Association of Heterozygous Hemochromatosis C282Y Gene Mutation with Hand Osteoarthritis

JAMES M. ROSS, ROMAN M. KOWALCHUK, JENNIFER SHAULINSKY, LORI ROSS, DANIEL RYAN, and PRADYUMNA D. PHATAK

ABSTRACT. ***Objective.*** To determine if there is an association between radiographic osteoarthritis (OA) of the hand and the presence of hemochromatosis HFE gene mutations.

Methods. One hundred seventy-six patients with radiographic OA of the hand were randomly selected from an academic rheumatology practice. We measured serum transferrin saturation (TS) and ferritin levels, and genotyped for the presence of the 2 common HFE mutations, C282Y and H63D. The prevalences of HFE mutations in these patients were compared to those in a hemochromatosis screening study from the same primary care patient base.

Results. There was a significantly increased prevalence of the C282Y mutation in the OA population compared to the unselected controls (12.5 vs 7.8%; p = 0.029). The prevalence of C282Y in OA was higher among older patients: 15.75% in the group older than 65 years versus 4.08% in the younger group. The mean TS level was higher among OA patients who were heterozygous for C282Y compared to those who lacked both HFE mutations (35.75 vs 25.93%; p < 0.0001). This difference was also found in the general population.

Conclusion. This is the first report to show an increased risk of OA among individuals who are heterozygous for the C282Y HFE mutation. The increase in this mutation in patients older than 65 suggests that this is associated with a late onset subset of OA. If this association is substantiated by larger randomized controlled studies, it could have major therapeutic implications in the development of specific therapy directed at individuals heterozygous for C282Y HFE mutation. (J Rheumatol 2003;30:121–5)

Key Indexing Terms:

HEMOCHROMATOSIS	OSTEOARTHRITIS	IRON
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The prevalence of osteoarthritis (OA) rises sharply with age and it is classified into primary and secondary forms. Primary OA can be divided into subsets, each possibly with a different etiopathogenesis. Some subsets of primary OA undoubtedly involve genetic factors and the heritability of OA has been described[1].

Hereditary hemochromatosis (HHC) is one of the most common genetic disorders among Caucasians, with a prevalence of 0.3%. The diagnosis of HHC is based on objective evidence of iron overload in screened individuals with a serum transferrin saturation (TS) of ≥ 45%. The HFE gene is a MHC class I-like gene that encodes a protein responsible for the regulation of iron metabolism[2]. Two common HFE mutations, C282Y and H63D, appear to account for the vast majority of HHC cases, with 60–90% of cases being homozygous for C282Y and a further 4–8% compound heterozygotes for both C282Y and H63D. The allele frequency of H63D is higher than that of C282Y but the penetrance of C282Y is higher, accounting for its higher prevalence among patients with HHC. The penetrance is higher in males than females, at least in part because premenopausal females are protected by iron losses associated with menses and childbearing. Other less common HFE mutations may also be associated with HHC[3]. It has been shown that heterozygotes for the HHC allele have higher total body iron stores and are relatively protected from iron deficiency[4]. In addition, associations between heterozygosity for the C282Y mutation and both cardiac disease and diabetes have been described[5,6].

Many patients with HHC develop a severe arthropathy that may be indistinguishable from calcium pyrophosphate dihydrate (CPPD) crystal deposition disease or OA[7-9]. One report found arthritis to be the most common symptom in

From the Departments of Medicine at the University of Rochester School of Medicine and Dentistry, and Rochester General Hospital, Rochester, New York; Department of Medicine, Pennsylvania State College of Medicine; and Lehigh Valley Hospital, Allentown, Pennsylvania, USA.

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J.M. Ross, MD, FACP, Assistant Clinical Professor, Department of Medicine, Pennsylvania State College of Medicine; R.M. Kowalchuk, MD, PhD, Clinical Assistant Professor of Radiology and Orthopedics; J. Shaulinsky, MD, Research Assistant, University of Rochester School of Medicine and Dentistry; L. Ross, RN, Research Assistant, Lehigh Valley Hospital; D. Ryan, MD, Professor of Pathology and Laboratory Medicine; P.D. Phatak, MD, Associate Professor, Department of Medicine, University of Rochester School of Medicine and Dentistry.

Address reprint requests to Dr. J.M. Ross, Lehigh Valley Hospital, 3600-1210 South Cedar Crest Blvd., Allentown, PA 18062, USA.

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HHC, occurring in up to 81% of patients[10]. However, a recent population screening study showed that the prevalence of common HHC symptoms including a history of arthritis among control subjects was similar to C282Y homozygotes[11]. The radiographic findings in HHC arthropathy are similar to the findings in generalized OA[7,12,13], with the exception of an increased prevalence of involvement of the 2nd and 3rd metacarpophalangeal (MCP) joints and the occurrence of chondrocalcinosis. Pathogenic mechanisms involved in HHC arthropathy, including the role of HFE mutations or iron overload, have not been established. One theory is that HHC arthropathy results from a different metabolic defect that accompanies but is independent from iron metabolism[7].

Since heterozygosity for both the HHC mutations is relatively common and is associated with increased body iron stores, we explored the possibility that individuals who are heterozygous for C282Y and H63D mutations may be prone to develop radiographic OA or chondrocalcinosis.

MATERIALS AND METHODS

Study design. We designed a random cross sectional study comparing the prevalence of HFE mutations and iron levels between 2 Caucasian populations from Rochester, NY. The study population comprised consecutive patients with radiographic hand OA recruited from an academic arthritis center who met study criteria and gave informed consent. The control group of 2138 random subjects, unselected by presence or absence of OA, was derived from an HHC screening study using the same primary care patient base. Only Caucasians were evaluated since both the HFE gene and genetic predisposition to OA have variable prevalence depending on race, and both are much more prevalent in Caucasians[3,14].

OA study population. Caucasian patients presenting with any complaint to an academic rheumatology unit in Rochester, NY, were screened for study entry with a hand joint examination and a history questionnaire. The presenting diagnoses included: fibromyalgia 27%, OA of the spine, hip, or knees 25%, systemic lupus erythematosus or Sjogren's syndrome 19%, polymyalgia rheumatica 15%, osteoporosis 5%, pseudogout 5%, and hand OA 4%. Entry into the study was based on screening with hand physical examinations using the American College of Rheumatology OA criteria requirement of hard tissue enlargement of finger joints[15]. Subject exclusion criteria, based on patient history and examination, included severe trauma, joint hypermobility, rheumatoid arthritis, seronegative arthritis, active alcohol abuse, tophaceous gout, hemodialysis, and hyperparathyroidism. One hundred seventy-six patients with radiographic hand OA out of 184 clinically identified patients were studied. Twenty additional patients refused enrollment.

Laboratory tests. Serum and whole blood were obtained from each patient and analyzed for serum TS and serum ferritin (SF) levels, and for the presence of the 2 common HFE mutations, C282Y and H63D. Serum samples for iron studies were frozen and analyzed in batches at the same laboratory. TS levels were analyzed using a Hitachi 747 analyzer. SF levels were determined with a standard Boehringer-Mannheim system with FerroZine as the reactive chromogen read at 570 nm.

The HFE genotyping was performed using a polymerase chain reaction (PCR) restriction digestion technique. DNA was extracted from buffy coats of EDTA anticoagulated blood samples using the Bio-Rad Instagene Whole Blood kit according to manufacturer's directions. Ten microliters of this preparation were used as a template for PCR. Genotyping for HFE C282Y and H63D mutations was performed by PCR restriction enzyme digestion as described[16], but using the similar enzyme BclI in place of MboI for the H63D enzyme digestion step. All samples genotyped as C282Y homozygote were confirmed with inner primers (sense: CAAGTGCCTCCTTTGGTGAA; antisense: TCACATACCCCAGATCACAA) to rule out potential false assignment of homozygosity due to a previously described G5569A polymorphism at the antisense primer binding site[17]. PCR contamination was monitored by simultaneous amplification of a negative control containing all reagents except template DNA.

Radiographic assessment. One PA radiograph of both hands was performed on each patient with OA using standard procedures. One trained observer read all radiographs blinded and in batches using the Kellgren-Lawrence (K-L) scale[18] and the score of each joint was recorded. Since the definition of radiographic hand OA has not been standardized, we utilized definitions described in other studies[19-21]. The diagnosis of radiographic hand OA was based on a K-L grade of $\geq$ 2 in at least one of the 2nd-5th distal interphalangeal (DIP), proximal interphalangeal (PIP), or MCP joints in either hand. Osteophyte involvement of the MCP joints was included in the definition of hand OA due to the predilection of HHC arthropathy for the MCP joints. Severe OA was characterized by having a grade of $\geq$ 3 in one or more of the above joints of either hand. For each subject the cumulative grades of the DIP and the PIP joints were determined and the highest score determined the preferential joint group. Confirmation of a diagnosis of CPPD deposition disease was not within the scope of this study and instead the radiographic presence of chondrocalcinosis at the triangular fibrocartilage of the wrist was determined. Twenty-five radiographs were re-read and also reviewed by a second trained observer and these intraobserver and interobserver radiographic readings had kappa correlations of 0.85 and 0.70.

Control population. This group was obtained from 16,031 subjects enrolled in a HHC screening study[22] and were not screened for the presence of arthralgia or arthritis. Serum samples were frozen during the latter part of this study and thereby available for genetic testing. A random subset of 2138 Caucasians were genotyped to determine the prevalence of the C282Y and H63D mutations in this general population.

Statistical analysis. Power calculations were determined using the prevalence rate for heterozygosity for the C282Y mutation of 0.08, as described[3]. Sample size calculations were based on detecting a relative risk of 2, which is equivalent to detecting a 14.8% prevalence rate for C282Y among people with OA. Assuming a 2 sided test with $\alpha = 0.05$ and using a standard formula for sample size calculation for one sample test of a proportion, 149 is the minimum number of subjects needed for 80% power and 210 for 90% power.

The chi-square test of association was used to compare prevalence of genotypes. Analysis of TS and SF was performed with paired t testing. Chi-square analysis was utilized for statistical analysis of historical and radiographic data in the OA population by comparison of each gene mutation type to normal HFE types.

RESULTS

Patient characteristics were as follows: 176 individuals with radiographic hand OA; 148 females and 28 males; mean age was 69.2 years; 19% had a history of previous iron supplementation; 12% had diabetes; and 27% had thyroid disease. The characteristics of men and women were similar except that only one man had previously used supplemental iron, none of the men was diabetic, and only one man was under the age of 60. Eight of 184 enrolled patients were excluded from the analysis because they did not meet the radiographic criteria of having at least one DIP, PIP, or MCP joint with a K-L grade of $\geq$ 2. All of these excluded patients had evidence of erosive OA or grade 1 osteophytes.

The prevalences of HFE genotypes are listed in Table 1.

Table 1. Prevalence of HFE genotypes.

Genotypes	General Population %	(n = 2138)	OA Population %	(n = 176)	p	Chi-square
wt/wt	63.61	(1360)	65.34	(115)	*	
C282y/wt	7.81	(167)	12.50	(22)	0.029	4.767
H63D/wt	23.80	(509)	18.75	(33)	0.128	2.319
H63D/H63D	2.43	(52)	2.27	(04)	*	
C282Y/H63D	1.64	(35)	1.14	(02)	*	
C282Y/C282Y	0.42	(09)	00.0	(00)	*	

wt: wild-type genotype; * not significant.
General population comprised of random Caucasian population genotypes with and without OA and had prevalence similar to prior publication[3].

The prevalence of HFE genotypes for the general population was similar to that described in previous population studies[3]. The OA study population had a significantly increased prevalence of the heterozygous C282Y genotype and the other OA genotypes were comparable to the general population. This difference was more striking among the subgroup of OA patients who were older than 65 years (15.75 vs 7.8% in the general population; p = 0.0079). The 28 men enrolled with OA had the following prevalence rates: wt/wt: 57.1%, C282y/wt: 21.4%, H63D/wt: 17.9%, and C282Y/H63D: 3.6%.

Table 2 shows the difference in prevalence rates of HFE genotypes by age group among the patients with OA. Those older than 65 years of age had a significant increase in the heterozygous C282Y mutation: 15.75 versus 4.08% in patients with OA 65 years or younger.

We also determined whether underlying rheumatic disease, or the presence of severe OA, MCP involvement, chondrocalcinosis of the wrist, onset of OA before age 55, and preferential involvement of PIP or DIP joints were influenced by the presence of the heterozygous state for either mutation (Table 3). None was found to be statistically significant. However, the prevalence of MCP involvement among our normal genotype (wild-type/wild-type) OA patients was much higher than the 12% that has been described in OA[23,24].

The mean TS level for the normal genotype OA population was 25.93% compared to 35.75% for C282Y/wt ($p < 0.001$). This difference was also found in the general population. SF levels in the OA population did not differ significantly by HFE genotype.

DISCUSSION

OA is a common rheumatic disorder of unknown etiology. The possibility that a heterozygous C282Y mutation may be involved in the pathogenesis in one subset of OA is suggested by the fact that HHC may be associated with a severe arthropathy that may be mistaken for OA[7,25]. In this regard, our results demonstrate a significant increase in the prevalence of the heterozygous C282Y mutation in Caucasians with radiographic OA compared to the general population. There was no evidence for an association of OA and the H63D mutation, but the small sample size does not preclude a possible association with homozygous H63D or the compound heterozygous state. Our study did not control for the occurrence of OA among our general population and therefore studying this prospectively may further strengthen our findings.

HHC arthropathy develops by the 4th or 5th decade after years of excess iron absorption. Increased iron absorption also has been observed in heterozygous C282Y mutation[4]. The pathogenesis of HHC arthropathy is unknown. However, it is possible that an OA subset associated with the heterozygous C282Y mutation would have the same mechanism as in HHC but with a delayed rate of onset, and it should therefore occur in older individuals. As we found the

Table 2. HFE genotype prevalence in patients with OA ≤ 65 and > 65 years of age.

Genotypes	OA Study Population ≤ 65 Years of Age %	(n = 49)	OA Study Population > 65 Years of Age %	(n = 127)	p	Chi-square
wt/wt	69.39	(34)	63.78	(81)	*	
C282y/wt	4.08	(02)	15.75	(20)	0.036	4.40
H63D/wt	22.45	(11)	17.32	(22)	*	
H63D/H63D	2.04	(01)	2.25	(03)	*	
C282Y/H63D	2.04	(01)	0.08	(01)	*	

wt: wild-type genotype; * not significant.

Table 3. Osteoarthritis characteristics bsed on genotype.

OA Characteristics	wt/wt* (n = 115)		C282Y/wt* (n = 22)		H63D/wt* (n = 33)	
Mean age (years)	70.57	SD 9.4	73.96	SD 8.0	71.03	SD 11.56
OA of ≥ 1 MCP joint	43.5%	(50)	36.4%	(08)	39.4%	(13)
Chondrocalcinosis of the wrist	18.3%	(21)	18.2%	(04)	24.2%	(08)
Severe OA	69.6%	(80)	63.6%	(14)	84.8%	(28)
History of OA onset before age 55	45.2%	(52)	36.4%	(08)	51.5%	(17)
Preferential DIP involvement	76.5%	(88)	77.3%	(17)	51.5%	(17)

* Number of subjects with homozygous and compound heterozygous genotypes was too small for meaningful comparisons.

heterozygous C282Y mutations were more prevalent in patients over age 65 years, our data support this hypothesis.

Even though HHC is an autosomal recessive disorder, phenotypic expression occurs later in women, usually after menopause. There are few data on HHC arthropathy in females, but this has been described. One report found similar manifestations between 176 age matched men and women with HHC including arthritis in 44.9% of women and 35.2% of men[26]. Therefore, it is not unreasonable to expect to find both males and females with HHC arthropathy or theoretically in an older subset of OA associated with the heterozygous C282Y mutation.

Individuals with HHC related arthropathy have a higher propensity for MCP involvement and chondrocalcinosis. We therefore compared the prevalence of chondrocalcinosis and MCP involvement in individuals with OA and the C282Y mutation to that of OA patients with a normal genotype. No difference was found. However, our population of OA subjects who lacked HFE mutations had more severe disease including a higher prevalence of MCP involvement than described in previous OA studies[23,24]. These findings may have been the result of an unrecognized selection bias that may have interfered with our ability to show a specific association of MCP involvement with the C282Y mutation. Therefore, it remains possible that future controlled studies on hand OA and heterozygous C282Y mutations will show a predilection towards severe 2nd and 3rd MCP involvement and chondrocalcinosis.

The majority of patients enrolled were seen for other rheumatic problems but also had concomitant hand OA. This reduced selection bias toward more severe cases of OA presenting to a rheumatology clinic. However, SF levels were not significantly different. SF is an acute phase reactant that could have been elevated due to underlying rheumatic disease. The increased TS seen with heterozygous C282Y mutation compared to the normal genotypes is an expected finding[3,4,11] and only suggests that iron may be a causative factor in our OA population. Our results do not exclude the possibility that the C282Y mutation is simply a marker for another gene that may be directly involved in one subset of OA.

In summary, this is the first report showing that a subset of OA appears to be associated with a heterozygous C282Y HFE mutation. In addition the increased prevalence of the C282Y mutation in patients older than 65 suggests that this subset is associated with late onset disease. The data from this HFE prevalence study are supportive of our hypothesis; however, a randomized controlled study, including patients without radiographic OA, is required to confirm these results. Further studies are also necessary to assess an association of this HFE mutation with OA at other sites and in other races. If the association of the heterozygous C282Y mutation with OA is substantiated, this could have significance since additional studies on the treatment and prevention of HHC arthropathy might also benefit this subset of OA.

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