scientific researches have demonstrated that some proteins such as insulin-like growth factor-1 (IGF-1) and IGF-binding protein-3 (IGFBP-3), as well as microRNAs (miRNAs) and metabolites could act great diagnostic indicators for OFs.

Proteins

Potential diagnostic protein molecules associated with OF risks were identified in cross-sectional studies, which are summarized and listed in **Table 3**. Serum IGF-I protein level was positively correlated with BMD and negatively associated with spinal OF in Japanese women.^[37] Moreover, Nakaoka *et al.*^[38] discovered that IGF-I protein was negatively correlated with the risk of spinal OFs in Japanese postmenopausal women [$HR=0.19$, 95% CI (0.07-0.59)]. Similar results were found for VFs [$OR=0.29$, 95% CI (0.15-0.57) per SD increase, $P=0.0003$].^[39] Besides, IGFBP-3 was also negatively associated with both spinal OFs ($r=-0.409$, $P<0.0001$)^[37] and VFs [$OR=0.31$, 95% CI (0.16-0.61), $P=0.0007$]^[39] in Japanese women. What's more, plasma periostin was a risk indicator of any fracture [$OR=1.50$, 95% CI (1.14-1.97), $P=0.003$], non-VF fracture [$OR=1.59$, 95% CI (1.12-2.24), $P=0.009$] in Koreans.^[40] The odds for non-VFs was 2.48-fold higher in subjects with the highest vs. lowest tertiles of plasma periostin level (95% $CI=1.10$ -5.61).^[40] Consistently, serum periostin level was positively correlated with femoral neck BMD ($r=-0.529$, $P<0.001$) in Chinese elderly females.^[41]

microRNAs

miRNA are a class of small (~22 nucleotides), single-stranded noncoding RNAs. They are considered "micro-managers" of gene expression, and their role in regulating biological functions has been increasingly recognized. The diagnostic miRNAs molecules associated with OFs risk are summarized in **Table 4**.

Seeliger *et al.*^[42] identified that nine serum miRNAs (miR-21, miR-23a, miR-24, miR-93, miR-100, miR-122a, miR-124a, miR-125b, and miR-148a) were significantly up-regulated in OFs, which presented modest diagnostic performance individually (AUC=0.61-0.77, $P<0.05$). Among the above nine miRNAs, miR-21, miR-23a, miR-24, miR-100 displayed a significantly higher expression level in bone tissues in OFs compared with controls. In 2015, Panach *et al.*^[43] showed that three miRNAs in serum (miR-122-5p, miR-125b-5p and miR-21-5p) were up-regulated in fracture cases vs. controls. Indeed, miR-21-5p was one of the few miRNAs, together with miR-223 and miR-155, which were directly related to osteoclast differentiation.^[44] In an explorative analysis of 175 serum miRNAs in patients with recent OFs and age-matched control, the down-regulation of miR-328-3p and let-7g-5p in

Table 2. Potential metabolite molecules capable of predicting OFs

Molecules	References	Study design	Sample	Sample size	Gender	Ethnicity	Age (yrs)	Follow-up (yrs)	Phenotype	Concentration	Effect size or difference	
											Male	Female
Free deoxyphenylalanine	Garnero <i>et al.</i> ^[20]	Prospective	Urine	7598 (HF: 109; Controls: 292)	Female	French	(mean±SD) HF: 82.6±4.6; Controls: 82.5±4.5	(mean) 22 months	OF	6.41±2.20 vs. 5.80±1.94 nmol/mmol Cr	NA	OR=1.9, 95%CI(1.1-3.2)
Deoxyphenylalanine	Yoshimura <i>et al.</i> ^[22]	Prospective	Urine	400 (Male: 199; Female: 200)	Male/female	Japanese	(mean±SD) Male: 59.4±11.4; Female: 59.8±11.6	10	Spinal OF	Male: 3.06±1.53 pmol/lmol Cr; Female: 4.76±1.91 pmol/lmol Cr	NS	HR=1.40, P<0.05
Homocysteine	Gjesdal <i>et al.</i> ^[25]	Prospective	Plasma	4766	Male/female=2127/2639	Norway	65-67 at enrollment	(mean) 12.6	HF	7-20 µmol/L	NS	HR=2.42, 95%CI (1.43-4.09)
Homocysteine	Ennenman <i>et al.</i> ^[26]	Prospective	Plasma	503	Female	Netherlands	(median) 68.5	(mean) 7	OF	9.4-11.6 µmol/L	NA	Adj. for age, BMI, FN-BMD, HR=1.82, 95%CI (1.02-3.22)
DMG	Øyen <i>et al.</i> ^[27]	Longitudinal	Plasma	3310	Male/female=1017/1204	Norway	71-74	(mean) 10.8	HF	4.4±1.5 µmol/L	Adj. for BMD, HR=1.70, 95%CI (1.28-2.26)	
S1P	Ardawi <i>et al.</i> ^[31]	Prospective longitudinal	Plasma	707 (Fracture: 138; No fracture: 569)	Female	Saudi Arabia	(mean) 61	(median) 5.2±1.3	OF	7.23±0.79 vs. 5.02±0.51 µmol/L	NA	Adj. HR=6.12, 95%CI(4.92-7.66) per SD increase.
24, 25(OH) ₂ D	Ginsberg <i>et al.</i> ^[32]	Longitudinal	Serum	890	Male/female=267/178	16% African-American	(mean) 78	(mean) 8.4	HF	1.7±1.0 ng/ml	HR=0.73, 95%CI (0.61-0.87) per SD increase	
VMR	Ginsberg <i>et al.</i> ^[32]	Longitudinal	Serum	890	Male/female=267/178	16% African-American	(mean) 78	(mean) 8.4	HF	6.84±2.23 ng/ml	HR=0.74, 95%CI (0.61-0.88) per SD increase	
25(OH)D	Swanson <i>et al.</i> ^[33]	Prospective	Serum	1000 (Q1: 144; Q2: 171; Q3: 113; Q4: 109)	Male	USA	74.6±6.2	(mean) 5.1	Non-VF	Q1: 3.13-20.90; Q2: 20.91-25.90; Q3: 26-31; Q4: 31.10-55.80 ng/ml	Q1 (Q2,Q3,Q4 as NA ref.): HR=2.03, 95%CI (1.25-3.29)	

Continued **Table 2.** Potential metabolite molecules capable of predicting OFs

Molecules	References	Study design	Sample	Sample size	Gender	Ethnicity	Age (yrs)	Follow-up (yrs)	Pheno- type	Concentration	Effect size or difference	
											Male	Female
1, 25(OH) ₂ D	Swanson et al. ^[33]	Prospective	Serum	1000 (Q1: 144; Q2: 171; Q3: 113; Q4: 109)	Male	USA	74.6±6.2	(mean) 5.1	Non-VF	Q1: 8.70-51.60; Q2: 51.70-62.00; Q3: 62.10-75.10; Q4: 75.20-142.00	Q1 (Q2,Q3,Q4 as NA ref.): <i>HR</i> =2.01, 95% <i>CI</i> (1.20-3.36)	
				ng/ml								
				NA								
				NA								
PUFAs	Harris et al. ^[36]	Longitudinal	Plasma	Fracture: 540; No fracture: 898	OF: male/fe-male=19/35; NF: male/fe-male=429/469	NA	OF:78.5±5.69; NF:76.5±5.48	(median) 7	OF	NA	<i>HR</i> =0.60, 95% <i>CI</i> (0.41-0.89)	Inverse association, <i>P</i> _{trend} =0.06
			Serum									

DMG: dimethylglycine; S1P: sphingosine-1-phosphate; 24, 25(OH)₂D: 24, 25-dihydroxy vitamin D; VMR: ratio of 24, 25(OH)₂D to 25(OH)D; 25(OH)D: 25-hydroxy vitamin D; PUFAs: polyunsaturated fatty acids; BMI: body mass index; FN-BMD: femoral neck-bone mineral density.

Table 3. Potential proteins having ability to diagnose OFs

Molecules	References	Study design	Sample	Sample size (n)	Gender	Ethnicity	Age (yrs)	Phenotype	Concentration	Effect size or difference
IGF-I	Sugimoto <i>et al.</i> ^[37]	Cross-sectional	Serum	165	Female	Japanese	(mean) 62	Spinal OF	NA	Lower in OF, $P<0.01$; cutoff 110 ng/ml provided a specificity of 81% with a sensitivity of 76%
IGF-I	Nakaoka <i>et al.</i> ^[38]	Cross-sectional	Serum	205	Female	Japanese	(mean) 64	Spinal OF	NA	$OR=0.19$, 95% CI (0.07-0.59), $P=0.003$
IGFBP-3	Sugimoto <i>et al.</i> ^[37]	Cross-sectional	Serum	165	Female	Japanese	(mean) 62	Spinal OF	NA	Lower in OF, $P<0.01$; cutoff 2.1 µg/ml provided a specificity of 81% with a sensitivity of 81%
IGFBP-3	Yamaguchi <i>et al.</i> ^[39]	Cross-sectional	Serum	193 (VF: 61; No fracture: 132)	Female	Japanese	(mean±SD) 62.5±8.0	VF	2.18±1.02 vs. 3.23±1.07 µg/ml	$OR=0.31$, 95% CI (0.16-0.61), $P=0.0007$
Periostin	Kim <i>et al.</i> ^[40]	Case-control	Plasma	266 (VF: 133; Non-VF: 133)	Female	Korean	VFs: 62.7±6.4; Non-VFs: 63.0±6.3	OF	Per SD increment	Any fracture: $OR=1.50$, 95% CI (1.14-1.97), $P=0.003$; non-VF: $OR=1.59$, 95% CI (1.12-2.24), $P=0.009$
Periostin	Yan <i>et al.</i> ^[41]	Case-control	Serum	367 (HF: 261; Controls: 106)	Female	Chinese	[median (IQR)]: HF: 80 (76-84); Controls: 78 (72-83)	HF	45.51 (28.77-79.70) vs. 32.87 (19.71-53.58) ng/ml	FN-BMD: $\beta=-0.390$, $P=0.004$; LS-BMD: $\beta=-0.158$, $P=0.190$

IGF-I: insulin-like growth factor-1; IGFBP-3: insulin-like growth factor binding-protein-3; IQR: interquartile range.

response to OFs was identified [Fold Change (FC)=-1.54, $P=0.034$ and FC=-1.24, $P=0.041$, respectively], and later confirmed in an independent case-control sample (FC=-2.04, $P=0.016$ and FC=-1.89, $P=0.027$, respectively).^[45]

Furthermore, Kocijan *et al.*^[46] quantified one hundred eighty-seven miRNAs in serum by qPCR, eight serum miRNAs (miR-152-3p, miR-30e-5p, miR-140-5p, miR-324-3p, miR-19b-3p, miR-335-5p, miR-19a-3p, miR-550a-3p) were found to be down-regulated in patients with low traumatic fractures, which excellently discriminated OFs cases and controls, regardless of age and sex (AUC>0.9, $P<0.05$). In this way, miRNAs might be directly linked to bone tissue homeostasis because of the significant correlations between miR-29b-3p and P1NP,^[47] miR-365-5p and iPTH, TRAP5b, P1NP and osteocalcin.^[48] Mandourah *et al.*^[49] reported that the circulating level of miR-4516 was significantly lower ($P<0.001$) in the OFs with low BMD than non-fracture subjects with low BMD or non-osteoporotic controls. Significantly, serum miR-140-3p and miR-23b-3p were detected to present high performances in relation to OFs (AUC=0.92, $P<0.0001$; AUC=0.88, $P<0.0001$).^[50]

To conclude, each miRNA has specific characteristics in predicting risk of OF. For example, miR-214 inhibits bone formation *via* ATF4 and is down-regulated in OF bone. As concluded by Chen *et al.*,^[51] there are biological differential expressions in some c-miRNAs between OF and non-OF individuals, but their study did not determine that these specific circulating miRNAs were diagnostic markers of OF.

Metabolites

Metabolites, including urinary mineral products and amino acid and lipid mediators, all play essential roles in bone metabolism as described above. Potential diagnostic metabolite molecules of OFs are summarized and listed in **Table 5** and presented in the following three categories.

Urinary mineral molecules

Elevated fasting urinary calcium/creatinine ratio mainly manifested the process of OF. The percentage of hypercalciuria in urine of OFs women (40%) was significantly higher compared to non-OFs women (18.8%) ($P=0.04$) in a Spanish cross-sectional study.^[52] 24-hour urinary calcium and fasting calcium/creatinine ratio might show some great characters as potential diagnostic molecules for OFs, to some extent.^[53] However, these results were

only obtained from epidemical studies other than clinical trials, further researches demonstrated these values of clinical application are warranted.

Amino acids

Homocysteine (Hcy) level in serum or plasma was a potential diagnostic molecular of OFs. Kuroda *et al.*^[54] discovered that plasma tHcy was a significant risk factor for severe VFs in postmenopausal Japanese women [OR (95%CI)=1.22 (1.03, 1.46)-1.27 (1.04, 1.58), $P<0.05$], and plasma tHcy level was significantly higher in moderate VFs (Grade 1, 9.2 ± 2.8 $\mu\text{mol/L}$) and severe VFs (Grade 2, 9.8 ± 3.7 $\mu\text{mol/L}$), as compared to Grade 0 (8.9 ± 2.9 $\mu\text{mol/L}$).^[54] Besides, plasma Hcy concentration differed between OFs group (17.21 ± 5.99 $\mu\text{mol/L}$), high-energy fracture (HEF) group (10.82 ± 3.60 $\mu\text{mol/L}$) and non-OF group (12.91 ± 4.32 $\mu\text{mol/L}$) ($F=9.483$). Furthermore, plasma Hcy was positively correlated with bone resorption markers [serum CTX and plasma levels of 25(OH)D], which were related to OF risks in elderly Chinese cohort.^[55] Consistently, Bahtiri *et al.*^[56] reported that serum Hcy level was inversely associated with both LS-BMD ($r=-0.163$, $P<0.05$) and femur neck BMD ($r=-0.164$, $P<0.05$), supporting the role of a risk for OFs. Of note, reducing plasma Hcy level can improve bone quality, thereby reducing the risk of OFs in the elderly. Thus, how to predict the occurrence of OFs is the direction for future research.

Lipid mediators

Lipid mediators, such as S1P and retinol, also play an important role in diagnosing OFs. Plasma S1P level was markedly higher in subjects with VFs (7.49 ± 3.44 $\mu\text{mol/L}$) than in those without VFs (5.58 ± 2.01 $\mu\text{mol/L}$) [OR=9.33, 95%CI (2.68, 32.49), $P=0.001$], as well as increased with the number of VFs in a dose-response manner ($P_{\text{for the trend}}<0.001$).^[57] Besides, an independent study reported that the adjusted OR (95%CI) of plasma S1P levels for OFs was 2.05 (1.03-4.00).^[58] Serum retinol, which was negatively correlated with BMD at lumbar spine ($r=-0.210$; $P<0.01$) and femoral neck ($r=-0.324$, $P<0.001$) after multivariable adjustment, could increase the likelihood of OFs.^[59] A particularly interesting finding is that the plasma S1P level was related with the high risk of incident fracture, even after additional adjustment for anti-osteoporotic medication. These results could be partially explained by the potential effects of S1P on the response to the anti-osteoporotic medication. Therefore, plasma S1P level, in combination with the BMD, could be a useful marker

Table 4. Potential microRNAs biomarkers to identify OFs

microRNAs	References	Study design	Sample size (n)	Sample	Expression trend in Case vs. Control	Fracture diagnostic value or difference	Phenotype
miR-21	Seeliger et al. ^[42]	Case-control	Serum: 30+30*; Bone: 20+20*	Serum, bone tissue	Up-regulated	AUC: 0.63, 95%CI (0.53-0.73), P = 0.013	OF
miR-23a	Seeliger et al. ^[42]	Case-control	Serum: 30+30*; Bone: 20+20*	Serum, bone tissue	Up-regulated	AUC: 0.63, 95%CI (0.53-0.73), P=0.015	OF
miR-24	Seeliger et al. ^[42]	Case-control	Serum: 30+30*; Bone: 20+20*	Serum, bone tissue	Up-regulated	AUC: 0.63, 95%CI (0.53-0.74), P=0.013	OF
miR-93	Seeliger et al. ^[42]	Case-control	Serum: 30+30*; Bone: 20+20*	Serum, bone tissue	Up-regulated	AUC: 0.68, 95%CI (0.58-0.78), P=0.001	OF
miR-100	Seeliger et al. ^[42]	Case-control	Serum: 30+30*; Bone: 20+20*	Serum, bone tissue	Up-regulated	AUC: 0.69, 95%CI (0.60-0.78), P=0.0003	OF
miR122a	Seeliger et al. ^[42]	Case-control	Serum: 30+30*; Bone: 20+20*	Serum, bone tissue	Up-regulated	AUC: 0.77, 95%CI (0.69-0.86), P<0.0001	OF
miR124a	Seeliger et al. ^[42]	Case-control	Serum: 30+30*; Bone: 20+20*	Serum, bone tissue	Up-regulated	AUC: 0.69, 95%CI (0.59-0.78), P=0.0005	OF
miR125b	Seeliger et al. ^[42]	Case-control	Serum: 30+30*; Bone: 20+20*	Serum, bone tissue	Up-regulated	AUC: 0.76, 95%CI (0.67-0.85), P<0.0001	OF
miR148a	Seeliger et al. ^[42]	Case-control	Serum: 30+30*; Bone: 20+20*	Serum, bone tissue	Up-regulated	AUC: 0.61, 95%CI (0.51-0.72), P=0.038	OF
miR-21-5p	Panach et al. ^[43]	Case-control	15+12*	Serum	Up-regulated	Fold change=1.57, P=0.002	OF
miR-122-5p	Panach et al. ^[43]	Case-control	15+12*	Serum	Up-regulated	Fold change=5.48, P=0.00008	OF
miR-125b-5p	Panach et al. ^[43]	Case-control	15+12*	Serum	Up-regulated	Fold change=4.62, P=0.0003	OF
miR-328-3p	Weilner et al. ^[45]	Cross-sectional	37	Serum	Down-regulated	Fold change=-1.54, -2.04, P<0.05	OF
let-7g-5p	Weilner et al. ^[45]	Cross-sectional	37	Serum	Down-regulated	Fold change=-1.24, -1.89, P<0.05	OF
miR-19a-3p	Kocjan et al. ^[46]	Case-control	36+39*	Serum	Down-regulated	AUC: 0.929, 95%CI (0.856-0.983)	Low traumatic fractures
miR-19b-3p	Kocjan et al. ^[46]	Case-control	36+39*	Serum	Down-regulated	AUC: 0.944, 95%CI (0.879-0.997)	Low traumatic fractures
miR-30e-5p	Kocjan et al. ^[46]	Case-control	36+39*	Serum	Down-regulated	AUC: 0.959, 95%CI (0.901-0.997)	Low traumatic fractures
miR-140-5p	Kocjan et al. ^[46]	Case-control	36+39*	Serum	Down-regulated	AUC: 0.947, 95%CI (0.900-0.983)	Low traumatic fractures
miR-152-3p	Kocjan et al. ^[46]	Case-control	36+39*	Serum	Down-regulated	AUC: 0.962, 95%CI (0.918-0.993)	Low traumatic fractures
miR-324-3p	Kocjan et al. ^[46]	Case-control	36+39*	Serum	Down-regulated	AUC: 0.950, 95%CI (0.885-0.994)	Low traumatic fractures
miR-335-5p	Kocjan et al. ^[46]	Case-control	36+39*	Serum	Down-regulated	AUC: 0.939, 95%CI (0.872-0.986)	Low traumatic fractures
miR-550a-3p	Kocjan et al. ^[46]	Case-control	36+39*	Serum	Down-regulated	AUC: 0.909, 95%CI (0.837-0.970)	Low traumatic fractures
hsa-miR-4516	Mandourah et al. ^[49]	Case-control	139	Serum, Plasma	Up-regulated	P=0.00014	OF
miR-140-3p	Ramírez-Salazar et al. ^[50]	Case-control	20+20*	Serum	Up-regulated	AUC: 0.92, P<0.0001	OF
miR-23b-3p	Ramírez-Salazar et al. ^[50]	Case-control	20+20*	Serum	Up-regulated	AUC: 0.88, P<0.0001	OF

*Combining OF cases with NF cases. AUC: area under curve.

Table 5. Potential metabolite molecules that diagnose OFs

Molecules	References	Study design	Sample	Sample size (n)	Gender	Ethnicity	Age [§] (yrs)	Pheno- type	Concentration	Effect size or difference in concentration or percentage	
										Male	Female
Urinary calcium	Rull <i>et al.</i> ^[52]	Cross- sectional	Urine	OFs:55; Non-OFs:32	Female	Spanish	OF: 70.1±13.8, Non-OF: 56.7±6.4	OF	8.95±0.73 vs. 9.19±0.38 mg/dl	NA	40% vs. 18.8% hypercal- ciuria, <i>P</i> =0.04
Homocys- teine	Kuroda <i>et al.</i> ^[54]	Cross- sectional	Plasma	Grade 0: 1052; Grade 1: 137; Grade 2: 124; Grade 3: 162	Female	Japanese	66.6±9.0	VF	Grade 0: 8.9±2.9, Grade 1: 9.2±2.8, Grade 2: 9.8±3.7, Grade 3: 11.3±5.3 μmol/L	NA	OR (95%CI): 1.22(1.03, 1.46)-1.27(1.04-1.58), <i>P</i> <0.05
Homocys- teine	Zhu <i>et al.</i> ^[55]	Cross- sectional	Plasma	OF: 39; HEF: 22; NF: 21	Male/ female =14/27	Chinese	80.61±5.61 (OF: OF: 81.82±5.49, HEF: 78.88±5.75, Non-OF: 79.75±5.47)	OF	OF: 17.21±5.99, HEF: 10.82±3.60, Non-OF: 12.91±4.32 μmol/L		<i>F</i> =9.483, <i>P</i> = 0.000
Homocys- teine	Bahtiri <i>et al.</i> ^[56]	Cross- sectional	Serum	Osteoporosis: 49; Normal: 33	Female	NA	56.53±5.84	OF	16.84±11.71 vs. 13.09±2.53 μmol/L	NA	LS-BMD (<i>r</i> =-0.163, <i>P</i> <0.05), FN-BMD (<i>r</i> =- 0.164, <i>P</i> <0.05)
SIP	Kim <i>et al.</i> ^[57]	Case-control	Plasma	VF: 69; Non-VF: 69	Female	Korean	VF: 65.1±6.7; Non-VF: 64.9±6.5	VF	7.49±3.44 vs. 5.58±2.01 μmol/L	NA	OR=9.33, 95%CI (2.68- 32.49)
SIP	Bae <i>et al.</i> ^[58]	Longitudinal	Plasma	Treated with antiosteo- porotic:172; Untreated: 76	Female	Korean	Treated: 58.4±6.3; Untreated: 59.1±7.7,	OF	4.90±0.2 vs. 5.27±0.4 μmol/L	Highest tertile: 5.90- 16.51 μmol/L	NA
Retinol	Navarro- Valverde <i>et al.</i> ^[59]	Cross- sectional	Serum	154	Female	Spanish	>65	OF	NA	NA	LS-BMD (<i>r</i> =-0.210, <i>P</i> <0.01), FN-BMD (<i>r</i> =-0.324, <i>P</i> <0.001)

§: Plus-minus values are means±SD.

HEF: high-energy fracture; LS-BMD: lumbar spine-bone mineral density.

for predicting the elevated likelihood of OFs.

CONCLUSIONS AND PROSPECTIVES

The mortality and morbidity of OFs are kind of high worldwide. Research progress on OFs risk biomarkers at gene/SNP level has been summarized by quite many reviews. In this study, we systematically reviewed the international research progress on the types of predictive and diagnostic molecules for OFs, and summarized significant molecules with potential clinical utility. Collectively, remarkable variations in protein, miRNAs and metabolite levels with the risk of OFs were principally identified through case-control studies and/or longitudinal cohort studies in various ethnicities.

The achievements, as reviewed above, from clinical observations to recent fundamental bone biology researches, represent private and public attention, investments, and efforts towards understanding the pathogenesis and preventing the occurrence of OFs. As reported in many clinical trials, some protein molecules, including IGFBP-1, periostin, adiponectin, CRP, BAP and FGF23, are positively associated with the incident OFs. Other proteins, such as IGF-1, IGFBP-3 and periostin, demonstrate great diagnostic values for OFs. Besides these significant protein molecules, metabolic products, including urinary mineral metabolite, amino acid and lipid mediators, might be crucial markers, as well. Furthermore, we have also summarized some reliable miRNAs molecules, which demonstrate great diagnostic values ($AUC > 0.9$, $P < 0.05$) for OFs. So far, few studies have been carried out to detect the interactions between various types of OF-associated molecules, and little is known about the complex neuroendocrine networks of protein, metabolic or miRNAs molecules.

In conclusion, great progress has been made in discovering OF-associated molecules in the past decade, and some novel and promising molecules have emerged. To be noted, clinical data on these novel molecules remained limited. For some molecules, the epidemiological results were controversial among independent study samples. For example, the miR-22-3p was found to be moderately up-regulated in the discovery cohort, but significant down-regulated in the validation cohort.^[45] Scientists have been aware of novel molecules involved in OFs, but the role and interactive mechanisms that these molecules play in them have yet to be investigated and elucidated fur-

ther. Therefore, it is necessary and would be important to affirm the potential clinical utility of the novel molecules through independent clinical studies with larger sample sizes. With global aging population increasing, senile OP and the resultant fractures incur heavy social and economic burden. New discoveries with no doubt will facilitate development of reliable molecules for OFs and inform development of novel tools and strategies for diagnosing, predicting, and preventing OFs.

Conflicts of interest statement

All authors have no conflicts of interest to declare.

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中文摘要

骨质疏松性骨折风险评估的潜在生物分子：一项综述回顾

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摘要

骨质疏松症是一种系统性的慢性骨骼疾病, 其特征为骨矿密度低、骨质疏松性骨折风险升高。骨质疏松症好发于中老年人群, 尤其是绝经后妇女。伴随着人口老龄化, 骨质疏松症已成为全球范围内严重的公共卫生问题。尽早识别高危人群、及时有效的干预和治疗对于预防骨质疏松性骨折至关重要。鉴于骨质疏松症的遗传率较高且发病机制复杂, 有必要综合考虑重要的生物分子、从而对骨质疏松性骨折进行准确的风险评估。为此, 我们总结回顾了可用于骨质疏松性骨折风险评估的潜在生物分子及其最新研究进展。未来对于这些生物分子的系统评估有助于研发新的方法和检测策略, 比如开发生物芯片, 以应用于骨质疏松症的早期识别和骨质疏松性骨折的预防。

关键词: 骨质疏松性骨折; 分子; 预测; 诊断

中文摘要

ATP1A3 基因突变致儿童交替性偏瘫：2 例病例报道

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摘要

儿童交替性偏瘫是一种罕见的神经发育性异常, 绝大多数为散发病例, 极少数有家族史。我院收治 2 例男性患儿, 6~7 月龄时开始癫痫发作, 表现为反复发作性全身无力或一侧肢体偏瘫。基因检测显示两患儿均携带 *ATP1A3* 基因杂合缺失突变 (OMIM:614820): c.3025 (exon 22) A>G (p.K1009E) 和 c.2443 (exon 18) G>A (p.E815K)。氟桂利嗪可以显著改善交替性偏瘫患儿的发作性运动症状。

关键词: *ATP1A3* 基因; 儿童交替性偏瘫; 癫痫