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Association of mutations in hemochromatosis genes with clinical severity of calcium pyrophosphate arthritis

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Abstract

Aims: To study factors associated with the development of calcium pyrophosphate (CPP) arthritis and the severity phenotype.

Methods: Transversal case-control study. Cases had to be confirmed by both X-ray chondrocalcinosis and CPP crystals in synovial fluid. Controls had neither chondrocalcinosis nor CPP crystals in synovial fluid. Patients and controls with hemochromatosis or primary hyperparathyroidism were not included. Mutations of hemochromatosis genes (*HFE*), magnesium (Mg), calcium (Ca), phosphate, iron (Fe), transferrin saturation, ferritin, parathyroid hormone (PTH), and calcifediol levels were studied.

Results: Three hundred patients and 300 sex and age matched controls were compared. Lower serum Mg (sMg) and higher ferritin levels were found among patients. Hypomagnesemia (HypoMg) and HFE mutations were more frequent among patients. Involvement of over one joint was observed in 199 (66.4%) patients whereas persistent joint inflammation was retrieved in 154 (51.4%) of the patients. Initial analysis showed that the frequency of polyarticular and inflammatory phenotypes seemed to be progressively overrepresented in patients with HFE mutations. Further bivariate and multivariate analysis adjusted for the time from onset disclosed that the presence of genotypes with C282Y mutations was associated with polyarticular disease (hazard risk 3.501, 95% confidence interval 1.862–6.581, $P < 0.001$). Although C282Y mutations also seemed to be associated with inflammatory patterns, the association did not reach statistical significance ($P = 0.173$).

Conclusions: Low sMg and high ferritin levels are associated with CPP arthritis (CPPA). In patients without hemochromatosis, *HFE* mutations, and specifically *C282Y* mutations seem to associate with the polyarticular disease phenotype, and plausibly with the chronic inflammatory phenotype.

Keywords

Pyrophosphate, arthritis, chondrocalcinosis, hemochromatosis genes

Introduction

Chondrocalcinosis, the presence of calcium pyrophosphate crystals (CPPC) within hyaline or fibrous cartilage, is very prevalent in the elderly population, even as much as 10% in subjects over 60 years [1]. Calcium pyrophosphate arthritis (CPPA), either as acute CPPA or chronic inflammatory CPPA [2], derives from the shedding of CPPCs from cartilage to synovial joints, inducing either acute flares, previously named as pseudogout, or a chronic inflammatory response deriving in progressive destruction of joint structures. The diagnosis of certain CPPA relies on the observation of CPPC in synovial fluid samples [2].

Factors associated with the presence of chondrocalcinosis, and therefore the risk of developing CPPA, include constitutional factors, such as aging [1], diseases inducing disturbances in calcium (Ca), iron (Fe), and magnesium (Mg) [3], and genetic such as polymorphisms of genes, ankylosis protein homolog human gene (*ANKH*) and hemochromatosis genes (*HFE*) [4]. Overexpression of the *HFE* allele *C282Y* has been associated with chondrocalcinosis [5] and hand osteoarthritis [6] and homozygosis for *HFE* allele *H63D* with chondrocalcinosis and osteoarthritis [7].

Most studies relate on the association of these factors to the presence of X-ray chondrocalcinosis or they limit acute pyrophosphate arthritis, retrieved from databases such as the Veteran Administration (VA) data warehouse [3] and The Health Improvement Network (THIN) [8] database. Such databases lack data on disease phenotype regarding the severity of the disease, genetic studies, or complete on associated factors such as Mg, Ca, Fe levels, or a dose of diuretics.

The objective of this study is to evaluate factors associated with the development and severity of CPPA in patients with definite, crystal-proved CPPA.

Materials and methods

Transversal, case-control unicentric study in a 3rd level university hospital with a referral population of 400,000, from January 2009 to December 2018. The study was included within a prospective inception gout cohort study approved by the Cruces University Hospital Ethics and Clinical Investigation Committee (CEIC Cruces CEIC07/15). Written, informed consent was obtained and signed by all patients.

Cases were defined by the presence of CPPC within leukocytes in synovial fluid samples in addition to the presence of chondrocalcinosis in conventional X-ray of joints not necessarily restricted to those clinically involved. Controls were defined as the absence of CPPC in synovial fluid and no chondrocalcinosis in conventional X-ray of the affected joint.

Clinical phenotypes were defined according to the European League Against Rheumatism (EULAR) classification [1] into acute pyrophosphate arthritis (unique or intermittent flares without chronic inflammation or joint structural damage), and chronic pyrophosphate inflammatory arthritis (presence of persistent inflammation or stablished joint structural damage not attributable to primary osteoarthritis). Further characterization of phenotype included joint distribution as monoarticular (1 joint ever involved), oligoarticular (2–4 joints ever involved), and polyarticular (> 4 joints ever involved).

Registers with a previous diagnosis of genetic hemochromatosis (*C282Y/YY* genotype), a family history of hemochromatosis, known Fe overload due to any cause, primary or tertiary hyperparathyroidism were excluded.

Metabolic factors studied included serum levels of Mg, Ca, phosphate, Fe, Fe saturation (SatFe%), ferritin, parathyroid hormone (PTH), 25-hydroxyvitamin D [25(OH)D], and high-sensitivity C-reactive protein. *HFE* testing included *C282Y*, *H63D* and *S65C* alleles. Hypomagnesemia (HypoMg) was defined as per the reference range to be < 1.70 mg/dL.

Results

The registers of 300 cases and 300 controls were analyzed and compared. Genetic studies were available for 284 cases and 281 controls. Three cases showed a double mutation of *HFE* allele *C282Y*, they were considered as genetic type 1 hemochromatosis cases and were not included in genetic analysis. Controls included different diseases, such as gout (n = 128), osteoarthritis (n = 102), and rheumatoid arthritis (n = 70).

The mean time from onset of the first symptom to recruitment was 4.5 years [interquartile range (IQR) 1–6] and 108 (36%) of the cases were recruited during their first year of clinical manifestations.

Monoarticular involvement was present in 101 (33.6%) patients, whereas oligoarticular and polyarticular involvement was present in 154 (51.4%) and 45 (15.0%) respectively. An only-acute clinical pattern was observed in 146 (48.6%) patients, whereas persistent joint inflammation was retrieved in 154 (51.4%) of the patients.

In cases recruited during the first year of symptoms, monoarticular (76/101, 75%) and only-acute presentation (99/146, 67.8%) were predominant, whereas multiple joint involvement and chronic inflammatory pattern were observed with increasing time from onset (Figure 1).

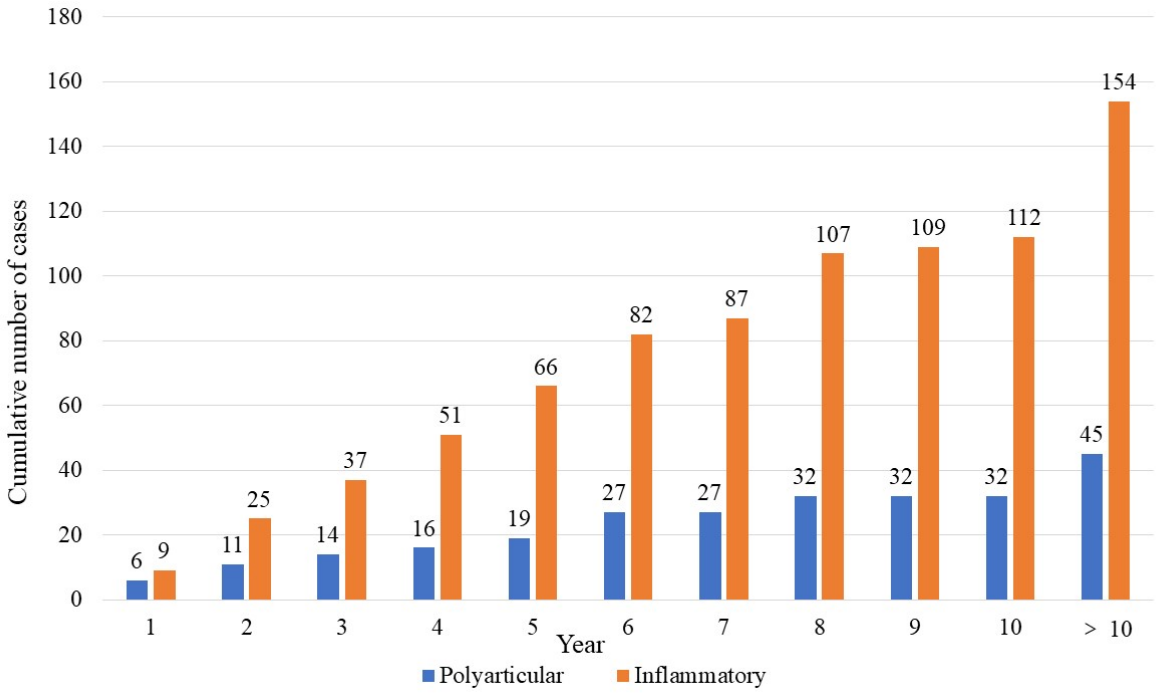

Figure 1. Cumulative number of cases (displayed in the y axis out of 300) developing polyarticular and inflammatory phenotypes from the time of onset of symptoms (displayed in the x axis in years)

Comparisons between cases and controls showed no differences in Ca, phosphate, Fe, SatFe%, PTH, or 25(OH)D. Lower serum Mg (sMg) levels and higher plasma ferritin levels were found in patients compared to controls. HypoMg, any *HFE* mutation, was also more common in cases than in controls (Table 1).

Only mutations in *C282Y* and *H63D* alleles were found more frequently in cases than in controls, but no difference in the *S65C* alleles was observed. The frequency of the different genotypes in cases and controls are displayed in Table 2.

The frequency of polyarticular and inflammatory phenotypes seemed to be progressively overrepresented in cases with HFE mutations, as may be observed in Figure 2.

Table 1. Comparisons between patients and controls

Variable	Cases (%)	Controls (%)	*P*
C-reactive protein (mg/L)	16 ± 8	4 ± 4	< 0.001
sMg (mg/dL)	1.99 ± 0.28	2.07 ± 0.23	< 0.001
HypoMg (< 1.70 mg/dL)	36/294 (12.2)	19/294 (6.5)	0.016
Ferritin (mg/dL)	257 ± 241	206 ± 173	0.004
Any *HFE* mutation	156/281 (55.5)	103/281 (35.6)	< 0.001
Any *C282Y* mutation	35/281 (12.5)	12/281(4.3)	0.001
Any *H63D* mutation	122/284 (42.9)	87/281 (30.9)	0.007
Any *S65C* mutation	6/284 (2.1)	9/281 (3.2)	0.658

The data is represented as means ± standard deviation for continuous variables, and number/total (percentage for discrete variables)

Table 2. Genotypes for statistically associated *HFE* alleles *C282Y* and *H63D* (%)

Population	*CCHH*	*CCHD*	*CCDD*	*CYHH*	*CYHD*	*N*
Controls	189 (67)	74 (26)	6 (2)	5 (2)	7 (3)	281
Cases	135 (47)	93 (33)	24 (8)	18 (6)	11 (4)	281
N	324	167	30	23	18	562

The data is represented as "*n* (%)" or "*n*". Chi-square (χ^2) test for distribution with Bonferroni adjustment $P < 0.001$. *CCHH:* non mutated; *CCHD:* heterozygote mutation for *H63D*; *CCDD:* homorozygote mutation for *H63D*; *CYHH:* heterozygote for *C282Y*; *CYHD:* combined heterozygote mutation for *C282Y* and for *H63D*

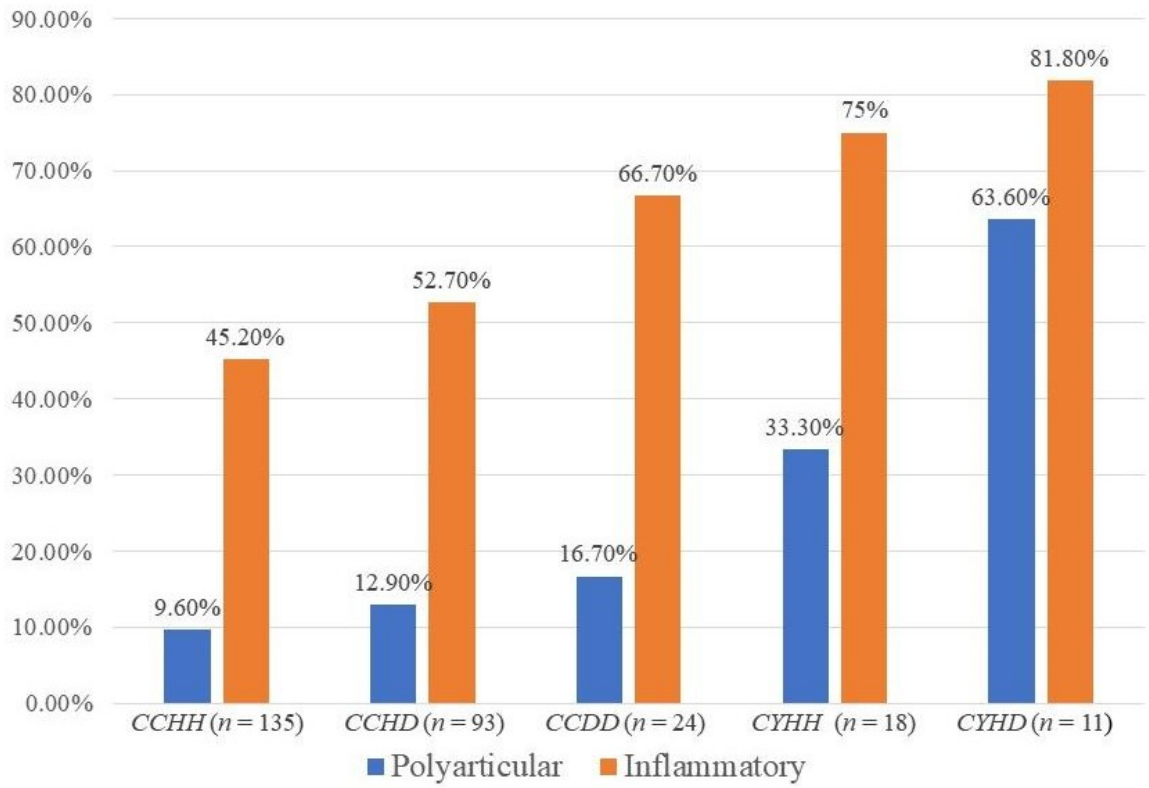

Figure 2. Frequency of polyarticular or chronic inflammatory phenotypes in cases. The "*n*" in the figure represents the number of cases

As the phenotype in a transversal study for a disease that can progress over time may be influenced by the course of the disease, further analysis was performed. In fact, as mentioned before, clinical phenotypes for polyarticular joint distribution and chronic inflammatory arthritis were more frequent with increasing time from the onset of the disease.

Therefore Kaplan-Meyer analysis was performed, with time from onset as exposure and polyarticular distribution and inflammatory pattern as outcomes. HypoMg, any *HFE* mutation, or *H63D* mutations were

not associated anymore with either polyarticular or inflammatory phenotypes but *C282Y* and combined *C282Y/H63D* mutations were associated with polyarticular joint distribution.

Multivariate Cox proportional hazard analysis showed that the presence of genotypes with *C282Y* mutations was the only variable associated with polyarticular disease (hazard risk 3.501, 95% confidence interval 1.862–6.581, $P < 0.001$). Although *C282Y* mutations also seemed to be associated with the inflammatory pattern, the association did not reach statistical significance ($P = 0.173$).

Discussion

CPPA is one of the most common inflammatory arthritis, especially in the elderly, and is caused by the formation of Ca pyrophosphate (CPP) crystals in cartilage structures that may induce both acute (also known as pseudogout) and chronic inflammatory arthritis when shed into synovial lined joints [2]. Disturbances in divalent cations, namely Ca, Mg, and Fe have been associated with chondrocalcinosis, as they influence the activity of pyrophosphatases [9, 10]. Therefore, along with aging, primary hyperparathyroidism, HypoMg, and hemochromatosis are clinically associated with CPPA [2].

Studies of factors associated with CPPA using a database and International Classification of Diseases-9th (ICD-9) coding have found associations between CPPA with HypoMg, hemochromatosis, and primary hyperparathyroidism [3], but also found association with diseases that may show similar clinical features, as osteoarthritis (OA), gout, and rheumatoid arthritis rheumatoid arthritis (RA) [3]. Therefore, diagnosis based on CPPC crystal identification is synovial fluid samples would be gold-standard [2] to avoid misclassification of cases. Other studies restricted to acute CPPA (formerly pseudogout) using the THIN databases only found associations with hyperparathyroidism and the use of loop diuretics, but not with RA, gout, OA, or thiazide diuretics [8]. This is a curious discrepancy, as in a prospective study of 9,820 patients, thiazide diuretics induced lower sMg levels and more frequently HypoMg than loop diuretics [11]. In addition to the risk of misdiagnosis, databases not designed specifically do not retrieve data on clinical phenotype.

To avoid these limitations, this study intended to evaluate factors associated with gold-standard definition of CPPA cases (CPP crystals in synovial fluid plus radiographic chondrocalcinosis) compared to controls with gold-standard controls (absence of both crystals in synovial fluid and chondrocalcinosis). That also allowed to obtain *HFE* testing, along with serum levels of divalent cations Ca, Mg, Fe, along with SatFe% and ferritin levels. More importantly, and to our knowledge first in literature, to determine clinical phenotype as only-acute CPPA and chronic inflammatory CPPA.

Common shared pathogenic pathways, not related directly to cartilage damage, but more to altered mechanisms in repair of the cartilage damage, have been considered to link hemochromatosis arthritis and CPPA [12].

Mutations of the *HFE* gene Fe overload and a common cause of hereditary (type 1) hemochromatosis. *HFE* gene expression induces the production of a protein that regulates the production of hepcidin, a protein that regulates the absorption of Fe by the intestinal cells, and its *C282Y* and *H63D* mutations may induce Fe overload [13].

We have found a higher prevalence of any *C282Y* and *H63D HFE* mutations in CPPA patients (55%) than in controls (35%). The high prevalence in controls is consistent with that published for newborns in Northern Spain [14] and adults in the Spanish Basque Country, where it reached 29% for mutations of the allele *H63D* and 13% allele *C282Y* [15].

The results from this case-controlled study support that both HypoMg and Fe overload, related to *HFE* gene mutations, are more frequent in patients with CPPD than in controls, supporting that inhibition of pyrophosphatases may be a common mechanism.

We have also observed, and reported for the first time, that in patients with CPPD, there is an apparent relationship between clinical phenotypes of polyarticular distribution and chronic inflammatory arthritis with *HFE* genotypes.

To avoid the bias of transversal design, where one-third of the patients had early (< 1 year) disease we used survival analysis with clinical phenotypes as outcomes with time from onset as time variable. It showed that only *C282Y*, but not *H63D* or combined heterozygous mutations, were associated with increased hazard ratio to develop polyarticular distribution as a surrogate of increased burden of CPP deposits. It may indicate that altered pyrophosphatase activity due to Fe overload may influence the extension of CPP crystal deposition.

Nevertheless, although close to significance, we could not demonstrate that *HFE* mutations were associated with the chronic inflammatory pattern. To that point, and considering that CPP crystal inflammation is derived from the nucleotide-binding oligomerization domain leucine-rich repeat-containing protein 3 (NRLP3)-caspase-interleukin-1 pathway [16], other factors may be at work that could be elucidated in the future.

There are limitations to the design of the study. Prospective recruitment from first clinical presentation and longitudinal follow-up would have been better, but would take decades. Also, hospital-based recruitment may induce a selection bias to the most severe phenotype.

In conclusion, *HFE* mutations, and especially *C282Y*, may increase the risk of CPPA and result in a more severe polyarticular disease. From the clinical point of view, study of associated factors such as Ca and phosphate disorders, HypoMg, and Fe overload should be performed, but *HFE* testing is of no practical use in the absence of Fe overload.

Abbreviations

Ca: calcium

CPP: calcium pyrophosphate

CPPA: calcium pyrophosphate arthritis

CPPC: calcium pyrophosphate crystals

Fe: iron

HFE: hemochromatosis genes

HypoMg: hypomagnesemia

Mg: magnesium

SatFe%: iron saturation

sMg: serum magnesium

Declarations

Author contributions

JASdB and FPR contributed eaqually to: Conceptualization, Data curation, Writing—review & editing. Nuria PH, Nerea PH, CVP, and MdCMC: Conceptualization, Writing—review & editing. All authors read and approved the submitted version.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

The study was included within a prospective inception gout cohort study approved by Cruces University Hospital Ethics and Clinical Investigation Committee (CEIC Cruces CEIC07/15), and it complies with the Declaration of Helsinki.

Consent to participate

Informed consent to participate in the study was obtained from all participants.

Consent to publication

Not applicable.

Availability of data and materials

Data can be obtained on request by contacting Fernando Perez-Ruiz (fernando.perezruiz@osakidetza.eus).

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References

1. Sanmartí R, Pañella D, Brancós MA, Canela J, Collado A, Brugués J. Prevalence of articular chondrocalcinosis in elderly subjects in a rural area of Catalonia. Ann Rheum Dis. 1993;52:418–22.
2. Zhang W, Doherty M, Bardin T, Barskova V, Guerne PA, Jansen TL, et al. European League Against Rheumatism recommendations for calcium pyrophosphate deposition. Part I: terminology and diagnosis. Ann Rheum Dis. 2011;70:563–70.
3. Balderrama CK, Rosenthal AK, Lans D, Singh JA, Bartels CM. Calcium pyrophosphate deposition disease and associated medical comorbidities: a national cross-sectional study of US veterans. Arthritis Care Res (Hoboken). 2017;69:1400–6.
4. Abhishek A, Doherty S, Maciewicz R, Muir K, Zhang W, Doherty M, et al. The association between *ANKH* promoter polymorphism and chondrocalcinosis is independent of age and osteoarthritis: results of a case–control study. Arthritis Res Ther. 2014;16:R25.
5. Timms AE, Sathananthan R, Bradbury L, Athanasou NA, Wordsworth BP, Brown MA. Genetic testing for haemochromatosis in patients with chondrocalcinosis. Ann Rheum Dis. 2002;61:745–7.
6. Ross JM, Kowalchuk RM, Shaulinsky J, Ross L, Ryan D, Phatak PD. Association of heterozygous hemochromatosis C282Y gene mutation with hand osteoarthritis. J Rheumatol. 2003;30:121–5.
7. Alizadeh BZ, Njajou OT, Hazes JM, Hofman A, Slagboom PE, Pols HA, van Duijn CM. The H63D variant in the *HFE* gene predisposes to arthralgia, chondrocalcinosis and osteoarthritis. Ann Rheum Dis. 2007;66:1436–42.
8. Rho YH, Zhu Y, Zhang Y, Reginato AM, Choi HK. Risk factors for pseudogout in the general population. Rheumatology (Oxford). 2012;51:2070–4.
9. Yang Q, Jian J, Abramson SB, Huang X. Inhibitory effects of iron on bone morphogenetic protein 2–induced osteoblastogenesis. J Bone Miner Res. 2011;26:1188–96.
10. Uribe S, Rangel P, Pardo JP, Pereira-Da-Silva L. Interactions of calcium and magnesium with the mitochondrial inorganic pyrophosphatase from *Saccharomyces cerevisiae*. Eur J Biochem. 1993;217:657–60.
11. Kieboom BCT, Zietse R, Ikram MA, Hoorn EJ, Stricker BH. Thiazide but not loop diuretics is associated with hypomagnesaemia in the general population. Pharmacoepidemiol Drug Saf. 2018;27:1166–73.
12. Mitton-Fitzgerald E, Gohr CM, Williams CM, Rosenthal AK. Identification of common pathogenic pathways involved in hemochromatosis arthritis and calcium pyrophosphate deposition disease: a review. Curr Rheumatol Rep. 2022;24:40–5.
13. Barton JC, Edwards CQ, Acton RT. *HFE* gene: structure, function, mutations, and associated iron abnormalities. Gene. 2015;574:179–92.

14. de Juan D, Reta A, Castiella A, Pozueta J, Prada A, Cuadrado E. HFE gene mutations analysis in Basque hereditary haemochromatosis patients and controls. Eur J Hum Genet. 2001;9:961–4.
15. Altes A, Ruiz A, Barceló MJ, Remacha AF, Puig T, Maya AJ, et al. Prevalence of the C282Y, H63D, and S65C mutations of the *HFE* gene in 1,146 newborns from a region of northern Spain. Genet Test. 2004; 8:407–10.
16. Martinon F, Pétrilli V, Mayor A, Tardivel A, Tschopp J. Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature. 2006;440:237–41.