

# Fragile X Syndrome

From Genetics  
to Targeted  
Treatment



Edited by  
**Rob Willemsen**  
**R. Frank Kooy**



# FRAGILE X SYNDROME

From Genetics to Targeted Treatment

---

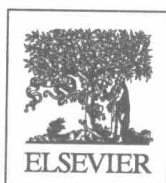
*Edited by*

ROB WILLEMSSEN

*Erasmus MC, Rotterdam, The Netherlands*

R. FRANK KOOY

*University of Antwerp, Antwerp, Belgium*



**ACADEMIC PRESS**

*An imprint of Elsevier*

Academic Press is an imprint of Elsevier  
125 London Wall, London EC2Y 5AS, United Kingdom  
525 B Street, Suite 1800, San Diego, CA 92101-4495, United States  
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States  
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom

Copyright © 2017 Elsevier Inc. All rights reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Details on how to seek permission, further information about the Publisher's permissions policies and our arrangements with organizations such as the Copyright Clearance Center and the Copyright Licensing Agency, can be found at our website: [www.elsevier.com/permissions](http://www.elsevier.com/permissions).

This book and the individual contributions contained in it are protected under copyright by the Publisher (other than as may be noted herein).

#### Notices

Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

#### Library of Congress Cataloging-in-Publication Data

A catalog record for this book is available from the Library of Congress

#### British Library Cataloguing-in-Publication Data

A catalogue record for this book is available from the British Library

ISBN: 978-0-12-804461-2

For information on all Academic Press publications visit our website at  
<https://www.elsevier.com/books-and-journals>



Working together  
to grow libraries in  
developing countries

[www.elsevier.com](http://www.elsevier.com) • [www.bookaid.org](http://www.bookaid.org)

*Publisher:* Mara Conner

*Acquisition Editor:* Melanie Tucker

*Editorial Project Manager:* Kathy Padilla

*Production Project Manager:* Chris Wortley

*Designer:* Maria Inês Cruz

Typeset by Thomson Digital

# FRAGILE X SYNDROME

---

# Contributors

---

**Han Bao** Emory University School of Medicine, Atlanta, GA, United States

**Mark F. Bear** Picower Institute for Learning and Memory, Massachusetts Institute of Technology, Cambridge, MA, United States

**Tamir Ben-Hur** The Agnes Ginges Center for Human Neurogenetics, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

**Nissim Benvenisty** The Azrieli Center for Stem Cells and Genetic Research, Silberman Institute of Life Sciences, The Hebrew University, Jerusalem, Israel

**Elizabeth Berry-Kravis** Rush University Medical Center, Chicago, IL, United States

**Aditi Bhattacharya** Center for Brain Development and Repair, Institute for Stem Cell Biology and Regenerative Medicine, National Centre for Biological Sciences, Bangalore, Karnataka, India

**Pietro Chiurazzi** Institute of Genomic Medicine, Catholic University, Rome, Italy

**Jeffrey Cohen** National Fragile X Foundation, Washington, DC, United States

**Lynda El-Hassar** Yale University School of Medicine, New Haven, CT, United States

**Douglas W. Ethell** Molecular Neurobiology, Graduate College of Biomedical Sciences, Western University of Health Sciences, Pomona; Developmental Immunology, La Jolla Institute for Allergy and Immunology, La Jolla, CA, United States

**Andreas Frick** Neurocentre Magendie, Pathophysiology of Neuronal Plasticity, INSERM U1215, University of Bordeaux, Bordeaux, France

**Christine M. Gall** University of California, Irvine, CA, United States

**Fabrizio Gasparini** Novartis Institutes for BioMedical Research, Neuroscience Discovery, Basel, Switzerland

**Inbal Gazy** National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, United States

**Melanie Ginger** Neurocentre Magendie, Pathophysiology of Neuronal Plasticity, INSERM U1215, University of Bordeaux, Bordeaux, France

**Christina Gross** Cincinnati Children's Hospital Medical Center and University of Cincinnati, Cincinnati, OH, United States

**Jacalyn Guy** University of Oxford, Oxford, United Kingdom; McGill University, Montréal, QC, Canada

**Randi Hagerman** MIND Institute, University of California Davis Medical Center, Sacramento, CA, United States

**Becky Hardiman** The Fragile X Society, Great Dunmow, Essex

**Charles Hoeffler** Institute for Behavioral Genetics, University of Colorado, Boulder, CO, United States

**Jessica E. Hunter** Center for Health Research, Portland, OR, United States

**Molly M. Huntsman** Skaggs School of Pharmacy and Pharmaceutical Sciences and School of Medicine, University of Colorado, Anschutz Medical Campus, Aurora, CO, United States

**Aia E. Jøneh** Odense University Hospital and University of Southern Denmark, Odense, Denmark

**Sébastien Jacquemont** Sainte Justine Research Institute, University of Montreal, Canada

**Peng Jin** Emory University School of Medicine, Atlanta, GA, United States

**Richard S. Jope** University of Miami School of Medicine, Miami, FL, United States

**Leonard K. Kaczmarek** Yale University School of Medicine, New Haven, CT, United States

**Peter Kind** Centre for Integrative Physiology and The Patrick Wild Centre for Research into Autism, Fragile X Syndrome and Intellectual Disabilities, The University of Edinburgh, Edinburgh, United Kingdom

**R. Frank Kooy** Department of Medical Genetics, University of Antwerp, Antwerp, Belgium

**Julie C. Lauterborn** University of California, Irvine, CA, United States

**Andrew Ligsay** Davis School of Medicine and MIND Institute, University of California, Sacramento, CA, United States

**Lothar Lindemann** Roche Pharma Research and Early Development, Neuroscience, Ophthalmology and Rare Diseases, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd., Basel, Switzerland

**Olivier J.J. Manzoni** INSERM, INMED and UMR, Aix-Marseille University Marseille, Marseille, France

**Henry G.S. Martin** INSERM, INMED and UMR, Aix-Marseille University Marseille, Marseille, France

**Montserrat Milà** Hospital Clinic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS); CIBER de Enfermedades Raras (CIBERER), Barcelona, Spain

**David L. Nelson** Baylor College of Medicine, Houston, TX, United States

**Giovanni Neri** Institute of Genomic Medicine, Catholic University, School of Medicine, Rome, Italy

**Daniela Neuhofer** INSERM, INMED and UMR, Aix-Marseille University Marseille, Marseille, France

**Emily K. Osterweil** Centre for Integrative Physiology/Patrick Wild Centre, University of Edinburgh, Edinburgh, United Kingdom

**Jörg Richstein** Interessengemeinschaft Fragiles-X e.V., Rostock, Germany

**Michael R. Santoro** Emory University School of Medicine, Atlanta, GA, United States

**Gaia Scerif** University of Oxford, Oxford, United Kingdom

**Sebastian S. Scharf** Roche Pharma Research and Early Development, Neuroscience,

Ophthalmology and Rare Diseases, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd., Basel, Switzerland

**Stephanie L. Sherman** Emory University, Atlanta, GA, United States

**Harpreet Sidhu** Molecular Neurobiology, Graduate College of Biomedical Sciences, Western University of Health Sciences, Pomona; The Scripps Research Institute, La Jolla, CA, United States

**Will Spooren** Roche Pharma Research and Early Development, Neuroscience, Ophthalmology and Rare Diseases, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd., Basel, Switzerland

**Laura J. Stoppel** Picower Institute for Learning and Memory, Massachusetts Institute of Technology, Cambridge, MA, United States

**Joshua Suhl** Emory University, Atlanta, GA; LabCorp, Variant Sciences Group, Westborough, MA, United States

**Elisabetta Tabolacci** Institute of Genomic Medicine, Catholic University, Rome, Italy

**Flora Tassone** University of California, Davis; MIND Institute, University of California Davis Medical Center, Sacramento, CA, United States

**Sally Till** Centre for Integrative Physiology and The Patrick Wild Centre for Research into Autism, Fragile X Syndrome and Intellectual Disabilities, The University of Edinburgh, Edinburgh, United Kingdom

**Karen Usdin** National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, United States

**Dan Vershkov** The Azrieli Center for Stem Cells and Genetic Research, Silberman Institute of Life Sciences, The Hebrew University; The Agnes Ginges Center for Human Neurogenetics, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

**Stephen T. Warren** Emory University School of Medicine, Atlanta, GA, United States

**Rob Willemssen** Erasmus MC, Department of Clinical Genetics, Rotterdam, The Netherlands

**Xiao-Nan Zhao** National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, United States

# Foreword

---

In 2016 it was 25 years since the identification of the *FMR1* gene and its new mutation mechanism. This book will give an overview of what has been achieved since then and gives an overview of the present knowledge of fragile X syndrome (FXS) and the gene involved. But let's go back first for a short visit into history.

In 1943 Martin and Bell described a pedigree of mental defect showing sex linkage. They showed in two generations of this family 11 males with imbecility. This term was typical for those days, but has since been evaluated into mental retardation and in this century into intellectual disabilities. In 1986 John M. Opitz described the burden in the families as follows:

And then as always, one stops to recollect with total astonishment and great reverence the massive burden of pain carried so patiently by the mothers, fathers, sibs, grandparents and the many others involved so closely on a daily basis with the apparent failure, defect, handicap, disability, and disappointment in the many thousands of Martin-Bell syndrome families throughout the world. *Am J Med Genet* 23:1-10

In 1969 Lubs noted a secondary constriction, referred to as a fragile site, which has been used to describe the syndrome as FXS. The presence of the cytogenetic expression of the fragile site was implemented as a diagnostic criterium but this was not a very reliable tool, in particular in the identification of carriers.

The development of recombinant DNA technology around 1980s made the cloning and identification of disease genes possible. The close association between the syndrome

in males and the fragile site at Xq27.3 indicated that the gene involved must be located at, or near to, the fragile site. Accordingly, the efforts of many different laboratories have been aimed at obtaining probes and fragments as close as possible to this fragile site, with the ultimate goal cloning the gene involved in FXS and the mutation in the disease gene.

The discovery by Verkerk and coworkers in 1991 that the disease is caused by a large-scale expansion of a highly unstable trinucleotide repeat in the *FMR1* gene has elucidated a new mutation mechanism of heritable unstable DNA. The gene was named *FMR1* (fragile X mental retardation 1), assuming that this was the first of an unknown number of future genes that might be isolated from the X chromosome involving fragility and mental retardation. The protein missing in fragile X patients was subsequently named FMRP. In the subsequent years more than 10 diseases with unstable repeat genes have been identified, all involved in neurological disorders. The presence of an unstable repeat in the *FMR1* gene has helped in direct testing in fragile X families and the identification of FRAX patients because the mutation is almost exclusively of the same type and there is an extremely low occurrence of other mutations in the disease. Since the identification of the unstable repeat in the *FMR1* gene much effort has been spent to get an answer to the following questions: What is the mechanism of repeat instability? What is the timing of repeat instability during embryonal life? What is the function of the repeat in the disease gene? What are the

functions of the (normal) gene product, with special focus on the brain?

Clues to the mechanisms that cause the abnormalities observed in FXS were limited. Initially research progress has been slow which is in part due to the lack of brain material of patients. To gain more insight in the pathological and physiological processes, researchers focused on animal models. No natural occurring animal models for FXS have been described. Therefore transgenic mouse models for FXS have been generated in Rotterdam and Houston. These mice show characteristics of FXS and have been made available to the research community. These mice might help to learn more about the function of the *FMR1* gene and the effect that the lack of the protein has on brain functioning. Furthermore, animal models might help in studying the timing and mechanisms of the repeat amplification. These mice have been instrumental in research to the understanding of the pathogenesis of FXS. Many chapters in this book are describing experiments using these animal models. Also models for FXS has been generated in flies, zebra fish, and rats. All of these have there own advantages and disadvantages. Rats (an animal widely used by the pharmaceutical industry) might be useful in testing

drugs while flies and zebra fish can be used for screening drugs.

The different contributions cover the progress in research in the field of FXS very well. They are subdivided into three different sections:

1. Clinics, diagnosis, epidemiology, molecular mechanisms, and models
2. Pathways involved
3. Clinical trials

Although we have learned a lot in the last 25 years, it will be clear to the readers that there is still more exciting work to do. In my view we still have to gain more insight in the pathological and physiological processes both at the level of (lack of) protein and the mechanism of repeat instability. So far progress in treatment of patients has been limited, not to say disappointing despite the fact that preclinical studies in mice were successful. We need to understand better differences within patient groups and we need better instruments to study the effect of treatment.

I hope this book will encourage readers and researchers to extend our knowledge further with regard to FXS.

Rotterdam, January 2016  
Ben A. Oostra

# Preface

---

Why a book? This is the first question you will ask upon seeing the nearly 500 pages of this book on Fragile X Syndrome. Isn't a book something very much of the past, something we used to be proud of since the invention of typography many centuries ago, an art now replaced by cybergraphy, providing us continuously with information free of charge wherever we are, even when watching a movie or relaxing on a sunny beach. Those who are satisfied with the information provided this way, please stop reading here. We are very much aware that even a simple search with the phrase "Fragile X Syndrome" on even the most amateurish of all search engines on any computer, laptop, or smartphone will result in thousands of hits within milliseconds, each of which will guide you through blogs, fora, papers, essays, etc. on this, or, in fact on any other topic. We felt however, that without the proper background on this complicated neurodevelopmental disorder, the "cyber only" reader would be congested with information within seconds and it will be impossible for him or her to filter out the relevant and reliable information. A classic example of too much of a good thing, that is, somewhat equivalent to going to a university without ever having seen inside of a secondary school. By providing the information on this genetic disorder in a highly structured and relevant format, our book is meant to serve as an anchor point for those in search of information.

Why on the Fragile X Syndrome? The simple fact that we choose this topic because both of us have been working on it for so long is not the answer, not at all. The reason

why we selected this topic is that the disorder keeps surprising us time after time after time by creating novel insights, by unraveling cellular mechanisms, and by its involvement in yet another molecular pathway. In the early years, immediately following the discovery of the gene, at that time novel mutational mechanism raised much attention. The many functions of the *FMR1* gene are another still not completely resolved mystery. Rather than a single function, the gene appears to play a role in a multitude of cellular processes and molecular pathways in the cell. Most interesting, several of these pathways are amendable to treatment with drugs that have been for many years on the shelf of various pharmaceutical companies that are eager to collaborate with the academic world to improve the condition of the patient. As such the Fragile X Syndrome has become the lead example of a monogenetic disorder that paved the way for the targeted intervention studies in many related neurodevelopmental disorders.

This book provides the state of the art in Fragile X Syndrome research, with an emphasis on the pathways amendable to treatment. It includes with an overview of the current clinical trials and reflects on those (What have we learned?). Of course we have not forgotten the input from the patients and their parents. The book, without exception written by long-term experts in the field, will appeal to a broad readership and is meant as a point of reflection for the "Nestors" in the field and at the same time as a point of inspiration for novel investigators that are eager to enter the field. For medical doctors,

patients, caregivers, and relatives it is meant to provide a realistic overview of what scientific research has achieved and what can be expected in the near future.

No book should ever be written without acknowledgments. We thank all contributors for their commitment and their eagerness to transform their expertise in written language. Only in retrospect this is easy. A specific thanks to our reviewers, many of whom felt they could have contributed to the contents of the book as well (and rightly

so!), who dedicated their time to improve the chapters for little more than this anonymous reward. Their efforts are immensely appreciated. And of course we thank our collaborators, students, and colleagues, for continuous inspiration over a long, long period of time. Finally we thank each other for our almost perfectly complementary expertises, professional networks, and characters. Together we made it work and it was fun to do so!

*Rob and Frank*

# Contents

---

List of Contributors ix

Foreword xi

Preface xiii

## I

### CLINICS, DIAGNOSIS, EPIDEMIOLOGY, MOLECULAR MECHANISMS, AND MODELS

#### 1. The Clinical Phenotype of the Fragile X Syndrome and Related Disorders

GIOVANNI NERI

Introduction 3

The Fragile X Syndrome 3

Fragile X Tremor Ataxia Syndrome 11

The Fragile X Premature Ovarian Insufficiency 13

References 13

#### 2. Fragile X Syndrome Genetics

DAVID L. NELSON, MICHAEL R. SANTORO,  
STEPHEN T. WARREN

Setting the Stage 19

Genetic Oddities 20

Positional Cloning of FRAXA and FMR1 22

FMR1 Structure and Function 25

FMRP and mRNA Metabolism 27

Resolving the Sherman Paradox 28

Premutation Disorders 30

Origins of FXS 31

Conclusions and Perspectives 31

References 34

#### 3. Molecular Diagnostics and Genetic Counseling in Fragile X Syndrome and FMR1-Associated Disorders

FLORA TASSONE, MONTSERRAT MILÀ

Fragile X Syndrome 41

The Diagnosis of Fragile X Syndrome 43

Genetic Counseling in FMR1-Associated  
Disorders 46

References 51

#### 4. Epidemiology of Fragile X Syndrome

STEPHANIE L. SHERMAN, JESSICA E. HUNTER

Introduction 57

Prevalence of FXS 59

Prevalence of FXS Among Subpopulations 61

Factors Related to Variation in Clinical  
Presentation Affect the Ability to Estimate  
Prevalence 67

Deletions and Point Mutations Leading to FXS 68

Conclusions 70

References 70

#### 5. Mechanisms of Repeat Instability in Fragile X Syndrome

KAREN USDIN, INBAL GAZY, XIAO-NAN ZHAO

Introduction 77

Potential Mechanisms for Repeat Expansion 81

Potential Mechanisms for Contraction and Error-  
Free Repair 89

Do Chromosome Fragility and Repeat Expansion  
Share a Common Mechanism? 90

Concluding Remarks and Future Directions 91

References 93

## 6. Modeling Fragile X Syndrome Using Human Pluripotent Stem Cells

DAN VERSHKOV, TAMIR BEN-HUR, NISSIM BENVENISTY

Human-Based Models for FXS	105
Modeling FXS in Human Pluripotent Stem Cells	106
Human ESCs as a Developmental Model for FXS	108
iPSCs in Modeling Fragile X Syndrome	109
Neural Differentiation of FXS-PSCs	111
PSC Modeling of CGG Repeat Instability	113
The use of FXS-PSCs for Targeted Drug Discovery	115
Conclusions	116
References	117

## 7. Animal Models of Fragile X Syndrome

R. FRANK KOOY, PENG JIN, HAN BAO, SALLY TILL,  
PETER KIND, ROB WILLEMSSEN

Introduction	123
Rodent Models of Fragile X Syndrome	124
Mouse Models of Fragile X Syndrome	125
The Phenotypic Spectrum of the Knockout Mouse	130
Rat Models of Fragile X Syndrome	134
Zebra Fish Models of Fragile X Syndrome	136
Concluding Remarks	141
References	141

# II

## PATHWAYS INVOLVED

### 8. RNA and Protein Targets of FMRP

JOSHUA SUHL, CHARLES HOEFFER

Introduction	151
Approaches to Defining the RNAs/Proteins Associated with FMRP	152
FMRP-Binding Determinants	160
References	167

### 9. The mGluR Theory of Fragile X: From Mice to Men

LAURA J. STOPPEL, EMILY K. OSTERWEIL, MARK F. BEAR

Introduction	173
FMRP Negatively Regulates Translation	175

### Animal Models of FXS

Dysregulation of Synaptic Protein Synthesis in the <i>Fmr1</i> KO Mouse	177
The mGluR Theory of FXS	178
Correcting FXS: Targeting mGlu <sub>5</sub>	179
Correcting FXS: Targeting Translation Control	179
Correcting FXS: Other Targets	187
From Mice to Men: Clinical Trials for FXS	189
Failure in the Clinic and What we can Learn	190
New Directions	191
Concluding Remarks	194
References	194

### 10. The GABAergic System Contributions to the Fragile X Syndrome Phenotype

MOLLY M. HUNTSMAN, R. FRANK KOOY

Introduction	205
Inhibitory Interneuron Dysfunction in FXS	206
Synaptic Components at GABAergic Synapses are Dysregulated in FXS	207
Targeting Deficiencies of the GABAergic System in FXS as Viable Treatment Options	210
Preventing Depolarizing GABAergic Potentials in Developing Circuits	211
Conclusions	212
References	212

### 11. Intracellular Signaling Networks in Fragile X Syndrome: Approaches to Drug Discovery and Therapeutics

CHRISTINA GROSS, ADITI BHATTACHARYA

Introduction	217
Dysregulated PI3K Signaling in FXS	218
Dysregulated ERK1/2 Signaling in FXS	221
Targeting the Signaling hub Ras to Correct Altered Signaling in FXS	223
TSC-mTORC1-S6K1-4EBP Nexus: a Major mRNA Translation Control Node in FXS	226
TSC 1-2 Complex is a Vital, but Understudied Signaling Node for FXS	226
mTOR is a Well-Studied Candidate in FXS, but may not be Suited for Direct Therapeutic Intervention	227
S6K1: A Signal Integrator and Translational Regulator with Therapeutic Potential in FXS	229

Modulation eIF4E via Mnk1 Offers an Alternative to Managing FXS Phenotypes	231
Challenges and Future Outlook	232
References	233

## 12. The Endocannabinoid System in Fragile X Syndrome

HENRY G.S. MARTIN, DANIELA NEUHOFFER,  
OLIVIER J.J. MANZONI

Introduction	241
Molecular Alterations in FXS	243
Inhibitory Neurotransmission	244
Excitatory Neurotransmission	249
Endocannabinoid System Interventions	251
Conclusions/Perspectives	253
References	254

## 13. Glycogen Synthase Kinase-3: Abnormalities and Therapeutic Potential in Fragile X Syndrome

RICHARD S. JOPE

Introduction	261
Fragile X Syndrome: Etiology and Animal Models	261
Glycogen Synthase Kinase-3	262
Morphological and Biochemical Effects of GSK3 Inhibition in <i>Fmr1</i> Knockout Mice	263
Behavioral Abnormalities in <i>Fmr1</i> Knockout Mice Improved by GSK3 Inhibitor Treatments	264
Cognitive Impairments in <i>Fmr1</i> Knockout Mice Rescued by Administration of GSK3 Inhibitors	267
Electrophysiological Abnormalities in <i>Fmr1</i> Knockout Mice Improved by GSK3 Inhibitors	269
Clinical Trials	270
Summary	270
References	271

## 14. Defects in Rho GTPase Signaling to the Spine Actin Cytoskeleton in FMR1 Knockout Mice

JULIE C. LAUTERBORN, CHRISTINE M. GALL

Introduction	277
Changes in the Spine Actin Cytoskeleton Support Synaptic Plasticity	280

FMR1 KO Defects in Rho GTPase Signaling Pathway Proteins	283
Conclusions and Future Directions	291
References	293

## 15. Matrix Metalloproteinases in Fragile X Syndrome

DOUGLAS W. ETHELL, HARPREET SIDHU

Introduction	301
FMR1-Deficiency and Dendritic Spine Morphology	303
Extracellular Matrix	306
Metalloproteinases	307
MMP-9 in FXS	310
Conclusions	313
Abbreviations	314
References	314

## 16. Ion Channel Dysfunction and FXS

ANDREAS FRICK, MELANIE GINGER, LYNDY EL-HASSAR,  
LEONARD K. KACZMAREK

Introduction	323
Voltage-Dependent Potassium Channels	324
Nonselective Cation Channels	331
Calcium Channels	332
Conclusions	334
References	334

## 17. Reactivation of the FMR1 Gene

ELISABETTA TABOLACCI, PIETRO CHIURAZZI

Introduction	341
Epigenetic Status of Premutated Alleles	343
Epigenetic Silencing of FMR1 Full Mutation	344
Rare Individuals with Unmethylated Full Mutation	349
Treatment Options for FXS	350
Reactivation of the FMR1 Gene	351
Future Perspectives	354
References	355

## III

## CLINICAL TRIALS

18. Drug Discovery for Targeted  
Pharmacotherapy of Fragile  
X SyndromeSEBASTIAN S. SCHARE, FABRIZIO GASPARINI,  
WILL SPOOREN, LOTHAR LINDEMANN

Introduction 363  
 Molecular Pathophysiology of Fragile X  
 Syndrome 364  
 Fragile X Disease Models 366  
 Targeted Interventions that have been Tested  
 Preclinically in FXS 372  
 Comparing Treatment Effects Observed in  
*Fmr1* Knockout Mice and FXS Patients: The  
 Example of mGlu5 NAMs 380  
 Future Directions for Drug Discovery  
 in FXS 381  
 References 389

19. Overview of Targeted  
Double-Blind, Placebo-Controlled  
Clinical Trials in Fragile  
X SyndromeANDREW LIGSAY, RANDI HAGERMAN,  
ELIZABETH BERRY-KRAVIS

Introduction 401  
 Clinical Trials in Young Children  
 with FXS 402  
 Clinical Trials of Agents Targeting Glutamate  
 Receptors in FXS 406  
 Clinical Trials of Agents Targeting GABA  
 Mechanisms in FXS 409  
 Clinical Trials of Agents Targeting Cellular  
 Signaling in FXS 412  
 Conclusions 414  
 References 415

20. Reflections on Clinical Trials in  
Fragile X Syndrome

AIA E. JØNCH, SÉBASTIEN JACQUEMONT

Introduction 419  
 Symptomatic Treatments 420  
 A Unique Targeted Drug Development Effort 424  
 Conclusions and Future Prospects in Clinical  
 Trials 435  
 References 437

21. Outcome Measures in Clinical  
Trials for Fragile X Syndrome: The Search  
for Sensitive Neurocognitive Assays

JACALYN GUY, GAIA SCERIF

Rethinking Fragile X Syndrome with a View to  
 Measuring Positive Treatment Outcomes: An  
 Overview 444  
 Beyond Brain-Behavior Links Through  
 Developmental Findings: Implications for  
 Treatment 445  
 Understanding Cognitive Underpinnings of Target  
 Symptoms: Insights from Autism 447  
 Understanding Cognitive and Neural Underpinnings  
 of Symptoms: Implications for Measure  
 Selection 448  
 Concluding Remarks on Current and Future Measure  
 Selection Choices 451  
 References 452

22. Fragile X Research From a Parental  
Perspective

JÖRG RICHSTEIN, JEFFREY COHEN, BECKY HARDIMAN

Introduction 457  
 Survey Methodology 458  
 Results/Data Discussion 460  
 Conclusions 464  
 Looking to the Future: Communication is Key 467  
 Some closing thoughts 469

Index 471

## SECTION I

# CLINICS, DIAGNOSIS, EPIDEMIOLOGY, MOLECULAR MECHANISMS, AND MODELS

- 1 *The Clinical Phenotype of the Fragile X Syndrome  
and Related Disorders* 3
- 2 *Fragile X Syndrome Genetics* 19
- 3 *Molecular Diagnostics and Genetic Counseling in Fragile  
X Syndrome and FMR1-Associated Disorders* 41
- 4 *Epidemiology of Fragile X Syndrome* 57
- 5 *Mechanisms of Repeat Instability in Fragile  
X Syndrome* 77
- 6 *Modeling Fragile X Syndrome Using Human  
Pluripotent Stem Cells* 103
- 7 *Animal Models of Fragile X Syndrome* 123

## SECTION I

# CLINICS, DIAGNOSIS, EPIDEMIOLOGY, MOLECULAR MECHANISMS, AND MODELS

- 1 *The Clinical Phenotype of the Fragile X Syndrome  
and Related Disorders* 3
- 2 *Fragile X Syndrome Genetics* 19
- 3 *Molecular Diagnostics and Genetic Counseling in Fragile  
X Syndrome and FMR1-Associated Disorders* 41
- 4 *Epidemiology of Fragile X Syndrome* 57
- 5 *Mechanisms of Repeat Instability in Fragile  
X Syndrome* 77
- 6 *Modeling Fragile X Syndrome Using Human  
Pluripotent Stem Cells* 103
- 7 *Animal Models of Fragile X Syndrome* 123

