

Summer 8-19-2016

Genetic Landscape of Pediatric Myelodysplastic Syndromes

Jennifer E. Grove

University of Nebraska Medical Center

Follow this and additional works at: <http://digitalcommons.unmc.edu/etd>

 Part of the [Genetics Commons](#), and the [Molecular Genetics Commons](#)

Recommended Citation

Grove, Jennifer E., "Genetic Landscape of Pediatric Myelodysplastic Syndromes" (2016). *Theses & Dissertations*. Paper 126.

This Dissertation is brought to you for free and open access by the Graduate Studies at DigitalCommons@UNMC. It has been accepted for inclusion in Theses & Dissertations by an authorized administrator of DigitalCommons@UNMC. For more information, please contact digitalcommons@unmc.edu.

GENETIC LANDSCAPE OF PEDIATRIC MYELODYSPLASTIC SYNDROMES

By

Jennifer E. Grove

A DISSERTATION

Presented to the Faculty of
the University of Nebraska Graduate College
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy

Medical Sciences Interdepartmental Area Graduate Program
(Pediatrics)

Under the Supervision of Professor Bhavana J. Dave

University of Nebraska Medical Center Omaha, Nebraska

June 2016

Supervisory Committee:

Bhavana J. Dave, Ph.D.

Tanner Hagelstrom, Ph.D.

James Eudy, Ph.D.

Gregory Bociek, M.D.

ACKNOWLEDGEMENTS:

My deep gratitude goes first to Prof. Bhavana J. Dave, who guided me throughout these years of my graduate career. Her unwavering dedication to her students and their work and her willingness to mentor throughout this process was unfailing. I will forever be grateful for her expert guidance, understanding, and encouragement.

A special acknowledgement to the late Dr. Warren Sanger who served on my committee until his untimely departure. Without Dr. Sanger's support and encouragement in the early years, I would never have made it to this point. I am also thankful to Dr. Tanner Hagelstrom, Dr. Gregory Bociek, and Dr. James Eudy for agreeing to serve on the committee of a non-traditional student. Their comments and questions were greatly valued.

I would also like to extend my appreciation to my colleagues in the Human Genetics Laboratory at UNMC. I would not have accomplished this research without their expertise and experience in the area of cytogenetics and microarray. I would like to specifically thank my fellow technologist and classmate, Rachel Utter. She has been my advocate every step of the way. I am lucky to call her an equal and a friend.

Last, but certainly not least, I would like to thank my family especially my husband Mike. His encouragement and support for me during this very long process contributed to my success. His willingness to take care of our children, the house, and our responsibilities, by himself at times, allowed me to accomplish my goals. I would never have completed this without him. I would also like to thank my young children, Tristan and Alice, for giving me the motivation I needed to fulfill this dream. Their understanding for all those evenings when Mommy had work to do will never be forgotten. Finally, I would

like to thank my parents and in-laws who supported me over these years and provided the extra hands to our family whenever and wherever we needed it.

GENETIC LANDSCAPE OF PEDIATRIC MYELODYSPLASTIC SYNDROMES

Jennifer E. Grove, Ph.D.

University of Nebraska Medical Center, 2016

Supervisor: Bhavana J. Dave, Ph.D.

ABSTRACT:

Myelodysplastic syndromes (MDS) are acquired heterogeneous hematopoietic clonal disorders primarily seen in the adult and elderly populations that presents a variety of cellular morphologies in cell lineages, varying prognoses, and differences in overall survival (OS) between individual patients. The occurrence of MDS in the pediatric and young adult population, or those between the ages of 0 and 29, is slowly on the rise. Pediatric and elderly cases exhibit diverse cytogenetic findings with differences in OS. The characterization of the genetic landscape of pediatric MDS is limited and most studies detailing genetic changes have been conducted in adult MDS cases. In order to aid in therapeutic stratification for pediatric cases, the key genes involved in hematopoietic transformation must be deciphered. This study utilized comprehensive analysis including cytogenetic karyotyping, FISH, and high-resolution microarray techniques. With the use of multiple techniques, this study confirmed the rarity of MDS in the pediatric population, characterized the frequencies of hallmark cytogenetic abnormalities, and identified key aberrations observed at the genetic level. With the use of microarray, we were able to detect genomic aberrations in 33 genes including novel copy number changes in more than one case in the *PRDM16*, *IRF4*, *MYH11*, *ALK*, *CDKN2B*, *PAX5*, *EXT2*, and *ERCC4* genes. The results from this study prove the importance of comprehensive testing utilizing a variety of techniques in distinguishing the most accurate genetic landscape of pediatric

MDS. This information can be used to better equip the medical community in accurately diagnosing and providing prognostic implications for therapy and treatment.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	i
ABSTRACT.....	iii
TABLE OF CONTENTS	v
LIST OF FIGURES.....	viii
LIST OF TABLES	xii
LIST OF ABBREVIATIONS	xiii
INTRODUCTION	1
Hematopoiesis	4
Pathogenesis of MDS	7
Epidemiology of MDS.....	11
Classification of MDS	13
<i>Refractory Cytopenia of Childhood (RCC)</i>	14
<i>Refractory Anemia with Excess Blasts and Refractory Anemia with Excess Blasts in Transformation (RAEB and RAEB-T)</i>	15
<i>Secondary MDS: Treatment Related and Occupational Exposure</i>	15
<i>Inherited Bone Marrow Failure Disorders (IBMF)</i>	17
<i>Aplastic Anemia (AA)</i>	18
Treatment for MDS	18
Genetic Testing in Myelodysplastic Syndromes	19
<i>Cytogenetics in Adult Populations</i>	21
<i>Cytogenetics in Pediatric Populations</i>	21

Prognostic Implications of Cytogenetics	22
Utility of Microarray Studies	23
Hypothesis and Specific Objectives	25
MATERIALS AND METHODS	27
Specimen Collection and Handling	28
Conventional Cytogenetic Studies	29
Fluorescence <i>in situ</i> Hybridization (FISH).....	35
Microarray	40
<i>DNA Extraction</i>	40
<i>DNA Quantification</i>	41
<i>CytoScan® HD Array Assay Technique</i>	41
<i>OncoScan® FFPE Assay Technique</i>	51
RESULTS.....	69
Patient Demographics.....	70
<i>Pediatric Patient Demographics</i>	70
Conventional Cytogenetic and FISH Analyses	74
Microarray Samples	82
Microarray Results	86
<i>Case Studies</i>	91
Comprehensive Testing Results.....	128
DISCUSSION	131
Demographics.....	132
Cytogenetics and FISH	133
Prognostic Implications in MDS.....	137
Microarray	138
Current Trends in Adult MDS Array Analysis	139

Current Trends in Pediatric MDS Array Analysis	145
Limitations of this Study.....	146
Gene Involvement in Pediatric MDS	147
Comprehensive Testing	165
SUMMARY AND FUTURE DIRECTIONS.....	168
Summary	169
Future Directions	171
BIBLIOGRAPHY	174
Appendix A. Reagent names and manufacturers	193
Appendix B. Equipment product names and manufacturers	198
Appendix C. Software product name and manufacturers.....	199

LIST OF FIGURES

Figure 1a. Schematic diagram of normal hematopoiesis	5
Figure 1b. Schematic diagram of abnormal hematopoiesis	6
Figure 2. Workflow of Cytogenetics and FISH	30
Figure 3. Workflow of cytogenetic culture setup	32
Figure 4. Workflow of FISH studies.....	36
Figure 5. MDS FISH Panel.....	38
Figure 6. Restriction enzyme digestion CytoScan® protocol	43
Figure 7. Ligation CytoScan® protocol	44
Figure 8. PCR CytoScan® protocol	45
Figure 9. Verification of the PCR product.....	47
Figure 10. Fragmentation CytoScan® protocol.....	49
Figure 11. Verification of the fragmentation product	50
Figure 12. Labeling CytoScan® protocol	52
Figure 13. Hybridization CytoScan® protocol	53
Figure 14. Anneal OncoScan® protocol.....	55
Figure 15. Gap fill OncoScan® protocol.....	56
Figure 16. First PCR OncoScan® protocol	58
Figure 17. Second PCR OncoScan® protocol	60

Figure 18. Verification of the first PCR product.....	61
Figure 19. HaeIII Digestion OncoScan® protocol	62
Figure 20. Verification of the HaeIII digestion product	64
Figure 21. Hybridization OncoScan® protocol	65
Figure 22. Smooth signal examination using ChAS.....	67
Figure 23. Demographic distribution of MDS	71
Figure 24. Percentage of MDS specimens per age group	72
Figure 25. Demographic distribution of pediatric and young adult MDS.....	73
Figure 26. Hallmark cytogenetic and FISH findings of MDS	75
Figure 27. Cytogenetic/FISH Findings of 2353 specimens from adult MDS (≥30 years of age) cases.....	76
Figure 28. Cytogenetic/FISH findings of pediatric and young adult MDS	79
Figure 29. Cytogenetic/FISH findings of pediatric MDS.....	81
Figure 30. Cytogenetic/FISH findings of young adult (19-29 years) MDS.....	83
Figure 31. Representative Images depicting normal cytogenetic karyotypes	87
Figure 32. Representative normal MDS FISH images	88
Figure 33. Microarray findings of pediatric and young adult MDS cases	89
Figure 34. Microarray results for Case 1	92
Figure 35. Microarray results for Case 4	94
Figure 36. FISH results for Case 5.....	95

Figure 37. Microarray results for Case 5.....	96
Figure 38. Microarray results for Case 6.....	98
Figure 39. Cytogenetic and FISH results for Case 7.....	99
Figure 40. Microarray results for Case 7.....	100
Figure 41. Cytogenetic and FISH results for Case 11.....	103
Figure 42. Cytogenetic and FISH results for Case 12.....	106
Figure 43. Microarray results for Case 12.....	107
Figure 44. Cytogenetic and FISH results for Case 13.....	108
Figure 45. Microarray results for Case 15.....	110
Figure 46. Microarray results for Case 16.....	111
Figure 47. Cytogenetic and FISH results for Case 17.....	112
Figure 48. Microarray results for Case 19.....	114
Figure 49. Microarray results for Case 20.....	115
Figure 50. Microarray results for Case 21.....	116
Figure 51a-b. Microarray results for Case 22.....	118
Figure 51c-d. Microarray results for Case 22.....	119
Figure 52. FISH results for Case 24.....	120
Figure 53. Microarray results for Case 25.....	122
Figure 54. Microarray results for Case 26.....	123

Figure 55. Cytogenetic and FISH results for Case 27.....	124
Figure 56. Microarray results for Case 28.....	126
Figure 57. Cytogenetic or FISH results in cases with abnormal microarray analyses .	141

LIST OF TABLES

Table I. Characterization of the MDS-related chromosomal abnormalities detected by cytogenetic and FISH analyses in adult MDS	78
Table II. Characterization of the MDS-related chromosomal abnormalities detected by cytogenetic and FISH analyses in the pediatric/young adult population	80
Table III. Comparison of MDS-related abnormalities observed in the pediatric and young adult MDS populations	84
Table IV. List of pediatric and young adult MDS specimens for microarray studies after karyotyping and FISH analyses	85
Table V. Characterization of genetic aberrations detected by microarray.....	90
Table VI. Microarray results for Case 10.....	102
Table VII. Microarray results for Case 11	105
Table VIII. Characterization of the cases with the <i>PRDM16</i> gene aberration	127
Table IX. Characterization of results by cytogenetics and/or FISH analyses.....	129
Table X. Characterization of results from comprehensive testing	130
Table XI. Details of abnormalities observed in pediatric MDS cases by microarray	140
Table XII. List of the most frequent gene alterations in adult MDS	143
Table XIII. List of genes that were detected in more than one case	148

LIST OF ABBREVIATIONS

AA	Aplastic anemia
ALL	Acute lymphoblastic lymphoma
AML	Acute myeloid leukemia
AN	Anemia
APL	Acute promyelocytic leukemia
ChAS	Affymetrix® chromosome analysis suite
CML	Chronic myelogenous leukemia
CNV	Copy number variations
DAPI	4,6-diamidino-2-phenylindole
dbVAR	NCBI database for genomic structural variants
DGV	Database of genomic variants
DIR	Direct culture
DON	Direct overnight
ET	Essential thrombocythemia
FA	Fanconi's anemia
FBS	Fetal bovine serum
FISH	Fluorescence in situ hybridization
G-banding	Giemsa banding

HBSS	Hank's balanced salt solution
HL	Hodgkins lymphoma
HSCs	Hematopoietic stem cells
HSCT	Hematopoietic stem cell transplant
IBMF	Inherited bone marrow failure disorder
IPSS-R	Revised international prognostic scoring system
ISCN	International system for human cytogenetic nomenclature
IST	Immunosuppressive therapy
JMML	Juvenile myelomonocytic leukemia
KCL	Potassium chloride
LOH	Loss of heterozygosity
MDS	Myelodysplastic syndromes
MIP	Molecular inversion probe
ML-DS	Myeloid leukemia of Down syndrome
MPD	Myeloproliferative disorders
MPN	Myeloproliferative neoplasms
NCBI	National Center for Biotechnology Information
NEU	Neutropenia
NHL	Non-Hodgkins lymphoma

NP-40	Nonidet P-40
OMIM	Online Mendelian Inheritance in Men®
OS	Overall survival
PCP	Pancytopenia
PCR	Polymerase chain reaction
PMF	Primary myelofibrosis
PV	Polycythemia Vera
RA	Refractory anemia
RAEB	Refractory anemia with excess blasts
RAEB-t	Refractory anemia with excess blasts in transformation
RBC	Red blood cell
RCC	Refractory cytopenia of childhood
SDS	Shwachman-Diamond syndrome
SNP	Single-nucleotide polymorphisms
SSC	Sodium chloride and sodium citrate
TCP	Thrombocytopenia
TdT	Terminal deoxynucleotidyl transferase
THC	Trypsin, hypotonic salts, and colcemid
UCS	Unknown clinical significance

WBC	White blood cell
WHO	World Health Organization

INTRODUCTION

INTRODUCTION

Myelodysplastic syndromes (MDS) are acquired hematopoietic clonal disorders primarily seen in the adult and elderly populations with an overall estimate of incidence at 14,000 new cases per year (Siegel, Ma et al. 2014). This group of heterogeneous bone marrow syndromes are characterized as stem-cell disorders with varying degrees of overall reduction in blood cell production. The heterogeneous nature of MDS presents as a variety of cellular morphologies in a number of myeloid cell lineages, varying prognoses, and differences in overall survival (OS) between individual patients. The majority of patients present a normocellular or hyperplastic bone marrow, however, up to 20% of patients have shown hypoplastic and myofibrotic bone marrow (Aul, Bowen et al. 1998). The overall numbers of myeloid cell lineages vary and morphological aberrations are observed in the clonal origin of hematopoietic cells. Hypercellular bone marrow displays morphological dysplasia and ineffective hematopoiesis in at least one of the three myeloid lineages (Aul, Bowen et al. 1998, Tefferi, Vardiman 2009, Whichard, Sarkar et al. 2010). Cellular bone marrow is unable to produce and deliver adequate numbers of mature cells to the peripheral blood during ineffective hematopoiesis.

Even though MDS is predominantly a disease of older populations, the frequency in the pediatric and young adult population, or those between the ages of 0 and 29, is slowly on the rise. Myelodysplastic syndromes in pediatric cases present diverse cytogenetic findings and differs in OS in comparison with the elderly. The amount of information on this rare group is limited. In order to aid in therapeutic stratification for pediatric patients more information is needed (Glaubach, Robinson et al. 2014, Ganapathi, Schafernak et al. 2015) . The use of high-resolution techniques including microarray can help with deciphering possible key aberrations that are observed at the genetic level.

Current molecular genetic studies have detailed key genes involved in adult MDS and the present study will be useful to compare similarities and differences between the elderly and pediatric populations (Silva, Maschietto et al. 2013, Shih, Abdel-Wahab et al. 2012, Bejar 2014).

The onset of this disease can be relatively benign in the form of refractory anemia (RA), typically observed as a decrease in red blood cells, but causes a decrease in the production of healthy platelets, red and white blood cells (Aul, Bowen et al. 1998, Tefferi, Vardiman 2009, Corey, Minden et al. 2007, Akhtari 2011). Red blood cells (RBC) transport oxygen to the rest of the body and brings carbon dioxide to the lungs. Having too few RBC, anemia, leaves the patient feeling tired and weak and can cause shortness of breath. White blood cells (WBC) are important as a line of defense against infection. The two major types are lymphocytes, which make antibodies, and granulocytes that destroy bacteria. Having too few WBC leads to severe infections in the body, or neutropenia. The small fragments of the megakaryocyte that enter the blood stream are called platelets. These are essential for blood clotting and without them can result in thrombocytopenia, which causes abnormal bleeding, and bruising (Brunning, RD. Bennett, JM. Flandrin, G. Matutes, E. Head, D. Vardiman, JW. 2001).

Myelodysplastic syndromes can be diagnosed as primary, or *de novo* MDS, or secondary MDS from past chemo- or radiation therapy or exposure to certain chemicals and heavy metals. Both result in dysplastic blood and bone marrow cells and cytogenetic abnormalities are observed in over 50% of MDS cases (Corey, Minden et al. 2007, Flandrin 2002). Hallmark genetic aberrations detected by conventional karyotyping or fluorescence *in situ* hybridization (FISH) include -5/del(5q), -7/del(7q), +8, or del(20q) (Cherian, Bagg 2006). The classification of these genetic findings has prognostic

implications and helps to stratify a more individualized treatment plan for MDS patients (Haase, Germing et al. 2007).

Hematopoiesis

Normal hematopoiesis gives rise to progressively more differentiated progenitor cells, which eventually differentiate into mature blood cells (Lobo, Shimono et al. 2007, Orkin, Zon 2008). The fundamental properties of hematopoiesis include proliferation, loss, and differentiation resulting in the development of over 500 billion blood cells per day. Normal adult hematopoietic stem cells (HSCs) are the common ancestors of all blood cells and are needed to maintain or repair their host tissue. These cell types have two functions of division that include symmetrical division, which yields two stem cells or two differentiated daughter cells, and asymmetrical division into either another stem cell or a more specialized cell. Self-renewal of HSCs produces a replicate stem cell that typically has the same development and replication fate. The production of specialized daughter cells is decided from biochemical signals and transcription factors and the HSC has the potential to generate cell types of each lineage (Kondo 2010, Whichard, Sarkar et al. 2010, Wilson, Laurenti et al. 2008).

During normal hematopoiesis the blood stem cell, or immature blast cells, make up 5% or less of the cells in the bone marrow and will develop into one of the three healthy blood cells (red blood cells, platelets, or white blood cells) from a homeostatic balance between proliferation, differentiation, and apoptosis (Figure 1a). The normal system efficiently replenishes the body when hematological stresses are encountered which includes infection and blood loss (Passegue, Wagers et al. 2005). Hematopoietic disorders, including MDS, are acquired when normal development is disrupted and blast cells lose proper function the way they should and die in the bone marrow or soon after

