Genetic and Expression Analysis of *CASP7* Gene in a European Caucasian Population with Rheumatoid Arthritis

VITOR HUGO TEIXEIRA, LAURENT JACQ, SANDRA LASBLEIZ, PASCAL HILLIQUIN, CATARINA RESENDE OLIVEIRA, FRANÇOIS CORNELIS, ELISABETH PETIT-TEIXEIRA, and The European Consortium on Rheumatoid Arthritis Families (ECRAF)

ABSTRACT.
Objective. To study the possible role of the caspase 7 (CASP7) gene in susceptibility to rheumatoid arthritis (RA) in a European Caucasian population.
Methods. CASP7 rs2227309 single nucleotide polymorphism (SNP) was genotyped in 197 French RA trio families and in 252 European RA families available for replication using Taqman allelic dis-crimination assay. Relative quantification of caspase 7 isoforms α and ß mRNA expression was per-formed from whole blood in 25 unrelated patients with RA and in 15 healthy controls by real–time quantitative reverse transcription-polymerase chain reaction. The genetic analyses for association and linkage were performed using the comparison of allelic frequencies, the transmission disequi-librium test, and the genotype relative risk.
Results. We observed, in the first sample, a significant association of rs2227309-AA genotype with RA [p = 0.03, odds ratio (OR) 2.11 (95% CI 1.0–4.6)]. The second sample did not show any signif-icant association of the AA genotype with RA [p = 0.6, OR 0.87 (95% CI 0.4–1.8)]. When the 2 sam-ples were combined, no significant association of the AA genotype [p = 0.3, OR 1.32 (95% CI 0.8–2.2)] was observed. CASP7 isoforms α and ß mRNA were expressed in patients with RA at lower level than in healthy controls (–89%, p = 0.003 and –47%, p = 0.01; respectively). Conclusion. CASP7 rs2227309 SNP was not associated with RA in a European Caucasian popula-tion. Nevertheless, CASP7 isoforms α and ß could have an involvement in the apoptosis process in RA.

Key Indexing Terms: RHEUMATOID ARTHRITIS, GENE EXPRESSION, CASP7 GENE , APOPTOSIS, ASSOCIATION STUDY, SINGLE NUCLEOTIDE, POLYMORPHISM

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by persistent synovial tissue inflammation associated with the destruction of affected joints[1]. An imbalance between cell proliferation and insufficient apoptosis of synovial macrophages, fibroblasts, and lymphocytes is one mechanism that might contribute to persistence of RA[2]. The increase of osteoblast apoptotic cell death may contribute to periarticular bone loss in patients with RA, while impaired apoptosis of rheumatoid synovial cells appears to cause hyperplasia of the synovial tissues[3]. Studies in animal arthritis models suggest that an increase of the induction of synovial cell apoptosis ameliorates synovial tissue hyperplasia[4,5]. Additionally, the dysregulation of apoptosis is involved in a large variety of human diseases including cancer, autoimmune diseases, and neurodegenerative disorders[6].

Apoptosis is a cell-suicide program, evolutionarily conserved, by which a cell undergoes an orderly demise. Apoptotic cell death is coordinated in cells by a family of the cysteine-aspartic acid proteases, the caspases. In mammals,

From GenHotel-EA3886, Evry-Paris VII Universities, Evry-Genopole, France; Faculty of Medicine, University of Coimbra, Coimbra, Portugal; Centre Hospitalier Sud Francilien, Corbeil-Essonnes; Service de Rhumatologie, Hôpital Lariboisière, Paris, France; Center for Neurosciences and Cell Biology, Faculty of Medicine, University of Coimbra; and Unité de Génétique Clinique, Hôpital Lariboisière, Paris, France.

Supported by Association Française des Polyarthritiques, Société Française de Rhumatologie, Association Rhumatisme et Travail, European Union for AutoCure, Association Polyarctique, Groupe Taitbout, Genopole®. V.H. Teixeira was supported by Foundation for Science and Technology, Portugal (grant SFRH/BD/23304/2005).

V.H. Teixeira, MSc, GenHotel-EA3886, Evry-Paris VII Universities and Faculty of Medicine, University of Coimbra; L. Jacq, MD, GenHotel-EA3886, Evry-Paris VII Universities and Centre Hospitalier Sud Francilien; S. Lasbleiz, MD, GenHotel-EA3886, Evry-Paris VII Universities and Service de Rhumatologie, Hôpital Lariboisière; P. Hilliquin, MD, PhD, Centre Hospitalier Sud Francilien; C.R. Oliveira, MD, PhD, Center for Neurosciences and Cell Biology, Faculty of Medicine, University of Coimbra; F. Cornélis, MD, PhD, GenHotel-EA3886, Evry-Paris VII Universities, Centre Hospitalier Sud Francilien, and Unité de Génétique Clinique, Hôpital Lariboisière; E. Petit-Teixeira, PhD, GenHotel-EA3886, Evry-Paris VII Universities.

Address reprint requests to V.H. Teixeira, GenHotel-EA3886, Laboratoire Européen de Recherche pour la Polyarthrite Rhumatoïde, 2 Rue Gaston Crémieux, 91057 Evry-Genopole Cedex, France. E-mail: vitor@polyarthrite.net

caspases involved in apoptotic responses are classified into 2 groups according to their structure and function. The first group is composed of initiator caspases (caspase-2, 8, 9, 10), which contain N-terminal adapter domains that allow for their autocleavage and the activation of downstream caspases. The second group is composed of effector or executioner caspases (caspase-3, 6, 7), which lack N-terminal adapter domains and are cleaved and activated by initiator caspases[7].

CASP7 gene is located in the chromosomal area 10q25.1-10q25.2. Caspase 7 exists as an inactive proenzyme, which undergoes proteolytic processing at conserved aspartic residues to produce 2 subunits, a large and small one, that dimerize to form the active enzyme. The precursor of this caspase is cleaved by caspase 3 and 10. Alternative splicing results in 4 transcript variants, encoding 3 distinct isoforms (α, ß, and Δ), the majority of which retain catalytic activity[8]. In the procaspase 7 isoforms α and ß, upon activation *in vivo*, a short N-terminal sequence is removed and an "IQADSG" site is cleaved, generating the active caspase 7 isoforms with catalytic activity[9]. Procaspase ß uses the same start codon as the variant α but has a distinct C-terminus compared with the other variants. Thus, the caspase 7 isoform ß lacks the active site of the enzyme and may act as a dominant inhibitor for active procaspase 7 isoforms[10]. *CASP7* gene mutations are present in human malignancies, and the inactivating mutations of *CASP7* gene might lead to the loss of its apoptotic function and contribute to the pathogenesis of human solid cancers[11]. Additionally, *CASP7* has been proposed as a positional candidate for susceptibility to insulin-dependent diabetes mellitus (IDDM)[12].

In the field of genetic factors analysis in RA, a previous study reported a significant association for an rs2227309 (A/G) single nucleotide polymorphism (SNP) [allele G, p = 0.001, odds ratio (OR) 1.32 (95% CI 1.11–1.56); genotype GG, p = 0.0005, OR 1.47 (95% CI 1.18–1.83)], located in exon 7 of the *CASP7* gene, with RA in a case-control Spanish Caucasian population[13]. Further, there was a statistically significant deviation in the relative expression of the mRNA-encoding *CASP7* isoform ß versus functional isoforms in healthy individuals stratified according to their rs2227309 genotypes[13].

Our aim was to confirm the role of the *CASP7* gene in susceptibility to RA in a European Caucasian population. We used RA familial material to test *CASP7* rs2227309 SNP for RA associations and linkages. Moreover, the expression level of *CASP7* isoform α and ß mRNA in unrelated French Caucasian patients with RA and in French Caucasian healthy controls and the relation between genotypes of the SNP tested and the level of expression in these 2 groups were studied.

MATERIALS AND METHODS

Study populations. The study was approved by the Ethics Committees of Hôpital Bicêtre and Hôpital Saint Louis (Paris, France) and all subjects provided informed consent. RA families were recruited through a national media campaign. All patients with RA satisfied the revised criteria of the American College of Rheumatology[14] according to the rheumatologist in charge of the patient. A rheumatologist university fellow reviewed all clinical data. Sample 1 and 2, used for association and linkage studies, consisted of 197 French Caucasian unrelated trio families (1 RA patient and both parents) with the 4 grandparents of French Caucasian origin and of 252 European Caucasian unrelated trio families (Caucasian families from France, Italy, Portugal, Spain, Belgium, and The Netherlands). Characteristics of the RA French Caucasian families (sample 1) and European Caucasian families (sample 2) are reported in Table 1. Among the 25 French Caucasian unrelated patients with RA used for the expression study, 19 were women [mean ± standard deviation (SD) age at enrolment 53.4 ± 11.1 yrs]. Among the 15 French Caucasian healthy controls used for the expression study, 11 were women (mean ± SD age at enrolment 46.9 ± 6.6 yrs).

Molecular genotyping method. Genomic DNA of the 449 European Caucasian RA trio families and the 25 French Caucasian unrelated patients with RA was isolated and purified from fresh peripheral blood leukocytes according to standard protocols. Genotyping of the *CASP7* rs2227309 SNP (A/G) was carried out with a Taqman 5' allelic discrimination assay on an ABI-7500 real-time polymerase chain reaction (PCR) machine (assay: C_500778_10, Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. Genotyping of each sample was automatically attributed using specific software for allelic discrimination. Ten percent of the samples chosen at random were genotyped again for quality control.

CASP7 isoform α/ß mRNA expression. Total RNA from whole blood was extracted from 25 French Caucasian unrelated patients with RA and 15 French Caucasian healthy controls using a PAXgene Blood RNA kit (Qiagen, Hilden, Germany). The measure of the RNA concentration was performed using the RNA RiboGreen dye (Invitrogen). RNA integrity was analyzed using the Agilent 2100 Bionalyzer (Agilent, Santa Clara, CA, USA). Reverse transcription (RT) was performed with SuperScript™ III First-Strand Synthesis SuperMix for quantitative RT–PCR (Invitrogen) according to the manufacturer's protocol. Real-time quantitative RT-PCR analysis was executed on an ABI-Prism 7500 machine, using TaqMan gene expression assay probes (Applied Biosystems) for *CASP7* isoform ß (Hs01032058_m1) and *CASP7* isoform α (Hs01029847_m1) and TaqMan endogenous controls probes (Applied Biosystems) for ß-actin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and $ß_2$-microglobulin. Each sample was tested in duplicate and a sample without template was included in each run as a negative control. Expression of the *CASP7* isoform α and ß mRNA was quantified using the threshold cycle (Ct) method and normalized to the amount of ß-actin, GAPDH, and $ß_2$-microglobulin. Samples showing Ct values > 35 and duplicates with a Ct > 0.3 were retested.

Statistical analysis. Using the previously reported frequencies of GG genotypes for CASP7 rs2227309 in Spanish patients with RA (56%) and in con-

Table 1. Characteristics of RA index cases.

Characteristic	Sample 1, n = 197	Sample 2, n = 252
Female, %	88.3	85.3
Mean age (± standard deviation, SD) at disease onset, yrs	31.5 (± 9.3)	30 (± 9)
Mean (± SD) disease duration, yrs	13 (± 8.1)	8.1 (± 7)
With bone erosions, %	81.5	70.2
Seropositive for rheumatoid factor, %	71.6	70.6
Seropositive for anti-cyclic citrulinated peptide antibodies, %	77.6	Not available
Carrying at least one HLA-DRB1 shared epitope allele, %†	79.7	Not available

† DRB1*0101, DRB1*0102, DRB1*0401, DRB1*0404, DRB1*0405, DRB1*0408, DRB1*1001.

trols (46.4%)[13], power to detect an association was calculated using the method described by Garnier, *et al*[15].

Hardy-Weinberg equilibrium was checked in the control group (constituted by the nontransmitted parental chromosomes from the trio subjects) prior to analysis.

The association analysis relied first on the Affected Family Based-Controls (AFBAC), which compares the allelic frequencies between chromosomes transmitted to the RA cases and nontransmitted parental chromosomes[16]. The linkage analysis relied on the transmission disequilibrium test (TDT), which compares, for a given allele, its transmission from heterozygous parents to patients with RA, with the transmission expected from Mendel's law (i.e., 50 %)[17]. Second, we used the genotype's relative risk (GRR), which compares the affected offspring's genotype with the control genotype derived from nontransmitted parental chromosomes, using the method proposed by Lathrop[18]. The OR and 95% confidence interval (CI) were estimated using the method of Woolf[19], as modified by Haldane[20]. The significance of the p value was assessed at 5%, leading to replication tests in sample 2.

Results of relative mRNA expression are presented as the mean ± SD percentage. Statistical analysis of the relative quantification of *CASP7* isoform α and ß mRNA expression in patients with RA and healthy controls was performed using the Mann-Whitney test, and $p < 0.05$ was considered significant. The association between genotypes of the SNP tested and the relative quantification of *CASP7* isoform α versus isoform ß mRNA expression in 25 unrelated patients with RA and in 15 healthy controls was assessed by the Mann-Whitney test. Data are expressed as the mean ± SD, and $p < 0.05$ was considered significant.

RESULTS

Power calculation. Using the reported frequencies of GG genotypes for *CASP7* rs2227309 in Spanish patients with RA (56%) and in controls (46.4%)[13], association analysis of 449 trio families (RA samples 1 + 2) provides 100% power to reach statistical significance ($p < 0.05$).

Hardy-Weinberg equilibrium. The *CASP7* rs2227309 SNP in the 2 samples investigated was in Hardy-Weinberg equilibrium in the control group.

Test for linkage and association in RA families. Sample 1: A total of 591 French Caucasian individuals from 197 trio families (1 RA case and both parents) were analyzed. The rs2227309-A allele frequency was slightly higher in RA index cases than in virtual controls (p = 0.5; Table 2A). There was a nonsignificant overtransmission of the rs2227309-A allele from heterozygous parents (p = 0.5; Table 3A). The GRR analysis of the *CASP7* rs2227309 showed a significant association of the homozygous AA genotype with RA (p = 0.03; Table 4A). The same tests were performed in subgroups stratified for rheumatoid factor positivity, anti-cyclic citrulinated peptide positivity, and presence of the *HLA-DRB1* shared epitope, and no significant associations/linkages were detected (data not shown).

Table 2. Affected family-based controls (AFBAC) analysis in rheumatoid arthritis trio families.

CASP7 rs2227309 (A/G) SNP	Allele	RA Cases	Controls	p
A. Sample 1, n = 197	A	0.26	0.24	0.5
	G	0.74	0.76	
B. Sample 2, n = 252	A	0.26	0.27	0.6
	G	0.74	0.73	
C. Sample 1+ 2, n = 449	A	0.26	0.26	1
	G	0.74	0.74	

SNP: single nucleotide polymorphism.

Sample 2: The significant association observed for *CASP7* rs2227309-AA genotype in Sample 1 led to a replication test in Sample 2 with the hypothesis of an AA genotype association with RA. A total of 756 European Caucasian individuals from 252 trio families were analyzed. One hundred thirteen families were of French origin and 139 were from continental Western European countries. In this sample, we did not observe a trend for association and linkage (AFBAC, p = 0.6; TDT, p = 0.6; Table 2B and 3B) of the allele A with RA. The GRR did not show a significant association of the AA genotype with RA (p = 0.6) (Table 4B). The same tests were performed in the 36 Spanish trio families from Sample 2 and no significant associations/linkages were detected (data not shown).

Samples 1 + 2: The combination of the 2 samples, indicated by the absence of any significant clinical difference between them, showed no significant association nor linkage of the allele A with RA (Table 2C and 3C). The GRR test did not show a significant association of the *CASP7* rs2227309-AA genotype with RA (p = 0.3; Table 4C).

Expression analysis. A relative quantification of *CASP7* isoform α and ß mRNA expression was performed in total RNA isolated from whole blood of 25 unrelated patients with RA and 15 healthy controls. *CASP7* isoform α and ß mRNA were expressed in patients with RA at lower level than in healthy controls (–89%, p = 0.003 and –47%, p = 0.01, respectively; Figure 1). The expression level of isoform α mRNA in each group was higher than the expression level of isoform ß mRNA (Figure 1), and the mRNA expression ratio (isoform α vs isoform ß) in healthy controls was higher compared to patients with RA (+32%).

A nonstatistically significant deviation was observed when comparing the mRNA expression ratio (isoform α vs isoform ß) in patients with RA (Figure 2A) and in healthy control samples (Figure 2B) stratified according to their rs2227309 genotypes (AA vs AG, AA vs GG, AG vs GG; $p > 0.05$).

DISCUSSION

Caspase 7 is crucial for apoptosis, and contributes to mitochondrial events such as the loss of mitochondrial membrane potential and the release of proapoptotic factors such as cytochrome c and apoptosis-inducing factor[21]. Caspase 7 is an executioner caspase that requires cleavage to turn into the active isoforms α or Δ^9. The caspase 7 isoform ß, which is not cleaved, may act as a dominant inhibitor of the active forms[10]. Deregulation of apoptosis synovial cells was one of the mechanisms suspected to be involved in RA physiopathology[2].

Table 3. Transmission disequilibrium test in RA trio families.

CASP7 rs2227309 (A/G) SNP	Allele	Transmitted	Non-transmitted	Transmission from Heterozygous Parents, %	p
A. Sample 1, n = 197	A	76	68	52.8	0.5
	G	68	76	47.2	
B. Sample 2, n = 252	A	96	104	48	0.6
	G	104	96	52	
C. Sample 1 + 2, n = 449	A	172	172	50	1
	G	172	172	50	

SNP: Single nucleotide polymorphism.

Table 4. Genotype relative risk analysis in RA trio families.

CASP7 rs2227309 (A/G) SNP	Genotypes	RA Cases	Controls	Odds Ratio (95% CI)	Lathrop p value (one genotype vs the others)
A. Sample 1, n = 197	AA	20	10	2.11 (1.0–4.6)	0.03 (AA vs AG + GG)
	AG	62	74	0.76	
	GG	115	113	1.04 (0.7–1.6)	0.94 (GG vs AA + AG)
B. Sample 2, n = 252	AA	15	17	0.87 (0.4–1.8)	0.5 (AA vs AG + GG)
	AG	99	103	0.94	
	GG	138	132	1.1 (0.8–1.6)	0.7 (GG vs AA + AG)
C. Sample 1 + 2, n = 449	AA	35	27	1.32 (0.8–2.2)	0.4 (AA vs AG + GG)
	AG	161	177	0.86	
	GG	253	245	1.07 (0.8–1.4)	0.7 (GG vs AA + AG)

SNP: single nucleotide polymorphism.

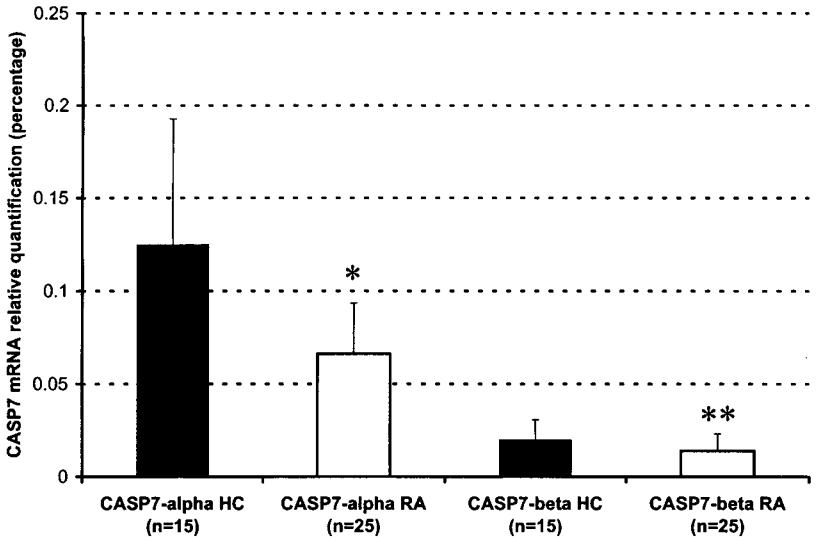


Figure 1. Levels of *CASP7* isoform α and ß mRNA expression in peripheral blood from unrelated RA patients (RA) and healthy controls (HC). *CASP7* isoform α and ß mRNA levels were determined by real-time quantitative RT-PCR and normalized to ß-actin, GAPHD, and $ß_2$-microglobulin levels. Expression of *CASP7* isoform α and ß transcripts was quantified using the threshold cycle (Ct) method. Data are presented as mean ± SD percentage of the *CASP7* isoform α and ß mRNA expression. *$p < 0.0001$ vs controls; **$p = 0.01$ vs controls; Mann-Whitney test.

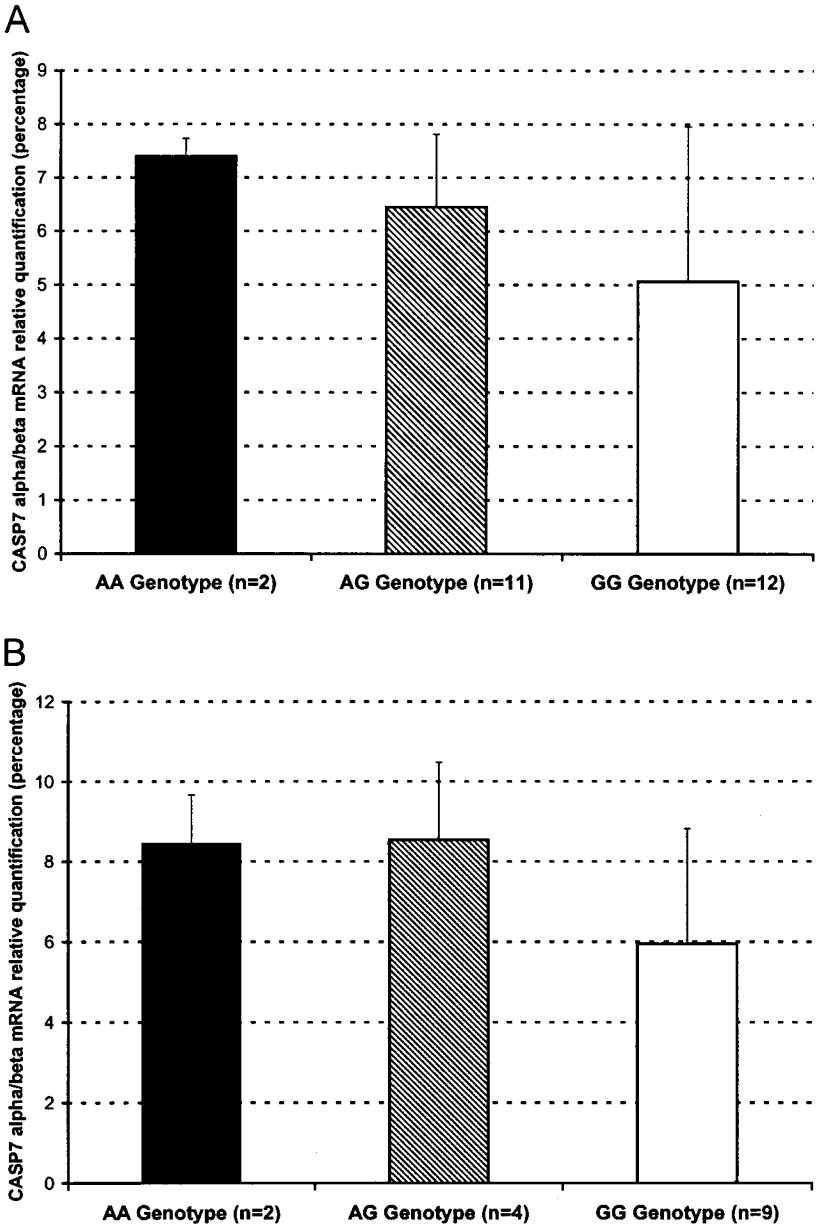


Figure 2. Levels of *CASP7* isoform α vs isoform ß mRNA expression in RA patients (A) and in healthy controls (B) stratified according to their rs2227309 genotypes. *CASP7* isoform α and ß mRNA levels were determined by real-time quantitative RT-PCR and normalized to ß-actin, GAPHD, and $ß_2$-microglobulin levels. Expression of *CASP7* isoform α and ß transcripts was quantified using the threshold cycle (Ct) method. Data are presented as mean ± SD percentage of the *CASP7* isoform α vs isoform ß mRNA expression in patients with RA and in healthy controls (AA vs AG, AA vs GG, AG vs GG: $p > 0.05$ by Mann-Whitney test).

A previous study reported a significant association ($p < 0.05$) for rs2227309-GG genotype of the *CASP7* gene with RA in a case-control Spanish Caucasian population study[13]. This SNP produces a K249R change in the isoform ß and a synonymous change in the isoform α. Further, this polymorphism is not located in the active site of the caspase 7 protein. Our results showed an overtransmission of the rs2227309-A allele from heterozygous parents and a significant association of the homozygous rs2227309-AA genotype with RA in Sample 1. This significant association led to a replication test in Sample 2 with the hypothesis of an AA genotype association with RA. In this sample, we observed neither a trend for association and linkage of the A allele nor a significant association of the AA genotype with RA.

Moreover, the combination of the 2 samples did not show any significant association of the *CASP7* rs2227309-AA genotype with RA. This family-based analysis avoids an imperfect population match between patients and controls, permitting the direct test of Mendel's law. The allele frequencies that we described in patients with RA (A = 26% and G = 74%) were the same as those observed in Spanish patients with RA[13]. However, the allele frequency inferred from parental nontransmitted chromosomes (A = 26% and G = 74%) was different from those observed in Spanish controls (A = 31.6% and G = 68.4%)[13]. Altogether, our results allowed us to exclude *CASP7* rs2227309 SNP as a major significant genetic factor in RA in a European Caucasian population. To exclude *CASP7* gene association with RA, other tagSNP should be tested. *CASP7* locus was not included among the 19 suggested non-HLA regions in the French Caucasian population linked to RA[22]. Further, the last genome-wide association study in a UK Caucasian population found 9 SNP that map to loci not associated previously to RA[23], and the *CASP7* locus was not present in these regions.

Our study is the first to show a statistically significant difference in the expression of *CASP7* isoform α and ß mRNA between patients with RA and healthy controls in peripheral blood cells. When we compared the expression of these 2 isoforms in healthy controls or in patients with RA, we observed higher mRNA expression of the α isoform compared to the ß isoform. Further, the decrease of mRNA *CASP7* isoform ß expression in patients with RA compared to healthy controls was more significant than observed for *CASP7* isoform α mRNA expression. Thus, in patients with RA, peripheral blood cells could have a lower apoptotic activity based on the lower relative quantity of the functional isoform α compared to healthy controls.

As the decrease of isoform ß mRNA expression in patients with RA was less significant than the decrease observed for isoform α, isoform ß may act as a dominant inhibitor of active forms. Thus, peripheral blood cells from patients with RA may have lower apoptotic activity based on the higher proportion of the nonfunctional isoform (ß isoform) compared to the active one (α isoform).

Reports have demonstrated that impaired apoptosis is a characteristic of several autoimmune diseases, and administration of factors that stimulate apoptosis decreases inflammation[3-5,24,25]. Further, in mice, the knockout of caspase 7 decreases the apoptotic process[21]. Nevertheless, to complete our observed data, further investigations should be done in specific cells such as T and B lymphocytes, monocytes, macrophages, and synoviocytes.

We cannot exclude the effects of methotrexate (MTX) in the apoptotic process of patients with RA because in our study, 92% of patients with RA have received MTX treatment. However, the mechanism of MTX action in autoimmune diseases remains unclear. An influence of MTX on apoptosis in peripheral blood mononuclear cells (PBMC) has been demonstrated only under nonphysiological conditions, for example after stimulation with mitogens[26,27]. Employing stimulation with conventional antigens, other studies have failed to demonstrate an apoptotic effect of MTX on PBMC, and instead attributed the effect of MTX on T cells to suppression of T cell activation and reduced expression of T cell adhesion molecules[27]. Finally, a recent study reported that MTX inhibits proliferation in *Candida albicans* (CA)- and tetanus toxoid (TT)-stimulated CD4+ T cells, and induces apoptosis in a subset of stimulated CD4+ T cells. These authors suggested that stimulation of T cells with the conventional antigens CA and TT more probably resembles stimulation with self-antigens occurring in autoimmune diseases, including RA, and the antiproliferative and proapoptotic effects of MTX reflect the actions of this drug in the treatment of rheumatic diseases[28]. Further investigations should be done in RA patients without MTX treatment to clarify this point and to identify the exact mechanism of MTX in RA.

Our findings provide evidence against the involvement of the *CASP7* rs2227309 SNP in the genetics of RA in a European Caucasian family population. Our study is the first to show that *CASP7* isoform α (functional isoform) and isoform ß (nonfunctional isoform) mRNA were expressed in patients with RA at lower levels than in healthy controls in peripheral blood cells. Further investigations of the regulation of *CASP7* expression in RA are required to prove the involvement of *CASP7* in disease susceptibility, more specifically in the signalling pathways of RA apoptosis.

ACKNOWLEDGMENT

We are grateful to the patients with RA, their families, and rheumatologists for participation in our study. We thank Dr. Valérie Chaudru (INSERM U794, France), Dr. Robert Olaso, and Tiphaine Oudot (CNG, France), Fréderique Bitton, and Samuel Mainguet (INRA, France).

The European Consortium on Rheumatoid Arthritis Families (ECRAF): F. Cornélis (coordinator), T. Bardin (France), P. Migliorini, S. Bombardieri (Italy), R. Westhovens, J. Dequeker (Belgium), A. Balsa, D. Pascuale-Salcedo (Spain), P. Barrera, L. Van de Putte, P. Van Riel, T.R. Radstake (The Netherlands), and H. Alves, A. Lopes-Vaz, M. Fernandes, C. Vaz (Portugal).

REFERENCES

1. Seldin MF, Amos CI, Ward R, Gregersen PK. The genetics revolution and the assault on rheumatoid arthritis (review). Arthritis Rheum 1999;42:1071-9.
2. Pope RM. Apoptosis as a therapeutic tool in rheumatoid arthritis. Nat Rev Immunol 2002;2:527-35.
3. Stanczyk J, Ospelt C, Gay RE, Gay S. Synovial cell activation. Curr Opin Rheumatol 2006;18:262-7.
4. Zhang H, Gao G, Clayburne G, Schumacher HR. Elimination of rheumatoid synovium in situ using a Fas ligand gene scalpel. Arthritis Res Ther 2005;7:1235-43.
5. Peng SL. Fas (CD95)-related apoptosis and rheumatoid arthritis. Rheumatology Oxford 2006;45:26-30.

6. Kuribayashi K, Mayes P, El-Deiry W. What are caspases 3 and 7 doing upstream of the mitochondria? Cancer Biol Ther 2006;5:763-5.
7. Degterev A, Boyce M, Yuan J. A decade of caspases. Oncogene 2003;22:8543-67.
8. National Center for Biotechnology Information (NCBI). Available from: http://www.ncbi.nlm.nih.gov/
9. Stennicke HR, Salvensen GS. Caspases — controlling intracellular signals by protease zymogen activation. Biochim Biophys Acta 2000;1477:299-306.
10. Fernandes-Alnemri T, Takahashi A, Armstrong R, et al. Mhc3, a novel human apoptotic cysteine protease highly related to CPP32. Cancer Res 1995;55:6045-52.
11. Soung YH, Lee JW, Kim HS, et al. Inactivating mutations of CASPASE-7 gene in human cancers. Oncogene 2003;22:8048-52.
12. Babu SR, Bao F, Roberts CM, et al. Caspase 7 is a positional candidate gene for IDDM 17 in a Bedouin Arab family. Ann NY Acad Sci 2003;1005:340-3.
13. García-Lozano JR, Torres B, Fernández O, et al. Caspase 7 influences susceptibility to rheumatoid arthritis. Rheumatology Oxford 2007;46:1243-7.
14. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
15. Garnier S, Dieudé P, Michou L, et al. IRF5 rs2004640-T allele, the new genetic factor for systemic lupus erythematosus, is not associated with rheumatoid arthritis. Ann Rheum Dis 2007;66:828-31.
16. Thomson G. Mapping disease genes: family-based association studies. Am J Hum Genet 1995;57:487-98.
17. Spielman R, McGinnis E, Ewens W. Transmission test for linkage disequilibrium: The insulin gene region and insulin-dependent diabetes mellitus (IDDM). Am J Hum Genet 1993;52:506-26.
18. Lathrop GM. Estimating genotype relative risks. Tissue Antigens 1983;22:160-6.
19. Woolf B. On estimating the relation between blood group and disease. Ann Hum Genet 1955;19:251-3.
20. Haldane JB. The estimation and significance of the logarithm of a ratio of frequencies. Ann Hum Genet 1956;20:309-11.
21. Lakhani SA, Masud A, Kuida K, et al. Caspases 3 and 7: key mediators of mitochondrial events of apoptosis. Science 2006;311:847-51.
22. Osorio Y, Fortéa J, Bukulmez H, et al. Dense genome-wide linkage analysis of rheumatoid arthritis including covariates. Arthritis Rheum 2004;50:2757-65.
23. The Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Naure 2007;447:661-78.
24. Mahoney JA, Rosen A. Apoptosis and autoimmunity. Curr Opin Immunol 2005;17:583-88.
25. Catrina AI, Trollmo C, af Klint E, et al. Evidence that anti-tumor necrosis factor therapy with both etanercept and infliximab induces apoptosis in macrophages, but not lymphocytes, in rheumatoid arthritis joints: extended report. Arthritis Rheum 2005;52:61-72.
26. Genestier L, Paillot R, Fournel S, Ferraro C, Miossec P, Revillard JP. Immunosuppressive properties of methotrexate: apoptosis and clonal deletion of activated peripheral T cells. J Clin Invest 1998;102:322-8.
27. Johnston A, Gudjonsson JE, Sigmundsdottir H, Ludviksson BR, Valdimarsson H. The anti-inflammatory action of methotrexate is not mediated by lymphocyte apoptosis, but by the suppression of activation and adhesion molecules. Clin Immunol 2005;114:154-63.
28. Nielsen CH, Albertsen L, Bendtzen K, Baslund B. Methotrexate induces poly(ADP-ribose) polymerase-dependent, caspase 3-independent apoptosis in subsets of proliferating CD4+ T cells. Clin Exp Immunol 2007;148:288-95.