INSTRUCTIONS FOR LETTERS TO THE EDITOR

Editorial comment in the form of a Letter to the Editor is invited; however, it should not exceed 800 words, with a maximum of 10 references and no more than 2 figures (submitted as camera ready hard copy per Journal Guidelines) or tables and no subdivision for an Abstract, Methods, or Results. Letters should have no more than 4 authors. Full name(s) and address of the author(s) should accompany the letter as well as the telephone number, fax number, or E-mail address.

Contact. **The Managing Editor, The Journal of Rheumatology, 365 Bloor Street East, Suite 901, Toronto, ON CANADA M4W 3L4. Tel: 416-967-5155; Fax: 416-967-7556; E-mail:jrheum@jrheum.com Financial associations or other possible conflicts of interest should always be disclosed.**

The Common HFE Variants C282Y and H63D Are Not Associated with Primary Osteoarthritis of the Hip or Knee

To the Editor:

In 2003, Ross, *et al*[1] reported a significantly increased frequency of the C282Y variant of the hereditary hemochromatosis gene HFE (chromosome 6p22.2) in a hand osteoarthritis (OA) cohort compared to a control group. To determine whether this effect was of a more general nature we have genotyped this variant, and the H63D variant of HFE, in a cohort of 557 OA cases and 557 age matched controls. The cases had each undergone total joint replacement of the hip (mono or bilateral), the knee (mono or bilateral), or the hip and knee for primary, idiopathic OA. The cases were ascertained through the Nuffield Orthopaedic Centre in Oxford, with their primary OA status supported by clinical, radiological, operative, and histological findings. The controls were members of the general population and had not undergone joint replacement or sought clinical treatment for OA. All cases and controls were aged 56 years or over and were of UK Caucasian origin. Ethical approval for the study was obtained from appropriate ethics committees and informed consent was obtained from all subjects. Further details relating to this case-control cohort have been published[2].

The C282Y variant is a G to A transition, whereas the H63D variant is a C to G transversion. The 2 variants were genotyped by PCR-restriction enzyme analysis using standard primers and the enzymes RsaI (C282Y) and MboI (H63D). Digestion products were electrophoresed through 3% agarose and scored after ethidium bromide staining. Genetic association was tested by chi-square contingency table analysis with Yates' correction. In the genotype association analysis if the cell number was ≤ 3 the 2 cells with the lowest numbers were combined and a 2 × 2 analysis was performed.

Table 1 lists the percentages and number of genotypes for the 2 variants in the cases and controls. There was no significant difference ($p \leq 0.05$) between the 2 cohorts for either variant. There was also no significant dif-

Table 2. Percentage of the A allele of C282Y and of the G allele of H6SD in cases and controls. Cases stratified by sex are compared to same-sex controls.

Cohort	C282Y		H63D	
	Frequency of A Allele	p	Frequency of G Allele	p
Cases				
All	8.0	0.42	14.7	0.67
Female	8.1	0.98	13.6	0.88
Male	7.8	0.37	16.5	0.28
Hip-only	7.0	0.96	15.3	0.49
Knee-only	9.2	0.27	14.7	0.85
Female hip-only	7.1	0.58	14.2	0.93
Male hip-only	7.0	0.74	17.1	0.24
Female knee-only	9.0	0.92	13.5	0.93
Male knee-only	9.5	0.28	16.4	0.58
Controls				
All	7.0		14.0	
Female	8.3		14.2	
Male	6.2		13.9	

Table 1. Percentage (number) of genotypes of the HFE variants C282Y and H6SD in cases and controls. When cases are stratified by sex, they are compared to same-sex controls.

Cohort	C282Y				H63D			
	GG	GA	AA	p	CC	CG	GG	p
Cases								
All, n = 557	85.1 (468)	13.8 (76)	1.1 (6)	0.61	72.5 (396)	25.5 (139)	2.0 (11)	0.56
Female, n = 342	85.0 (288)	13.9 (47)	1.2 (4)	0.97	74.8 (252)	23.1 (78)	2.1 (7)	0.86
Male, n = 215	85.3 (180)	13.7 (29)	0.9 (2)	0.53	68.9 (144)	29.2 (61)	1.9 (4)	0.27
Hip-only, n = 390	86.2 (330)	13.6 (52)	0.3 (1)	0.99	72.1 (274)	25.3 (96)	2.6 (10)	0.71
Knee-only, n = 136	83.8 (114)	14.0 (19)	2.2 (3)	0.54	71.3 (97)	27.9 (38)	0.7 (1)	0.52
Female hip-only, n = 244	85.9 (207)	14.1 (34)	0 (0)	0.77	74.6 (179)	22.5 (54)	2.9 (7)	0.99
Male hip-only, n = 146	86.6 (123)	12.7 (18)	0.7 (1)	0.89	67.9 (95)	30.0 (42)	2.1 (3)	0.17
Female knee-only, n = 78	84.6 (66)	12.8 (10)	2.6 (2)	0.89	73.1 (57)	26.9 (21)	0 (0)	0.94
Male knee-only, n = 58	82.8 (48)	15.5 (9)	1.7 (1)	0.43	69.0 (40)	29.3 (17)	1.7 (1)	0.47
Controls								
All, n = 557	86.4 (475)	13.3 (73)	0.4 (2)		74.5 (412)	23.0 (127)	2.5 (14)	
Female, n = 215	84.4 (179)	14.6 (31)	0.9 (2)		74.4 (160)	22.8 (49)	2.8 (6)	
Male, n = 342	87.6 (296)	12.4 (42)	0 (0)		74.6 (252)	23.1 (78)	2.4 (8)	

ference when the cases were stratified by sex, by joint replaced (hip or knee), or by sex combined with joint replaced. Table 2 lists the percentages of the A allele of C282Y and the G allele of H63D in the cases and controls. Again, there were no significant differences.

OA is a heterogeneous disorder and there is evidence from epidemiological and genetic studies that genes predisposing to OA at one joint site may not predispose to OA at all other joint sites[3,4]. Ross, *et al*[1] reported an association in a hand OA cohort. Our data imply that the common HFE variants C282Y and H63D are not risk factors for OA of the hip or knee.

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Dr. Ross replies

To the Editor:

Dr. Loughlin and Dr. Chapman report that HFE variants C282Y and H63D are not risk factors for hip or knee osteoarthritis (OA). I previously reported a possible increased risk of hand OA in Caucasians heterozygous for C282Y HFE mutation as well as an increased prevalence in subjects over age 65 years[1]. Loughlin and Chapman report that the prevalence was not statistically different for heterozygous C282Y mutation in knee and hip OA cases versus controls. However, there are some study design issues that may have affected their outcome. Also, they did not comment on an increased prevalence of homozygous C282Y mutation that may be indicative of the presence of hereditary hemochromatosis. Although their number of cases is small, the prevalence of homozygous C282Y mutation in knee-only cases of 2.2% is much higher than the control population's 0.4%. It would have been useful to see the percentage iron saturation and ages of these homozygous cases. If hemochromatosis was confirmed in these homozygous cases, then this could be a justification for routine screening for iron overload in severe knee OA.

The above studies compare the prevalence of heterozygous C282Y mutation in subjects with OA to the general population. Loughlin and Chapman's small sample from the general population appears to accurately represent C282Y prevalence since it is similar to other general population reports from England of 12%–13% for heterozygotes and 0.3%–0.5% in homozygotes[2,3]. However, since a majority of an older general population will have radiographic evidence of OA, this group is not a true control group. They attempt to address this flaw in design by using the case subjects' asymptomatic spouses as control subjects. Even though this resulted in a low frequency of clinical OA, a significant percentage of this control group might have had radiographic OA. Since both the above studies did not utilize a true control group, it is difficult to assess the true significance of their outcomes.

I also reviewed Loughlin and Chapman's reference of their prior study[4] for details of their case-control cohort. A potential selection bias for severe OA is present, since the cases were identified after joint replacement and it is possible that inclusion of milder cases might have altered the results. Also they report that their subjects were at least 56 years of age at enrollment. However, age at the time of joint replacement ranged from 47 to 85 years, with an average age of 64 years in women and 67 years in men. So some of the cases had early onset of severe primary OA. Since I reported that subjects over age 65 years had a possible increased prevalence of heterozygous C282Y mutation, it would have been useful to see their results stratified by age. In addition, power calculations are not reported, so the sample size of their knee OA subjects may be of inadequate number to support their conclusion about knee OA.

There appears to be a possible association of C282Y heterozygous mutation with hand OA but not with knee or hip OA. Neither of these studies is definitive due to the absence of adequate comparison controls. A controlled population study of elderly subjects, including subjects without radiographic OA, is required to confirm these results. As well, Loughlin and Chapman's results are suggestive of an increased prevalence of hemochromatosis in severe knee OA.

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Dietary Glutamate and Fibromyalgia

To the Editor:

Dr. Geenen and colleagues' article[1] entitled "Dietary Glutamate Will Not Affect Pain in Fibromyalgia" captured our interest. We have speculated whether ingestion of gram quantities of monosodium glutamate could potentially alter the sensitivity of muscle nociceptors[2]. However, after reading the article from Geenen, *et al* we feel compelled to comment on some of their statements. First, the reader is left with the impression that muscle pain evoked by intramuscular injection of glutamate into the human masseter muscle has not been compared with a control. In fact, it has been demonstrated that 0.2 ml injections of both 0.5 M and 1.0 M glutamate into the human masseter muscle evoke a similar intensity of pain that is significantly greater than that evoked by isotonic saline injections[3,4]. Perhaps of greater relevance to any potential association of fibromyalgia (FM) pain and monosodium glutamate intake is our repeated demonstration that muscle pain evoked by glutamate is significantly greater in women than in men[3,5,6]. As about 80% of FM sufferers are women, we were surprised that Geenen and colleagues overlooked mentioning this finding, since it may imply that women are more sensitive to the effects of elevated levels of glutamate in muscle tissue than are men.

We would also like to correct the impression left by the article that only animal studies indicate a role for peripheral excitatory amino acid receptors in the mechanism whereby elevated levels of muscle glutamate evoke pain in human subjects. In a recent article it was shown that injection of glutamate into the human masseter muscle evokes pain in part through activation of peripheral NMDA receptors[7]. In addition, animal studies clearly show that elevation of muscle glutamate both excites and sensitizes cutaneous and muscle nociceptors through activation of peripheral NMDA and non-NMDA receptors[1,7,8]. Collectively, these findings point to the potential for elevated tissue levels of glutamate to act via a *peripheral* mecha-

nism to alter pain perception. Unfortunately, the authors fail to develop this concept in their article, and instead conclude that without elevation of central nervous system levels of glutamate there can be no effect of monosodium glutamate ingestion on ongoing muscle pain. It is our opinion that in doing this, the authors have overlooked the important potential contribution of peripheral excitatory amino acid receptor mechanisms to the development and maintenance of chronic musculoskeletal pain such as that in patients with FM.

While we agree with these authors that the current evidence does not support a recommendation of decreased glutamate intake for patients with FM or other chronic muscle pain, we feel that it is premature to draw strong conclusions about the effect of dietary glutamate on muscle pain syndromes. We prefer to await the publication of more definitive research on this topic before reaching such conclusions.

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Dr. Geenen, et al reply

To the Editor:

Dr. Cairns and Dr. Svensson discuss several issues that were implicated in their interesting research of glutamate-evoked pain in the human masseter muscle. Our hypothesis article discussed a related but different question. Our main concern related to possible pain-modulating effects of glutamate in *diet*. We concluded that a dietary reduction of glutamate will not accomplish pain relief in fibromyalgia (FM)[1]. Cairns and Svensson agree with our conclusion that current evidence does not support the recommendation of decreased glutamate intake for FM, but they feel that our conclusion is premature and prefer to await more definitive research before reaching such a conclusion. We do not argue that future research may refute our hypothesis, but it is reassuring that there is no discussion about our recommendation based on current research findings.

It was not our intention to suggest that muscle pain evoked by intramuscular injection into the human masseter has not been compared with a control. We referred in the introduction of our article to the study of Svensson, *et al*[2] because it demonstrated that an increase of glutamate in muscles enhances pain. Indeed, this study was not controlled. We referred to another study of the same group[3] to support the statement that this increase might be accomplished through peripheral excitatory amino acid receptors. Two other studies from this group, one of which was published after our article, also support this conclusion. Thus, there was and is no disagreement with Cairns and Svensson about the conclusion that intramuscular injection of glutamate in the human masseter muscle will enhance pain.

The higher prevalence of FM in women than in men attracts attention to many physiological and psychosocial sex differences that may play a role in pain. Cairns and Svensson tentatively suggest that the repeated observation that muscle pain evoked by glutamate is significantly greater in women than in men may be of relevance to a potential association of FM pain and monosodium glutamate intake. The finding that women are more sensitive to the effects of elevated levels of glutamate in muscle tissue than men, however, does not prove that this is a crucial pathogenic mechanism in FM. Moreover, it definitely does not prove that increased glutamate in muscles due to dietary intake of glutamate plays a role in FM, the topic of our report. It remains to be experimentally verified whether there is a link between elevated muscle tissue glutamate levels and FM or between daily dietary glutamate and muscle tissue glutamate levels.

Cairns and Svensson state that our conclusion about the effects of dietary intake of glutamate on pain is based on the idea that without an elevation of central nervous system levels of glutamate there can be no effect of monosodium glutamate ingestion on ongoing muscle pain. As outlined in their letter, the research group consistently demonstrated that peripheral excitatory amino acid receptor mechanisms matter in pain. This very observation was also a crucial starting-point of our article. In addition, we cited a study that described a short-term 2-hour increase of intramuscular glutamate after bolus ingestion of monosodium glutamate[4]. However, such a bolus ingestion cannot be compared with common daily dietary glutamate intake. We consider that research into a potential role for peripheral excitatory mechanisms in FM is important, but we do not see in the literature or in Cairns and Svensson's letter proof of a significant role of common dietary glutamate. To paraphrase, "it is not clear how or under what natural conditions tissue glutamate levels could be sufficiently elevated to cause muscle pain"[5].

Our article[1] put forward a hypothesis. We indicated that nonscientific literature on the Internet about a potential role of dietary glutamate by far outweighs the scientific literature. We appreciate sound scientific studies, as have been done by Cairns and Svensson and coworkers. Research is needed to verify and refute hypotheses. Our hypothesis, based on present knowledge about glutamate consumption and its metabolism in humans, is that a reduction of glutamate in diet will not accomplish pain relief in FM. But we encourage experimental research that will refute this hypothesis by showing that glutamate ingestion affects pain; for instance, through peripheral excitatory amino receptor mechanisms.

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