HLA Has Strongest Association with IgA Nephropathy in Genome-Wide Analysis

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ABSTRACT

Demographic and family studies support the existence of a genetic contribution to the pathogenesis of IgA nephropathy, but results from genetic association studies of candidate genes are inconsistent. To systematically survey common genetic variation in this disease, we performed a genome-wide analysis in a cohort of patients with IgA nephropathy selected from the UK Glomerulonephritis DNA Bank. We used two groups of controls: parents of affected individuals and previously genotyped, unaffected, ancestry-matched individuals from the 1958 British Birth Cohort and the UK Blood Service. We genotyped 914 affected or family controls for 318,127 single nucleotide polymorphisms (SNPs). Filtering for low genotype call rates and inferred non-European ancestry left 533 genotyped individuals (187 affected children) for the family-based association analysis and 244 cases and 4980 controls for the case-control analysis. A total of 286,200 SNPs with call rates >95% were available for analysis. Genome-wide analysis showed a strong signal of association on chromosome 6p in the region of the MHC (P = 1 × 10⁻⁹). The two most strongly associated SNPs showed consistent association in both family-based and case-control analyses. HLA imputation analysis showed that the strongest association signal arose from a combination of DQ loci with some support for an independent HLA-B signal. These results suggest that the HLA region contains the strongest common susceptibility alleles that predispose to IgA nephropathy in the European population.

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IgA nephropathy (IgAN) is the most common form of glomerulonephritis and is an important cause of ESRD. Clinical presentation is usually with hematuria with a variable degree of proteinuria; progression to ESRD occurs in ~25% of those affected in the 20 years after diagnosis.1 Pathologically, IgAN is characterized by deposition of polymeric IgA1 in the renal mesangium, accompanied by mesangial hypercellularity, mesangial matrix expansion, and varying degrees of glomerulosclerosis and interstitial fibrosis. The pathogenesis is unclear, and diversity in the clinico-pathologic disease spectrum has suggested etiologic heterogeneity.
A number of observations suggest that there is a significant genetic contribution to the pathogenesis of IgAN. These include differences in the prevalence of IgAN across ancestry groups, increased disease prevalence in relatives of affected individuals, and reports of large pedigrees containing multiple affected individuals. To date, the strongest direct evidence for the existence of genetic factors in the development and/or progression of IgAN has been provided by linkage analyses in such families. A major disease locus designated \( IGAN1 \) on chromosome 6q22–23 was defined in a study of white families. However, only 60% of the families in the study were linked to this locus, and, to date, no disease-specific genes associated with disease/susceptibility have been identified within the linkage interval. This locus was excluded by linkage analysis in a Japanese family, whereas in Italian families, linkage has been reported at two different IgAN loci on 4q26–31 and 17q12–22. A Canadian family study has localized another IgAN susceptibility locus to chromosome 2q36, whereas analysis of a large Lebanese kindred did not provide evidence for linkage at 6q22–23, 2q36, or 4q22–31.

These studies therefore suggested that at least part of the genetic basis of IgAN is specified by variants that are confined to specific families or discrete populations (and therefore might not be fundamental to the disease process as a whole), and it is unclear whether there are common IgAN susceptibility haplotypes that operate across large populations. This led to increasing interest in defining the existence and extent of IgAN susceptibility loci in large populations using genetic association. Most studies conducted to date examined small numbers of candidate genes, although some surveyed candidate genes more extensively or conducted limited genome-wide analyses. Although a number of potential associations with polymorphisms at candidate gene loci were reported (reviewed in references 2, 13, 14), these associations have not all been reproduced, and there is no systematic genome-wide analysis of susceptibility to IgAN.

To evaluate evidence for genetic association with IgAN, we undertook a genome-wide analysis in patients with European ancestry using a panel of \( \sim 300,000 \) tagging single nucleotide polymorphisms (SNPs), designed to maximize coverage of common human haplotypes based on linkage disequilibrium intervals.

We performed both family-based and case-control association studies, using cases and nuclear families ascertained from the UK MRC/Kidney Research UK National DNA Bank for Glomerulonephritis and controls from the 1958 British Birth Cohort and the UK National Blood Service. Strong signals of association were observed in the MHC on chromosome 6p but not other loci, indicating that, in the UK population, the MHC contains the strongest common susceptibility alleles for IgAN.

RESULTS

Genome-Wide Association Analysis

In total, 318,127 autosomal and X-chromosomal SNPs were typed in 914 samples that self-reportedly comprised 181 complete parent-child trios, 24 parent-child pairs, and 323 unrelated samples. Control genotypes for 5069 individuals from the 1958 British Birth Cohort and UK Blood Service that have been genotyped using the same platform were obtained for the 314,656 SNPs that were typed in both studies.

We next applied a series of quality controls to the data to ensure reliable population structure and genotyping. Principal components analysis identified 37 putative non-Europeans in the IgA nephropathy samples compared with the North West European, East Asian, South Asian, and West African reference populations (see Supplementary Figure S1). After quality control filtering of individuals with low genotype call-rates and non-European ancestry, and revision of family structures based on identity-by-state information, there were 187 affected children available for family-based association analysis and 244 cases with 4980 controls for case-control analysis. After removal of SNPs with \( < 95\% \) call rates, a minimum allele frequency (MAF) \( < 5\% \), or major deviation from Hardy-Weinberg equilibrium (\( P \) value \( < 1 \times 10^{-6} \) in controls), a total of 286,200 SNPs were available for analysis in both the family-based and case-control data; 3 SNPs that showed extreme heterogeneity of association (\( P < 1 \times 10^{-6} \)) in the two studies were eliminated from further analysis.

In the family-based study, testing for linkage and association was performed using the program TRANSMIT; in the case-control study, logistic regression analysis was performed using Stata\textsuperscript{TM} (version 9.2). To maximize the power to detect association signals, we also combined the results from the family-based and case-control samples using meta-analysis techniques. The distribution of the association statistics (asymptotically a one degree of freedom, \( x^2 \) distribution) showed little evidence of overdispersion (Supplementary Figure S2, left panel) with a genomic control parameter \( \lambda \) equal to 1.02; this indicates that there was no evidence of population stratification (admixture) or cryptic relatedness or other sources of systematic bias that might inflate the type 1 (false positive) error. The \( \lambda \) value for the family-based statistics alone was 1.02 and for the case-control statistics was 1.02, which confirms that the type 1 error was tightly controlled in both arms of the study.

The genome-wide association plot of the association statistics for the combined data are shown in Figure 1. There is a strong signal of association on chromosome 6p defined by a group of strongly associated SNPs (minimum \( P \) value \( = 1 \times 10^{-9} \)). The most strongly associated SNPs in this group, rs3115573 and rs3130315, showed clear and consistent evidence for association in both the family-based and case-control data (Table 1). These two SNPs were in absolute linkage disequilibrium, and the small differences in association detected by each SNP were explained by minor differences in genotype call-rates (Supplementary Table S1).

A conditional association analysis of the combined family-based and case-control data was performed to search for signals secondary to that detected by the lead SNP (rs3115573) across the 997 SNPs that mapped to the greater MHC region.
on chromosome 6p (29.0 to 33.4 million bp). Three SNPs (rs13209234, rs11244, and rs2857106) showed significant conditional associations ($P < 0.05$ after correction for multiple testing); these SNPs showed very little linkage disequilibrium with the lead SNP ($r^2 \approx 0.02$) or with each other ($0.03 \leq r^2 \leq 0.32$). These results suggest that multiple discrete loci conferring susceptibility to IgAN map to the greater MHC region.

Further review of the genome-wide analysis showed no evidence of statistically significant association outside the MHC. Supplementary Table S2 lists the association statistics for the combined datasets at 72 loci, which were selected because of previous reported associations in case control studies or because of plausible candidacy based on current understanding of the pathogenesis of IgAN. None of these statistics was close to the significance of the signal at HLA.

Post hoc power calculations suggested that the study was powered ($\geq 80\%$) to detect common susceptibility alleles ($\text{MAF} \geq 0.2$) with genome-wide levels of statistical significance ($P < 5 \times 10^{-8}$) that increase risk by at least 70\% (i.e., odds ratio $\geq 1.7$) and low-frequency alleles ($\text{MAF} \geq 0.05$) that increase risk by at least 120\% (i.e., odds ratio $\geq 2.2$).

**Table 1. Genotypic and statistical data for SNPs on chromosome 6p that were most strongly associated with IgAN**

<table>
<thead>
<tr>
<th>SNP</th>
<th>Location (bp)</th>
<th>Alleles Major/Minor</th>
<th>MAF</th>
<th>Family Data (186 Nuclear Families)</th>
<th>Case-Control Data (244 Cases; 4980 Controls)</th>
<th>Family + Case-Control Meta-Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OR (95% CI) $P$ Value</td>
<td>OR (95% CI) $P$ Value</td>
<td>OR (95% CI) $P$ Value</td>
</tr>
<tr>
<td>rs3115573</td>
<td>32,326,821</td>
<td>A/G</td>
<td>0.45</td>
<td>1.88 (1.37 to 2.57) $6 \times 10^{-5}$</td>
<td>1.55 (1.29 to 1.85) $2 \times 10^{-6}$</td>
<td>1.62 (1.39 to 1.90) $1 \times 10^{-9}$</td>
</tr>
<tr>
<td>rs3130315</td>
<td>32,328,663</td>
<td>G/A</td>
<td>0.45</td>
<td>1.87 (1.37 to 2.56) $7 \times 10^{-5}$</td>
<td>1.55 (1.29 to 1.86) $2 \times 10^{-6}$</td>
<td>1.62 (1.39 to 1.90) $1 \times 10^{-9}$</td>
</tr>
</tbody>
</table>

**HLA Imputation Analysis**

Multiple SNPs were associated with IgAN across the MHC region with the strongest support ($P < 10^{-8}$) in the interval 31.7 to 32.5 million bp (Figure 2). To explore the chromosome 6p association signal further, we applied HLA imputation techniques to infer SNP association results in terms of classic HLA alleles, pooling independent (i.e., founder) haplotypes inferred in families with those inferred in cases and controls. Imputed counts of HLA alleles for HLA-A, HLA-B, HLA-C, DQ-A, DQ-B, and DR-B in cases and controls are shown in Supplementary Table S3. Analysis of the individual HLA loci showed strong association at HLA-B ($P < 10^{-8}$), HLA-DRB ($P < 10^{-9}$), HLA-DQA ($P < 10^{-8}$), and HLA-DQB ($P < 10^{-9}$) (Figure 2; Supplementary Table S3). There is strong linkage disequilibrium between these loci, so a series of simultaneous analyses of the six HLA loci was performed (Table 2). Models 1 to 6 show that the effects of dropping a single HLA locus are similar, consistent with individual effects being masked by strong linkage disequilibrium between the loci. Analysis of nested models suggested that the strongest association was detected by a combination of DRB, DQ, and HLA-B loci (models 7 to 9; Table 2) However, the strongest association...
was with HLA-DQ, and imputation of alleles at this locus showed significant positive association with DQB1*0501 (odds ratio [OR] = 1.51; 95% confidence interval [CI], 1.14 to 2.01) and negative association with DQB1*0201 (OR = 0.66; 95% CI, 0.5 to 0.87; Table 3).

DISCUSSION

Here we report the results of the first genome-wide association study of IgAN. The study encompassed both family-based and case-control methodology, using affected individuals and families ascertained from the UK Glomerulonephritis DNA Bank. The diagnosis of IgAN was supported in every case by renal biopsy. Rigorous genetic criteria were used to remove a small number of individuals from the analysis who, despite ascertainment as white Europeans, most likely contained non-European genetic admixture; analysis of the association statistics following these exclusions showed no evidence of population stratification.

A strong SNP association signal was observed in both the family-based and case-control studies in the MHC, demonstrating the existence of a reproducible, statistically robust association of IgAN with HLA loci in the UK white population. Imputation techniques were used to infer classic HLA alleles, which showed that association signals were observed across the HLA-B, DRB1, DQA, and DQB loci.

Previous studies of HLA association with IgAN in Europeans have sometimes, but not always, identified associations with the HLA class II genes but not consistently with any allele. Both the family-based and the case-control components of this analysis were larger than any previous study in Europeans, and both components showed very similar and highly significant associations with HLA genes; the strongest signals have sometimes, but not always, identified associations of this analysis were larger than any previous study in Europeans, and both components showed very similar and highly significant associations with HLA genes; the strongest signals were observed at the HLA-DQ locus. An association of IgAN with HLA-DR4 and HLA-DQ4, which showed that association signals were observed across the HLA-B, DRB1, DQA, and DQB loci.

An association of IgAN with HLA-DR4 and HLA-DQ4, which are in strong linkage equilibrium, has been consistently identified in Japanese cohorts using both serologic and molecular techniques.

Table 3. Association analysis of HLA-DQB imputed alleles

<table>
<thead>
<tr>
<th>HLA-DQB</th>
<th>OR</th>
<th>Linearized</th>
<th>SE</th>
<th>t</th>
<th>P &gt; t</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>*0201</td>
<td>0.66</td>
<td>0.09</td>
<td>−2.89</td>
<td>0.004</td>
<td>0.50 to 0.87</td>
<td></td>
</tr>
<tr>
<td>*0301</td>
<td>1.26</td>
<td>0.17</td>
<td>1.69</td>
<td>0.091</td>
<td>0.96 to 1.65</td>
<td></td>
</tr>
<tr>
<td>*0303</td>
<td>0.85</td>
<td>0.18</td>
<td>−0.76</td>
<td>0.448</td>
<td>0.55 to 1.30</td>
<td></td>
</tr>
<tr>
<td>*0402</td>
<td>0.59</td>
<td>0.20</td>
<td>−1.56</td>
<td>0.118</td>
<td>0.30 to 1.15</td>
<td></td>
</tr>
<tr>
<td>*0501</td>
<td>1.51</td>
<td>0.22</td>
<td>2.86</td>
<td>0.004</td>
<td>1.14 to 2.01</td>
<td></td>
</tr>
<tr>
<td>*0502</td>
<td>0.59</td>
<td>0.17</td>
<td>−1.86</td>
<td>0.062</td>
<td>0.33 to 1.03</td>
<td></td>
</tr>
<tr>
<td>*0503</td>
<td>1.30</td>
<td>0.30</td>
<td>1.13</td>
<td>0.257</td>
<td>0.83 to 2.02</td>
<td></td>
</tr>
<tr>
<td>*0602</td>
<td>0.80</td>
<td>0.13</td>
<td>−1.41</td>
<td>0.157</td>
<td>0.58 to 1.09</td>
<td></td>
</tr>
<tr>
<td>*0603</td>
<td>0.91</td>
<td>0.18</td>
<td>−0.5</td>
<td>0.614</td>
<td>0.62 to 1.33</td>
<td></td>
</tr>
<tr>
<td>*0604</td>
<td>0.88</td>
<td>0.16</td>
<td>−0.73</td>
<td>0.468</td>
<td>0.62 to 1.25</td>
<td></td>
</tr>
</tbody>
</table>

Reference allele = *0302.

The results are derived from a series of logistic regression analyses comparing a general model that includes all six classic HLA loci with nested models to show the effects of dropping one or more loci. Haplotypes for classic HLA loci were imputed (predicted) using chromosome 6p SNP genotype data. df1 and df2 denote the numerator and denominator degrees of freedom associated with the F-statistic comparing general and nested model.
ular typing strategies\textsuperscript{26,27} (for review see reference\textsuperscript{28}). Furthermore, a more recent SNP-based study of IgAN in the Japanese population identified an association with the DRA locus that is in strong linkage disequilibrium with DQ, but DQ loci were not directly probed by any of the SNPs used.\textsuperscript{29} There are differences in the prevalence and gender distribution of IgAN in East Asians and Europeans; IgAN is more common in East Asians in whom it occurs equally in males and females, whereas it occurs predominantly in males in Europeans. Despite these differences, our study suggests that similar loci within the HLA region may predispose to IgAN in both populations. Further studies will be required to test this directly and to determine the molecular basis of predisposition.

Conditional analysis of the SNP associations across the greater MHC in our study suggested the presence of more than one susceptibility locus within this region. The two most strongly associated SNPs, rs3115573 and rs3130315, map within 1.6 kb of each other, \textasciitilde27 kb upstream of the \textit{neurogenic locus notch homolog protein 4} gene. Of the three secondary signals, rs13209234 maps to an intergenic interval 3.1 kb downstream of HLA-DRA and 69 kb downstream of HLA-DRB, rs11244 maps to the 3’ UTR of HLA-DQB, and rs2857106 maps to an intergenic interval 2.7 kb upstream of HLA-DQB and 2 kb downstream of the TAP2 gene encoding antigen peptide transporter 2. Further studies will be required to determine the mechanisms underlying these associations.

Survey of the association statistics across the genome (Figure 1) did not reveal any other association of comparable strength to that in the MHC. In keeping with this, the excess of $\chi^2$ association statistics $>10.8$ ($P < 0.001$) in the $\chi^2$ distribution plot can be seen to be almost entirely caused by the chromosome 6p association signal (Supplementary Figure S2, right panel). This indicates that, despite reported evidence of larger gene-specific risks at a number of loci in smaller candidate gene studies, at least in the UK population, any common susceptibility haplotypes at extra-HLA loci are likely to have substantially smaller effect sizes. We did not observe significant associations across any of the IgAN loci identified in linkage analyses of multiplex pedigrees, suggesting that, consistent with evidence of heterogeneity in these studies, linkage does not arise from co-inheritance of a common susceptibility haplotype but more likely discrete predisposing genotypes that are specific to certain of these pedigrees. None of the loci selected on the basis of putative mechanistic involvement in the pathogenesis of IgAN or previously reported genetic association (Supplementary Table S2) manifested an association statistic that was significant, the smallest values being of the order of $P = 10^{-3}$, well below that at the HLA locus ($P = 10^{-7}$). Although the size of the study places limits on the ability to detect or exclude small associations, we found little support for association at loci that have been reported in previous studies of IgAN. Although the Hap300 BeadChip provides good coverage of common genetic variation (75\% coverage at $r^2 \geq 0.8$ evaluated in HapMap2),\textsuperscript{30} it is inevitable that some disease-associated variants were neither genotyped nor reliably tagged in this study. In the future, meta-analyses of combined datasets including ours, and further genotyping of this and other large collections will aid to confirm our conclusions of lack of strong association with IgAN at these loci.

Overall, our data provided clear evidence for the association of IgAN with genes at the HLA locus but not other regions of the genome. The full set of association results, and the genotype dataset, available at the European Genome-phenome Archive www.ebi.ac.uk/ega, should be of use in combination with future studies to define the existence or otherwise of extra-HLA common susceptibility loci and to analyze the HLA association in greater detail.

### CONCISE METHODS

This study was conducted in accordance with the Declaration of Helsinki and approved by a UK multicenter research ethics committee (MREC) and local research ethics committees. Informed consent was obtained from all participants.

### Study Design, Patients, and Controls

The MRC/Kidney Research UK National DNA Bank for Glomerulonephritis established collections in five common glomerular diseases, of which one is IgAN. Participants of presumed European ancestry were recruited through four UK centers (Glasgow, Leicester, London, and Oxford) through probands with renal biopsy-proven IgAN <50 years of age at the time of diagnosis and >18 years of age at the time of recruitment; gender distribution and recruitment by center are shown in Supplementary Table S4. Individuals with evidence of liver disease or Henoch-Schönlein purpura were excluded. Diagnosis was confirmed in all cases by direct review of renal biopsy histopathology reports and clinical case records. DNA was extracted from venous blood samples by standard methods.

Where available, samples were also collected from the parents of affected individuals and, in some cases, other nuclear family members, to enable a study of family-based association. Individuals for whom suitable family DNA samples could not be obtained were entered into a separate case-control analysis, using control genotypes for individuals from the 1958 British Birth Cohort and UK Blood Service that have been genotyped using the same platform. The cases used in the family-based and case-control studies did not overlap; the two studies were analyzed separately, after which the data were combined by meta-analysis.

### Genotyping

Nine hundred and fourteen individuals were genotyped for 318,127 autosomal and X-chromosomal SNPs with the Illumina Sentrix HumanHap300 BeadChip\textsuperscript{31}; genotypes were automatically called using the proprietary BeadStudio software. This chip is composed of a panel of tagging SNPs based on the phase I HapMap analysis (www.HapMap.org). Control genotypes for 5069 individuals (from the 1958 British Birth Cohort and UK Blood Service) were determined using a custom Illumina 1.2M Beadchip (www.wtccc.org.uk/ccc2/); genotypes were automatically called using the Illuminus algorithm.\textsuperscript{32} This chip in-
cludes 314,656 SNPs that are available on the HumanHap300 BeadChip.

Statistical Analysis
SNPs with genotype call-rates <95% and samples with call-rates <95% were eliminated from the analysis. Incompatibilities between recorded gender and that inferred from X-chromosome genotype data were resolved in favor of the genotype data. Family relationships were confirmed or revised based on the results of an identity-by-state (IBS) analysis. An ancestry analysis was carried out using the EIGENSTRAT2.0 software using HapMap data (CEU, YRI, GIH, JPT, and CHB) as representatives of West European, West African, South Asian, and East Asian populations to infer ancestry informative principal components, which were projected onto the case and control samples. Putative non-European samples were flagged as "outliers" and eliminated from subsequent analyses.

Autosomal SNP Association Analysis
A family-based test of linkage and association was performed using the computer program TRANSMIT. TRANSMIT calculates a score-test statistic that omits terms that are sensitive to population stratification (admixture) and incorporates nuclear families with incomplete genotype data and multiple affected offspring. Estimates of the genetic effect size (per-allele odds ratio and the SE of this estimate) were calculated from the estimated transmission proportion of alleles to affected offspring and the equivalent number of informative transmissions. Case-control data were analyzed using the logistic regression function in Stata, with indicator variables defined to model a multiplicative genetic risk model (which models additive genetic effects on the log risk scale). Finally, the estimates of the per-allele odds ratio from the family-based (TRANSMIT) and case-control (logistic regression) analyses were combined in a fixed-effects meta-analysis using inverse-variance weighting with standard formulae.

X-Linked SNP Association Analysis
Because TRANSMIT does not compute appropriate statistics for X-linked SNP data, the family-based and case-control X-linked SNP data were pooled for logistic regression analysis with indicator variables defined to model a multiplicative genetic risk model, with an assumption of common male-hemizygote and female-homozygote risks. Robust sandwich estimation of the variance was used to model nuclear family level clustering of genotypes; this approach has previously been shown to efficiently absorb variance inflation (overdispersion) caused by relatedness.

Hardy-Weinberg equilibrium was tested in unrelated controls using an exact algorithm implemented in the Stata procedure genhwi. X-linked SNP data in trios and cases and controls were pooled and analyzed with logistic regression models with indicator variables defined to model a multiplicative genetic risk model and assuming common male-hemizygote and female-homozygote risks. Estimates of the per-allele odds ratio from family-based and case-control analyses were combined in a fixed-effects meta-analysis using inverse-variance weighting with standard formulae. Variance inflation (overdispersion) of the test statistics, which under the null hypothesis are asymptotically distributed as $\chi^2$ statistics with 1 degree of freedom, was assessed by inspection of quantile-quantile plots and by calculation of a genomic control parameter $\lambda = \min\{1,\text{median}(\chi^2)/0.456\}$.

HLA Imputation
Genotypes for a set of 999 SNPs spanning the greater MHC region on chromosome 6p (29.0 to 33.4 million bp) were extracted for HLA imputation. Initially, missing SNP genotypes were imputed and SNP haplotypes were phased using the Beagle program; this method can simultaneously accommodate both family-based and unrelated data. HLA prediction was performed on each haplotype output from Beagle, with 30 CEPH North West European (CEU) families from the HapMap (www.HapMap.org) used as training data. Subsets of SNPs were chosen for prediction based on cross-validation performance within the training data. Prediction accuracy within the training data were 94.86, 91.62, 96.63, 92.49, 95.56, and 98.87%, using 20, 21, 13, 51, 15, and 40 SNPs for HLA-A, HLA-B, HLA-C, DRB, DQA, and DQB, respectively. We used these same SNPs to predict HLA types in our data, with the predicted alleles being the ones with maximum posterior probability. For families, four founder haplotypes, two corresponding to the transmitted (to the affected child) and two corresponding to the untransmitted haplotypes, were recorded. For unrelated cases and controls, two independent haplotypes were recorded for each individual.

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DISCLOSURES
None.

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Supplemental information for this article is available online at www.jasn.org.