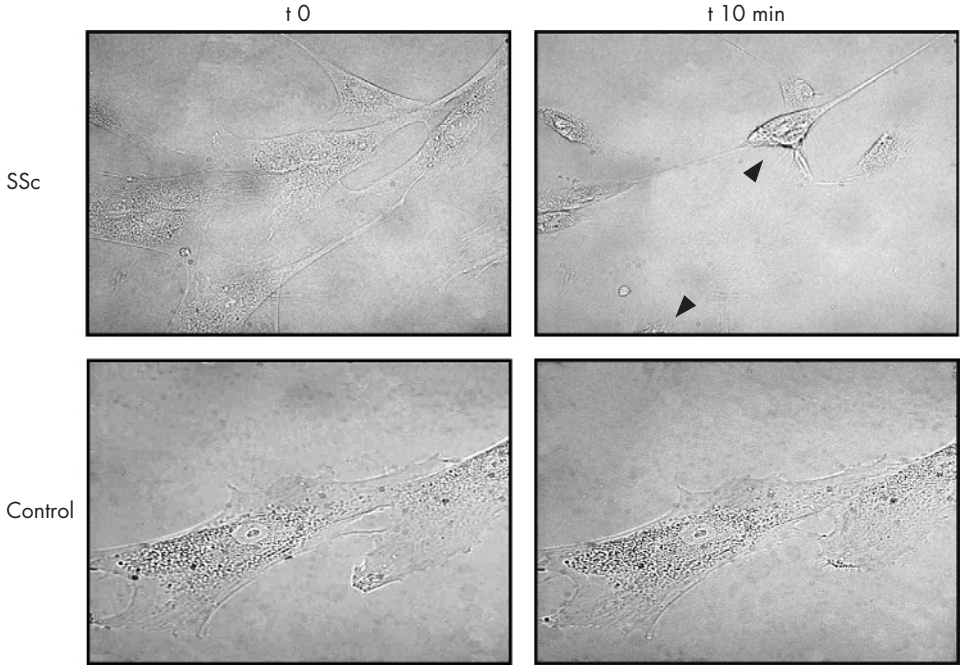

Figure 2 ATP causes cell shrinkage and rounding in SSc but not in control fibroblasts. Cells were seeded in DMEM onto 24 mm round glass coverslips and incubated for 24 h. Coverslips were then transferred to the thermostatically controlled stage of a Nikon Eclipse T300 microscope equipped with a back-illuminated CCD camera. ATP was added at a concentration of 3 mmol/l.

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REFERENCES

1 **Steen VD**, Medsger TA Jr. Epidemiology and natural history of systemic sclerosis. *Rheum Dis Clin North Am* 1990;**16**:1–10.
2 **LeRoy EC**. Systemic sclerosis. A vascular perspective. *Rheum Dis Clin North Am* 1996;**22**:675–94.
3 **Strehlow D**, Korn JH. Biology of the scleroderma fibroblast. *Curr Opin Rheumatol* 1998;**10**:572–8.
4 **Solini A**, Chiozzi P, Morelli A, Fellin R, Di Virgilio F. Human primary fibroblasts in vitro express a purinergic P2X7 receptor coupled to ion fluxes, microvesicle formation and IL-6 release. *J Cell Sci* 1999;**112**:297–305.
5 **Wang X**, Arcuino G, Takano T, Lin J, Peng WG, Wan P, *et al*. P2X7 receptor inhibition improves recovery after spinal cord injury. *Nat Med* 2004;**10**:821–7.
6 **LeRoy EC**, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TA Jr, *et al*. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol* 1988;**15**:202–5.
7 **Solini A**, Chiozzi P, Morelli A, Adinolfi E, Rizzo E, Baricordi O, *et al*. Enhanced P2X7 activity in human fibroblasts from diabetic patients: a possible pathogenetic mechanism for vascular damage in diabetes. *Arterioscler Thromb Vasc Biol* 2004;**24**:1240–5.
8 **Gachet C**. Regulation of platelet functions by P2 receptors. *Annu Rev Pharmacol Toxicol* 2006;**46**:277–300.
9 **Angiolillo DJ**, Fernandez-Ortiz A, Bernardo E, Ramirez C, Sabate M, Jimenez-Quevedo P, *et al*. Clopidogrel withdrawal is associated with proinflammatory and prothrombotic effects in patients with diabetes and coronary artery disease. *Diabetes* 2006;**55**:780–4.

In rheumatoid arthritis, a polymorphism in the HLA-G gene concurs in the clinical response to methotrexate treatment

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We read with interest the editorial ''Methotrexate pharmacogenomics'' by Kremer, recently published in the *Annals of Rheumatic Diseases*.[1] Several reports have proposed a fundamental role of the folate pathway in the clinical effects of methotrexate (MTX) treatment in rheumatoid arthritis, mainly due to genetic variations in the methylene tetrahydrofolate reductase (MTHFR) gene. The observations by Hughes *et al* of ethnic differences in the frequencies of

Abbreviations: MTHFR, methylene tetrahydrofolate reductase; MTX, methotrexate; sHLA-G, soluble HLA-G; SNP, single-nucleotide polymorphism; UTR, untranslated region

single-nucleotide polymorphisms (SNPs) in the MTHFR coding region suggest possible links between other specific genotypes and MTX response.[2]

We recently identified an association between the 14 bp deletion/insertion polymorphism in exon 8 at the 3′ untranslated region (UTR) of the HLA-G gene and the clinical response to MTX treatment in rheumatoid arthritis.[3] The HLA-G antigens are non-classic MHC class Ib molecules with limited allelic polymorphisms,[4] restricted tissue distribution,[5–7] and alternative splicing mechanisms for mRNA that allow the production of both membrane-bound and soluble isoforms.[8] HLA-G molecules are associated with the development or persistence of several autoimmune diseases because of their tolerogenic capacity against innate and adaptive responses.[7 9] The 14 bp insertion/deletion polymorphism in the HLA-G gene influences mRNA stability and quantitative protein production. Furthermore, the 14 bp insertion allele (+14 bp) destabilises mRNA and decreases soluble HLA-G (sHLA-G) protein production.[10]

In this study, we showed that MTX can induce the in vitro production of circulating HLA-G molecules by peripheral blood monocyte cells from healthy subjects and patients with rheumatoid arthritis, with interindividual differences. Moreover, the highest quantitative in vitro production of sHLA-G molecules was associated with the presence of the deletion (−14/−14 bp) genotype.

To have in vivo confirmation of the pharmacogenetic role of the 14 bp polymorphism in MTX response, we also performed a retrospective study of the genotype distribution in 156 patients with rheumatoid arthritis, who were subdivided into two cohorts on the basis of their clinical response to MTX. The data obtained indicated a significantly higher frequency of the −14/−14 bp genotype in ''responder'' patients compared with ''non-responders'' ($\chi^2 = 6.12$; df = 1; $p = 0.02$ (χ^2 test); odds ratio 2.46, 95% CI 1.26 to 4.84; $p<0.009$).

These results suggest a pharmacogenetic role for the HLA-G 14 bp polymorphism, and that there is clinical advantage of the −14/−14 bp genotype in response to MTX. The HLA-G 14 bp polymorphism should be investigated in association with other MTHFR SNPs that predominantly show a role in MTX toxicity.[1] The combined analysis of such MTHFR SNPs and the HLA-G 14 bp polymorphism could help in assessing the likelihood that patients will experience MTX-related toxicity or benefits.

In conclusion, in rheumatoid arthritis it seems to be important to consider the concurrence of different genetic polymorphisms to help predict the clinical response to MTX treatment.

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REFERENCES

1 **Kremer JM**. Metotrexate pharmacogenomics. *Ann Rheum Dis* 2006;**65**:1121–3.
2 **Hughes LB**, Beasley TM, Patel H, Tiwari HK, Morgan SL, Baggott JE, *et al*. Racial or ethnic differences in allele frequencies of single-nucleotide polymorphisms in the methylenetetrahydrofolate reductase gene and their influence on response to methotrexate in rheumatoid arthritis. *Ann Rheum Dis* 2006;**65**:1213–18.
3 **Rizzo R**, Rubini M, Govoni M, Padovan M, Melchiorri L, Stignani M, *et al*. HLA-G 14bp polymorphism regulates the methotrexate response in rheumatoid arthritis. *Pharmacogenet Genomics* 2006;**9**:615–23.
4 **Hylenius S**, Andersen AM, Melbye M, Hviid TV. Association between HLA-G genotype and risk of pre-eclampsia: a case-control study using family triads. *Mol Hum Reprod* 2004;**10**:237–46.
5 **Crisa L**, McMaster MT, Ishii JK, Fisher SJ, Salomon DR. Identification of a thymic epithelial cells subset sharing expression of the class Ib HLA-G molecule with fetal trophoblast. *J Exp Med* 1997;**186**:289–98.
6 **Seliger B**, Abken H, Ferrone S. HLA-G and MIC expression in tumors and their role in anti-tumor immunity. *Trends Immunol* 2003;**24**:82–7.
7 **Fainardi E**, Rizzo R, Melchiorri L, Castellazzi M, Paolino E, Tola MR, *et al*. Intrathecal synthesis of soluble HLA-G and HLA-I molecules are reciprocally associated to clinical and MRI activity in patients with multiple sclerosis. *Mult Scler* 2006;**12**:2–12.
8 **Fujii T**, Ishitani A, Geraghty DE. A soluble form of the HLA-G antigens is encoded by a messenger ribonucleic acid containing intron 4. *J Immunol* 1994;**153**:5516–24.
9 **Rizzo R**, Mapp CE, Melchiorri L, Maestrelli P, Visentin A, Ferretti S, *et al*. Detective production of soluble HLA-G molecules by peripheral blood monocytes in patients with asthma. *J Allergy Clini Invest* 2005;**115**:508–13.
10 **Rizzo R**, Hviid TVF, Stignani M, Balboni A, Grappa MT, Melchiorri L. Relationship between HLA-G genotype and IL-10 levels in soluble HLA-G expression. *Immunogenetics* 2005;**57**:172–81.

A ''new'' technique for the diagnosis of chondrocalcinosis of the knee: sensitivity and specificity of high-frequency ultrasonography

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According to the criteria proposed by Ryan and McCarty,[1] the diagnosis of calcium pyrophosphate dihydrate (CPPD) deposition disease has been based on radiological evidence of the characteristic calcifications and on verification of the synovial liquid of CPPD crystals.

Joint ultrasonography is an innocuous diagnostic technique that is well tolerated by patients, and is the elected method for observing calcified deposits in soft tissues.[2]

Abbreviation: CPPD, calcium pyrophosphate dehydrate