# New SNP Loci Found

**SLE** *from page 1*

More than 5,000 systemic lupus erythematosus (SLE) patients participated in the studies altogether, "but even that is probably not enough to get further details. … It really underscores the necessity for patients to be involved and for collaborative efforts of investigators," Dr. Craft said in an interview. Dr. Craft reported having no relevant conflicts of interest.

In two largest studies, investigators performed genome-wide association scans with hundreds of thousands of single nucleotide polymorphisms (SNPs) on DNA samples from SLE patients and controls with European ancestry in collections around the world. SNPs are DNA sequence variations that occur when a nucleotide in the genome sequence is altered.

In all four studies, each of the SNPs associated with SLE and located in or near candidate susceptibility genes increased the odds of developing the disease by 20%-70%.

Geoffrey Hom, Ph.D., of Genentech Inc., San Francisco, and his colleagues used 502,033 SNPs to identify genetic loci associated with SLE in 1,311 patients with the disease and 3,340 control subjects. They found multiple SNPs that were very significantly associated with SLE near the B lymphoid tyrosine kinase (BLK) gene and in an area that contains the integrin alpha M (ITGAM) and integrin alpha X (ITGAX) genes, none of which have previously been associated with susceptibility to SLE.

Samples from 793 SLE patients and 857 matched controls in Sweden confirmed the new findings (N. Engl. J. Med. 2008 Jan. 20 [Epub doi:10.1056/NEJM0a0707865]).

Dr. Hom and his coinvestigators also confirmed the existence of many significant SNPs in three previously established risk loci in the human leukocyte antigen (HLA) class II region and in genes that encode interferon regulatory factor 5 (IRF5) and signal transducer and activator of transcription 4 (STAT4).

One particular SNP allele close to BLK that was highly associated with SLE appeared to reduce the level of expression of BLK messenger RNA. Another candidate susceptibility gene called C8orf13, which has an unknown function, also lay close to this SNP and BLK. The same SNP allele was associated with higher expression of C8orf13 messenger RNA. But this SNP and other variants that are strongly associated with it did not modify known sites of transcription-factor binding in BLK and C8orf13.

"We speculate that altered protein levels of BLK might influence tolerance mechanisms in B cells, predisposing persons to systemic autoimmunity," wrote the researchers, many of whom are employees of Genentech or have conflicts of interest with other SLE drug makers.

One of the possible SNPs that could underlie the association within the ITGAM-ITGAX region was not associated with the messenger RNA levels of either ITGAM or ITGAX. "Although we cannot exclude ITGAX because of the strong linkage disequilibrium in the region that extends into its 5' region, we think that variants of ITGAM are driving the association," Dr. Hom and his associates wrote.

The ITGAM protein (also known as CD11b) combines with another molecule to mediate adhesion between cell types in the immune system and the adhesion of myeloid cells to the endothelium. In several models of autoimmunity (including SLE) in mice, ITGAM deficiency leads to enhanced disease progression and inflammation. Patients with active SLE also have been reported to have elevated ITGAM protein expression on neutrophils.

Dr. Chris T. Derk, director of the lupus center at Thomas Jefferson University Hospital, Philadelphia, said the identification of ITGAM as significantly associated with SLE susceptibility may be one of the more interesting discoveries because it may help researchers understand if atherosclerosis in SLE is unique or if it shares some of the same features of atherosclerotic heart disease.

"If that can be understood better, maybe we can treat the vascular aspect of lupus better," he commented in an interview. Dr. Derk said that he conducts research for Aspreva Pharmaceuticals, which is marketing and developing mycophenolate mofetil (CellCept) for autoimmune diseases.

In another genome-wide association scan reported by the International Consortium for SLE Genetics (SLEGEN) and other contributing investigators, 3 of the 317,501 SNPs used in the analysis were significantly associated with SLE in or near ITGAM in an analysis of 720 women with SLE and 2,337 controls, all of European ancestry. The investigators confirmed this finding by conducting a smaller analysis of 8,230 SNPs that had been identified in the first analysis, involving two additional groups totaling 3,671 women with and without SLE (Nat. Genet. 2008 Jan. 20 [Epub doi:10.1038/ng.81]).

In analyses involving the combined 6,728 women in all the groups, other individual SNPs that were significantly associated with SLE were located in the gene KIAA1542, near the gene IRF7, and in the gene PXK.

The SLEGEN researchers also confirmed previously reported links between SLE and specific SNPs in the HLA region and in the genes PTPN22, FCGR2A, and STAT4.

The ITGAM findings were independently confirmed in another study conducted by Swapan K. Nath, Ph.D., of the Oklahoma Medical Research Foundation, Oklahoma City, and colleagues. After locating ITGAM as a plausible candidate susceptibility gene for SLE, Dr. Nath found that 11 of 26 common SNPs in ITGAM were significantly associated with SLE in a set of 732 SLE patients and 747 unaffected control individuals with European ancestry. All 11 were confirmed in another set of 1,184 patients with and 1,155 patients without SLE.

Altogether, 33% of the samples of patients and controls in this study also were included in the study conducted by the SLEGEN consortium (Nat. Genet. 2008 Jan. 20 [Epub doi:10.1038/ng.71]).

One particular SNP within ITGAM seemed to explain the entire association between the 11 SNPs and the risk of SLE. This SNP also was significantly associated with SLE in two independent sets of SLE patients and control subjects of African descent. The investigators found that this SNP is "predicted to alter the structure and function of the ITGAM protein, making this sequence variant a plausible candidate for a causal polymorphism leading to the development of SLE."

Another study that searched for SNPs associated with SLE found nine within BANK1, which encodes a B-cell adaptor protein and is expressed primarily in CD19-positive B cells. In the study, Sergey V. Kozyrev, Ph.D., of Uppsala (Sweden) University and his coinvestigators genotyped 279 Swedish patients with SLE and 515 controls. After locating the nine specific SLE-associated SNPs, the researchers chose three that could lead to changes in the structure or function of the BANK1 protein. They were able to corroborate their original results for these SNPs after genotyping four additional sets of SLE cases and controls from Argentina, Germany, Italy, and Spain (Nat. Genet. 2008 Jan. 20 [Epub doi:10.1038/ng.79]).

"Further experiments are required to fully understand if, and how, BANK1 polymorphisms lead to B-cell hyperactivity, breakage of B-cell tolerance and production of autoantibodies," Dr. Kozyrev wrote. Two of his colleagues are employees of MerckSerono Inc., which performed and financed the SNP genotyping in the study. ■

**Genes Significantly Associated With Systemic Lupus Erythematosus Risk**

| Gene | Observed function |
|---|---|
| **New genes*** | |
| ITGAM | Aiding adhesion of leukocytes to each other and to endothelium |
| BLK | Activating B cells |
| BANK1 | Encoding B-cell adaptor protein |
| KIAA1542 | Encoding elongation factor necessary for ribosomal translation |
| PXK | Encoding a serine-threonine kinase of unknown function |
| C8orf13 | Unknown |
| **Confirmed Genes*** | |
| HLA region | Presenting antigens |
| STAT4 | Modulating production of cytokines in T cells and natural killer cells; mediator of macrophages' response to interferon-α |
| FCGR2A | Clearing immune complexes |
| IRF5 | Producing interferon-α |
| PTPN22 | Inhibiting lymphocyte activation |

* Genes that contain or are near single nucleotide polymorphisms significantly associated with SLE.
Sources: New England Journal of Medicine and Nature Genetics



# Salivary Gland Ultrasound Sensitive for Sjögren's Diagnosis

BY DENISE NAPOLI
*Assistant Editor*

Ultrasonography is a noninvasive, inexpensive technique for evaluating the salivary glands in patients with suspected Sjögren's syndrome, according to a recent study.

Other methods of assessing salivary gland involvement—sialography, for one, or scintigraphy of the salivary glands— are invasive and have low specificity, respectively, wrote the researchers.

Dr. Dirk Wernicke of the Clinic for Rheumatology Berlin-Buch, Berlin, and colleagues looked at 316 consecutive patients with rheumatic diseases. Overall, 57 had primary Sjögren's syndrome (SS); 33 had secondary SS due to the presence of other inflammatory diseases; 78 had sicca syndrome involving symptoms of dry mouth and eyes in the absence of other evidence of SS; and 148 were asymptomatic controls who had other inflammatory conditions, including rheumatoid arthritis, lupus, or undifferentiated connective tissue disease. In all groups, the majority was female, and the mean ages were 51, 53, 48, and 45, respectively (J. Rheumatol. 2008 Jan. 15 [Epub ahead of print]).

"In comparison with asymptomatic controls, the mean volume of the submandibular glands in the women with primary and secondary SS was reduced by 33% and 40%, respectively" on ultrasound, wrote the authors, from 4.8 mL in the controls to 3.2 mL and 2.9 mL in primary and secondary SS patients, respectively. There was also a significant difference in men with primary SS—the mean volume was reduced by 28%, compared with controls—but, likely due to the small number of men in the cohort, no significance was seen between controls and secondary SS.

The researchers also looked at parenchymal inhomogenicity. In this study, a diagnosis of SS was made when"at least two major salivary glands showed evident grade II parenchymal inhomogenicity," wrote the researchers. In an interview, Dr. Wernicke said grade II parenchymal inhomogenicity refers to "diffuse hypoechoic areolae larger than 2 mm." (Grade 0 is normal homogenous parenchyma, and grade I mild parenchymal inhomogenicity is seen on ultrasound as diffuse hypoechoic areolae smaller than 2 mm.) This yielded a specificity of 96.1% in sicca symptom patients and 100% in the asymptomatic controls. "SS was diagnosed in our cohort with a sensitivity of 63%" (in both primary and secondary groups), they added.

"The use of imaging techniques is very different in [various] countries," said Dr. Wernicke. In the U.S., he said, it is "so far, not widely used by rheumatologists. The experience is good in Germany and Italy, less in the U.K. and in North America." He believes ultrasound should be included in the diagnostic criteria of the disease.

Dr. Wernicke and his associates disclosed no conflicts of interest. ■

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