

Summary of the 2012 International Consensus Conference from DBAF's Research Director, Steven Ellis, PhD:



The 12th Diamond Blackfan Anemia International Consensus Conference was held March 17-19th in Battery Park in New York City. The conference began with a kickoff dinner held in a restaurant club room which was actually an old vault in a former bank in lower Manhattan. The intimate setting set the stage for a meeting known for its blend of physicians, scientists and other interested parties who freely exchange ideas in a highly collaborative environment. The keynote speaker at the dinner was Jeff Bond, father of four-year old Angus Bond, who was diagnosed with DBA at 8 weeks of age. Like many, Mr. Bond told of the initial anguish he and his wife Jessica had in trying to understand his son's diagnosis and how from this experience the seeds were sown for the Captain Courageous Foundation of Australia, one of a growing number of international foundations supporting research on Diamond Blackfan anemia. The Bonds were warmly welcomed into the broader DBA community.

Sunday, March 18th

Plenary Session "Assessment of iron loading in Diamond Blackfan anemia".

This session was particularly timely given recent deaths in the DBA community from complications of iron overload in transfusion dependent patients.

Dr. Thomas Coates (Los Angeles) gave the first of two talks on iron loading relying on his considerable experience with thalassemia patients. He described studies indicating that certain tissues, like the heart, take up iron by mechanisms different from that in the bone marrow and the liver, and the rates at which tissues accumulate iron also differ. **Consequently, a surrogate measure for tissue iron like serum ferritin is a totally unsatisfactory way to**

monitor iron content of different tissues. Instead, new imaging technologies are improving the way iron content in individual tissues can be monitored allowing much better assessment of the effectiveness of chelation therapies.

Adherence to treatment regimes continues to be a major problem in older patients. Effective iron monitoring and close cooperation between patients, parents, and physicians is essential for reducing mortality.

His talk was followed by Dr. Ellis Neufeld (Boston) who spoke on the strengths and weaknesses of current chelation therapies. He indicated that there is still a need for improved chemical chelation therapies. He provided some encouraging data on a new chelator currently undergoing early phases of testing.

[Note: the ICC also had a session where research was presented in a poster format. I will not discuss the posters separately, but instead describe them briefly if they complement one of the session talks. Posters will be presented in italic.]

Dr. Josu de la Fuente (London) reiterated themes raised by Drs. Coates and Neufeld in his poster on iron loading in the UK DBA population. He showed data indicating a number of patients had clinically significant iron overload even though their ferritin measurements indicated adequate iron chelation. Again, these data indicate the need for more effective means of monitoring tissue iron content in DBA patients receiving transfusion therapy.

Developmental Biology

This session focused on the timing of DBA presentation during mammalian development.

Dr. Lydie Da Costa (Paris) gave a talk on DBA presentation in a developing fetus. One of the more puzzling aspects of DBA is why it typically presents in the first year of life, or in other words, why it doesn't present in the developing fetus. Previously, published studies on fetal deaths thought to be as a result of DBA lacked a definitive genetic diagnosis. Dr. Da Costa's study provided the first documented evidence of a ribosomal protein mutation associated with signs of anemia in the developing fetus. These studies indicate that DBA should be considered in cases where there is evidence of fetal anemia, but

also raise the question of why many DBA patients escape anemia during fetal development and present after birth.

Dr. Johnson Liu (New York) spoke on work using mouse embryonic stem cell models of DBA comparing the differentiation of two cell lines each of which was mutated in a different mouse gene known to be affected in DBA. The results presented indicated that depending on the gene mutated there could be different effects on differentiation, providing a potential rationale for the heterogenous clinical presentation in humans affected in DBA. Trying to link clinical features of DBA with the underlying genetic defect has been a holy grail in DBA research. Dr. Liu's presentation gave a very nice segue into the session on genetics which followed.

Genetics

his session focused on gene discovery, new approaches to gene discovery in DBA, and the identification of genes that may modify the manner in which DBA presents and responds to therapy in different patients.

Dr. Hanna Gazda (Boston) began this session by describing the first DBA gene that does not encode a ribosomal protein. As has been this case in all my meeting reports, I am unable to go into too much detail on the talks given as unpublished work is frequently presented. Suffice it to say, that this discovery raised quite a stir and set the stage for large scale projects interrogating the entire genomes of DBA patients for possible causative genes. One of the questions after Dr. Gazda's talk was whether this new gene somehow links in to the ribosome defect in other DBA patients representing some type of biological continuum in disease pathophysiology or whether this represents a distinct subclass of DBA. The answer to these questions awaits further study.

Dr. Jason Farrar (Baltimore) presented his work on scanning the genomes of DBA patients for larger deletions containing genes whose loss would have been missed by traditional DNA sequencing technology. One of the more fascinating aspects of his study was finding that in some of these patients these deletions were found in mosaic cell populations where some of the cells

had the deletion and others not. This mosaicism appears to be linked to remission in certain DBA patients.

Dr. Adrianna Vlachos (New York) presented another outgrowth of the deletion studies described by Dr. Farrar. One of the deletions indicated that a patient originally diagnosed with classic DBA in fact had a more non-classical form of DBA that can be effectively managed with a novel treatment. These studies illustrated the power of defining the underlying genes affected in all DBA patients, pointing out that gene discovery is not simply an academic exercise.

Dr. Paula Quarello (Turin) presented work on gene deletions in the Italian DBA population supporting the view that gene deletions are a relatively frequent occurrence in DBA patients.

Dr. Elizabeth Chao (Aliso Viejo) presented work on the diagnostic testing Ambry Genetics has begun offering to DBA patients in its CLIA (Clinical Laboratory Improvement Amendments) lab. Ambry Genetics currently offers testing for 11 possible DBA genes in a cost effective manner.

Dr. Annarita Migliaccio (New York) presented work on the tremendous amount of structural variation in the glucocorticoid receptor in humans. Since this receptor mediates many of the biological and clinical effects of steroid hormones, their laboratory is currently investigating whether these sequence variants might somehow be linked to the different ways in which DBA patients respond to steroid therapy. Thus, it may be possible someday to be able to predict a patient's response to steroids depending on the nature of their glucocorticoid receptor gene.

This session ended with a talk by Dr. Marcin Wlodarski (Freiburg) who described their studies on the genetics of DBA in the German DBA Registry. He, like others described above, is using various whole genome interrogation methods to identify genes affected in DBA patients. Clearly, these are very exciting times in the gene discovery field.

Basic Mechanisms and Translation

This session explored the molecular mechanisms whereby defects in ribosome synthesis influence cell death signaling pathways and how these pathways may be targeted in developing more effective therapies for DBA.

Dr. Teng Teng (Cincinnati) presented work on how reducing expression of the DBA protein Rpl11 influences how cells divide. Previous studies have reported that Rpl11 plays a central role in p53 activation in response to ribosome stress. Therefore, it came as somewhat of a surprise when RPL11 was identified as a DBA gene. The studies reported here indicate that alternative mechanisms may come into play in cells depleted of Rpl11.

Dr. Alan Warren (Cambridge) discussed what he feels may be a more primordial form of ribosome stress signaling in *Drosophila* which he plans to exploit in a strategy to identify therapeutics that target elements of this pathway.

Dr. Akiko Shimamura (Seattle) presented a poster that suggested additional complexity in signaling pathways responding to ribosome stress.

Dr. Pierre-Emmanuel Gleizes (Toulouse) outlined his studies on a newly identified DBA gene which appears to regulate p53 activity through various mechanisms. As I'm sure the reader is by now aware, there appear to be numerous mechanisms operating in terms of signaling ribosome stress to cell growth and death signaling pathways. This should come as no surprise however, given the ubiquitous and fundamental role the ribosome plays in all cell types.

Dr. Fabrizio Loreni (Rome) presented studies on one of these additional signaling pathways that appears to integrate aspects of cellular energy metabolism with ribosome stress and how it may be relevant to DBA.

Nadia Danilova (Palo Alto) presented work on how small molecule effectors of energy metabolism pathways influence phenotypes in their zebrafish model of DBA.

Dr. Alison Taylor (Boston) backed into one of these alternative signaling pathways when she was screening chemicals that reversed a DBA-like phenotype in her zebrafish model of DBA. These studies have obvious implications for potential therapeutic development.

Dr. Lingbo Zhang (Boston) discussed his work on protein that is required for the ability of glucocorticoids to stimulate erythropoiesis. Identification of factors critical for the effects of glucocorticoids may ultimately lead to

alternative strategies to influence the activity of these factors with less toxic therapeutics.

Dr. Janice Abkowitz (Seattle) presented her work on the role of heme toxicity as a potential mediator of the erythroid selective nature of DBA clinical presentation. Her work is based on the DBA-like phenotypes in mouse where the heme exporter FLVCR is knocked out.

Two posters presented from the laboratory of Dr. Chetankumar Tailor linked ribosomal protein mutations to defective expression of FLVCR through an effect on RNA splicing. These studies again might relate to the continuum whereby defective ribosome synthesis can influence other cellular processes that ultimately give rise to the distinct clinical features of DBA.

Along these same lines, Dr. Marieke von Lindern (Rotterdam) presented her work on two genes that influence erythroid development whose expression is preferentially affected by ribosomal protein haploinsufficiency. Dr. Irma Dianzani (Novara) presented work on various transcriptional regulatory pathways that appear to be affected in cells expressing suboptimal amounts of ribosomal proteins.

Finally, Dr. Adrianna Vlachos (New York) closed out the session with her analysis of cancer incidence in the North American DBA Registry. While DBA appears to be a cancer predisposition syndrome, the cancer risk appears lower than with other bone marrow failure syndromes. While Dr. Vlachos' talk at first blush may appear out of context with all the other talks in this session on different signaling mechanisms, it is worth pointing out that many of the signaling pathways that appear to be involved in DBA pathophysiology also play important roles in tumorigenesis.

Monday, March 19th

Clinical Treatment and Drug Development

This session focused on translating basic science discoveries into more effective treatments for DBA.

Dr. Anu Narla (Boston) presented her work on the effects of leucine in reversing phenotypes in zebrafish and human cellular models of DBA.

Dr. Dagmar Pospislova (Olomouc) presented a poster on her encouraging work on the effects of leucine in the Czech DBA population and Pekka Jakko (Lund) presented a poster on the effects of leucine in his mouse model of DBA.

Ms. Sara Sjögren (Lund) presented two high throughput strategies for DBA therapeutic development currently underway in the laboratory of Dr. Johan Flygare.

Dr. Johnson Liu (New York) described a new clinical trial for adult patients with DBA evaluating a drug initially developed for osteoporosis which had the undesirable side effect (at least in non-anemic patient populations) of stimulating red cell production. The safety and efficacy of this drug will be evaluated in this study. This fascinating drug is also undergoing clinical trials to stimulate red cell production in patients receiving cancer chemotherapy.

The last scientific talk of the meeting was given by Dr. Carol Mercer (Cincinnati) who discussed her work on the effects of a p53 inhibitor in a mouse model of DBA.

Summary

This was another outstanding DBA conference! I hope you can tell from the foregoing discussion that I think we have really turned the corner on potentially identifying genes affected in all DBA patients, which in turn will dramatically influence our ability to correlate clinical features of the disease with underlying genetic changes. The basic science discoveries of the past few years also appear to be leading to many different and novel approaches to therapeutic development. We hope that the fruits of the endeavors reported here will soon lead to improving the lives of patients and families affected by DBA.

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