Personal factors

By Chris Cain, Senior Writer

A multi-institute team collaborating with the **FDA** has determined why patients with a form of severe hemophilia rarely develop neutralizing antibodies against factor VIII replacement therapies. The team is also developing an algorithm to predict the likelihood of antibody development against other recombinant protein–based therapies.[1] Based on the results, one of the investigators has cofounded **Haplomics Inc.** to develop new factor VIII replacement therapies.

Hemophilia A is caused by inherited alterations in the *F8* gene that encodes factor VIII, a critical component of the coagulation cascade. Standard of care for the disease is treatment with plasma-derived or recombinant factor VIII protein.

At least eight companies market factor VIII replacement products.

Because these products are given to patients whose normal factor VIII is mutated, the immune system can deem the replacement protein a foreign antigen and produce neutralizing antibodies against it.

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—Vincent LaTerza, Haplomics Inc.

These neutralizing antibodies, commonly known as inhibitors, develop less frequently in patients with *F8* missense mutations, who have mild to moderate forms of the disease. The inhibitors develop more frequently in patients with large deletions in *F8*, who have severe forms of the disease, because their immune systems are not tolerized to the normal form of the protein.

A long-standing question has been why development of inhibitors is infrequent in a subset of patients with severe disease—those carrying an inversion in intron 22 (I22) of *F8*. Like patients with *F8* deletions, these patients lack antigenically cross-reactive material in their plasma. But only about 20% of patients with the inversion develop inhibitors, whereas up to 88% of patients with deletions develop inhibitors.

To investigate this discrepancy, a team led by Tom Howard, director of the Hemostasis Laboratory and co-director of the Hemostasis and Transfusion Medicine Consult Service at the **VA Greater Los Angeles Healthcare System**, and Zuben Sauna, a visiting scientist at the Division of Hematology in the FDA's Center for Biologics Evaluation and Research, set out to characterize *F8* and factor VIII in these patients in greater detail.

Howard is also director of the pharmacogenetics section of the VA's Molecular Pathology Laboratory and an associate professor of pathology and laboratory medicine at the **Keck School of Medicine of the University of Southern California**.

RT-PCR and protein expression analysis of cell lines derived from a patient with the I22 inversion showed that the entire protein was produced within cells as 2 separate polypeptides, 1 comprising the first 2,143 amino acids of the protein and 1 comprising the last 16 amino acids.

This meant that although factor VIII was not functional in these patients, the full-length sequence of the protein was still being produced. The researchers used immunohistochemistry to confirm the expression of these factor VIII polypeptides in numerous cell types and showed that the lack of factor VIII function was likely because the peptides could not be secreted by cells.

RT-PCR of blood samples from 25 additional patients found that both factor VIII polypeptides were produced in all cases.

Valder Arruda, an associate professor of pediatrics at the **Perelman School of Medicine at the University of Pennsylvania** and **The Children's Hospital of Philadelphia**, told *SciBX* that these results clarified a long-standing question in the hemophilia community.

"This was a puzzle for the field until this paper came out. We have called patients cross-reactive material [CRM] positive or CRM negative based on an analysis of plasma from patients. What this group is saying is that in people with this I22 inversion, they are CRM negative in the plasma but CRM positive intracellularly, and their immune system does see pieces of factor VIII," he said.

Having established a rational explanation for why these patients often do not develop inhibitors, the research team next set out to better understand why some I22 inversion carriers develop inhibitors and others do not.

First, the group sequenced *F8* in the patients with I22 to identify regions of potential mismatch between each patient and factor VIII replacement therapy. They expected that some of this mismatch would be due to differences between the replacement protein and the junction region that normally bridges the two halves of the separate factor VIII polypeptides made in these patients.

An additional source of mismatch would be nonsynonymous polymorphisms within the *F8* gene found in different ethnic populations, which carry slightly different haplotypes of the gene.

The team identified all overlapping 15-mer peptides that include these mismatched positions—this is the size of peptides that are presented by major histocompatibility complex class II (MHCII) proteins. This information was combined with genotyping of each individual's *MHCII DR β1* (*HLA-DRB1*) locus to predict foreign peptides with high affinity for *HLA-DRB1* that might induce an immune response.

The group found that, as expected, the number of high-affinity foreign peptide–HLA-DRB1 complexes could significantly predict inhibitor development and was more effective than simply counting the number of predicted foreign peptides alone. This suggests that polymorphisms within the genes could indeed affect the risk of inhibitor development.

Results were published in *Nature Medicine*. Howard was previously a visiting professor of hematology and oncology in the Department of Medicine at the **University of California, Los Angeles David Geffen School of Medicine**.

Niche populations

Howard and Sauna said that the findings could help explain why African-American patients are more likely to develop inhibitors than other ethnic groups.

In a 2009 study published in *The New England Journal of Medicine*, Howard led a team that showed African Americans more frequently carry an *F8* haplotype that is different from the haplotype used to generate recombinant factor VIII replacement products.[2] Moreover, the frequency of inhibitor development was higher in patients with those haplotypes.

In the *Nature Medicine* paper, an analysis of an independent cohort of 313 patients with known race, I22 status and inhibitor status showed a statistically significant increase in inhibitor development in African-American patients with the I22 inversion compared with European-American patients with I22.

Howard told *SciBX*, "These nonsynonymous *F8* polymorphisms, which occur much more frequently in African-American patients, represent additional determinants of immunogenicity risk that may explain why the prevalence of antidrug antibodies in this population is twice that observed in European Americans."

In 2004, Howard cofounded Haplomics based on early findings related to this work.

"What distinguishes our approach is that we are examining what factor VIII protein material the patient is making internally before making a decision as to which product is optimal for the patient," Haplomics cofounder and CEO Vincent LaTerza told *SciBX*. "We want to develop a predictive, diagnostic tool that would facilitate the treater's ability to make more informed choices about factor VIII replacement products. Ideally, factor VIII products that closely match what factor VIII the patient expresses—for example, their haplotype—could be administered to as many patients as possible."

LaTerza acknowledged that the market is a subset of an already small patient population but said that the business case can be made given the high cost required to treat patients with hemophilia who develop anti–factor VIII antibodies. "In our view, failing to take into account what factor VIII material a patient will likely tolerate is a major missed opportunity for treaters and, more importantly, for hemophilia A patients at risk for inhibitors," he said.

Glenn Pierce, SVP of global medical affairs and CMO of **Biogen Idec Inc.**'s hemophilia therapeutic area, told *SciBX* that he wants to see more clinical data to back up the utility of the predictive algorithm.

"The numbers of people with I22 inversion and the specific haplotype variants discussed in this paper are very small. In order to test the hypotheses generated, it would be important to conduct a clinical trial just in these patients and particularly in a subset of them who had never been treated with a factor VIII therapy, if feasible. Until that kind of work can be done, it would be difficult to know if the algorithm developed could have significant clinical utility. However, the algorithm shows initial promise and indicates a variety of directions for further research," he said.

Arruda agreed. "While there is an association between haplotype and risk of inhibitor development, it has not been proven that it is due to the haplotype. To demonstrate that, you have to show that patients with inhibitors have T cell epitopes with haplotype-specific amino acids," he said.

Pierce was a coauthor on the *Nature Medicine* study. Biogen's long-acting Eloctate rFVIIIFc has completed Phase III testing and is under FDA review. The company is also collaborating with multiple not-for-profit organizations including the **National Hemophilia Foundation** to provide financial and scientific support for genotyping patients with hemophilia A and B.

Howard and Sauna both agreed that the key next step for their pharmacogenomic analysis is to examine whether the peptide predictions hold up in a clinical trial.

"We hope to establish clinical collaborations to identify actual peptides presented by major histocompatibility complex proteins of hemophilia A patients with the I22 inversion," said Sauna. "If the peptide sequences are those that would be predicted based on the current work, this will support our hypothesis. This, in turn, may lead to development of new strategies for products to minimize the risk from immunological reactions for at-risk subpopulations or ethnicities."

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>
> ***—Zuben Sauna, Food and Drug Administration***

Broad applications

Sauna thinks that the team's approach has applications outside of hemophilia A. "Numerous bioengineered proteins are entering the drug development pipeline. Such engineered proteins always have neoepitopes that are not found in nature. These can sometimes trigger immune responses. We are currently applying our methods to understand whether neoepitopes generated during the design of clotting factors may cause them to be immunogenic," he said.

"If we can validate our approaches, at-risk individuals could potentially be identified in early stages of drug development, opening the door to clinical trials based on pharmacogenetic characteristics," he added. "This paradigm would ultimately facilitate the development of drugs by identifying and excluding the few individuals for whom the drug is unsuitable."

Søren Erik Bjørn, VP of hemophilia R&D at **Novo Nordisk A/S**, agreed that the algorithm is promising, contingent on further clinical validation.

"In principle, this approach could be generally applicable, not only to benefit the hemophilia population but also in other areas where you have peptide drugs prone to an immunogenic response. Clinical studies within hemophilia will be challenging due to the small number of patients available, and it needs to be validated in larger cohorts, like rheumatoid arthritis," he said.

He said that the algorithm is particularly interesting for its incorporation of information about the HLA genotype of patients and said that Novo Nordisk uses similar types of computational and *in vitro* analyses to assess the potential immunogenicity of the recombinant protein products it develops.

Novo Nordisk markets NovoSeven, a recombinant human coagulation factor VIIa, to treat patients with factor VII deficiency and those who have acquired hemophilia or factor VIII or factor IX

deficiency and who have developed inhibitors. In October, the FDA approved Novoeight turoctocog alfa recombinant factor VIII to treat hemophilia A patients with factor VIII deficiency.

Pierce and Bjørn both sounded notes of caution on whether there is sufficient evidence to engage in the development of personalized therapies for hemophilia A.

"Currently, we know that certain gene mutations carry increased risk of inhibitors, but we cannot predict with a high degree of accuracy who will develop an inhibitor and who will not. It is important to understand with more certainty who is likely to form an inhibitor, as well as develop new therapeutic approaches, before envisioning a time when clinicians could optimally guide treatment selections for their patients," said Pierce.

He added, "For this small subset of the hemophilia population, the contribution of haplotype risks remains unclear; it may be one of a number of factors that could potentially affect inhibitor risk. Larger NIH-sponsored clinical studies are ongoing to assess haplotype mismatch between patients and replacement therapies to determine if there is a higher risk for inhibitor development. Developing haplotype-specific treatments would be challenging given the current significant regulatory steps required for the development of all hemophilia therapies."

According to Bjørn, "There might be a need for several factor VIII molecules to significantly reduce inhibitor formation, and such development would be a huge task for any company. We should also look into other technologies and single products that more broadly prevent inhibitor development in patients with hemophilia."

VA Los Angeles has filed for patents covering the algorithm, and the IP is licensed to Haplomics.

Cain, C. *SciBX* 6(42); doi:10.1038/scibx.2013.1181
Published online Oct. 31, 2013

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