Association of sickle avascular necrosis with bone morphogenic protein 6

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LETTER TO THE EDITOR

Association of sickle avascular necrosis with bone morphogenic protein 6

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Dear Editor,

Avascular necrosis (AVN) of femoral and humeral heads is a frequent and debilitating complication in patients with sickle cell disease (SCD), its prevalence being highest in individuals with SCD-Hb SS and coincidental α-thalassemia. Family and sibling studies suggest a genetic predisposition, and recently, variation in genes involved in bone modeling or the vasculature have been proposed as significant factors in the development of AVN [1]. Baldwin et al. [1] investigated single nucleotide polymorphisms (SNPs) in candidate genes involved in vascular function, inflammation, oxidant stress, and endothelial cell biology for association with AVN. SNPs in *bone morphogenic protein 6* (*BMP6*), *annexin A2* (*ANXA2*), and *klotho* (*KL*) genes were suggested to be associated with sickle cell AVN. We attempted to replicate this association in 244 adult patients with SCD, 39 of whom had joint symptoms with radiological and/or magnetic resonance imaging (MRI) evidence of AVN (AVN group; cases), whereas the remaining 205 had no clinical symptoms of AVN (non-AVN group; controls). Those with AVN, on average, were 3 years older than the controls (Table 1). In addition, individuals with AVN had a higher prevalence of coincidental α-thalassemia, higher hematocrit levels, and lower fetal hemoglobin (Hb F) levels when compared to the non-AVN group, though these observations were not statistically significant (Table 1). All patients were of West African or African Caribbean descent. The study was approved by the King's College Hospital Research Ethics Committee (LREC 01-083).

Based on the visual inspection of phased haplotypes in the Yoruba population from Ibadan, Nigeria (YRI), two haplotype blocks were defined in *KL*, one block in *ANXA2*, and three blocks in *BMP6*. Tag SNPs with population frequency information were then selected using the HapMap Project data provided for the Yoruba community, as this ethnic group relates most closely to our sample population. Of the selected tag SNPs, one per gene was identical to that used by Baldwin et al. (Table 2).

The selected tag SNPs were genotyped using the TaqMan® allelic discrimination assay. Most assays performed well, but *ANXA2-2* failed repeatedly. Genotype data generated from the SNP assays for the remaining six markers ranged from 95.2% to 100% complete, and markers displayed no deviation from the Hardy–Weinberg equilibrium.

To test for trait association with the candidate SNPs, allele frequencies between cases (AVN group) and controls (non-AVN group) were compared using Pearson's chi-square statistic with a significance threshold of $p \leq 0.05$ (Table 2). Characterization of the six SNP markers revealed that only one (*BMP6-3* or *rs3812163*) showed significant evidence of association ($p = 0.021$) with AVN.

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Table 1 Comparison of AVN and non-AVN patients

	AVN		Non-AVN	
N	39	Percentage	205	Percentage
Male/Female	17:22	43.6:56.4	92:113	45:55
SCD genotype				
Hb SS	30	76.9	128	62.4
Hb SC	7	17.9	62	30.2
Hb S/β^+	2	5.1	10	4.9
Hb S/β^0	–	–	5	2.4
Age (mean, range)	38.82 (19–65)		35.9 (15–84)	
α-Thalassemia genotype				
αα/αα	19	48.7	130	64.3
αα/α–	14	35.9	56	27.7
α–/α–	2	5.1	11	5.4
α–/ααα	1	2.6	–	–
αα/ααα	–	–	3	1.4
Unknown	3	7.7	5	2.5
Hb (mean, range, g/dL)	8.80 (7.3–12.0)		7.94 (5.0–11.5)	
P value	0.092			
HCT (mean, range)	0.256 (0.209–0.348)		0.229 (0.138–0.339)	
P value	0.069			
Hb F% (mean, range)	7.57 (1.1–20.1)		10.73 (6.6–23.4)	
P value	0.020			

'Unknown' indicates missing data "%" denotes percentage of patients
Hb hemoglobin, *HCT* hematocrit, *Hb F%* percentage fetal hemoglobin

This case–control study on the association between the occurrence of sickle AVN and a small set of genetic polymorphisms was designed to replicate recent findings implicating the involvement of *KL*, *BMP6*, and *ANXA2* in the occurrence of AVN in patients with SCD. Six tag SNPs covering the major haplotypes representing the three genes were selected. Our study confirmed association of a single SNP, *BMP6-3* (*rs3812163*), suggesting a potential role for *BMP6* in the development of AVN in SCD patients.

Members of the BMP family are unique in their ability to induce endochronal bone formation when implanted at ectopic sites [2]. In the past, *BMP6* mRNA has been localized in hypertrophic cartilage [3]. More recently, when Bobacz et al. [4] investigated the role of *BMP6* in cartilage homeostasis, they discovered a crucial involvement of this gene in the maintenance/repair of human articular cartilage. In AVN, the progressive degeneration of bone eventually leads to its collapse and destruction of the articular

Table 2 Selected tag SNPs for typing in *bone morphogenic protein 6* (*BMP6*), *annexin A2* (*ANXA2*), and *klotho* (*KL*)

SNP ID	Given name	Chromosomal position	Minor allele	Minor allele frequencies in YRI	Minor allele frequencies in SCD		Association *p* values
					AVN	Non-AVN	
rs267196[a]	*BMP6-1*	Chr6: 7794334	A	0.24	0.29	0.30	0.895
rs9392924	*BMP6-2*	Chr6: 7740099	G	0.48	0.35	0.38	0.600
rs3812163	*BMP6-3*	Chr6: 7670759	A	0.30	0.18	0.31	0.021
rs9526990	*KL-1*	Chr13: 32510826	G	0.25	0.29	0.29	0.966
rs211239[a]	*KL-2*	Chr13: 32494189	C	0.19	0.22	0.21	0.763
rs8028846	*ANXA2-1*	Chr15: 58472000	G	0.22	0.36	0.33	0.295
rs7170421[a]	*ANXA2-2*	Chr15: 58421440	C	0.28	–	–	–

Allele frequencies between the AVN group and the non-AVN group were compared using Pearson's chi-square statistical analysis with one degree of freedom; '–' denotes that the SNP assay did not work
[a] The SNP was also genotyped by Baldwin et al. [1]

cartilage, supporting a role for *BMP6* in the pathogenesis of this complication.

Association studies which examined polymorphisms in *MTHFR* [5], platelet glycoprotein IIIa (*ITGB3*) [6], and plasminogen activator inhibitor-1 (*PAI-1*) [7] with AVN in SCD in small numbers were non-conclusive. Another study sought association of combined vaso-occlusive complications including stroke, acute chest syndrome, AVN, and priapism with polymorphisms in candidate genes [8]. In this small study (34 cases and 63 case controls), association was found with a SNP in human platelet antigen-5 (*HPA-5b* allele) gene. Most of the events were AVN, with only four individuals having more than one complication. Similarly, numerous association studies with candidate genes for the other complications of SCD–stroke [9], priapism [10], and pulmonary disease [11] have been published, but to our knowledge, none of the associations have been replicated.

We acknowledge that our study was limited by the small sample size. While absence of AVN in case controls was not verified by radiologic exam in all cases, and thus may have included some patients with early AVN who were still symptom-free, the AVN cohort included only those with radiological and/or MRI evidence of AVN.

This study did not replicate the association between variants in *KL* and *ANXA2*, and AVN, shown in earlier studies [1]. *KL*, *ANXA2*, and *BMP-6* were also shown to be associated with other vascular complications in SCD such as priapism [10] and stroke [12], suggesting a common underlying molecular basis for these vascular complications.

Future clinical investigations should aim at replicating these findings including higher resolution genotyping in the region of the *BMP6* gene to identify the causal variant involved in sickle AVN. Large and appropriately powered studies designed for genetic analysis are required for such investigations.

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