

Complement Factor H
29-Apr-06 - 1:00 pm - 4:30 pm

Rando Allikmets: Thank you very much, Paul, and it's a real pleasure to be here. I really want to thank Emily who included my presentation at the very last minute. She also changed the title of the presentation to a much simpler one that I appreciate from what I had suggested. As Greg already mentioned, this study is the result of three-way collaboration of like a three-headed monster of Columbia, like Dean at National Cancer Institute, and, of course, Greg, but pretty much spits out one major AMD gene per year, as we know now. And also I have to mention that only people from _____ helped us out with statistical analysis. We don't have any commercial relationship, which means that we're not only productive, but also we're all philanthropists. Now as you understand that there are policies of disclaimers and statements that has really gotten out of hand, so I thought I'd throw in one of my own, so if you see a smiley face, so don't really take it too seriously.

Now what is the genetic cause of AMD, and we all heard about that, so just quickly to summarize: We suggested, when I was still working in Mike's lab, that ABCR is the first gene involved in AMD, where we found that very rare variants, usually less than one percent, are associated with AMD in heterozygous carriers, and of course, you know, all what happened last year were common variants, in general, more than 10 percent frequency were associated with AMD, so this year we came up with another gene in the same alternative complement cascade where we have pretty much the same situation.

Before I go and talk about the factor B story, since this event is called a course, I thought I should give a few pointers, especially to younger colleagues, what should they do when they carry out their research? So those who know what happened with our ABCR story know that my first warning is that if you come up with an important discovery, don't assume that everybody will really greet that well. There can be major difficulties. In fact, from a personal perspective, every time I come to ARVO and enter the Convention Center, I remember the year of 1998 when I first came here and presented the ABCR story. So things have, of course, gotten a little better over the years, and I really am happy to show you the last piece of evidence that I think would put the discussion on ABCR story to rest.

When I was searching the CRISP database on AMD grants, so I came up with a grant that was funded last year that belongs to Dr. Stone from Iowa and I'm not going to talk about the grant, that makes no sense, but I really love the very specific aim that states they are using ABCR knockout mice as the tool, as the model for AMD. So that's a very strong statement. I would personally not make a statement as strong as that; however, if Ed says that, and if any eye study section agrees, who am I to argue this situation.

Well, moving ahead, and actually if I would have known that last year, I probably would have retired. Now I didn't, but in factor H things are much, much better and as you will see, and as you have seen, and you will see at the upcoming ARVO meeting pretty much everybody says that whatever we said is true. Now I think the same is also true for factor B and it should be pretty straightforward, just for the main reason that we already have typed and published the Iowa cohort.

Now you all heard what happened last year, that many groups discovered this major influence of the alternative complement cascade in CFH in AMD, so we thought that, and we were always going from biological perspective, we thought that if you have found something like that, you don't really want to steer off the course and we presented with the hypothesis that probably other genes and proteins entered the complement cascade, especially those that regulated also have a role in modulating the disease, and we came up with factor B during those studies.

Now here is just a summary of what we had found. We had found many variants in factor B and also in complement component 2 genes. They are adjacent genes just next to each other on human chromosome 6; therefore, they are in very good linkage disequilibrium. And here you can see that two pairs, one is in blue and the other is in white; one SNP is in C2, the other is in factor B, are really highly associated with AMD. In this case they are both protective as documented by chi-square, and P-values, and odds ratios, and confidence intervals. I didn't quite understand what Al mentioned here about not being that significant or minor, because I think that P-values of minus six to minus nine are pretty substantial. Of course, factor B does not have the same impact on the AMD as factor H does, but I think it's a pretty substantial part of the disease. Now to illustrate it more graphically, here are the two genes and human chromosome 6 right in the MHE locus, and therefore, the SNPs, there's linkage equilibrium, across all this region, there is a risk haplotype, as always; you know, if you find the protective, you also find the risk and vice versa. There is a risk haplotype that moderately increases ones risk to develop the disease, and then there are two major protective haplotypes that are really highly significant and reduce risk two, three, or four times of getting the disease.

Now Mike Dean, in his next talk will open up this figure and graph a little bit better. I just want to point out to the lower right hand corner this last figure here, so when we did the analysis of the two genes together, the factor H and factor B, and you can say factor BC2 locus, so we came up with the following numbers, and these are not estimates; these are actual physical numbers of screening a cohort, so 74 percent of all patients, and as you recall it was a large cohort, about a thousand patients, two cohorts joined together; 74 patients have a risk allele in either factor H or factor B, and 58 percent of controls have at least one protective allele in one of the two genes. So this is the major outcome of this, and Mike will explain this to you a little bit more how this was achieved.

Now there's always a question and here the question is that is this a true association or is it due to the MHC, multiple histocompatibility locus, because as we know, genes and markers in that locus are often associated with late-onset complex traits to a certain extent, so there are a few point that we want to make showing that it's really the FC2 haplotypes and not the MHC locus. There is modest linkage disequilibrium with this locus and the adjacent loci and the HapMap data suggests that there are high re-combination rates that flank the C2/BF locus. An interesting observation was here, that when we're looking at the genome scan that was talked about a lot today by Josephine Hoh's group, they did not find an association in that locus. Now why, since the Affymetrix 100k array has a pretty good coverage; it has eighty SNPs across the MHC locus, but the array did not have any of those SNPs that were in the C2/BF place, or locus. So this study really proves two things: first, why the association was not found,

and that it is not due to linkage disequilibrium with the rest of MHC. And I also want to point out a study that was done a year ago from Henry _____'s group that did find some protective association with some alleles in one and two loci; however the association was at a much lower level, so therefore, we also think that might be, that really the C2/BF is the culprit.

Okay, so is it C2 or BF? And you all heard today it's really difficult to say and, strictly speaking, we don't know. There are a couple of ideas why we think it's really BF and not C2, but genetically we can't really distinguish since the genes are right next to each other. The two points we want to make is that there are non-conservative and functional changes in BF, and one of the two changes, for example, we analyzed as a proven function, has a lower hemolytic activity; whereas the C2 SNPs are _____ and conservative. Again, this is not direct evidence. And the other evidence, when Greg showed you pictures of the proteins in drusen and so alternative complement cascade proteins are often found there where classical cascade proteins are not.

So the study came to three major conclusions. The first is that this study unequivocally nailed down the fact that the alternative complement cascade is at the bottom of the majority of AMD, because here we have an independent locus from the same pathway that is highly associated, and the proteins, of course, interact, by definition; so that's the first major conclusion. The second is, that if you analyze only these two genes, you can explain up to three out of four cases, what is really incredible for any complex trait. Of course, I'm not saying that analysis of the two genes only would be enough to explain three out of four, but pretty much that is what it does. And it also now allows diagnostic screening, because there's been a lot of discussion in terms of should we do it or should we not do it? Just the factor H data, it made no sense, now it makes absolute sense. And, of course, the third is it defines specific therapeutic target, as we all heard, the alternative complement cascade, the localized immune response, and inflammation, so it can really do early intervention on pretty much everybody, I think, in some time when we figure out how.

Now, so what is the very simplistically proposed disease mechanism? So inflammation is triggered, as Greg said. So, then, it persists if a person has a genetic susceptibility. So the alternative complement cascade has really evolved to efficiently fight the trigger, and it doesn't care what happens to your eyes, you know, when you're 60 and way past the reproductive age, so it's not under any evolutionary pressure. So that's, if you have susceptibility, you may have persistent inflammation, so that results in early AMD or drusen, as we all heard; and it may develop into late stages if other conditions are met; and several studies have pointed out that there are other conditions needed, although I haven't seen any direct evidence to that fact, because, I think, and Greg already mentioned, that it might not be the case; maybe just the alternative complement cascade proteins are enough for one to develop an AMD.

So I want to finish my talk today with a couple of notions. I mean, there was quite a bit and very substantial discussion of what the methods are that one uses to dissect the complex trait, and I really don't have to go into this in greater detail. I just wanted to throw in a word in defense of the Candidate Gene-Based Case Control Association Study. And it's a very simple setup. I mean, this picture looks much simpler than what John Haines showed. You just collect, you know, two cohorts, and cases and controls, affected and unaffected. So you take a

gene, type a SNP, so depending on which way it falls, so it's either associated with the disease or it's protective. In this case, it's associated. The you pick another SNP and, you know, so you can modify the disease or not; then you throw in a couple of environmental factors, like smoking and sunlight exposure, or find the triggers, what really – so it's a very straightforward and simple thing. So, why then, it's often called a fishing expedition, so why nobody believes in that until recently we all didn't believe much at all. Well, let's look at the facts. So, this is, again, my opinion; this is not a scientific fact here, so I think there are five genes right now that are reliably associated with AMD, and here they are in historic order. Well, the fact is that we found three out of the five by the Candidate Gene-based Association Study, and the fourth gene was found by the same method. So, four out of five, I think, is a pretty good efficiency. So I would say it's a very good method, and why is it a good method? I could give you another hour of lecture why we think it is, but I think I'll finish it in layman's terms, that if you go fishing, you have to pick your fishing buddies who will show you where to cast the line.

Thank you. I'm leaving a minute short.

Q: In terms of targeted therapies toward the complement system, then, to treat early stages of the disease, since the complement system's involved in so many other tissues besides the eye, do you envision, sort of, a targeted therapy that would have to be specific into the eye, injected into the eye, or could you envision a systemic therapeutic approach?

Rando Allikmets: You really should have asked Greg that question when he was up here the last time, because he knows much more about this than I do. Well, the answer is probably both could be applicable, because targeted would be definitely better, and I can tell you several ideas that are maybe probably too invasive. You could try something in terms of gene therapy, but you can try something just to deliver by eyedrops to the eyes. So, if we would find out the way to do that, that would be ideal, because you can really go straight to the eye and treat the disease, especially when we want to do that as a preventative measure, and I think that that's a possibility. Another interesting notion is that if you control inflammation, even systemically, by taking, say, over the counter anti-inflammatories, there have been a couple of studies out there, although I probably really call them studies, but articles that say that if you'll, say, pop an aspirin or an ibuprofen every day for 10 years, you will not develop AMD. So, I don't know; it may happen, and maybe that's one way to go, so there's a humorous note. So I think both ways can work, so we'll have to figure out what is the exact one.

So everybody wants to go to the break.