ANNUAL REPORT
2014/2015

THE HESS B. AND DIANE FINESTONE LABORATORY
IN MEMORY OF
JACOB AND JENNY FINESTONE

Submitted by: Dr. David S. Rosenblatt

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MEMBERSHIP

PHYSICIANS AND SCIENTISTS
David S. Rosenblatt
David Watkins

ADMINISTRATIVE SUPPORT STAFF
Camilah Arbabian-Urguilla
Alexandre Michaud
Tracy Wang

CLINICAL SUPPORT STAFF
Laurence Baret
Maria Galvez
Leah Ladores

RESEARCH SUPPORT STAFF
Wayne Mah (with James Coulton)
Tania Cruz

GRADUATE STUDENTS
Jordan Chu
Mihaela Pupavac

ANNUAL REPORT 2014/2015

The Hess B. and Diane Finestone Laboratory in Memory of Jacob and Jenny Finestone, was first established in 1988. The intent of the donor was to honour the memory of Jacob and Jenny Finestone, and the 80th birthday of Mr. Hess B. Finestone. A permanent endowment was created at McGill University devoted to the advancement of medical genetics. The specific objectives of the endowment are to a) fund research projects related to the field of medical genetics; b) fund trainees in the field of medical genetics; and c) publicize the field of medical genetics through the support of special lectures, visiting professorships and other appropriate means. Dr. David S. Rosenblatt has been Director of the laboratory since its inception, and this annual report describes the activity of his laboratory. Material relating to members of the Department of Human Genetics at McGill and the Departments of Medical Genetics at the McGill University Health Centre and the Jewish General Hospital should be sought in the respective university or hospital annual reports.

The Hess B. and Diane Finestone Laboratory in Memory of Jacob and Jenny Finestone, is located on Livingston 3 of the Montreal General Hospital Site of the McGill University Health Centre. In February 2015, the laboratory moved to the new Glen Site of the MUHC Research Institute. This is one of two major international referral laboratories for the diagnosis of patients with inherited disorders of folate and vitamin B_{12} transport and metabolism. It is involved in studying the biochemical and molecular bases of these diseases. Since Dr. Rosenblatt directs a certified molecular diagnostic laboratory adjacent to the research laboratory, advances in knowledge from research can be immediately translated to clinical diagnostics.

As this report coincides with the renewal of Dr. Rosenblatt's appointment as Dodd Q. Chu & Family Chair in Medical Genetics, it will exceptionally focus on this activity.

Dr. Rosenblatt was appointed as the inaugural Chair holder of the Dodd Q. Chu & Family Chair in Medical Genetics for a five year term commencing January 1, 2011. This chair was initially established on November 9, 2003 with the purpose of furthering the study of Medical Genetics.

At the time of his appointment, Dr. Rosenblatt was also the Chair of the Department of Human Genetics at McGill, a position he had held since 2001. He remained Chair of the Department of Human Genetics until the spring of 2013. The present report will outline his activities in the areas of research, teaching, clinical, and service.

RESEARCH:

Our laboratory has been a world leader in the area of inherited disorders of vitamin B_{12} and folate metabolism for forty years. It has been able to define novel steps in intracellular cobalamin metabolism and to identify three novel complementation groups *(cblE, cblF, cblG)* and the first patient with a defect in the transcobalamin receptor. Since 2000, my collaborators

and I have identified the genes for 7 disorders: *cblA* (*MMAA*), *cblB* (*MMAB*), *cblC* (*MMACHC)*, *cblD* (*MMADHC*), *cblF* (*LMBRD1*), *cblJ* (*ABCD4*), and *cblX* (*HCFC1*). Since 2011 activity in the laboratory has resulted in the publication of 42 articles in peer-reviewed journals. Selected examples are described below:

cblC and *cblD*: Using surface plasmon resonance, we showed that MMACHC and MMADHC interact. We demonstrated cobalamin binding to MMACHC with micromolar affinity. We discovered that MMACHC localizes exclusively to the cytosol, whereas MMADHC localizes to both the cytosol and mitochondria. We have shown that MMACHC is capable of removing the alkyl groups from alkylcobalamins, and have conducted the first studies on the MMACHC proteome, showing alterations in cytoskeletal and muscular proteins. Using RNA *in situ* hybridization, we showed tissue and cell type specific expression of *Mmachc* during mouse organogenesis.

cblJ: We performed whole exome sequencing on a patient whose cellular phenotype mimics that of the *cblF* disorder caused by mutations in the *LMBRD1* gene, but whose cells complemented those from all known complementation groups. We found causal mutations in the *ABCD4* gene; transfection of patient fibroblasts with wild type *ABCD4* led to rescue of the cellular phenotype. We have expanded on the clinical presentation of this disease with the description of a patient with late onset. We demonstrated that detergent-solubilized LMBD1 and ABCD4 exist as homodimers and that the two proteins interacted with high affinity. Consistent with our phage display predictions, MMACHC also interacted with both LMBD1 and ABCD4 with high affinity. Our results indicate that a multi-protein complex is involved in the transport of vitamin B_{12} across the lysosomal membrane.

MTHFD1: An infant referred with megaloblastic anemia, atypical hemolytic uremic syndrome, and severe combined immune deficiency had a selective decreased synthesis of methylcobalamin in cultured fibroblasts. Whole exome sequencing performed on patient genomic DNA resulted in identification of the first described mutations in the *MTHFD1* gene. This patient had severe combined immunodeficiency which responded to treatment with cobalamin and folate]. Studies of cultured patient fibroblasts demonstrated that the clinical phenotype of severe combined immunodeficiency was mainly mediated through the effect of mutations in *MTHFD1* on thymidine metabolism. Investigation of four additional patients revealed clinical heterogeneity but a pattern of hematological, immunological, and renal symptoms.

cblX: We discovered that a number of patients with the cellular phenotype seen among patients with *cblC*, have mutations in the *HCFC1* gene on the X chromosome. This is the first description of a defect in a transcription factor leading to an inborn error of intracellular vitamin metabolism. It is also clinical importance as the inheritance is as an X-linked rather than an autosomal recessive. Loss of function studies in the developing zebrafish showed that loss of the *hcfc1b* ortholog of *HCFC1* results in defects in craniofacial development.

Dr. Rosenblatt held CIHR operating funds continuously from 1975-2014. CIHR funding has become difficult with the re-organization of CIHR. Dr. Rosenblatt received a one-year no cost extension of his CIHR grant.

Dr. Rosenblatt received 3 major honours for his work, since 2011. In 2013, he was named a "Champion of Genetics" by the Canadian Gene Cure Foundation. In 2015, he was asked to deliver the Aaron Michael Graham Lecture in Metabolic Diseases at the Children's Hospital of Los Angeles. In the summer of 2015, he served as the Honorary President of the 10th International Conference: one carbon metabolism, vitamins B and homocysteine, held in Nancy, France.

TEACHING

Dr. Rosenblatt has supervised the following graduate students during the time of holding the Dodd Q. Chu and Family Chair in Medical Genetics:

Alison Brebner	M.Sc.	2012-2014
	Title: Search for non-coding mutations in patients with *cblC* and *mut* inborn errors of cobalamin metabolism Currently Medical Student at McGill	
Mihaela Pupavac	Ph.D.	2012-
	Title: Next Generation Sequencing to Discover Genes for Mendelian Diseases	
Jordan Chu	M.Sc.	2014-
	Title: Study of patients with atypical inborn errors of cobalamin metabolism	

Classroom teaching has varied from year to year with alterations in the undergraduate medical curriculum, but for most years until the current year, Dr. Rosenblatt delivered an introductory lecture on Medical Genetics to the second year medical students and a lecture on Huntington Disease. He has also usually been a tutor for small group teaching in prenatal diagnosis and hereditary cancer.

In the hospital, Dr. Rosenblatt has been a supervisor for residents in Medical Genetics and trainees in the Canadian College of Medical Genetics program in Biochemical Genetics, who have rotated through the molecular and biochemical genetics laboratory at the Montreal General Hospital.

David Watkins

Biology 575

Department:	Biology/Human Genetics
Format:	Lecture
Title:	***Inborn Errors of Folate and Cobalamin Transport and Metabolism***
Role:	Lecturer
Level:	Undergraduate/Graduate

Time: 3 1.5-hour class lectures

CLINICAL

At the McGill University Health Centre (MUHC), Dr. Rosenblatt is a member of the Department of Medical Genetics and it is at the MIUHC that he holds "actif" status. Until the move to the new Glen Site, he has also been head of the Division of Medical Genetics in the Department of Medicine. With the consolidation of administrative structures, the Division of Medical Genetics in the Department of Medicine of the MUHC will be closed, and he will have a hospital cross-appointment in the Division of Medical Biochemistry in the Department of Medicine. The clinical laboratory is CLIA certified and the only referral centre for the diagnosis of inborn errors of vitamin B_{12} metabolism in North America. At the MUHC, he also is responsible for the clinical interpretation of results of molecular genetics testing for Huntington disease and hereditary breast cancer.

At the Jewish General Hospital (JGH), Dr. Rosenblatt is Chief of the Department of Medical Genetics. Clinical activity in medical genetics at the JGH is restricted to the areas of Hereditary Cancer and Prenatal Diagnosis. He is directly responsible for supervision of the genetic counsellors working in the area of prenatal diagnosis. At the JGH, he has spent the past two years re-organizing the Department and defining its mission. In 2015, there has been great progress in obtaining new positions for genetic counsellors and moving part time administrative staff into full time hospital positions.

SERVICE

Dr. Rosenblatt is a member of the Faculty of Medicine promotions committee.

Dr. Rosenblatt serves as a corresponding editor for two journals: *Molecular Genetics and Metabolism Reports* and *Human Mutation.*

He is a member, and the only Medical Geneticist, on the INNESS Comité SEVAB (Comité Scientifique Analyses de Biologie Médical. This committee is responsible for the evaluation of the appropriateness of new tests for the province of Quebec.

Since being named a "Champion of Genetics", he was asked to serve on the Board of Directors of the Canadian Gene Cure Foundation.

He is a member of the Conseil d'Administration of the Association of Medical Geneticists of Quebec.

ORIGINAL PUBLICATIONS

Atkinson C, Miousse IR, Watkins D, Rosenblatt DS, Raiman JAJ. Clinical, biochemical, and molecular presentation in a patient with the cblD-variant 1 inborn error of cobalamin metabolism. *JIMD Rep* 17:77-81, 2014

Stockler S, Corvera S, Lambright D, Fogarty K, Nosova E, Leonard D, Steinfeld R, Ackerly C, Shyr C, Au N, Selby K, Van Allen M, Vallance H, Wevers R, Watkins D, Rosenblatt D, Ross CJ, Conibear E, Wasserman W, van Karnebeek CDM. Single point mutation in rabenosyn-5 in a female with intractable seizures and evidence of defective endocytic trafficking. *Orphanet J Rare Dis* 9:141, 2014

Field MS, Kamynina E, Watkins D, Rosenblatt DS, Stover PJ. Human mutations in methylenetetrahydrofolate dehydrogenase 1 impair nuclear de novo thymidylate biosynthesis *Proc Natl Acad Sci (USA)* 112:400-405, 2014

Deme JC, Hancock MA, Xia X, Plesa M, Kim JC, Carpenter EP, Rosenblatt DS, Coulton JW. The putative human lysosomal vitamin B12 transporters LMBD1 and ABCD4 interact in vitro. *Mol Membrane Biol* 31:250-261, 2014

Awan Z, Aljenedil S, Rosenblatt DS, Cusson J, Gilfix BM, Genest J, Severe hyperhomocysteinemia due to cystathionine beta synthase deficiency and factor V Leiden mutation in a patient with recurrent venous thrombosis. *Thrombosis* J 12:30, 2014

Quintana AM, Geiger EA, Achilly N, Rosenblatt DS, Maclean KN, Stabler SP, Arlinger KB, Appel B, Shaikh TH. *Hcfc1*, a zebrafish ortholog of *HCFC1*, regulates craniofacial development by modulating *mmachc* expression. *Dev Biol* 396:94-106, 2014

Burda P, Kuster A, Hjalmarson O, Suormala T, Bürer C, Lutz S, Roussey G, Christa L, Asin-Cayuela J, Kollberg G, Andersson BA, Watkins D, Rosenblatt DS, Fowler B, Holme E, Froese DS, Baumgartner MR. Characterization and review of MTHFD1 deficiency: four new patients, cellular delineation and response to folic and folinic acid therapy. *J Inher Metab Dis* 38:863-872, 2015

Field MS, Kamynina E, Watkins D, Rosenblatt DS, Stover PJ. New Insights into the Metabolic and Nutritional Determinants of Severe Combined Immunodeficiency. *Rare Diseases* 3:e1112479, 2015.

ABSTRACTS

A Brebner, H-C Yu, D Watkins, V Adoue, T Pastinen, F Skovby, TH Shaikh, DS Rosenblatt. A homozygous mutation in the transcription factor THAP11 in a patient with methylmalonic aciduria and a severe neurological phenotype. Canadian Human and Statistical Genetics Meeting, 3-6 May 2014, Victoria BC.

A Brebner, H-C Yu, D Watkins, V Adoue, T Pastinen, F Skovby, TH Shaikh, DS Rosenblatt. A homozygous mutation in the transcription factor THAP11 in a patient with methylmalonic aciduria and a severe neurological phenotype. Réseau de Médecine Génétique Appliquée Journées Génétiques, 14-16 May 2014, Montreal QC.

A Brebner, H-C Yu, D Watkins, V Adoue, T Pastinen, F Skovby, TH Shaikh, DS Rosenblatt. A homozygous mutation in the transcription factor THAP11 in a patient with methylmalonic aciduria and a severe neurological phenotype. AMGQ Reunion Scientifique 2014, May 23 2014, Montreal QC.

Pupavac M, Petrella F, Watkins D, Fahiminiya S, Muenzer J, Majewski J, Rosenblatt DS. "Investigating a potentially novel inborn error of cobalamin metabolism". RMGA Journées Génétiques. Montreal, Canada. May 2014. Poster Presentation.

Pupavac M, Sloan JL, Johnston JJ, Dempsey-Nunez L, Jung J, Wynter J, Manoli I, Biesecker LG, Rosenblatt DS, Venditti CP. "ACSF3 mutations in unclassified patients with apparently isolated methylmalonic aciduria". Canadian Human and Statistical Genetics Meeting. Victoria, Canada. May 2014. Poster Presentation.

A Brebner, H-C Yu, D Watkins, V Adoue, T Pastinen, F Skovby, TH Shaikh, DS Rosenblatt. A homozygous mutation in the transcription factor THAP11 in a patient with methylmalonic aciduria and a severe neurological phenotype. European Society of Human Genetics, 31 May-3 June 2014, Milan Italy.

Moreno-Garcia MA, Pupavac M, Rosenblatt DS, Tremblay M, Jerome-Majewska LA. "Mmachc is required for pre-implantation in the mouse". European Human Genetics Conference. Milan, Italy. 2014.

A Brebner, D Watkins, DS Rosenblatt. Molecular Analysis of Patients Diagnosed as cblC. FASEB Folic Acid, Vitamin B12 and One-Carbon Metabolism, 3-8 August 2014, Steamboat Springs CO.

Pupavac M, Petrella F, Watkins D, Fahiminiya S, Muenzer J, Majewski J, Rosenblatt DS. "An infant with hyperhomocysteinemia, methylmalonic aciduria, and an atypical cellular distribution of protein-bound cobalamin". FASEB – Folic Acid, Vitamin B12 and One-Carbon Metabolism. Steamboat Springs, USA. August 2014.

Stockler S, Corvera S, Lambright D, Fogarty K, Nosova E, Leonard D, Steinfeld R, Ackerley C, Shyr C, Au N, Selby K, Van Allen M, Vallance H, Wevers R, Watkins D, Rosenblatt D, Ross C, Conibaer L, Wasserman W, Van Karnebeek C. Rabenosyn-5 (ZFYVE20) deficiency in a female with intractable seizures and evidence of defective endocytic trafficking. Annual Symposium of the Society for the Study of Inborn Errors of Metabolism, Innsbruck. J Inher Metab Dis 37 (Suppl 1): S2, 2014.

Waters PJ, Clarke JTR, Watkins D, Rosenblatt DS, Lévesque S. A patient with evidence of methylmalonyl-coA epimerase (MCE) deficiency, presenting with severe metabolic acidosis and biochemical profiles initially interpreted as propionic acidemia. Annual Symposium of

the Society for the Study of Inborn Errors of Metabolism, Innsbruck. *J Inher Metab* Dis 37 (Suppl 1): S92, 2014.

Gavrilov D, Bishop L, Sellars E, Schimmenti L, Gallant N, Hopkin R, Leslie N, Berry SA, Rinaldo P, Rosenblatt D, Fair A, Matern D, Raymond K, Oglesbee D, Tortorelli S, Wong D. Successful prospective treatment of patients with remethylation defects detected by newborn screening – Cobalamin E deficiency, cobalamin G deficiency, and methylenetetrahydrofolate reductase deficiency. Annual Symposium of the Society for the Study of Inborn Errors of Metabolism, Innsbruck. *J Inher Metab* Dis 37 (Suppl 1): S54, 2014.

Pupavac M, Chu J, Watkins D, Tian X, Feng Y, Chen S, Fenter R, Zhang VW, Wang J, Wong LJ, Rosenblatt DS. Diagnosing patients with methylmalonic aciduria – comparison of somatic cell and next generation sequencing panel testing. SIMD 2015

Chu J, Pupavac M, Watkins D, Tian X, Feng Y, Chen S, Fenter R, Zhang VW, Wang J, Wong LJ, Rosenblatt DS. Challenging findings in patients with methylmalonic aciduria confirmed as *mut* by somatic cell complementation analysis and studied with a novel next generation sequencing panel. SIMD 2015

Pupavac M, Chu J, Watkins D, Tian X, Feng Y, Chen S, Fenter R, Zhang VW, Wang J, Wong LJ, Rosenblatt DS. Diagnosing patients with methylmalonic aciduria – comparison of somatic cell and next generation sequencing panel testing. SIMD Meeting, Salt Lake City, 2015

Chu J, Pupavac M, Watkins D, Tian X, Feng Y, Chen S, Fenter R, Zhang VW, Wang J, Wong LJ, Rosenblatt DS. Challenging findings in patients with methylmalonic aciduria confirmed as *mut* by somatic cell complementation analysis and studied with a novel next generation sequencing panel. SIMD Meeting, Salt Lake City, 2015

Pupavac M, Janer A, Watkins D, Brebner A, Petrella F, Fahiminiya S, Shaikh T, Muenzer J, Majewski J, Shoubridge E, Rosenblatt DS. An infant with hyperhomocysteinemia, methylmalonic aciduria, and an atypical cellular distribution of protein-bound cobalamin. CHSGM Meeting, Vancouver 2015

Chu J, Pupavac M, Watkins D, Tian X, Feng Y, Chen S, Fenter R, Zhang VW, Wang J, Wong LJ, Rosenblatt D. Challenging atypical findings in two patients diagnosed as *mut* by somatic cell complementation analysis and studied with a novel NGS panel. CHSGM Meeting, Vancouver 2015

Pupavac M, Janer A, Watkins D, Petrella F, Fahiminiya S, Muenzer J, Majewski J, Shoubridge E, Rosenblatt DS. An infant with hyperhomocysteinemia, methylmalonic aciduria and an atypical intracellular cobalamin distribution. OCM 2015

Chu J, Pupavac M, Watkins D, Tian X, Feng Y, Chen S, Fenter R, Zhang VW, Wang J,

Wong LJ, Rosenblatt DS. Search for the molecular basis of two complementation-confirmed atypical *mut* patients. OCM 2015

FINANCIAL REPORT – 2014/15

Starting Balance		**$** 97, 476
*Salary Support and Benefits	$ 50, 792	
Materials and Supplies	$ 2, 628	
Conferences, Travel, Special Events	$ 18, 970	
Memberships	$ 6,104	
Phones, Pagers, Computer, Printing, Couriers	$ 3,142	
Miscellaneous	$ 269	
Total Expenses		**$ 81, 905**
**Balance		**$ 15, 571**

** Arbabian-Urquilla, Baret, Chu, Michaud, Notte, Pupavac, Rosenblatt, Veyre, Wang*