Podcast 31: How Are Blood Groups Discovered?

Tony Casina:

Welcome to QuidelOrtho Science Bytes. We're proud to sponsor this podcast as a continuing commitment to transform the power of diagnostics into a healthier future for all. Today our topic is How Are Blood Groups Discovered? I am Tony Casina, and today I am joined by Dr. Jill Storry. Jill is a professor at the Division of Hematology and Transfusion Medicine, Department of Laboratory Medicine, Lund University, Sweden, and responsible for the immunohematology laboratories within the Department of Clinical Immunology and Transfusion Medicine, Lund. She's an AABB National Blood Foundation Scholar for her discovery of the genetic basis of the Vel blood group system, and her awards include the BBTS Margaret Kenwright, and Race and Sanger Awards, the AABB Sally Frank Award, and an ISBT Award for services to education. She has authored over 60 original papers, reviews, and textbooks, and given over 100 talks at international and national conferences and courses. She is a member of the editorial boards of Transfusion Medicine Reviews, Transfusion, and Immunohematology, and section editor for Vox Sanguinis. Thank you so much for joining us today, Jill.

Jill Storry:

Well, thanks, Tony. It's really a pleasure to be here. Yeah, I've been looking forward to doing this podcast for some time.

Tony Casina:

Great. Let's start with the basics. How many blood group systems and blood group antigens are there?

Jill Storry:

Well, as recently as two weeks ago, the ISBT Working Party for Red Cell Immunogenetics and Blood Group Terminology had its annual meeting, so I can proudly report that we added a blood group system at that meeting, and so we now have 44 blood group systems. There are 385 known blood group antigens, but the majority, 355 of that 385, are in those 44 blood group systems. As you probably can do the math, that leaves 30 blood group antigens with no homes, and those are divided into two series, one of low prevalence antigens and another of high prevalence antigens, and also a group of collections. And then recently, one of those collections was elevated to a blood group system. So, yes, it's a continuing process.

Tony Casina:

Great. Okay. So, what challenges have researchers encountered throughout the years in trying to link blood groups to certain blood group systems?

Jill Storry:

Well, that is a good question, because there've been a number of challenges. I think all our discovery in blood group systems or in natural science research is all based on what techniques you have, and the new generations of techniques bring with them new discoveries. In the early days of blood group discovery, then if you look at some of those papers, a lot of it's just looking at family studies and trying to connect it. For a blood group antigen to be called a blood group antigen, it must have a human antibody that defines it, so that it's been made in a patient or a pregnant woman. It must be shown to be inherited from one generation to the next, and be independent of the other blood group systems. So, there's quite a lot of work that goes into them. In the early days it was literally doing a lot of family

studies. In some of those early Vox Sanguinis papers or Transfusion papers, you can see these very elaborate tests between the different generations.

Now we can use a bit more modern techniques, but often the, well, I wouldn't say more recently, but often the challenge or one of the major challenges is that they can be very rare phenotypes. You may be looking at the absence of a high-frequency antigen or the presence of a low-frequency antigen. And so getting enough material can be difficult to show inheritance and to show, serologically, that these antibodies are not related to other blood group systems. So, it is challenging, but it's more puzzle work if anything else. I think I've always enjoyed puzzles myself and I think that that's really what attracts me to the whole field of trying to identify new blood group antigens, is seeing how you can piece them together.

Tony Casina:

Thank you. Since you have been involved and recognized in several publications for discovering new blood groups, what triggers the idea that a new blood group might be involved when you're doing serological work? Can you share how this process works?

Jill Storry:

We can use serological fingerprinting, I like to call it. So, you have your patient's plasma in front of you and it reacts with a whole panel and you've been able to, by testing the patient's red cells with antibodies to high-prevalence antigens, for instance, or by testing your library of rare cells, you haven't been able to find a match for that particular plasma. So, that can indicate that perhaps you have something new that you want to see where it's going. You can do some serological testing of different enzymes to see if you can give some indication as to what sort of protein it might be carried on and if maybe that fits a pattern that really does tie it into one of the existing blood group systems. So, at the serological level, that's probably the way to go. But as I see the way forward with new blood group systems, I see three main paths.

And the most common I would say is the discovery of the genetic basis of an already known antigen. And I can take from my own work the example of Vel, but also JR and LAN from about 10 years ago and then more recently PEL and MAM and the Er antigens, that collection I talked about. But even there there was some completely new antigens discovered when they made their discovery. And so often, as I mentioned, it's the techniques that make the difference. And you can have blood or you can have red cells, for instance, that are Vel-negative. Then so what did we do in that particular case? And this was true for JR and LAN as well. Well, we took DNA from people that we knew were Vel-negative and also from a couple of families that we had from northern Sweden, in our case.

And we took their DNA and then we tested them using a technique called a single nucleotide SNP array, which really looked at different single nucleotide polymorphisms across the entire genome. And what that means is that we tried to match their DNA for existing polymorphisms that were found. And we were able to identify, narrow our search really, not to a specific molecular basis, but to an area of a chromosome, in this case chromosome 1, and then narrow down our search by looking, "Okay, in that region where the SNP, this single nucleotide variant seems to be heavily represented in these people, what genes are present there?" And then once we'd narrowed that down to a couple of the 124 genes, then we asked the question, "Okay, which one of those genes encodes a protein on the red cell surface?"

Because we knew that it had to be on the red cell and we knew it had to be a membrane protein. So, then once you have a potential candidate, and you may have more than one candidate, then you can start sequencing that gene and seeing is there something in common in that gene to all the Vel

negatives, for instance? And we were really lucky in that particular case because we found a 17 base pair deletion that was common to all Vel-negatives, but sometimes you're not so lucky and you may identify the gene, but then it could be a giant gene with over 30 exons that you've got to sequence. Or it could be that there are many different molecular bases, JR and LAN are good examples of that, for instance, and you just have to methodically sequence through the gene. SNP array is a bit old fashioned now.

That was quite a long time ago. A lot of people have gone directly to something called whole exome sequencing or whole genome sequencing. So, these are techniques that fall under the umbrella of massively parallel sequencing where you can take DNA from a couple of people that lack in the high-preference antigen. You could take, for example, PEL, which was discovered by the French group in Paris, or MAM that was discovered by the Bristol group and our group together. And what the Bristol group did with MAM or the French group did with PEL is that they took this DNA and performed whole exome sequencing. So, in this case you take the person's DNA and then you're just using this massive parallel sequencing technique. You sequenced all the genes in the human genome looking for something that might stand out.

So, it's still needle in a haystack work, but with powerful computers to help you sort all the data you can often, again, narrow it down to one or two genes that you can then work on. That's one of the parts. So, you're looking for the genetic carrier of a already known antigen or known phenotype. And then the second path is perhaps when a lab finds a home for previously undescribed antigens. And I must say I've been so impressed with the French group in Paris recently as they're particularly good at this. And they recently described a blood group called CL2, which is actually the carrier molecule. And there they had in their freezer, they had plasma from five unrelated Moroccan women that contained antibodies to high-prevalence antigen and they were all mutually compatible. And then they're also compatible with red cells from a person of European descent.

But that lady's plasma was not compatible with their red cells. So, she reacted with their red cells. So, it suggested that, "Okay, we've got a shared protein perhaps, but there may be different molecular bases for that." So, again, they performed exome sequencing and identified CL2 in that case and were able to show that all the Moroccan women had the same mutation and the person from Europe had another mutation, that she in fact lacked the whole protein. So, it's really fitting them together piecemeal. And then the third category, discovering new antigens. I call emerging antigens and they can sneak into our blood group catalog from all sides. But one that I think is quite a good example is CD59, which is, as you know, it's a complement regulatory proteins. It's a very clinically important protein.

It's involved in a lot of different processes. But there was a young child who was identified to be CD59-deficient who required transfusion, and she produced antibodies to CD59, which then gave it the status of a blood group antigen because it fulfilled the criteria. It was antigen lacking from her cells. She had made antibodies to it and it was definitely an inherited phenotype. That was shown by testing DNA from her mom and mom and dad. So, yeah, you can really discover them from many different ways. The traditional one is having these antigens or having these plasma samples in your freezer and then finding a gene that actually encodes it. That's the discovery bit of it, I think. Seems quite a wordy way of describing it, but there are these three tracks.

Tony Casina:

Thank you, Jill. What role does demographics or geographic location play in these discoveries?

Jill Storry:

Well, I think sometimes it plays no role at all. You can find the phenotype distributed across the world. Again, I use Vel all the time, but I mean, that's because it's the one that's closest to my heart. But if we

look at that particular phenotype, being Vel-negative is more common where I am in Sweden. But there are examples of Vel-negative people all over the world, and more commonly in Europe and also Africa, very much less so in Asia. But they do occur, and that is a single molecular background. And I'll talk to you about CTL2. That was a case where you have the one antigen called RIF in the CTL blood group system. The RIF-negative phenotype is only found or has only been found in people from Morocco or from Northern Africa. So, there you have a very, very, very confined phenotype.

P1PK-negative phenotype again has certain pockets where it's found in the Amish group in Pennsylvania and around there. It can also be found here in northern Sweden. And also there's a mutation that seems to have a Pakistani restriction. So, the locale can have quite an important impact. And it can be just that it is a rare phenotype and perhaps people are living in a remote area and it's a small population that may be slightly more inbred, but not always. Not always. Sometimes it's just there may have been other factors for why it's popped up. And there are other blood group systems like JR. JR's quite interesting because it's carried on ABCG2, which is one of these big transporters on the red cell membrane. But there you have a couple of mutations that seem to be restricted to a particular demographic group.

For instance, in the Roma people in Europe, they have one particular mutation giving rise to the JRA-negative phenotype. In the Japanese it's another. But then there are more than 20 or so other variants that cause the JR phenotype that don't show any particular reason or group. It does mean though, I think practically for us in the lab, that if you want to screen for a particular blood group type, if it does belong to a particular group of people, then that's where you should be screening your blood donors and not wasting SirA or PCR or whatever you're using to screen.

Tony Casina:

Okay, thanks. What are the implications of discovering more and more blood groups? What does this mean for the patients receiving transfusions when these new blood groups happen to come up and be a problem?

Jill Storry:

Well, I think that the indications of discovering more blood groups, they're twofold. I think if you can discover the background to a rare sample that you've had in your freezer, it means that you then stand a much better chance of finding blood for that particular patient because you know what you're looking at. You know the gene. You know the mutation. If it is found in a particular demographic, you can search the donors from that area. But these are extremely rare phenotypes. So, perhaps you don't need a fridge full of blood of that type. But we know, for instance, from the MAM-negative phenotype where Nicole Thornton's group, have identified the molecular basis in 10 individuals in that paper.

There's been one more found. That means there's 11 people that we know with that MAM- negative phenotype, and we know of only one donor. And then that person happens to be a group B. So, you've got all the problems of not only finding blood for the person, but also the barriers of A, B, O that we constantly are reminded of each day. So, the implication is really that with more and more knowledge than we can perhaps help find donors for patients. And I mean, I'm just assuming here that all these antibodies are clinically significant, but in many cases they really are. We really do have to face the problem of, can we transfuse against an antibody because we don't have blood or can we screen and find them among our donors?

So, by understanding the basis or the carry behind the blood group phenotypes, it means that we can screen for more blood donors. It means we can have more in stock. And this has been shown to be very important in the long term when looking at the length of patients stay. Delayed blood to a patient obviously means that they have to stay in hospital longer. They may be discharged with a slightly lower

hemoglobin than is good for them because you can't find the blood. So, there's a lot of important clinical reasons for really taking the time to understand what our blood groups are all about.

Tony Casina:

Well, Jill, that's been quite informative. To close out our conversation, how can transfusion medicine laboratories stay up-to-date on the latest discoveries?

Jill Storry:

Well, I would love to say that's an easy thing. I think what we try to do with the Working Party for red cell genetics and blood group technology, we try to keep our webpage updated on the ISBT website. And it's all very manual work. So, I mean, it's not that it's always up-to-date straight after we've had a meeting. We also report our findings in a general report every two years. And I realize that that's not the most swift method of doing things, but we are trying to be better at that too. And I think the ISBT is really trying to put a lot more work into their own podcasts so that if we do have a discovery, maybe we can get the people that discover the blood group to describe it a bit more. And that's been one idea that's been thrown up.

But I think yes, the website is the best source for all the combined information. And there are tables on there that you can click on links to find, "Okay. Well, how many blood group systems do we have? How many antigens do they have?" and so on and so forth. And there's also people that you can contact if you really feel like you'd more information. Then of course PubMed is always good if you're the sort of person that has the time and energy to scan the PubMed website. Or even Google Scholar will give you some information. But the disadvantage of course for those sources is that you're not really getting a single collected source of information. So, I would follow the ISBT. The Working Party is the organ that assigns names and numbers to blood group systems and blood group antigens. And we in turn will try and be much better at keeping the information up-to-date.

Tony Casina:

Well, thank you. Could you share with us the most recent blood group system that you talked about that you discussed two weeks ago?

Jill Storry:

Absolutely. Yeah. The new blood group system is called Er, which I think will appeal to many people. And we elevated a collection, the Er collection, which was Era, Erb, and Er3, which I'm sure many people might recognize. And this was after Dr. Vanja Crew and her colleagues identified that these antigens were carried on a molecule called PIEZO1. And for those of you good at remembering who got the Nobel Prize last year, one of the two Nobel Prize winners for medicine actually won the Nobel Prize for his discovery of the function of PIEZO1 and PIEZO2 on cells of the body.

And so it makes it very relevant with some of these blood group discoveries that we are discovering important proteins. So, the blood group system's called Er. It's carried on PIEZO1. And in unraveling this blood group system, then Dr. Crew and her colleagues also identified two more antigens in the blood group system. So, it went from being a collection of three blood group antigens to a blood group system with five blood group antigens. So, very exciting work. And they did a tremendous amount of confirmatory testing to show that they genuinely knew what they were talking about. Very exciting.

Tony Casina:

Thanks so much, Jill, for sharing that. Very interesting. Jill, I really want to thank you for taking the time with us today and giving us your experiences and insights on this fascinating topic. It's been a pleasure to talk with you, Jill, and again, thank you so much for your time today on this podcast.

Jill Storry:

It's always a pleasure to talk to you too. Thank you.

Tony Casina:

It's been my pleasure too, as always. Thank you for listening and I hope you all enjoyed this episode of QuidelOrtho Science Bytes about how are blood groups discovered. Make sure to review the sessions within the podcast description for any reading materials that we've suggested. Based on today's podcast, I'll leave you with this pop quiz question. What might be a key indicator you are working with a new blood group? You can go back and listen again at any time. And please subscribe to QuidelOrtho Science Bytes, brought to you by QuidelOrtho Corporation, where we are transforming the power of diagnostics into a healthier future for all. Take care. Stay healthy and safe.