RESEARCH NOTE

Investigations on possible role of *MIF* gene polymorphism in progression of chikungunya infection into cases of acute flaccid paralysis and chronic arthropathy

SHWETA RAMDAS FULSUNDAR[1], SUBARNA ROY[1], SATHYA PRAKASH MANIMUNDA[1], SHIV SHANKAR SINGH[2], A. P. SUGUNAN[1] and PALURU VIJAYACHARI[1]*

[1]*Regional Medical Research Centre, Indian Council of Medical Research, Post Bag No. 13, Port Blair 744 101, India*
[2]*Govind Ballabh Pant Hospital, Port Blair 744 101, India*

Introduction

Chikungunya fever re-emerged in India during 2005–2006 after a calm of 32 years causing an epidemic of fever with rash and polyarthralgia. Few unusual cases of acute flaccid paralysis (AFP) and chronic arthropathy (CAR) following chikungunya fever were noticed in the Andaman Islands. DNA amplification and sequencing analyses of blood samples from these patients and controls were carried out for screening the size of a CATT microsatellite repeat at −794 and presence of a mutation at −173* in the macrophage migration inhibitiory factor (*MIF*) gene, recently shown to be associated with a plethora of different diseases, in an attempt to explore the possibility of these genes playing a role as a host genetic factor in development of these complications. Except for the presence of homozygous tetra-CATT or penta-CATT repeat in all AFP samples, we did not find any association between AFP and CAR cases with polymorphisms in the *MIF* gene. These incidental observations made on a small number of cases warrants larger studies on the role of polymorphisms in the *MIF* genes and other host genetic factors for acquisition of rare complications following chikungunya fever.

A large outbreak of chikungunya fever started in the islands of Indian Ocean in early 2005, spreading through adjoining islands and appeared in peninsular India by late 2005. After spreading through the southern states of Andhra Pradesh, Tamil Nadu, Karnataka and Kerala, it appeared in central and western states of India: Madhya Pradesh, Maharashtra and Gujarat, and finally reached the Andaman Islands in July–August 2006 (Manimunda *et al*. 2007). The Andaman and Nicobar is an archipelago of more than 500 islands and islets situated about 1200 km south of peninsular India. Port Blair, the administrative capital of the islands has a population of about 99,984 (Government of India, Census data, 2001, available at http://www.and.nic.in/Know%20Andaman/ecostat2007/area.pdf). In Port Blair, the attack rate of chikungunya fever was about 60% (Manimunda *et al*. 2007). Isolation, serology and RT-PCR were used for confirmatory / laboratory diagnosis. The disease presented as high-grade fever with multiple joint pain and maculopapular rash as the commonest symptoms. We reported an unusual neurological presentation of acute flaccid paralysis (AFP) that was observed during the outbreak. Four adults had febrile illness associated with joint pain that developed in all four limbs 3–15 days after onset of illness. All the four patients had areflexic quadriplegia and one required ventilatory support, but all of them recovered within a week (Manimunda *et al*. 2007). Also in the Andaman Islands, some patients developed chronic arthropathy (CAR: joint pain greater than three months duration) following chikungunya fever. Since there was no apparent difference in the sequences of the partial genomes of the infecting CHIKV (Subarna Roy, N. Murgamandam and P. Vijayachari; unpublished data) and all AFP and CAR cases were observed during the same outbreak, the possibility of different genotypes of the virus causing the different presentations is unlikely and host factors might have been the contributing factor. As in the case of any infectious diseases, chikungunya virus infection also triggers inflammatory response in various organs. In most cases, it resolves within a few days to a few weeks. The exact nature of pathogenesis of complications in chikungunya virus infection is not clearly understood. Since the discovery of *MIF* as a key regulatory cytokine acting

*For correspondence. E-mail: pblicmr@sancharnet.in; vijayacharip@yahoo.com.

Keywords. chikungunya; arthritis; acute flaccid paralysis; chronic arthropathy; *MIF* gene; human genetics.

within both the innate and adaptive immune response about a decade ago, a plethora of diverse disease associations have been described with increased MIF protein expression, more so in case of adult inflammatory arthritis (Barton *et al*. 2002) and juvenile idiopathic arthritis (Donn *et al*. 2002) wherein a CATT microsatellite repeat and mutation at *MIF*-173*C has been demonstrated to confer the subjects with an increased risk of acquiring the diseases. As the first effort in our hunt for a host genetic factor responsible for development of AFP as complications of chikungunya infection, we chose to explore the possibility of finding any association between any polymorphism in the CATT microsatellite repeat in the *MIF* promoter with these complications. Detection of expressed levels of this cytokine would require synovial fluids of the patients and controls obtained through invasive procedures involving ethical issues and, therefore, was not attempted. Instead, we chose to look for the known polymorphisms in the *MIF* gene, viz. the number of CATT repeats in the ~ 119 bp promoter region, single nucleotide polymorphism (SNP) at *MIF*-173, G>C or any new SNPs in these regions as have been done for several other infections, like malaria (Zhong *et al*. 2005).

Materials and methods

DNA was isolated from the leucocytes of the peripheral blood obtained from three laboratory confirmed cases of chikungunya infection exhibiting AFP and four of CAR. In addition DNA from five apparently healthy individuals were also taken. The DNA were subjected to PCR amplification for the segments of the *MIF* gene and its promoter following the protocol described by Zhong *et al*. (2005). PCR was done in ABI Gold GeneAmp 6700 thermal cycler (Applied Biosystems, Foster City, USA) and the PCR regime was 94°C for 5 min followed by 35 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 1 min, and a final denaturation of 72°C for 10 min. PCR products were purified and sequenced using BigDye™ chemistry (Applied Biosystems, Foster City, USA) in ABI 3130 Automated DNA sequencer (Applied Biosystems 3130 Genetic Analyzer, Foster City, USA). ABI trace files thus generated were analysed using the PHRED software http://www.mbt.washington.edu/phrap.docs/phred.html) which assigns quality scores to each base. The PHRED outputs for any given PCR amplicon were then aligned and assembled using SeqMan software of DNAstar (Lasergene, available at http://dnastar.com). The assembled sequences were analysed for SNP detection and repeat analysis. The assembled sequences were BLAST searched in the databases like GenBank and dbSNP and analysed.

Results and discussion

Five to six tandem repeats of the CATT element (5-CATT) were found in position −794 in all three AFP cases in homozygous condition. Two of the AFP cases carried six CATT repeats at position MIF-794 (6-CATT/6-CATT) and one individual showed the presence of five (5-CATT/5-CATT). In the four CAR cases, the CATT repeat was found in 5/6 heterozygous condition in two individuals and in 5/7 heterozygous condition in the other two. All five healthy individuals showed a 5/6 heterozygous repeat (table 1). At position *MIF*-173, the base was found to be guanine for all samples showing *MIF*-173*G allele including those in the apparently healthy controls (table 1).

Table 1. Comparison of the *MIF* genotypes of the CATT tetranucleotide microsatellite repeat and the −173 G/C SNP in a selection of human DNA samples.

Cohort	Sample ID	CATT repeat	−173 G/C SNP
AFP	AFP02	5/5	G/G
	AFP03	6/6	G/G
	AFP04	6/6	G/G
CAR	CAR02	5/6	G/G
	CAR03	5/6	G/G
	CAR04	5/7	G/G
	CAR05	5/7	G/G
Control	NOR01	5/6	G/G
	NOR02	5/6	G/G
	NOR03	5/6	G/G
	NOR04	5/6	G/G
	NOR05	5/6	G/G

There is growing interest surrounding the MIF molecule (Baugh and Bucala 2002). It has been shown to have proinflammatory, enzymatic and hormonal activities (Lolis 2001; Baugh and Bucala 2002). Raised MIF concentration in tissues in several diseases of an endocrine or chronic inflammatory basis suggests its role in pathogenesis (Niino *et al*. 2000; Yabunaka *et al*. 2000; De Jong *et al*. 2001; Matsumoto and Kanmatsuse 2001; Murakami *et al*. 2001; Donn *et al*. 2002; Meazza *et al*. 2002; Morand *et al*. 2002; Sakai *et al*. 2003). Increased expression of *MIF* in these sites could also be consequential rather than causative. Baugh *et al*. (2002) showed that the short CATT repeat (5-CATT) was associated with less severe rheumatoid arthritis in a cohort of hospital-derived patients. Plant *et al*. (2005) reported that 5-CATT repeat allele exhibited lowest MIF promoter activity *in vitro* and patients carrying this allele had less aggressive cystic fibrosis. Barton *et al*. (2003) found an association of a specific MIF promoter haplotype composed of 7-CATT-*MIF*-173*C and susceptibility to adult inflammatory polyarthritis. More specifically, De Benedetti *et al*. (2003a) found that carriage of the *MIF*-173*C polymorphism was correlated with raised serum and synovial fluid levels of MIF protein. Again, the duration of clinical response to the steroid treatment (months with no clinical evidence of synovitis) was significantly shorter in patients carrying a *MIF*-173*C allele than in the *MIF*-173*GG homozygotes (De Benedetti *et al*. 2003b). It has been shown that there is functional interac-

tion between the number of CATT repeat and *MIF*-173*C polymorphism in cell-type specific manner (Donn and Ray 2004).

In our study however, we noticed that all the three cases of chikungunya fever that presented with severe AFP exhibited the homozygous CATT repeat of either five or six times. The common *MIF*-173*G allele that is believed to be associated with low MIF production and less aggressive disease (Donn and Ray 2004) was found in all the subjects in our study. Our study shows that presence of *MIF*-173*G does not confer an individual with any protection against arthritis, at least due to chikungunya fever. However, although lesser number of CATT repeat has been shown to be associated with less severe forms of other arthritis, in the limited number of cases that we saw in our study, the presence of homozygous condition in the CATT repeat of five or six times might play a role in development of AFP as a complication of CHIKV infection. Interestingly, in two out of the four cases of CAR, haplotypes showed the presence of seven microsatellite repeats of the CAAT tetranucleotide. Since many number of CAAT repeat has been shown to be more vulnerable to certain forms of arthritis-like-rheumatoid arthritis, the presence of this long repeat in cases of chronic arthropathy might have some implication.

Our study comprised of only three AFP and four CAR cases that presented with a rare complication of chikungunya fever and therefore its scope is limited only for observations rather than generalizations. However, the leads obtained in the study warrants further studies on the role of polymorphisms in the *MIF* genes and on other host genetic factors for acquisition of rare complications following chikungunya fever.

References

Barton A., Lamb R., Symmons D., Silman A., Thomson W., Worthington J. and Donn R. 2003 Macrophage migration inhibitory factor (*MIF*) gene polymorphism is associated with susceptibility to but not severity of inflammatory polyarthritis. *Genes Immunol.* **4**, 487–491.

Barton A., Salway F., Thomson W., Silman A., Ollier W., Worthington J. *et al*. 2002 *MIF* polymorphism is associated with susceptibility to inflammatory arthritis. *Rheumatology* **41** (suppl. 1), 126.

Baugh J. A. and Bucala R. 2002 Macrophage migration inhibitory factor. *Crit. Care Med.* **30**, 27–35.

Baugh J. A., Chitnis S., Donnelly S. C., Monteiro J., Lin X., Plant B. J. *et al*. 2002 A functional promoter polymorphism in the macrophage migration inhibitory factor (*MIF*) gene associated with disease severity in rheumatoid arthritis. *Genes Immun.* **3**, 170–176.

De Benedetti F., Meazza C., Vivarelli M., Rossi F., Pistorio A., Lamb R., Lunt M. *et al*. 2003a Functional and prognostic relevance of the -173 polymorphism of the macrophage migration inhibitory factor gene in systemic-onset juvenile idiopathic arthritis. *Arthritis Rheum.* **48**, 1398–1407.

De Benedetti F., Vivarelli M., Lamb R., Meazza C., Muratori F., Cioschi S. *et al*. 2003b Association of the -173 SNP of the macrophage migration inhibitory factor (MIF) gene with response to intra-articular glucocorticoids in oligoarticular JIA. *Arthritis Rheum.* **48**, 254.

De Jong Y. P., Abadia-Molina A. C., Satoskar A. R., Clarke K., Rietdijk S. T., Faubion W. A. *et al*. 2001 Development of chronic colitis is dependent on the cytokine MIF. *Nat. Immunol.* **2**, 1061–1066.

Donn R., Alourfi Z., De Benedetti F., Meazza C., Zeggini E., Lunt M. *et al*. 2002 Mutation screening of the macrophage migration inhibitory factor gene: positive association of a functional polymorphism of macrophage migration inhibitory factor with juvenile idiopathic arthritis. *Arthritis Rheum.* **46**, 2402–2409.

Donn R. P. and Ray D. W. 2004 Macrophage migration inhibitory factor: molecular, cellular and genetic aspects of a key nueroendocrine molecule. *J. Endocrinol.* **182**, 1–9.

Lolis E. 2001 Glucocorticoid counter regulation: macrophage migration inhibitory factor as a target for drug discovery. *Curr. Opin. Pharmacol.* **1**, 662–668.

Manimunda S. P., Singh S. S., Sugunan A. P., Singh O., Roy S., Shriram A. N. *et al*. 2007 Chikungunya fever, Andaman and Nicobar Islands, India. *Emerg. Infect. Dis.* **13**, 1259–1260.

Matsumoto K. and Kanmatsuse K. 2001 Increased production of macrophage migration inhibitory factor by T cells in patients with IgA nephropathy. *Am. J. Nephrol.* **21**, 455–464.

Meazza C., Travaglino P., Pignatti P., Magni-Manzoni S., Ravelli A., Martini A. *et al*. 2002 Macrophage migration inhibitory factor in patients with juvenile idiopathic arthritis. *Arthritis Rheum.* **46**, 232–237.

Morand E. F., Leech M., Weedon H., Metz C., Bucala R. and Smith M. D. 2002 Macrophage migration inhibitory factor in rheumatoid arthritis: clinical correlations. *Rheumatology* **41**, 558–562.

Murakami H., Akbar S. M., Matsui H. and Onji M. 2001 Macrophage migration inhibitory factor in the sera and at the colonic mucosa in patients with ulcerative colitis: clinical implications and pathogenic significance. *Eur. J. Clin. Invest.* **31**, 337–343.

Niino M., Ogata A., Kikuchi S., Tashiro K. and Nishihira J. 2000 Macrophage migration inhibitory factor in the cerebrospinal fluid of patients with conventional and optic-spinal forms of multiple sclerosis and neuro-Behcet's disease. *J. Neuroimmunol.* **179**, suppl. 127–131.

Plant B. J., Gallagher C. G., Bucala R., Baugh J. A., Chappell S., Morgan L. *et al*. 2005 *Am. J. Respir. Crit. Care Med.* **172**, 1412–1415.

Sakai Y., Masamune A., Satoh A., Nishihira J., Yamagiwa T. and Shimosegawa T. 2003 Macrophage migration inhibitory factor is a critical mediator of severe acute pancreatitis. *Gastroenterology* **124**, 725–736.

Zhong Xiao-bo., Leng L., Beitin A., Chen R., McDonald C., Hsiao B. *et al*. 2005 Simultaneous detection of microsatellite repeats and SNPs in the macrophage migration inhibitory factor (*MIF*) gene by thin-film biosensor chips and application to rural field studies. *Nucleic Acids Res.* **33**, e121.

Yabunaka N., Nishhira J.,Mizue Y., Tsuji M., Kumagai M., Ohtauka Y. *et al*. 2000 Elevated serum content of Macrophage migration inhibitory factor in patients with type 2 diabetes. *Diabetes Care* **23**, 256–258.

Received 29 August 2008; accepted 15 December 2008
Published on the Web: 24 March 2009