OXFORD MONOGRAPHS ON MEDICAL GENETICS NO. 13

Fragile Sites on Human Chromosomes

GRANT R. SUTHERLAND

FREDERICK HECHT

OXFORD MONOGRAPHS ON MEDICAL GENETICS NO. 13

Fragile Sites on Human Chromosomes

GRANT R. SUTHERLAND
Chief Cytogeneticist
Adelaide Children's Hospital
North Adelaide, South Australia

and

FREDERICK HECHT
President and Director
The Genetics Center
Southwest Biomedical Research Institute
Tempe, Arizona

With Contributions by

John C. Mulley
Adelaide Children's Hospital
North Adelaide, South Australia

and

Thomas W. Glover and Barbara K. Hecht
The Genetics Center
Southwest Biomedical Research Institute
Tempe, Arizona

NEW YORK OXFORD
OXFORD UNIVERSITY PRESS
1985

Oxford University Press
Oxford London New York Toronto
Delhi Bombay Calcutta Madras Karachi
Kuala Lumpur Singapore Hong Kong Tokyo
Nairobi Dar es Salaam Cape Town
Melbourne Auckland

and associated companies in
Beirut Berlin Ibadan Mexico City Nicosia

Published by Oxford University Press, Inc.,
200 Madison Avenue
New York, New York 10016

Published in Great Britain by Oxford University Press,
Walton Street, Oxford OX2 6DP

Library of Congress Cataloging in Publication Data

Sutherland, Grant R.
Fragile sites on human chromosomes
(Oxford monographs on medical genetics; no. 13)
Includes index.
1. Fragile X syndrome. 2. Human chromosome abnormalities. I. Hecht, Frederick. II. Title. III. Series.
[DNLM: 1. Chromosome Fragile Sites. 2. Sex Chromosome Abnormalities. QS 677 S966f]
RJ506.F73S87 1985 611'.01816 84-14874
ISBN 0-19-503542-9

Printing (last digit): 9 8 7 6 5 4 3 2 1

Printed in the United States of America

Preface

Fragile sites are a frontier in human genetics. Discovered in 1965, they were a minor curiosity for more than a decade. Active exploration of fragile sites began in the late 1970s when the fragile X was recognized to be a common chromosome abnormality in mental retardation.

Fragile sites have drawn attention for their apparent association with the origin of chromosome rearrangements in cancer. They have also come under consideration as possibly predisposing to new heritable chromosome rearrangements.

Highly specific tissue culture conditions are needed to demonstrate the fragile X and other fragile sites on chromosomes. Cytogenetic laboratories have to adjust their methods to insure the detection of fragile sites. Clinicians have to contend with a new type of chromosome anomaly. Investigators have a new array of phenomena to study.

The genetic behavior of fragile sites is extraordinary. Only now is this behavior beginning to be understood through the application of classical genetics and DNA methods. New questions about fragile sites are arising faster than old questions are being answered.

The knowledge in this book can be viewed from two perspectives that are diametrically opposed. One perspective is to look at what has been learned about fragile sites. From next to nothing, much has accumulated. As Pascal put it, "How many stars have telescopes revealed to us which did not exist for the philosophers of old?" (*Les Pensées,* 1670). The second perspective is to see how little we really understand and still need to fathom. In T. S. Eliot's words: "All our knowledge brings us nearer to our ignorance." (*The Rock,* 1934).

This book takes these two perspectives. It provides information, some published, some unpublished, about fragile sites. The unpublished data come from our laboratories and those of many colleagues. The book tries, too, to point out where gaps in knowledge lie, gaps far greater in dimension than all the published and unpublished particles of knowledge about fragile sites.

The many sides of fragile sites are the subject of this book. It moves

from the laboratory bench to the patient to concepts. After a general introduction (Chapter 1), the laboratory section (Chapters 2–5) considers the cytogenetics of fragile sites, the tissue culture conditions required to see them, and the biochemistry of fragile site expression. The clinical section (Chapters 6–10) is devoted to X-linked mental retardation, the fragile X phenotype, and genetic counselling with fragile sites. The genetics section (Chapters 11–15) concerns a number of intriguing properties of fragile sites, including population cytogenetics and segregation analysis, linkage and fragile sites in the etiology of cancer, and constitutional chromosome changes. The final chapter (Chapter 16) outlines part of what needs to be learned about fragile sites. Every part of the book touches on other parts to make the whole.

Contrary to premature announcements of the death of cytogenetics, this book gives evidence that it is alive and well. Cytogenetics provides many problems worthy of study such as fragile sites. For a number of years to come, there will be a need for classical cytogeneticists as well as for the new molecular cytogeneticists to investigate fragile sites on chromosomes and other mysterious areas in cytogenetics.

As a guide to the detection of fragile sites, this book may be of practical use in diagnostic cytogenetics. As a companion to the investigation of fragile sites, the book may also serve research cytogenetics.

To those who care for patients, we hope we have presented a clear picture of the fragile X cytologically, biologically, and clinically, since it is the most common chromosome abnormality next to trisomy 21 in mental retardation. Persons with the fragile X chromosome range from being of normal intelligence to being profoundly mentally retarded. They vary from normal to abnormal in behavior, and physically their features may appear normal or abnormal. There is not always a family history of X-linked mental retardation to point the way. Diagnosis is a challenge, as are management and genetic counseling.

To those concerned with cancer, the current concept is that fragile sites set the stage for chromosome changes in cancer: changes in chromosome structure, gene function, and cell behavior. Until fragile sites are clearly shown not to be connected to cancer, this book may be important in studies of oncogenesis.

For those concerned with constitutional chromosome abnormalities, the same considerations apply. Fragile sites may predispose to heritable chromosome rearrangements which can cause pregnancy wastage, congenital malformations, and mental deficiency.

This book is not a single-authored monograph nor a series of diverse chapters by many authors. It is the product of our two centers, one in South Australia and one in Arizona, with persons from each center contributing materials. Thomas W. Glover contributed to Chapter 3 and wrote Chapter 5; John C. Mulley wrote Chapter 12; Barbara K. Hecht contributed to Chapters 14 through 16 and participated actively in the general production of the book; part of the work reported in Chapter 13 was

carried out in collaboration with P. B. Jacky. The two senior authors took full liberties to revise, revamp, add and subtract materials, fuse styles, avoid unwanted repetitions, emphasize key points, and speed the book's completion in the brief (four-month) span of time available to us.

Fragile sites are revolutionary in concept and nature. Chromosome breakage, once thought to be random in distribution, is not random. It tends to occur at certain hereditary points on chromosomes, namely, at fragile sites. The precise nature of these weak links in DNA is conjectural, but it is clear that fragile sites are unique in nature. They may well be transposable elements, movable segments of DNA that insert into the genome, are susceptible to breakage and rearrangement and are capable of exerting position effect, altering the action of genes nearby.

Look at some of what has been learned in the past year or so about fragile sites. There are many more fragile sites than had been envisioned. Their locations tend to be in bands with cancer and constitutional chromosome breakpoints. The segregation of the fragile X is odd.

For fragile sites the past is prologue to the future. Look ahead a little. Excess thymidine induces fragile sites sensitive to thymidine deprivation. Fragile sites are in particular places, usually in light G-bands. The linkage relations of the fragile X are becoming clear.

The search for knowledge of fragile sites is afoot. This search is bringing many fields into contact, including cytogenetics, classical genetics, biochemistry, dysmorphology, psychology, clinical and behavioral genetics, oncology and molecular genetics. These act as individual forces contributing to a final vector, which it is hoped will lead to full knowledge and understanding of fragile sites on human chromosomes.

G.R.S.
F.H.

Acknowledgments

We would like to thank the many people whose assistance helped turn the idea for this book into the volume you are reading. Many authors provided us with preprints of their articles and personal communications, which should mean that the book is not as far out of date at the time of its publication as it might otherwise have been. Most of those who provided assistance to us with various aspects of the studies contained in this book have been thanked in the publications from our laboratories. In particular, however, we would like to thank Elizabeth Baker for her help in collating much of the previously unpublished data from Adelaide, which she has played a major part in generating, and for her assistance in preparing many of the figures. We thank Fred Flohrschutz for preparing figures in Arizona. Our typists, Ruth Allen in Tempe and Athalie Nation in Adelaide, stoically dealt with the numberless revisions of the manuscript which took place during writing and editing. Since this book is the joint effort of our two centers we would like to thank our professional colleagues in Arizona and South Australia who contributed chapters and covered for us while we were writing rather than doing.

Support for the studies reported in this book came in Australia from the National Health and Medical Research Council, the Adelaide Children's Hospital Trust, the Anticancer Foundation of the Universities of South Australia, and the Channel 10 Children's Medical Research Foundation.

In the United States we thank the National Cancer Institute especially and the National Institute of Child Health and Human Development and the Bureau of Community Health Services, Bureau of Health Care Delivery and Assistance for the Arizona and Nevada Genetics Programs.

This book was conceived in 1982 while Frederick Hecht was in Adelaide as a guest of the Human Genetics Society of Australasia. The manuscript was then drafted during a stay of Grant R. Sutherland in Tempe made possible by a Fulbright Senior Scholarship and support from the Adelaide Children's Hospital and The Genetics Center of Southwest Biomedical Research Institute. Revision and new writing continued on

identical twin word processors in Adelaide and Tempe. Jeffrey W. House and Susan Meigs and their colleagues at Oxford University Press in New York helped bring the manuscript into publishable form. The distances involved 21.5 hours in time zones from Adelaide to Tempe to New York. The book was a collaborative project and we would appreciate your additions and corrections so that it can be updated as a continuing, reliable source about fragile sites on human chromosomes.

Contents

Fragile Sites on Human Chromosomes

1

Fragile Sites on Chromosomes

Fragile sites on chromosomes are points at which the chromosome is liable to break. They are of interest to cytogeneticists because of the unusual chromosomal aberrations they produce, and to geneticists because they represent a unique situation whereby what is apparently a gene is visible at the cytological level.

The first fragile site on a chromosome was described in 1965, although the nature of this finding was unknown at that time (Dekaban). In 1968, fragile sites were shown to be inherited chromosomal features (Lejeune et al.). These chromosomal phenomena were the subject of sporadic case reports until the late 1970s when two separate events rekindled interest in them.

First came the demonstration that the fragile site on the X chromosome was not the isolated curiosity it had been considered since it was initially reported (Lubs, 1969), but was in some way associated with a common form of X-linked mental retardation (Giraud et al., 1976; Harvey et al., 1977).

The second event was the discovery that certain fragile sites were only expressed if lymphocytes were grown in culture medium TC 199 as opposed to other commercially available media (Sutherland, 1977a,b). The essential feature of TC 199 was shown to be its lack of folic acid and thymidine (Sutherland, 1979a). Consequently, the fragile sites expressed under such conditions are known as folate sensitive fragile sites. There are other fragile sites for which folic acid and thymidine are not important, but which require special tissue culture conditions for expression.

Fragile sites are seen as features of chromosomes when viewed at the metaphase stage of mitotic cell division. Chromosomes may be examined after plain staining (unbanded) when fragile sites are most clearly seen, but when most individual chromosomes cannot be specifically identified and the exact location of the fragile site cannot be determined. Alternatively, any of the now numerous chromosome banding techniques may be applied before the chromosomes are examined. These usually make the fragile sites harder to see, but allow for their precise localization. A combination of both approaches is normally used.

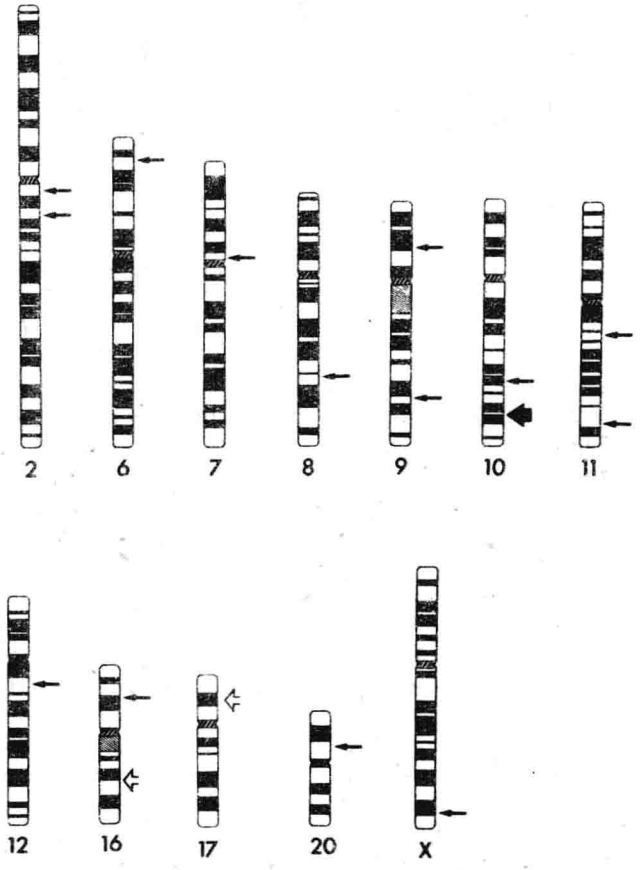

Fig. 1.1a Ideogram of the known rare heritable fragile sites. Group 1 *(small arrows)* at 2q11, 2q13, 6p23, 7p11, 8q22, 9p21, 9q32, 10q23, 11q13, 11q23, 12q13, 16p12, 20p11, and Xq27; Group 2 *(open arrows)* at 16q22 and 17p12; Group 3 *(broad arrow)* at 10q25.

Classification of Fragile Sites

Fragile sites can be divided into two major groups, those which are rare or relatively so and those which are common. Paradoxically, less is known about the common sites than about the rare ones. The rare fragile sites (Fig. 1.1) can be classified according to the conditions of tissue culture under which they are expressed. One guideline is whether they are expressed spontaneously. However, spontaneous expression of fragile sites is an inconsistent phenomenon and as such is *not* a useful criterion for classification. *All* known fragile sites are inducible. Induction leads to their expression or elevates the proportion of cells in which they are seen if they

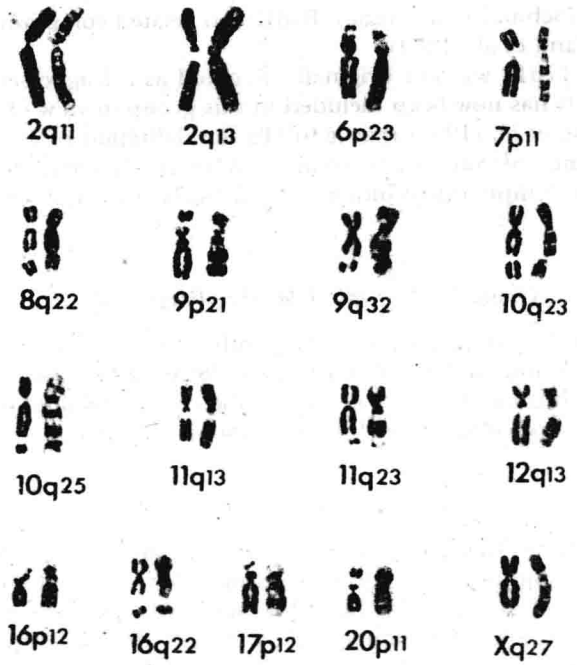

Fig. 1.1b Composite partial karyotype of the known rare heritable fragile sites, showing an unbanded chromosome on the left of each pair and a G-banded chromosome on the right of each pair.

are spontaneously expressed. The mode of induction provides a basis for classification.

Seventeen rare fragile sites are known and, of these, 14 are folate sensitive. Two come into a second group, the distamycin A inducible fragile sites, and a solitary bromodeoxyuridine (BrdU) requiring fragile site constitutes a third group. The common fragile sites form the fourth group.

Group 1: The Folate Sensitive Fragile Sites

The folate sensitive fragile sites are those at 2q11, 2q13, 6p23, 7p11, 8q22, 9p21, 9q32, 10q23, 11q13, 11q23, 12q13, 16p12, 20p11 and Xq27. All these fragile sites are expressed under the same tissue culture conditions.

Group 2: The Distamycin A Inducible Fragile Sites

The fragile site at 16q22 was found not to be folate sensitive. It was originally thought to be expressed independently of tissue culture conditions (Sutherland, 1979a), but was subsequently proved to be inducible with