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Research article

Functional variants of the *P2RX7* gene, aseptic osteolysis, and revision of the total hip arthroplasty: A preliminary study

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ABSTRACT

Periprosthetic osteolysis (OL) is a major long-term complication of the total hip arthroplasty (THA), which can result in aseptic loosening and revision surgery. Purinergic receptor P2X, ligand-gated ion channel 7 (P2RX7) is an important regulator of inflammation and bone turnover. We were therefore interested in whether functional variants of the *P2RX7* gene may be associated with OL and risk of THA failure. A total of 205 unrelated Czech patients with cementless-type THA were stratified according to the severity of acetabular OL and revision of THA. Four "loss-of-function" *P2RX7* single nucleotide polymorphisms (SNPs), namely Glu496Ala, Ile568Asn, Arg307Gln, and null allele (rs35933842), were genotyped by polymerase chain reaction with sequence-specific primers (PCR-SSP). No significant association of *P2RX7* variants with severity of OL was observed. The carriers of rare variants *P2RX7* 568Asn, 307Gln and null allele, all causing complete loss of P2RX7 function, tended to be overrepresented among patients with THA revision (9.6%) by comparison with those with unrevised functional prosthesis (2.1%, $p = 0.09$). Furthermore, the carriage of the *P2RX7* 307Gln allele was associated with greater cumulative hazard of THA revision ($p = 0.02$). In this preliminary study, we could nominate but not clearly demonstrate rare *P2RX7* loss-of-function variants being associated with THA failure. Investigation in large THA cohorts is therefore warranted.

1. Introduction

Total hip arthroplasty (THA) has progressed in the last decades into the highly efficient curative strategy which restore mobility in patients with critically damaged hip joint [1]. Nevertheless, indispensable proportion of patients after THA still faces the complications that may lead to the premature prosthesis failure and revision surgery with significant impact on quality of life and also with increased burden on any health care system [2]. Aseptic loosening is considered a dominant long-term complication of THA requiring revision surgery [3,4]. Although not completely elucidated, it is believed that at least in part wear particles deliberated from the prosthetic surfaces are beyond the aseptic loosening. These should stimulate mesenchymal cells to inflammatory response and osteoclast accumulation, leading eventually to excessive resorption, *i.e.*, osteolysis [5,6]. As a result, with increasing bone loss there is increasing probability to loss of prosthetic fixation to a bone bed. However, the relationship between the dose (here, wear rate) and effect (extension of osteolysis) is not direct; therefore, there is some place for another factors [7]. Among them, individual susceptibility to such complications was postulated, assuming that this could help to explain differences between individuals with similar wear rate (exposition).

Purinergic receptor P2X, ligand-gated ion channel 7 (P2RX7), activated by extracellular adenosine triphosphate (ATP), is considered an important regulator of inflammation and bone turnover in response to mechanical stimuli [8,9]. In immune response, P2RX7 participates in the processing and release of cytokines such as interleukin (IL)–1β [10]. Furthermore, activation of P2RX7 may initiate immune cells death via both apoptotic and necrotic pathways [8]. A regulatory role of P2RX7 in inflammation has also been demonstrated in *P2RX7* knockout mice, where attenuated inflammatory response was observed [11]. By contrast, P2RX7 may prevent tissue damage, in particular inflammatory processes, by promoting release of anti-inflammatory cytokine IL-1Ra [12].

In bone tissue, the activation of P2RX7 affects functional activity of cells via multiple signaling pathways [9]. In osteoblast lineage, the P2RX7, *e.g.*, stimulates phospholipases D and A2 to the increased production of bioactive lipids [13]. This pathway was hypothesized to participate in the osteoblast differentiation and enhanced bone mineralization. In osteoclasts, P2RX7 activates nuclear factor nuclear factor–κB (NF-κB), which is essential for osteoclast development [14]. Nevertheless, in mature osteoclasts P2RX7 enhances apoptosis, which is consistent with the increase of osteoclast numbers in *P2RX7* knockout mice [9]. Although the role of P2RX7 in bone metabolism and inflammation is complex and not

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completely clarified, animal models strongly suggest that overall P2RX7 effect is pro-osteogenic and that it promotes bone anabolic effects of mechanical stimuli.

Importantly, the *P2RX7* gene is polymorphic, and several variants that impair signaling up to complete loss of its function have been described previously [15,16]. We therefore hypothesized that substantial reduction of P2RX7 function conferred by loss-of-function (LOF) polymorphisms of its gene may deviate the balance between the bone resorption and formation at the bone–prosthesis interface in favor of osteolysis and bone loss. In the present study, we therefore investigated four candidate *P2RX7* LOF polymorphisms in the group of well-characterized patients after THA and were interested in whether these variants could be associated with severity of acetabular osteolysis and THA failure (defined as revision because of aseptic loosening and/or aseptic osteolysis).

2. Subjects and methods

2.1. Study population

Our local register was scrutinized for patients who did and did not undergo revision for acetabular osteolysis; the latter were chosen from among patients with the longest follow-up, assuming that those are of "resistant genotype" against premature failure and development of severe osteolysis (phenotypically no or mild osteolysis). All patients were contacted and invited for clinical and radiographic examination and blood sampling. In patients with bilateral THA who did not undergo revision on either side by the day of blood sampling, the data for hips with longer follow-up were included whereas in the case of revision, the data for hips with shorter follow-up were recorded. The reasons for that were similar to those above, *i.e.*, hypothesized risk genotype for severe acetabular osteolysis development. Accordingly, between February 2004 and June 2007, blood samples were collected by venipuncture from 205 patients with mild and severe acetabular bone defects around THA. The subjects were divided into these two groups according to the size of their acetabular bone defects. This was determined by the classification of Saleh *et al.* from preoperative radiographs and was confirmed intraoperatively [17]. Briefly, if patients fulfilled the criteria for acetabular bone defects of type I and II they were considered as having mild osteolysis ($n = 89$), whereas others with more extensive bone defects (types III–V) were classified as having severe osteolysis ($n = 116$). The study included only Czech Caucasian patients who were operated on at a single institution and those with an identical cementless prosthesis (ABG, Howmedica, Inc., Staines, England). Basic demographic and clinically relevant data for THA patients classified according to the severity osteolysis are shown in Table 1.

All hips included in the study had stable prosthesis at the first year after index surgery. Interpretation of final radiographs consisted of evaluation of implant stability, occurrence, and extent of osteolysis. This was performed according to well-known and validated criteria [18–20]. In the revised cases ($n = 157$), the radiographic findings were supplemented with intraoperative findings. In more than two-thirds of patients, the polyethylene wear measurement was performed on using a Universal-type measuring microscope [20].

All blood specimens were collected under the same conditions. Written informed consent was obtained from each subject and the study was approved by the Ethics Committee of Palacky University and Teaching Hospital in Olomouc.

2.2. Genetic analysis

Four single nucleotide polymorphisms of the *P2RX7* gene have been selected based on their functional effect on P2RX7 molecule. SNP rs3751143 confers the amino acid change Glu496Ala in the carboxyl terminus of the P2RX7 molecule, which abolishes ATP-induced ethidium uptake of P2RX7, but without affection of ATP-induced opening of the cation-selective channel [15]. The second SNP, rs1653624, encoding for Ile568Asn mutation in the P2RX7 trafficking motif, prevents normal expression and function of the P2RX7 molecule. The third SNP, rs28360457 (Arg307Gln), within ATP-binding pocket, is associated with complete lack of channel and pore function of the expressed P2RX7. Finally, the rarest investigated variant rs35933842 (splice site mutation at the first intron) causes null allele, which is not expressed on the cell surface.

The genotyping of all investigated SNPs was performed by polymerase chain reaction with sequence-specific primers (PCR-SSP) under the conditions described elsewhere [21]. The sequence-specific primers used for genotyping are described in the Table 2.

Table 1
Basic characteristics of the total hip arthroplasty (THA) patients included in the study stratified according to the severity of osteolysis at the acetabular site

	Mild osteolysis (types I and II)	Severe osteolysis (types III–V)	*p* Value[a]
Patients (*n*)	89	116	
Gender (male/female)	35/54	33/83	0.101
Age at index surgery (y)	48 (27–58)	45 (24–68)	0.128
Primary diagnosis			<0.001
Osteoarthritis	35	13	
Dysplastic hip	23	62	
Other diagnoses	31	41	
Bod mass index (kg/m^2)	28.1 (20.3–35.7)	27.2 (16.0–42.6)	0.062
Revision (yes)	44	113	<0.001
Age at event[b] (y)	55 (34–69)	52 (29–77)	0.005
Time to event[b] (y)	9 (2–13)	6 (3–12)	<0.001
Harris hip score	78 (14–96)	65 (28–98)	<0.001
Prosthesis stable (yes)	82	86	<0.001
Linear wear rate (mm/y)	0.22 (0.04–0.92)	0.34 (0.04–2.52)	0.009

Data presented as median and range (minimum to maximum) in parentheses.
[a] *p* Values for comparison between the groups of patients with mild/severe osteolysis were calculated by χ^2-test or Mann–Whitney *U* test as appropriate.
[b] The "event" was defined as THA revision for revised patients or the final visit for nonrevised patients.

2.3. Study design and statistical analysis

This is a case-control association study investigating possible relevance of functional SNP variants of the *P2RX7* gene for the long-term outcome of THA. The concordance of distribution of *P2RX7* genotypes to the Hardy-Weinberg equilibrium was tested by χ^2 goodness-of-fit test. The genotype frequencies and carriage rates of investigated *P2RX7* SNPs were compared between the subgroups of THA patients stratified according to the two main endpoints of the study, *i.e.,* severity of osteolysis and THA revision. Odds ratios (OR) were calculated for carriers of *P2RX7* alleles compared with noncarriers. Because all investigated gene variants affect P2RX7 function in the same manner (loss-of-function [LOF]), we performed an additional combined analysis that considered carriers of any LOF variant versus patients with multiple wild-type genotype. The revision of THA was considered as the end point in this analysis, although it is known that the decision making on the timing of revision depends on several factors, including those unrelated to the prosthesis failure [22]. The Cox regression analysis was used for comparison of cumulative hazard of THA failure (THA revision) according to the carriage of *P2RX7* variants. Statistical analysis was performed using the SPSS 15.0 (SPSS, Inc., Chicago, IL). A *p* value less than 0.05 was considered as significant.

3. Results

3.1. Distribution of investigated P2RX7 variants in THA patients

The group of 205 well-characterized unrelated Czech patients after THA was genotyped for four selected functional (LOF) *P2RX7* polymorphisms using the PCR-SSP technique. The distribution of

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Table 2
Sequences of oligonucleotide primers used for genotyping of particular *P2RX7* SNPs by polymerase chain reaction with sequence-specific primers

P2RX7 SNP	Primer	Primer sequence
rs3751143 A/C	S1	5′ TTTTTCCGGCACAGCAGCT
	S2	5′ TTTTTCCGGCACAGCAGCG
	C	5′ ACAAGCCGTCTTCAAAGGCCT
rs1653624 T/A	S1	5′ GACATGGCTGACTTTGCCAA
	S2	5′ GACATGGCTGACTTTGCCAT
	C	5′ GTACAGAGAAGGTTTCGCCT
rs28360457 G/A	S1	5′ AGGAAAAACTGTTGAAAGCGG
	S2	5′ AAGGAAAACTGTTGAAAGCGA
	C	5′ CTCTTTATCCCTGCAAAGAA
rs35933842 G/T	S1	5′ GATCATCTTTTCCTTGGT
	S2	5′ TGACTCTTTTCCTTGGT
	C	5′ GCCTGAAAGTAGCAGAAAGGA

S1, allele-specific primer 1; S2, allele-specific primer 2; C, constant primer.

genotypes agreed to the Hardy-Weinberg equilibrium for all investigated SNPs. The frequencies of minor alleles in the whole group of THA patients were as follows: rs3751143*C: 0.207, rs1653624*A: 0.022, rs28360457*A: 0.012, rs35933842*T: 0.007. Observed frequencies of *P2RX7* alleles were similar to those reported for Caucasian population by others [15] and in public genetic databases (http://www.ncbi.nlm.nih.gov/SNP).

3.2. Association of P2RX7 variants with THA outcome

3.2.1. P2RX7 loss-of-function variants and severity of osteolysis

To reveal any possible association of investigated *P2RX7* SNPs with severity of periprosthetic osteolysis, the proportion of *P2RX7* variants was compared between the groups of patients with severe and mild or no osteolysis. The genotype and phenotype frequencies of *P2RX7* SNPs in these subgroups are listed in the Table 3. No significant association of individual *P2RX7* SNPs and severity of osteolysis was found. The carriers of any of investigated *P2RX7* LOF variants were moderately overrepresented among the patients with severe periprosthetic osteolysis (OL) (47% of carriers vs 53% wild-type genotypes) by comparison to those with mild/no osteolysis (38% of carriers vs 62% wild-type genotypes); however this difference did not attain statistical significance.

3.2.2. Association of P2RX7 variants with THA revision

To determine whether any of investigated *P2RX7* SNPs might be related to the THA failure, we compared the frequencies of *P2RX7* LOF variants between patients with THA revision and those with unrevised functional prosthesis. Interestingly, two rare variants associated with complete loss of *P2RX7* receptor function were observed only among patients with THA revision (*P2RX7* 568Asn: 5.7%, 307Gln: 3.2% of revised cases; Table 3) and were completely absent among those with unrevised functional prosthesis. We therefore compared the proportion of carriers of any *P2RX7* variant causing complete loss-of-function (*P2RX7* 568Asn, 307Gln, rs35933842 null allele). In this combined analysis, the carriers of these LOF variants tended to be overrepresented among revised patients (15/157, 9.6%) by comparison to those with unrevised functional prosthesis (1/48, 2.1%, $p = 0.09$, Fig. 1A).

Finally, because THA failure and the need for revision should better be considered as time-dependent variable, we analyzed cumulative hazard of THA failure (THA revision) stratified according to the presence of particular *P2RX7* LOF variants. Importantly, in this analysis we observed significantly higher cumulative hazard of THA revision in carriers of *P2RX7* 307Gln LOF variant ($p = 0.019$, Fig. 1B). The cumulative hazard of THA failure was not associated with the presence of other *P2RX7* variants when analyzed either separately or in combined analysis (carriage of any of investigated variants, data not shown).

4. Discussion

This preliminary study was conducted to reveal possible association between loss-of-function variants of the gene encoding for purinergic signaling receptor, namely *P2RX7,* with the long-term outcome of THA. We did not observe any significant relationship between *P2RX7* LOF variants and periprosthetic osteolysis. Nevertheless, the rare variants causing complete loss of P2RX7 function tended to be overrepresented among patients with THA revision. In

Table 3
Distribution of *P2RX7* genotypes and carriage of *P2RX7* minor alleles in patients with total hip arthroplasty (THA) stratified according to the severity of osteolysis (severe periprosthetic osteolysis [OL], $n = 116$ vs mild OL, $n = 89$) and according to revision of THA (patients with revised THA, $n = 157$ vs patients without revision, $n = 48$)

P2RX7 SNP	Function	Group	Genotype 1	Genotype 2	Genotype 3	Carriage minor allele
rs3751143 A/C	Glu496Ala	Severe OL	68 (0.586)	45 (0.388)	3 (0.026)	48 (0.414)
		Mild OL	59 (0.663)	26 (0.292)	4 (0.045)	30 (0.337)
		Revision yes	98 (0.624)	54 (0.344)	5 (0.032)	59 (0.376)
		Revision no	29 (0.604)	17 (0.354)	2 (0.042)	19 (0.396)
rs1653624 T/A	Ile568Asn	Severe OL	109 (0.940)	7 (0.060)	0 (0.000)	7 (0.060)
		Mild OL	87 (0.978)	2 (0.022)	0 (0.000)	2 (0.022)
		Revision yes	148 (0.943)	9 (0.057)	0 (0.000)	9 (0.057)
		Revision no	48 (1.000)	0 (0.000)	0 (0.000)	0 (0.000)
rs28360457 G/A	Arg307Gln	Severe OL	114 (0.983)	2 (0.017)	0 (0.000)	2 (0.017)
		Mild OL	86 (0.966)	3 (0.034)	0 (0.000)	3 (0.034)
		Revision yes	152 (0.968)	5 (0.032)	0 (0.000)	5 (0.032)
		Revision no	48 (1.000)	0 (0.000)	0 (0.000)	0 (0.000)
rs35933842 G/T	Null allele	Severe OL	115 (0.991)	1 (0.009)	0 (0.000)	1 (0.009)
		Mild OL	87 (0.978)	2 (0.022)	0 (0.000)	2 (0.022)
		Revision yes	155 (0.987)	2 (0.013)	0 (0.000)	2 (0.013)
		Revision no	47 (0.979)	1 (0.021)	0 (0.000)	1 (0.021)

Data are presented as absolute numbers with proportions in parentheses.

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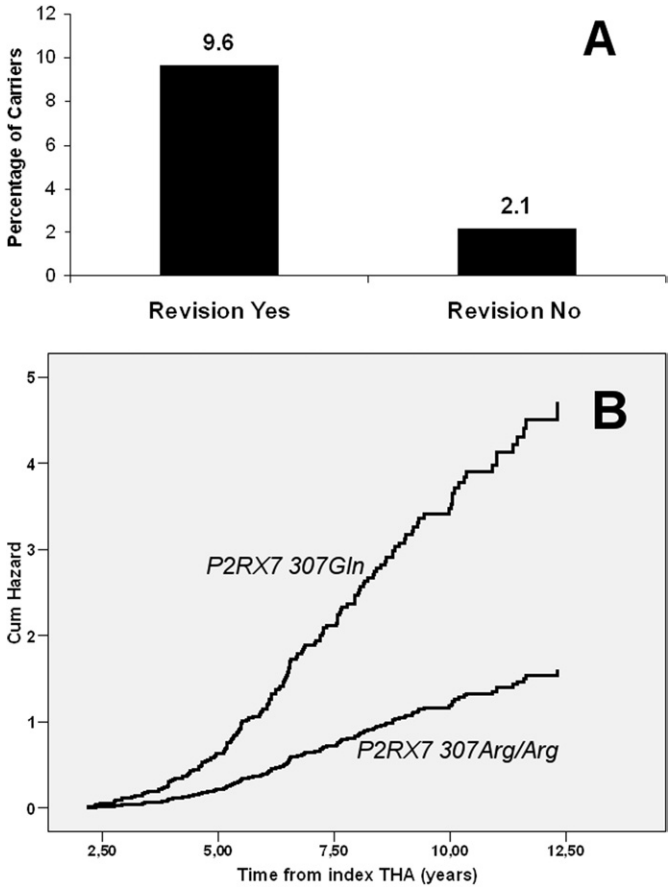

Fig. 1. Association of investigated *P2RX7* variants with total hip arthroplasty (THA) failure (THA revision). (A) Comparison of proportion (percentage) of individuals carrying any of three rare variants causing complete loss of function of P2RX7 (*P2RX7* 568Asn, 307Gln, null allele rs35933842*T) between patients with THA revision (Revision Yes) and those with unrevised functional prosthesis (Revision No, $p = 0.09$, OR = 4.96, 95% CI = 0.64–38.60). OR, odds ratio; CI, confidence interval. (B) Comparison of cumulative hazard of THA failure (THA revision) between the carriers (*P2RX7* 307Gln, upper curve) and noncarriers (*P2RX7* 307Arg/Arg, lower curve) of the *P2RX7* 307Gln loss-of-function variant assessed by Cox regression analysis ($p = 0.019$).

particular, *P2RX7* 307Gln allele increased cumulative hazard of THA revision. Our results may therefore indicate that *P2RX7* LOF variants act in favor of processes leading to the premature prosthetic failure.

The genetic component of THA long-term outcome has so far been studied using a candidate gene approach. Accordingly, variants of genes encoding for the pathways (*e.g.*, receptor activator of NF-κB ligand [RANKL]/receptor activator of NF-κB [RANK]/osteoprotegerin [OPG]) and mediators (*e.g.*, proinflammatory cytokines) important for the development of osteolysis and aseptic THA loosening have already been investigated [23–28]. By analogy, *P2RX7* gene was selected for this study as an important mediator of bone turnover and inflammation, especially as variants that demonstrably abrogate *P2RX7* function have been described [9,15]. P2RX7 mediates purinergic signal into the many cell types via binding with extracellular ATP released in response to mechanical or inflammatory stimuli. It has been shown in an animal model that disruption of *P2RX7* gene leads to impaired periosteal bone formation [29], increased bone resorption, and substantial reduction of the skeletal response to mechanical stimuli [30]. Recently, several pathways associated with P2RX7 activation in bone cells (both osteoblasts and osteoclasts) have been identified that further clarified molecular basis of the P2RX7 effects on osteogenesis stimulation [13,31,32]. Apart from its pro-osteogenic role in mediating the response of skeleton to mechanical stimuli P2RX7 has been identified as an important regulator of immune response and inflammation [8,33]. In this regard, P2RX7 is specifically involved in the secretion of the IL-1 cytokines (namely IL-1β and and IL-1Ra) [10,12]. It has also been implicated in the initiation of immune cell death via both apoptotic and necrotic pathways [8].

Despite the fact that our preliminary results are far from clear evidence of the *P2RX7* gene being associated with THA long-term outcomes, the results still suggest that carriage of *P2RX7* LOF variants may promote THA failure. The above-mentioned roles of the P2RX7 in bone metabolism may provide an explanation supporting our preliminary results. Because P2RX7 signaling is rather pro-osteogenic, a decline in its activity caused by LOF variants may confer periprosthetic bone loss, osteolysis, and aseptic loosening. Importantly, this speculation has indirectly been supported by the observation in clinical conditions: Ohlendorff *et al.* reported that *P2RX7* LOF SNPs (namely Glu496Ala and Ile568Asn) associate with the fracture risk in postmenopausal women and response to hormone replacement therapy [34]. Furthermore, these authors observed that LOF 496Ala/Ala genotype reduced osteoclast apoptosis, which *in vitro* may suggest a correlation with increased fracture risk related to *P2RX7* LOF variants [34]. Collectively, these observations combined with our findings support the hypothesis that P2RX7 LOF variants might, under particular conditions, contribute to the deviation of bone turnover to bone resorption and loss.

In this study, we confirmed that *P2RX7* SNP variants causing complete loss of function are rare in the Caucasian population. Accordingly, three of four investigated *P2RX7* variants have been present in less than 5% of THA patients. This fact has two important consequences for this study: First, the uncommon occurrence of *P2RX7* variants markedly reduced the statistical power to detect a potential moderate association between the *P2RX7* variants and THA failure, which may be considered as a major limitation of the present study. However, because we did not restrict our analyses in THA outcomes only to the role of individual *P2RX7* LOF variants, but considered them also in combination, we partially compensated for this limitation [15]. Second, an overall contribution (population attributable risk) of *P2RX7* variants to the genetic component of aseptic periprosthetic osteolysis and THA failure would rather be limited in the Caucasian population. Nevertheless, in case that the substantial impact of *P2RX7* LOF polymorphisms for the THA outcome is further confirmed, these variants may prospectively be studied together with other described genetic markers to assess their real clinical importance. Future testing of genetic markers predicting THA outcome might then identify patients at high risk for premature THA failure in whom modified clinical and prophylactic approaches could be applied.

Despite the fact that several associations were reported with increasing recognition on the genetic component of the premature THA failure, at the same time there has been a growing awareness of the limitations of some studies. The problematic issues have included small sample size, lack of agreement about the primary outcome and conditions interfering with it, or incorrect estimation of the effect size [35]. In this regard, here we present a study on an identical implant that was previously found to be prone to premature failure because of excessive polyethylene wear [20]. Therefore, most cases under study had to resist to increased exposition of the wear particles, assuming better distinction between patients with osteolysis resistant and susceptible phenotype, respectively. In addition, all included hips were stable at 1 year postoperatively, and almost all of the revision surgeries were performed by a single surgeon. Throughout this work, we adhered to rules for proper conductance of genetic association studies, including caution not to overinterpret our data [36].

In conclusion, the rare *P2RX7* loss-of-function polymorphisms tended to be associated with THA failure in this preliminary study.

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Despite the fact that the suboptimal statistical power resulting from the rare occurrence of *P2RX7* LOF variants prevents us from clearly demonstrating (or excluding) the effect of the *P2RX7* variants on periprosthetic osteolysis and THA failure, we believe that further studies of the role of P2RX7, a key molecule of bone turnover and an important regulator of inflammation, are highly desirable in regard to THA conditions. Therefore, extension of our preliminary findings in larger, multicenter THA cohorts is needed.

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