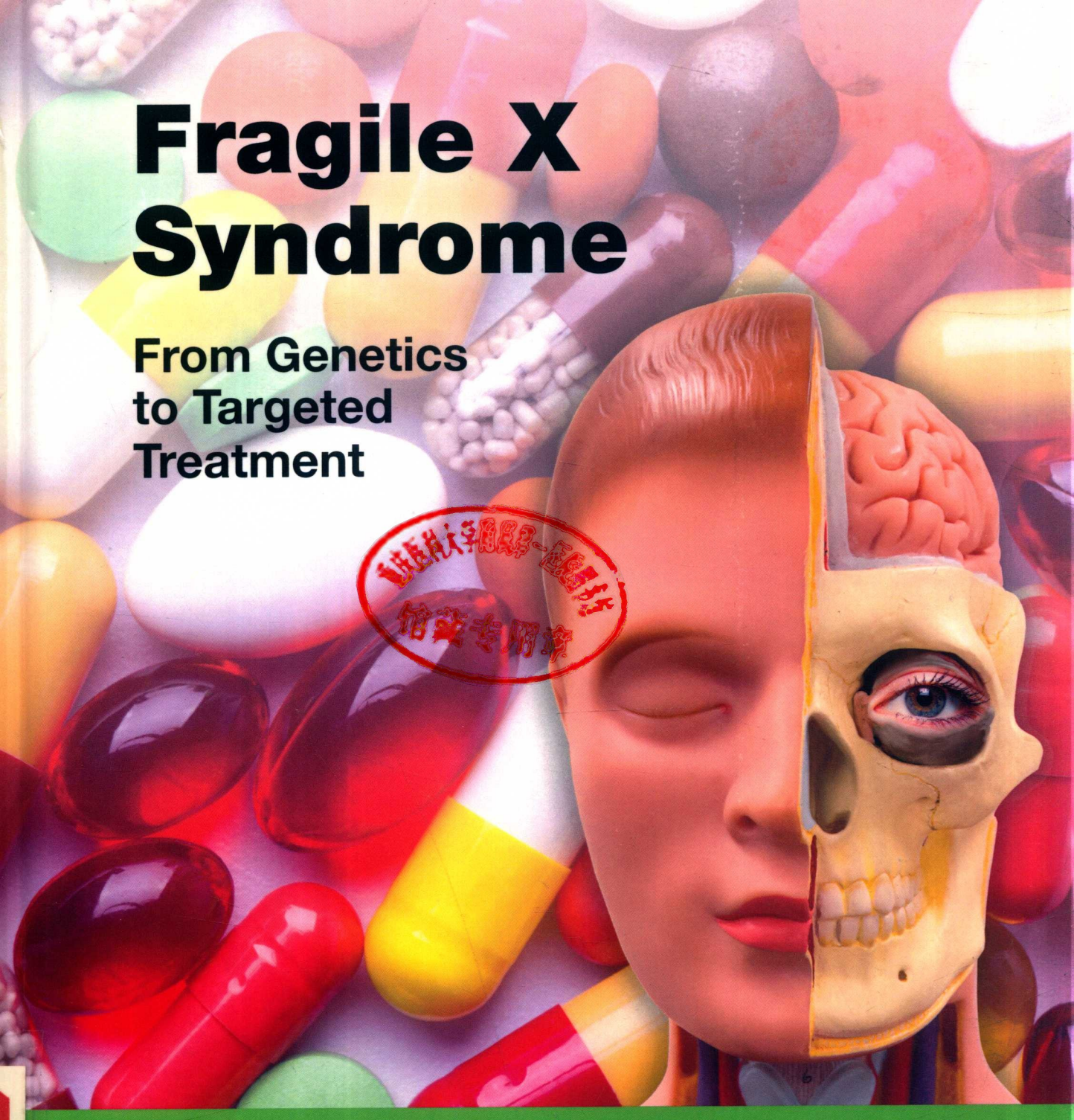
Fragile X Syndrome

From Genetics to Targeted Treatment



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FRAGILE X SYNDROME

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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

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