

Contents

Contributors	xix
Foreword	xxix
<i>Francis S. Collins and Alan E. Guttmacher</i>	
Acknowledgments	xxxii
Introduction ... The Journey Inward	xxxiii
<i>Betty S. Pace</i>	
PART I: THE HUMAN GENOME ERA, A HISTORIC PERSPECTIVE	
1. Sickle Cell Disease: Demystifying the Beginnings	1
<i>Clarice Reid and Griffin Rodgers</i>	
The Precursors	1
The National Sickle Cell Disease Program	2
Clinical Advances	5
Summary	10
References	10
2. Sponsorship of Sickle Cell Disease Research by the National Institutes of Health: A Brief History and Projections for the Future	13
<i>Gregory L. Evans and David G. Badman</i>	
Introduction	13
Sickle Cell Disease	13
The National Institutes of Health	14
The National Heart, Lung, and Blood Institute (NHLBI)	15
The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)	15
NHLBI Funding of Sickle Cell Disease Research	16
Clinical Care Guidelines	16

Clinical Research — Multicenter Clinical Trials	17
The New NHLBI SCD Clinical Research Network	19
NHLBI-Supported Translational SCD Research — Pulmonary Complications of SCD, and Programs of Excellence in Gene Therapy	19
Translational Research — NHLBI Comprehensive Sickle Cell Centers	21
Structure and Main Purpose	21
Clinical Research — Change in Focus Toward Multicenter Clinical Trials	21
Training Programs	22
Basic Research	22
Other NIH RFAs and Program Announcements	23
<i>National Heart, Lung, and Blood Institute</i>	23
<i>National Institute of Diabetes and Digestive and Kidney Diseases</i>	24
<i>National Human Genome Research Institute</i>	24
Investigator-Initiated Grants	24
Joint Efforts between NHGRI, NHLBI, NIDDK, and other NIH Institutes and Centers — 2003 Conference	25
The Future	26
3. The Human Genome Project	27
<i>Betty S. Pace</i>	
Introduction	27
The Pre-Genomic Era	27
DNA: The Molecule of Heredity	27
The National Institutes of Health (NIH)	28
The Department of Energy (DOE)	28
Birth of the Human Genome Project	28
The First Five Years: 1990–1994	28
The International Human Genome Sequencing Consortium	29
Goals of First Five Years	30
Budget Allocations	30
A New Five-Year Plan: 1993–1998	30
Goals of the Extended Period	30
The Final Phase of the Human Genome Project: 1998–2003	31
Goals for the Last Phase	31
The Human Genome Draft Sequence	32
Technological Advances	33
The Genome Era	33
The Human Genome	33
Model Organisms	34
The National Center for Biotechnology Information (NCBI)	34
A Vision for the Future	34
The Impact of Genome Era Research in Health and Disease	36
DNA Variations Related to Human Disease	36

Human Diseases and Haplotypes	37
The International HapMap Project	37
Pharmacogenomics	38
Renaissance of Sickle Cell Disease Research in the Genome Era	39
The Genome Era	40
References	41

PART II: CLINICAL RESEARCH PERSPECTIVES

4. Sickle Cell Disease: A Phenotypic Patchwork 45

Kim Smith-Whitley and Betty S. Pace

Introduction	45
Origin of Sickle Gene	46
Sickle Mutation	46
Malaria	46
Classification of Sickle Hemoglobin Syndromes	47
Hemoglobin Genes	47
Hemoglobin Disorders	47
Diagnosis of Sickle Cell Disease	48
<i>Protein-Based Techniques</i>	48
<i>Solubility Test</i>	50
<i>Molecular-Based Techniques</i>	50
<i>Genome Era Techniques</i>	51
Pathophysiology of Sickle Cell Disease (SCD)	52
Cooperative Study of Sickle Cell Disease (CSSCD)	53
Painful Episodes	54
Globin Gene Effects	54
Exacerbation of Anemia	54
Acute Chest Syndrome	55
Central Nervous System	55
“Silent” Central Nervous System (CNS) Infarcts	56
Priapism	57
Other Complications	57
Growth and Development	57
Genetic Disease Modifiers	57
Predictors of Disease-Severity	57
Newborn Cohort	59
Treatment Strategies	59
Hydroxyurea	59
Transfusions Therapy	60
Alloimmunization and Delayed Hemolytic Transfusion Reactions	60
Health Maintenance	60
Medical Home	60
Health Maintenance	60

Family and Patient Education	61
Transition to Adult Care	61
Sickle Cell Trait	62
Future Perspectives	62
References	63
5. Preventive Care and Advances in the Treatment of Sickle Cell Disease	69
<i>Charles T. Quinn and George R. Buchanan</i>	
Introduction	69
Bacterial Infection	69
Newborn Screening (NBS)	71
Routine Health Maintenance	72
Acute Vaso-Occlusive Complications	72
Painful Episodes	73
Acute Chest Syndrome	73
Acute “Hematologic” Crises	73
Priapism	74
Stroke	74
Specific Pharmacotherapy of Sickle Cell Disease	74
Clinical Trials	75
Summary	75
References	76
6. Sickle Cell Disease in Adults	79
<i>Johnson Haynes, Jr. and Ardie Pack-Mabien</i>	
Introduction	79
Medical Complications	79
Long-term Survival	79
Vaso-Occlusive Episodes	80
<i>Acute Pain</i>	80
<i>Chronic Pain</i>	81
Acute Chest Syndrome	81
Etiology of Acute Chest Syndrome	81
<i>Treatment</i>	82
Sickle–RBC Pulmonary Vascular Interactions	82
Sickle Chronic Lung Disease	83
<i>Pulmonary Artery Hypertension</i>	83
<i>Lactate Dehydrogenase</i>	84
<i>Endothelial Dysfunction</i>	84
<i>Sildenafil</i>	84
<i>Arginine</i>	84
Stroke	84
<i>Stroke Treatment</i>	85

Avascular Necrosis	85
Renal Failure	86
Retinopathy	86
Other Clinical Complications	87
<i>Infections</i>	87
<i>Cardiovascular</i>	87
<i>Priapism</i>	87
<i>Liver and Gallbladder</i>	87
<i>Skin Ulcers</i>	88
Genetic Counseling and Pregnancy	88
Hemoglobin-SC Disease	88
Hydroxyurea	88
Other Treatments	89
Preventive Health Maintenance	89
Adult Healthcare Maintenance	89
Adult Comprehensive Clinic Components	90
Unmet Needs	90
Quality of Life	90
Education and Employment	91
Social and Psychosocial Needs	91
Workshop on Adults with Sickle Cell Diseases: Meeting Unmet Needs	91
Sickle Cell Adult Provider Network (SCAPN)	91
A Sickle Cell Disease Clinical Research Network	91
Future Directions	91
References	92

7. Pain in Sickle Cell Disease: A Multidimensional Construct 99

Lennette J. Benjamin and Richard Payne

Introduction	99
The Nature of Pain in Sickle Cell Disease (SCD)	99
Pathophysiology of Sickle-Related Pain	100
Appraisal of Risk Factors	100
Pain Mechanisms and Biopsychosocial Integration	101
<i>Nociceptive Pain</i>	101
<i>Neuropathic Pain</i>	102
<i>Biopsychosocial Integration</i>	102
Treatment-Related Issues	104
Assessment of Pain and Symptom Management	104
Access to Quality Care	105
Home Care	106
Outpatient Day Hospital and Day Care Centers	106
Disease-Specific Therapy	106
Disparities in Access to Care	106
Inadequate Pain Treatment: A Major Public Health Problem	107

Physiological and Pharmacological Consequences	107
Pain Under Treatment and Pseudoaddiction	108
Opioid-Induced Hyperalgesia: An Emerging Neurotoxicity Syndrome	108
NMDA and Opioid Receptor Interactions	108
Physical Dependence and Opioid Withdrawal-Related Pain	109
Pharmacologic Tolerance and Tolerance-Associated Hyperalgesia	109
<i>NMDA Receptor Antagonists</i>	110
Opioid Rotation	110
<i>Fentanyl or Sufentanil</i>	110
Combine Opioid Rotation and NMDA Receptor Antagonist Therapy	111
<i>Methadone</i>	111
Caution in the Use of Methadone	111
Practical Matters: Post Script	112
Summary	112
References	113
8. Transfusion Therapy in Sickle Cell Disease	117
<i>Carolyn Hoppe, Robert Adams and Elliot Vichinsky</i>	
Introduction	117
Stroke in Sickle Cell Disease	117
Epidemiology of Stroke	117
Diagnosis of Stroke	118
Neurocognitive Functioning and Silent Infarction	118
Pathophysiology of Stroke	119
Risk Factors for Ischemic Stroke	119
Primary Prevention of Ischemic Stroke in Children	120
Prevention of Recurrent Stroke	121
Alternative Treatments for Stroke	123
Advances in Transfusion Medicine	124
Transfusion-Transmitted Infection	124
Alloimmunization	125
Iron Overload	125
Erythrocytapheresis	127
Summary	127
References	127
PART III: BASIC RESEARCH PERSPECTIVES	
9. Hemoglobin S Polymerization, Just the Beginning	135
<i>Frank A. Ferrone</i>	
Introduction	135
Equilibrium	135

Hemoglobin Function	135
Polymer Structure	136
Polymer Stability	137
pH and Ionic Dependences	139
Hemoglobin Mixtures	139
Fiber and Gel Rigidity	141
Kinetics	141
Mechanism of Hemoglobin S Polymerization	141
The Thermodynamic Control of Nucleation	144
Kinetics of HbF Mixtures	146
Depolymerization	148
Inhibition by Design	148
Drugs	148
Hemoglobin	148
References	149

10. Damage to the Red Blood Cell Membrane in Sickle Cell Disease 153

Steven R. Goodman and Clinton Joiner

Introduction	153
The RBC Surface and Vaso-Occlusion	154
Altered Phospholipid Asymmetry in the RBC Membrane	155
Membrane Transport and Volume Regulation in Sickle RBC Membrane	
Transport	156
Cell Volume Regulation	156
Consequences of Sickle RBC Dehydration	156
Mechanisms of Sickle RBC Dehydration	157
Sickling-Induced Cation Pathway	157
Gardos Pathway	158
KCl Cotransport	159
Cation Uptake Pathways	160
Interaction of Cation Transport Pathways and the Kinetics of Cellular	
Dehydration	160
Beyond Dehydration–Pathological Rehydration in Sickle RBCs	161
Pharmacological Intervention in Dehydrated Sickle RBCs	161
The Membrane Skeleton and ISC Formation	163
The Proteomics of the Sickle RBC Membrane	166
References	167

11. Fetal Hemoglobin for What Ails Sickle Hemoglobin 173

Solomon F. Ofori-Acquah and Betty S. Pace

Introduction	173
Developmental Expression and Cellular Distribution of HbF	173

Composition of Fetal Hemoglobin	174
Interactions between Hemoglobin F and S	174
Globin Gene Regulation: The Locus Control Region	175
β -Globin Cluster Restriction Fragment Length Polymorphisms	177
The Role of HS2 Polymorphisms in HbF Synthesis	178
Regulation of γ -Globin Gene Expression	178
Hereditary Persistence of Fetal Hemoglobin	179
Deletional HPFH	179
Non-deletional HPFH	179
Emerging Cell Signaling Mechanisms for Drug-Mediated HbF Induction	180
Mitogen Activated Protein Kinase Signaling Pathway	181
Signal Transducer and Activators of Transcription (STAT) Signaling Pathways	181
Nitric Oxide and Cyclic Guanosine Monophosphate (cGMP) Signaling Pathways	181
Pharmacological Fetal Hemoglobin Induction	182
Cytokines and Growth Factors	182
Cytotoxic Agents	182
DNA Methyltransferase Inhibitors	184
Histone Deacetylase Inhibitors	184
Short-Chain Fatty Acids	185
Future Perspectives	186
References	186

12. Genetic Modulation of Sickle Cell Disease 193

Martin H. Steinberg and Swee Lay Thein

Introduction	193
Variation in the Human Genome — Similarities and Differences	194
Identification of Genetic Variants, Haplotypes and Linkage Disequilibrium	194
A Genetic Approach Towards the Study of SCD	195
Established Predictors of Complications in Sickle Cell Anemia	196
Fetal Hemoglobin (HbF)	196
Genetic Regulation of Fetal Hemoglobin	196
β -globin Gene Cluster Haplotypes	197
α -Thalassemia	198
Genetic Variants as Predictors of Disease Severity	198
Painful Episodes	198
Stroke	198
Priapism	200
Osteonecrosis	200
Pulmonary Disease	201
Gallstones	201
Compound Phenotypes	202
Future Directions	202
Summary	202
References	203

13. Molecular Framework of Hemoglobin Switching	207
<i>Steven Fiering</i>	
Introduction	207
Developmental Patterns of β -like Globin Gene Expression	207
Model Systems for Study of Hemoglobin Switching	209
The LCR and Its Role in Hemoglobin Switching	210
Activation of γ -globin Expression after Birth	212
Erythroid Progenitor Commitment to γ or β Globin Expression	213
<i>Trans</i> -factors Involved in Globin Switching	213
New Approaches	214
Acknowledgments	215
References	215
14. Dynamic Nucleoprotein Structure of the β-Globin Locus: Establishing a Rational Molecular Basis for Therapeutic Modulation of Hemoglobin Switching	219
<i>Emery Bresnick, Hogune Im, Kirby D. Johnson, Meghan E. Boyer and Jeffrey A. Grass</i>	
Introduction	219
Transcriptional Mechanisms within Endogenous Chromatin Domains: The β -Globin Locus System	219
Experimental Systems to Analyze Mechanisms of β -like Globin Gene Activation Versus Developmental Regulation	222
Factor Occupancy/Recruitment Module	223
“Histone Modification Pattern” Module	225
Targeting the “Histone Modification Module”	227
Summary	228
References	228
15. Vertebrate Models for Sickle Cell Disease Research	237
<i>Barry H. Paw, Seong-Kyu Choe, Flavia C. Costa, Shirin V. Sundar and Kenneth Peterson</i>	
Introduction	237
Transgenic Mice	237
Globin Gene Switching	238
Early Transgenic Models	238
YAC and BAC Transgenesis	239
Knockout Models	241
Sickle Cell and Thalassemia Models	242
S Antilles and S + S Antilles Models	242
SAD Model	243
Berkeley Model	243

Birmingham Model	244
San Francisco Model	244
Enhanced γ -globin Expression Models	244
Thalassemia Models	245
<i>In Vivo</i> Drug Screening	245
Hydroxyurea	245
Short Chain Fatty Acid Analogs	245
Zebrafish	246
Zebrafish as a Model System for Organogenesis	246
Zebrafish as a Model for Hematopoiesis	246
Zebrafish Globins	250
Zebrafish Transgenesis to Study Hematopoiesis	251
High Throughput Small Molecule Screens for Drug Discovery	252
Summary	253
References	253
16. Stem Cell Biology	259
<i>Wei Li and Alan W. Flake</i>	
Introduction	259
Hematopoiesis	260
Primitive Hematopoiesis	260
Definitive Hematopoiesis	260
Characterization of Hematopoietic Stem Cells	260
Cell Surface Markers	260
Fetal Hematopoietic Stem Cells	261
Adult Hematopoietic Stem Cells	261
The Side Population	262
Methods for Isolating HSCs	262
Non-Antibody-Based Isolation	262
Antibody-Mediated Isolation	263
Stem Cell Assay Systems	263
In Vitro Assay Systems	263
In Vivo Assay Systems	264
Competitive Repopulation	264
Regulation of Stem Cell Self-Renewal	265
Erythroid Lineage Commitment	266
Globin Gene Expression	267
Cell Signaling Pathways	267
Erythropoietin	268
Stem Cell Factor	269
Hematopoietic Stem Cell Plasticity	269
Mechanisms of Stem Cell Plasticity	270

Clinical Use of Hematopoietic Stem Cells	270
Summary	270
References	271
17. Bone Marrow Transplantation	277
<i>Robert I. Raphael and Mark C. Walters</i>	
Introduction	277
Current Indications for Hematopoietic Cell Transplantation (HCT)	278
Current Results of HCT for Sickle Cell Disease (SCD)	278
Complications after HCT	280
Growth and Development after HCT for SCD	282
Stable Mixed Chimerism after HCT	283
Non-Myeloablative HCT for Sickle Cell Disease	285
Alternative Stem Cell Sources	286
Summary	288
References	288
18. Genetically Engineered Cures: Gene Therapy for Sickle Cell Disease	295
<i>Punam Malik and Philippe Leboulch</i>	
Introduction	295
Complexity of the β -Globin Gene	295
Prerequisites for Effective Gene Therapy of SCD	296
Difficulties Related to Human β -Globin Gene Transfer Using	
Oncoretroviral Vectors	296
Renaissance of Gene Therapy for the β -Hemoglobinopathy Disorders with	
HIV-Based Lentiviral Vectors	297
Production of HIV-1 Based Lentiviral Vectors	297
Gene Therapy of Murine β -Thalassemia	298
Gene Therapy of SCA in Transgenic Mouse Models	299
Globin Gene Transfer into Human Hematopoietic Cells	300
Safety from Randomly Integrating Lentiviral Vectors	300
Lentiviral Vectors Modifications to Improve Safety and Efficacy: SIN Deletions and	
Chromatin Insulators	301
Chromatin Insulators	301
HSC Selection Strategies	302
Ex Vivo Expansion and Selection of HSC	303
In Vivo Expansion and Selection of HSC	303
Target Number of HSCs for Genetic Correction of SCD	303
First Clinical Trial of a Lentiviral Gene Therapy Vector for Hemoglobinopathy	
Disorders	304
Alternative Vectors and Gene Therapy Strategies	304

Impact of the Human Genome Project (HGP)	304
Summary	305
References	305

PART IV: COMMUNITY PERSPECTIVES

19. Sickle Cell Disease: The Past, Present and Future Social and Ethical Dilemmas 311

Vence L. Bonham, Jr., Carlton Haywood, Jr. and Vanessa Northington Gamble

Introduction	311
The Creation of a “Black Genetic Disease”	311
Eliminating Racial and Ethnic Disparities in Health and Health Care: The Role of SCD	313
Race and Disease in the Genome Era: Lessons from the History of Sickle Cell Disease	314
Ethical, Legal and Social Implications Research of SCD in the Genome Era	316
SCD Research: Trust, Trustworthiness, & Distrust	316
Race, Trust, and Medical Research	317
Race, Trust, and Genetics	318
Attitudes toward Research Participation among Persons with SCD	318
Lessons for the Conduct of SCD Research in the Genome Era	320
Summary	321
Acknowledgments	321
References	321

20. It Takes a Village to Cure Sickle Cell Disease 325

Rosie Peterson and Denise Davis-Maye

Introduction	325
Social and Cultural Context of Sickle Cell Disease (SCD)	325
Inconsistencies in Information Dissemination	326
Healthcare Communication Needs Assessment Surveys	327
Inclusion of Adolescents and Young Adults in the Healthcare Process	327
The Impact of Previous Healthcare and Research Practices	328
The Tuskegee Syphilis Study	328
Mistrust of the Healthcare Community	329
The Role of National Institutes of Health (NIH) in Promoting Participation in Clinical Trials	329
Summary	330
References	331

**21. Beyond National Borders: A Global Perspective
on Advances in Sickle Cell Disease Research and Management, and New
Challenges in the Genome Era** **333**

Solomon F. Ofori-Acquah and Kwaku Ohene-Frempong

Introduction	333
Global Health Burden of Sickle Cell Disease (SCD)	334
Africa	334
America	336
Asia	338
Europe	338
Middle East	338
Micromapping	338
Genetic Background of the Sickle Cell Mutation	339
“Hybrid” and Functional Haplotypes	339
Progress in Health Management: From the Outside Looking in	340
Pharmacologic Intervention	340
Blood Transfusions	340
Transplantation	341
Global Collaborative Research	341
Capacity-Building in Epicenters of the Sickle Cell Mutation	342
The HapMap Project and International Collaborations	343
Summary	343
References	344

Index **347**