SickKids
THE HOSPITAL FOR
SICK CHILDREN

UNIVERSITY OF TORONTO
FACULTY OF MEDICINE

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Mr. and Mrs. Carmine and Judi Tullio
Nicola Kids' Triathlon

RE: Research Report

Dear Carmine and Judi,

On behalf of the Marrow Failure and Myelodysplasia Program at SickKids, SickKids Foundation and myself I would like to deeply thank you, your family and the many volunteers of Nicola Kids' Triathlon for the support of our research in bone marrow failure and myelodysplasia. Over the years, your support was critical for genetic and stem cell research in bone marrow failure at SickKids. Below is a short summary of several genetic projects that have been supported by your the funds.

Nicola Kid's Triathlon Funding Support to Marrow Failure and Myelodysplasia Research, at The Hospital for Sick Children, Toronto

Report for the study: Copy number variants underlying inherited bone marrow failure syndromes
Principle Investigator: Dr. Yigal Dror

I. Summary of Project 1: Bone marrow failure and developmental delay caused by mutations in poly(A)-specific ribonuclease.

Santhosh Dhanraj, Sethu Madhava Rao Gunja, Adam P. Deveau, Mikael Nissbeck, Boonchai Boonyawat, Andrew J. Coombs, Alessandra Renieri, Mafalda Mucciolo, Annabella Marozza, Sabrina Buoni, Lesley Turner, Hongbing Li, Ameer Jarrar, Mathura Sabanayagam, Melanie Kirby, Mary Shago, Dalila Pinto, Jason N. Berman, Stephen W. Scherer, Anders Virtanen, Yigal Dror (Published in the Jounral of Medical Genetics 2015)

Developmental delay is a common disability in children aged 0 to 4 years in Canada, with 1.1% experiencing some degree of developmental delay. In many cases the cause is unknown. We identified large deletions of one copy of a gene called *PARN* in four patients with developmental

delay or mental illness. In addition, we identified one patient in whom one copy of *PARN* was affected by a large deletion and the second copy by a smaller but damaging mutation. This patient not only had profound development delay, but also severely low blood counts due to bone marrow failure. The disease had unique features, but based on both, the clinical findings and short telomeres it could be classified as a very severe form of dyskeratosis congenita. We performed multiple genetic, cell culture and biochemical experiments to prove that the mutations in *PARN* are the causal of the disease. We also showed that the role of *PARN* in production of normal amount of blood cells is more global in nature since inhibition of the gene in zebrafish impaired blood cell formation. Our study revealed a new gene that is critical for normal development of blood cell and the brain. Clinically, the results of our study facilitate early diagnosis and family counseling of a group of patients with bone marrow failure and developmental delay.

II. Summary of Project 2: copy number variants underlying inherited bone marrow failure syndromes.

Santhosh Dhanraj, Nicolas Waespe, Manju Wahala, Tom Enbar, Bozana Zlateska, Hongbing Li, Robert Klaassen, Conrad V Fernandez, Rochelle A Yanofsky, John Wu, Yves Pastore, Mariana Silva, Jeff H Lipton, Josee Brossard, Bruno Michon, Sharon Abish, MacGregor Steele, Roona Sinha, Mark Belletrutti, Vicky R. Breakey, Lawrence Jardine, Lisa Goodyear, Liat Kofler, Ibrahim Ghemlas, Michaela Cada, Lillian Sung, Mary Shago, Stephen Scherer, Yigal Dror (In Preparation for Publication)

Inherited bone marrow failure syndromes (IBMFSs) comprise a group of over 25 diseases. The diseases are often diagnosed in early childhood. Patients with IBMFSs suffer from low blood cell counts because of failure of the bone marrow to produce blood cells. Patients can also suffer from varying degrees of birth defects, and many of them have a high risk of cancer. The diagnosis of an IBMFS and determining the specific syndrome critically impact on clinical care; including the type of treatment delivered, the type of tests done during regular check ups and the type of counselling given to the patients and families, Unfortunately, determining whether the patients have an IBMFS and what is the specific syndrome are commonly challenging and rely on genetic testing. The process of genetic testing is complex since each syndrome can be caused by mutations in several different genes and overall there are over 80 IBMFS genes have been identified. Therefore, the best strategy to establish a diagnosis in a timely and cost effective manner is unknown. The aims of this study were to evaluate whether large pieces of DNA are sometimes lost or appear in excess in patients with IBMFSs and whether such copy number variants (CNVs) are associated with more severe disease than small mutations in genes. To do so, we analyzed patients from the Canadian Inherited Marrow Failure Registry (CIMFR) who were genetically investigated by comprehensive CNV analysis or comprehensive advanced gene analysis (next generation sequencing) for a panel of 72 genes in our laboratory, as well as patients who were genetically investigated in clinical laboratories. Among 328 patients from the CIMFR who underwent genetic investigation, the genetic cause was identified in 185 cases (56.4%). Overall 89.8% had small mutations and 10.2% had CNVs. In many cases, the identified CNVs or small mutations were critical for establishing the diagnosis. We also found that patients with CNVs tended to have significantly more birth defects, developmental delay and short stature compared to patients with small mutations. We concluded that although most patients with IBMFSs have small mutations, a significant proportion of patients large CNVs. Therefore, comprehensive CNV analysis should be considered in IBMFS cases, where small mutations are

not found. Patients with IBMFS and large CNVs have higher number of birth defects, and more frequently have developmental delay and short stature.

III. Summary of Project 3: Improving diagnostic precision, care and syndrome definitions using comprehensive next generation sequencing for the inherited bone marrow failure syndromes. Ghemlas I, Li H, Zlateska B, Klaassen R, Fernandez CV, Yanofsky RA, Wu J, Pastore Y, Silva M, Lipton JH, Brossard J, Michon B, Abish S, Steele M, Sinha R, Belletrutti M, Breakey VR, Jardine L, Goodyear L, Sung L, Dhanraj S, Reble E, Wagner A, Beyene J, Ray P, Meyn S, Cada M, Dror Y. (Published in the Journal of Medical Genetics 2015).

Over 25 different inherited marrow failure syndromes have been described. The clinical features of the various IBMFSs frequently overlap and many times limits the ability to establish a diagnosis based solely on clinical features. Since over 70 IBMFS genes have been identified, genetic testing is often prolonged and costly. Since correct diagnosis, treatment, monitoring for cancer and family counseling often depend on identifying the mutated gene, strategies that enable the identification of the genetic mutation in timely fashion is essential. To overcome these challenges, we developed a comprehensive assay using an advanced technology (called next generation sequencing) to analyze a panel of 72 known IBMFS genes.

A total of 158 patients with unknown mutations were studied. Of 75 patients with known IBMFS categories (*e.g.* Fanconi anemia), 59% had causal mutations. Among 83 patients with unclassified IBMFSs, we found causal mutations and established the diagnosis in 18% of the patients. The assay detected mutant genes that had not previously been reported to be associated with the patient clinical features. In other cases, the assay led to amendments of clinical diagnoses. In 20% of genotype cases the results indicated a need to implement a cancer surveillance program for the patients. We concluded that the novel assay that we developed is efficient, accurate and has a major impact on patient care.

IV. Summary of Project 4: Molecular characteristics of a pancreatic adenocarcinoma associated with Shwachman-Diamond syndrome.
Dhanraj S, Manji A, Pinto D, Scherer SW, Favre H, Loh ML, Chetty R, Wei AC, Dror Y. (Published in Pediatr Blood Cancer 2013).

Shwachman-Diamond syndrome (SDS) is characterized by reduced number of cells in the bone marrow and exocrine pancreas. The disease is associated with a high risk of bone marrow cancer (leukemia). It is unknown whether solid tumors are part of the disease phenotype. An SDS patients who were enrolled in our Canadian Inherited Marrow Failure Registry developed pancreatic cancer. Among 41 patients with SDS who enrolled in the registry, we identified one male patient with a solid tumor: pancreatic cancer. Detailed genetic characterization revealed 41 copy number variants (CNVs). None of these CNVs were exclusive to the tumor, and all appeared also in a blood sample from the patient. One copy of several genes that are important for protecting people from cancer was lost. These included CTNNA3 and LGALS9C. Direct sequencing of genes and commonly involved in pancreatic cancer (TP53, KRAS, and NRAS) revealed no mutations. The levels of several proteins relevant to pancreatic cancer (cyclin D1, E-cadherin, p53 MLH1 and MSH2 and β-catenin) were similar to that seen in non-hereditary pancreatic cancer. In summary, our case raises the possibility that solid tumors are associated with SDS, thereby broadening the clinical phenotype of the disease. The relatively young age at cancer diagnosis and the specific involvement of the pancreas make the possibility of an association with SDS likely. Similar to leukemia in SDS, the pancreatic cancer developed in a

tissue with paucity of cells. This observation and the relative minor genetic changes in the tumor raises the possibility that cancer cells grow in SDS because they better adapt compared to the surrounding disadvantaged cells of the patient.

V. Summary of Project 5: Combined de-novo mutation and non-random X-chromosome inactivation causing Wiskott-Aldrich syndrome in a female with thrombocytopenia. Boonyawat B, Dhanraj S, Al Abbas F, Zlateska B, Grunenbaum E, Roifman CM, Steele L, Meyn S, Blanchette V, Scherer SW, Swierczek S, Prchal J, Zhu Q, Torgerson TR, Ochs HD, Dror Y. (Published in J Clin Immunol. 2013).

Chromosomes are elongated structures in the cells that carry the genetic material (DNA). Humans have 23 pairs of chromosomes (one set from the father and one set from the mother). 22 pairs comprise identical chromosomes. One of the pair comprise of one X-chromosome and one Y chromosome in males and two X chromosomes in females. Disorders caused by mutations in genes on the X chromosomes typically affect males, since they have only one X-chromosome. A female with thrombocytopenia, eczema and mild lymphocyte abnormalities with extensive negative diagnostic testing, was suspected to have Wiskott-Aldrich syndrome (WAS), a disease caused by mutations in the gene WAS, which is on the X-chromosome. The aim of the study is to decipher the mechanism of disease expression in a female patient with a heterozygous mutation on the X-chromosome. Due to a clinical features that may suggest WAS, the WAS gene was analyzed. Indeed, the girl had a mutation (c.397G > A, p.E133K) in one of her X-chromosome. However, this should not cause her a disease unless the gene on the other X-chromosome is not active. Typically the X-chromosomes in females undergo random process of inactivation so about half of the cells have one chrosmosome (from the father) active, and in about half the other chromosome (from the mother) is active. We indeed found that the patient had abnormal non-random inactivation all the X-chromosome from her father, while the mutation was on the X-chromosome from the mother. Copy number analysis showed excluded deletions and amplifications of DNA pieces as a cause for the non-random inactivation of one X-chromosome. Our study emphasizes the need to test selected female patients with complete or incomplete disease expression for X-linked disorders even in the absence of a family history.

Others:
Novel other genetic changes that have been identified in the present study are under study in our follow-up projects.

Again, thank you so much for you support,
Kindest Regards,
Yigal

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