On the etiology of mental retardation: the clinical genetic approach

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On the etiology of mental retardation

The clinical genetic approach

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The clinical genetic approach

PROEFSCHRIFT

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en volgens het besluit van het College van Decanen,

in het openbaar te verdedigen
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1 INTRODUCTION

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1. INTRODUCTION

1.1 Definition and epidemiology of mental retardation

Mental retardation (MR) is a lifelong disability with a major impact on an individual's life. The condition also determines the life of the whole family to a large extent, as well as the interaction of the affected individual with society. As it affects a considerable proportion of the population, and demands a substantial part of health care services, MR is of major concern to society as a whole.

The understanding of MR has undergone substantial changes during the 20th century. The institution model aimed at separating mentally retarded persons from society, and was discarded for the community model which aspired to integrating them as far as possible. The support model favoured today focuses on the limitations in individual functioning, and the resulting needed supports (Bradley & Knoll, 1995; Schalock and Luckasson, 2004).

Definitions of MR have been given by the American Association on Mental Retardation (AAMR), the Diagnostic and Statistical Manual of Mental Disorders (4th version, DSM-4) of the American Psychiatric Association (1994) and the International Classification of Diseases (ICD) of the World Health Organisation (WHO). Each of these definitions are widely accepted, and all include three basic criteria (Luckasson et al., 1992):

- significantly subaverage intellectual functioning (IQ <70);
- significant adaptive limitations in several skill areas, such as communication, selfcare, the ability to live independently, social skills, the ability to use public services, self direction, academic skills, work, leisure, health, and safety;
- manifestation before 18 years of age.

The most recent AAMR definition is *"Mental retardation is a disability characterized by significant limitations both in intellectual functioning and in adaptive behavior as expressed in conceptual, social and practical adaptive skills. The disability originates before age 18"* (AAMR 2002). In addition, the DSM-4 classification defines various levels of severity which are widely used in practice:

- Mild MR: IQ (50-55) – 70
- Moderate MR: IQ (35-40) – (50-55)
- Severe MR: IQ (20-25) – (35-40)
- Deep MR: IQ below (20-25)

The DSM-4 classification involves ranges of IQs, which indicates that the levels are overlapping sections of a spectrum of grades of severity.

Published rates of prevalence of MR vary considerably. That established by the WHO is close to 3%. However, several studies have found lower rates: 1.2% in Canada (MacLaren & Bryson, 1987); 1.14% on average in the USA, with a signif-

ificant variation between the individual states (Curry et al., 1997; King et al., 1997); 1.13% in South Carolina (Stevenson & Schroer 1996); 1.2% in Atlanta (Murphy et al., 1995); 0.77% in British Columbia (Baird & Sadovnick, 1985); and 0.7% in Sweden (Hagberg & Kyllerman, 1983). In January 2001, at least 111,750 people with MR were living in the Netherlands, consistent with a prevalence of MR of about 0.7%–0.8% (Van Schrojenstein Lantman-de Valk et al., 2002a).

Most studies on prevalence distinguish between mild (IQ 50–70) and severe (IQ <50) MR. Roeleveld et al. (1997) reviewed critically 43 studies performed since 1960, and determined a relatively constant prevalence of 0.38% for severe MR; in contrast, the prevalence rate for mild MR was widely variant, with an average of 3%. The method of identifying people with MR, the age of the study population with a peak prevalence of MR usually around age 10 years, and the assessment criteria are all variables which critically influence the outcome of the various studies (Roeleveld et al., 1997). These differences preclude an objective determination of the prevalence of mild MR.

In most studies on MR, an excess of male individuals has been documented in particular for mild MR, with a male/female ratio ranging from 1.25:1 to 2.1:1 (Murphy et al., 1995; Stevenson & Schroer, 1996; Roeleveld et al., 1997). This male predominance is (partly) caused by genes on the X-chromosome. During the last few years, knowledge on X-linked causes of syndromic and non-syndromic MR has dramatically increased.

1.2 Care of mentally retarded people in the Netherlands

In 1996, about 25 % of the mentally retarded persons in the Netherlands lived in an institutional residential setting, 17 % lived in small community-based residential supports (e.g. GVT, Gezins Vervangend Tehuis), and 58 % lived at home with their families (Kommer et al., 1999). In 2001, five years later, the proportion of individuals living at home had decreased slightly to 53 %; the remaining 47 % lived in a residential setting, but with a remarkable increase in the proportion living in various forms of small community-based groups (van Schrojenstein Lantman-de Valk et al., 2002a). It is expected that an additional 10,000 individuals with MR will move from institutions to small-scale housing in the coming years.

In the Netherlands, a general practitioner (GP) is most often the central medical care provider for persons with MR, in particular when these live at home or in a small-scale community-based housing. In contrast, specialised Physicians for Persons with Intellectual Disabilities (Arts voor Verstandelijk Gehandicapten, AVG) often serve institutions for mentally retarded people in the Netherlands. This medical specialisation was founded in the Netherlands in 2000. It requires a

training period of three years, which includes a facultative residence for several months at a Department of Clinical Genetics.

Mentally retarded individuals have twice as many health problems as people without MR (van Schroyensteen Lantman-de Valk et al., 2000b; Jansen et al., 2004). This may be complicated by communication problems which can be due to sensory impairment but can also have many other causes (Meijer et al., 2004). The specific health needs of individuals with MR require both specialised and adapted primary health care. Recently, the core elements of adequate primary health care for individuals with MR were proposed in a first European Manifesto (Meijer et al., 2004).

The health problems of mentally retarded persons are, however, partly related to the underlying causes of their individual MR. Consequently, the provision of appropriate preventive management depends strongly on an understanding of the cause of MR. In addition, the nature of the psycho-social care provided may depend on knowledge of the cause of the MR, particularly as developmental and behavioral profiles become known for an increasing number of MR syndromes. The clinical genetic evaluation of MR thus is an important part of specialist care for mentally retarded persons.

1.3 Aim of the Thesis

This thesis aims at providing an overview of the current state of the art of diagnostic investigation in MR, focusing on the clinical genetic approach. The goal is to illustrate the clinical evaluation as well as representative parts of cytogenetic, molecular and metabolic investigations. In Chapter 2, recent studies on the etiology of MR are critically reviewed, and the term “diagnosis” is discussed. A novel and dynamic method of classification of the causes of MR is proposed. A protocol for the clinical genetic evaluation in MR is presented in Chapter 3. The goal is to highlight: (i) a multi-disciplinary approach; and (ii), the particular significance and importance of clinical examinations for defining syndromes, for determining further diagnostic procedures by laboratory testing, and for research purposes. This approach is illustrated by the presentation of a particular syndrome (oculocerebrocutaneous syndrome) and its associated pattern of brain malformations. Chapter 4 focuses on chromosome analysis in the mentally retarded. It gives a summary of the evolution of different techniques and illustrates the benefit of combined techniques. It also reviews cryptic subtelomeric chromosome abnormalities found at the Department of Clinical Genetics in Maastricht. Causes of MR which can be diagnosed by molecular studies are discussed in Chapter 5. Fragile X and other X-linked syndromes, as well as important autosomal MR syndromes are briefly presented. The relationship between syndromic and non-syndromic X-linked MR is discussed, and is exemplified by a study on *MECP2*

related disorders in male patients. In Chapter 6, the contribution of metabolic studies to the evaluation of adult persons with MR is examined. Completing the considerations of different aspects of diagnostic evaluation of MR, Chapter 7 deals with the benefits of the investigations discussed in previous chapters, and discusses future perspectives.

This thesis is thus based both on research which has been previously published, and on new research which will be presented here. It is hoped that it will serve as a practical tool for care-providers for mentally retarded persons, such as Physicians for Persons with Intellectual Disabilities (AVGs), clinical geneticists, GPs, and pediatricians.

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2 ETIOLOGY OF MENTAL RETARDATION

The outcome of diagnostic studies on the etiology of mental retardation: Considerations on the classification of the causes

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2 ETIOLOGY OF MENTAL RETARDATION

The outcome of diagnostic studies on the etiology of mental retardation: Considerations on the classification of the causes

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ABSTRACT

In the different studies on the etiology of mental retardation (MR), a diagnosis has been reached in a remarkably variable proportion of cases. This is due to differences in methodology (population examined, age of the patients, degree of MR, time and protocol of investigation, expertise), method of classification (according to timing of defect, type of defect or both), and, in particular the different use of the term diagnosis. The latter varies with the employed criteria (causal vs pathomorphological vs pathophysiological), with the degree of exclusiveness (monocausal vs multifactorial/predisposing background factors) and particularly with the degree of implied certainty (definite vs provisional). All these factors contribute to the fact that in general, the results of different clinical studies on the etiology of MR are not strictly comparable and cannot be pooled. A dynamic method of classification is proposed which combines both time of occurrence and type of defect, and includes the degree of certainty of a diagnosis. The method allows identification of patients which need follow-up and reconsideration of their cases.

Key words: classification of causes; diagnostic yield; etiological studies; mental retardation

INTRODUCTION

In the different studies on the etiology of developmental delay (DD) or mental retardation (MR), a diagnosis has been reached in a widely varying proportion of cases. Two examples illustrating the spectrum of outcome are the studies by, on the one hand, Battaglia et al. who "reached a causal/pathogenetic diagnosis in 80.8% in a cohort of children referred for evaluation of DD/MR" [1999] and, on the other hand, the study performed by Hunter in 2000 who "made a specific genetic/syndrome diagnosis in 19.9% of cases". The remarkably variable yield of the diagnostic studies on MR has been discussed repeatedly [Anderson et al., 1996; Curry et al., 1997; Stromme & Hagberg, 2000] and was most often attributed to methodological differences, such as differences in population, time and protocol of investigation. However, as these differences are relatively small between the two cited studies – both being performed in the same period, by experts in the field, and in a cohort of children with DD of variable degree – it is likely that other factors play an important role. Hunter stated that the rate of established diagnoses depends significantly on the definition of a diagnosis [2000]. A comparison of different clinical studies on the etiology of MR performed since 1981 [Hagberg et al., 1981; Fryns et al., 1986; McQueen et al., 1986; Dereymaeker et al., 1988; Lamont et al., 1988; Fryns et al., 1990; Wellesley et al., 1991; Ohdo et al., 1992; Majnemer et al., 1995; Anderson et al., 1996; Root et al., 1996; Saul et al., 1996; Battaglia et al., 1999; Hunter, 2000; Stromme & Hagberg, 2000; Van Buggenhout et al., 2001; Wilska et al., 2001; Devriendt et al., 2003] shows that apart from methodological aspects in general, different classification systems, and in particular a remarkably differing use of the term diagnosis contribute to the extremely varying results.

In the first section of this paper, the factors influencing the outcome of diagnostic studies on the etiology of MR will be discussed. This is followed by the introduction of an adapted method of classification of the causes of MR which includes the degree of certainty of a diagnosis.

DISCUSSION

General aspects of methodology

Clinical studies on the etiology of MR vary with:

- the selection and ascertainment source of cases. Most studies are either community or institution based. The former are mostly based on registration charts whereas the latter are ideally based on a complete and uniform investigation including history, physical examination and laboratory tests. On the

other hand, institution based studies often investigate a highly selected group of patients, e.g. patients with severe MR [e.g. Fryns et al., 1986]. A review of previous studies, in particular of institutionalized mentally retarded patients was given by Van Buggenhout et al. [2001] and includes a synopsis of populations and outcomes. Furthermore, there are some studies reporting on a population seen at a specialized diagnostic center or a general genetic clinic; in these studies, the referring specialist plays an important role [Hunter, 2000].

- the age and degree of MR of the patients. It is well known that a younger age increases the likelihood of establishing a specific diagnosis [Curry et al., 1997], in part because of the availability of information about pre-, peri- and postnatal development, medical and family history, and the fact that underlying genetic causes may lead to increased mortality at a young age. An IQ roughly <50 (moderate, severe and profound MR) probably also increases the proportion of established diagnoses [Anderson et al. 1996; Raynham et al., 1996].
- the time and protocol of investigation. The progress of laboratory and imaging techniques, and clinical syndrome delineation has led to slowly increasing numbers of causal, well established diagnoses. With chronological follow up of patients, this development not only reduces the number of cases with unknown etiology, but it also eliminates previous diagnoses without or with reaching new ones, or confirms previously clinical diagnoses by laboratory test and gives a pathogenetic explanation.
- the expertise of the investigator(s).

Variability in all these aspects contributes to the vast differences in outcome.

Aspects of classification

The causes of MR can be classified basically according to

1. the type of defect in genetic, environmental and unknown causes,
2. the timing of defect in pre-, peri-, postnatal and unknown causes,
3. the timing and type of defect, thus, a combination of both.

Admittedly, this is an arbitrary academic categorization, and, as will be shown, it is not a sharp categorization. Its purpose is to elucidate underlying classification aspects and their influence on the outcomes.

The first category (classification according to the type of defect) is illustrated by the large South Carolina population study by Anderson et al. [1996] who classified the causes of 7600 persons with MR. In this study the category "genetic causes" is subdivided into chromosomal, single gene, multifactorial, culturofamilial disorders and known syndromes, and the category "environ-

mental causes" splits into pre-, peri- and postnatally acquired causes. The study thus approaches the third (combined) category.

An example of the second category (classification according to the timing of defect) is the community based Western Australia study by Wellesley et al. [1991] who classified the causes of DD of 1480 children with an IQ <70. As in other studies [Stromme and Hagberg, 2000], prenatal causes are subdivided into genetic, acquired and unknown prenatal causes, thus again approaching the third category. In these and other studies results are subsequently translated into results fitting the second category in order to determine the proportions of genetic and environmental causes. Unfortunately, at this point the flow of data can not be followed. In other terms, in order to distil the proportion of genetic causes of MR, classifications according to timing or type of defect are not per se translatable.

A more sophisticated use of the first category has led to the third one which combines the classifications based on timing and type of defect. A recent example is the classification method delineated by Wilska and Kaski [2001]. The causes of MR are divided into genetic causes, CNS malformations, or syndromes of unknown origin, external prenatal factors, perinatally acquired, postnatally acquired and untraceable/unclassified/unknown disorders. The method takes into account differences between causal genetic diagnoses, as chromosomal or single gene disorders, and pathomorphologically or pathophysiologically descriptive constitutional diagnoses (see below): a CNS malformation might be purely genetic but it might also be caused by teratogenic factors or a disruption. The Belgian studies dating from the late '80s [Fryns et al. 1986; Dereymaekers et al., 1988; Fryns et al., 1990] are yet less elaborated precursors of the same method.

The use of the term diagnosis

The use of the term diagnosis varies considerably between the studies in question. Firstly, it varies with the employed criteria: the diagnosis may be a causal (e.g., chromosome aberrations, single gene disorders as fragile X syndrome), a pathomorphological (e.g., holoprosencephaly of unknown cause, syndromes of unknown origin) or a pathophysiological diagnosis (e.g., culturofamilial MR). Pathomorphological and pathophysiological diagnoses should be considered as provisional ones, open to reclassification when the underlying cause(s) become(s) known. So far, the different studies classify CNS malformations and/or syndromes of unknown origin differently: as a constitutional disorder [Fryns et al., 1986; Dereymaeker et al., 1988; Fryns et al., 1990], pathogenetic diagnosis [Battaglia et al., 1999] or as a condition of unknown (prenatal) cause [Wellesley et al., 1991; Ohdo et al., 1992; Stromme & Hagberg, 2000].

Secondly, the term diagnosis varies in particular with the degree of implied certainty: the diagnosis can be definite or provisional. In general, chromosome aberrations are definite causes of MR but have to be coded as provisional when the meaning is still unclear or subject to further research (e.g., seemingly balanced *de novo* structural rearrangements, insufficiently delineated deletions found by the use of microarray techniques). Also single gene disorders most often are definite diagnoses but should be open for reconsideration if a strong clinical suspicion cannot be confirmed by molecular testing (e.g., Rett syndrome without *MECP2* mutation). The degree of certainty of a diagnosis plays an even more important role with regard to external, particularly to perinatal factors. The majority of studies include perinatal risk factors into the end result of reached diagnoses [Hagberg et al., 1981; Fryns et al., 1986; Dereymaeker et al., 1988; Lamont et al., 1988; Fryns et al., 1990; Wellesley et al., 1991; Majnemer et al., 1995; Anderson et al., 1996; Battaglia et al., 1999; Van Buggenhout et al., 2001; Devriendt et al., 2003]. As perinatal risk factors are highly subjective and often insufficiently documented, in most studies poorly standardized and may arise secondary to an underlying genetic disorder, studies are not strictly comparable in this point. To a certain degree, the same holds true for other external causes (infections).

Thirdly, the term diagnosis varies with the degree of exclusiveness: an identified cause can be the only or at least the predominant cause of MR, but it can also be part of a multifactorial genesis or it may contribute to predisposing background factors. The term “cultural-familial MR” in a diagnostic sense does not only lack causality and clarity, it often describes only some of several factors contributing to MR. Thus, it should be used with caution and awareness of its limitations. In general, the degree of exclusiveness of an identified or suspected cause is difficult to integrate into any classification method other than by including the category “multifactorial”. At this point, a genetic method of classifying the somatic causes of MR has a common surface with broader classifications as handled by the American Association on Mental Retardation [2002].

In summary, methodological factors, different classification methods, and in particular a different use of the term diagnosis contribute to the fact that, in general, results of different clinical studies on the etiology of MR are not strictly comparable and cannot be pooled.

Consequently, summarizing the data of different studies should be avoided. The much appreciated review of several diagnostic studies on MR by Curry et al. [1997], set up to provide guidelines for the evaluation of MR, emphasizes in the discussion the limitations of pooling data (“...it is difficult, if not impossible, to compare clinical series...”) and points to methodological differences and “vastly different classification methods”. However, what will be remembered (and often cited) is the suggestion that “a diagnosis/cause of the MR can be identified in 40–60% of all patients undergoing evaluation”. It is noteworthy that in this study, the

table presenting the "general distribution by causation" pools causal, pathomorphological and pathophysiological diagnoses as well as definite and provisional ones.

Despite the present progress in delineating the genetic determinants of MR, it is likely that a group of patients will remain unexplained. Thus, it seems appropriate to focus on the qualitative aspects of the evaluation of MR and to assign more adequately a different diagnosis methodology. The following suggestion of a classification method is meant to contribute to resolving this problem.

Dynamic classification method: a proposal

The proposed classification method is shown in Table I and basically distinguishes genetic causes, unknown causes including a group of malformative disorders, and acquired causes. In addition, to the diagnoses established as genetic or acquired, a degree of certainty is assigned. Category I (definite) is assigned only in case of a clinically or by laboratory testing definitive diagnosis. In all other cases, the diagnosis belongs to category II (provisional). Provisional means that the security in establishing the diagnosis is more than 50%, e.g., it is more likely that the considered diagnosis is correct than incorrect. Provisional diagnoses include possible and probable ones with a security of 51 to 79% and 80% or more, respectively. Diagnoses proposed with a security of less than 50% should not be classified as diagnoses. Per definition, cases belonging to "unknown" (2.1–3.5) are categorized as having a provisional diagnosis (II).

Genetic causes (1.1–1.4) are categorised as: chromosomal disorders (1.1), monogenic disorders including nonsyndromic X-linked MR (MRX) (1.2), well-delineated known syndromes (1.3), and multifactorial causes including cultural-familial MR (1.4). The known syndromes group (1.3) is represented by a recognizable pattern of anomalies without established etiology, and by syndromes that can be caused by different pathogenetic mechanisms or in which a monogenic cause has been shown for a part of the patients only. By convention, microdeletion syndromes are also categorized as known syndromes and not as chromosomal. Following the proposed approach, code 1.1 I would be assigned to a patient with a *de novo* deletion 1p36.3, or with a chromosomal imbalance resulting from a familial translocation; code 1.1 II to an individual with a *de novo* reciprocal translocation, or a with a submicroscopic terminal deletion 2q and parents unavailable for testing; code 1.2 I to a person with fragile X syndrome proven by FMR1 analysis, or to a patient with Sanfilippo A syndrome based on biochemical and enzyme analysis; code 1.2 II to a patient with MRX based on the phenotype and family history, or to a person with clinically probable Smith-Lemli-Opitz syndrome in whom no cholesterol screening could be performed; code 1.3 I to a patient with Angelman syndrome confirmed by molecular analysis,

or to an individual with a clinically convincing diagnosis of Costello syndrome; code 1.3 II to a patient considered clinically as having probably Rubinstein-Taybi syndrome in whom no *CREBBP* mutation or chromosomal anomaly could be found, or to a girl with a Rett variant and negative *MECP2* analysis; code 1.4 I to a child with mild developmental delay, normal phenotype and normal results on testing, born to parents of borderline intelligence, with further borderline or mildly retarded family members; code 1.4 II to the same child when adopted with only restricted information on the parents.

It is evident that a growing number of disorders theoretically can be included in group 1.2 or group 1.3, e.g., Cornelia de Lange or Noonan syndromes. Assignment to 1.2 is advisable when the diagnosis has been confirmed by molecular studies or, in general, for a recognizable pattern of anomalies caused by a specific monogenic defect in the majority of the patients. Although arbitrary to a certain degree, the distribution in groups 1.2 and 1.3 is not crucial as both belong to genetic causes and as the classification in definite and provisional diagnoses is more important.

Cases with MR of unknown origin comprise an important subgroup of malformative disorders (2.1-2.3), splitting up into MR with pure CNS malformations, e.g., holoprosencephaly of unknown origin (2.1), MR with CNS malformations and other congenital anomalies (2.2), and MR with congenital anomalies other than CNS malformations and/or dysmorphism (2.3), all of whom do not fit any known syndrome or monogenic disorder. It is strongly recommended that patients with newly described, insufficiently delineated syndromes of unknown origin are included in one of these groups and not in group 1.3.

Groups 3.1-3.5 are for the unknown cases *sensu strictu* with unexplained MR without congenital malformations other than micro- or macrocephaly. This group is subdivided into: MR only (3.1), MR in association with microcephaly or macrocephaly (3.2), MR in association with neurological symptoms as spastic paresis, seizures, hypotonia, dystonia, athetosis or ataxia (3.3), MR in association with psychiatric illness and/or serious behavioral problems (3.4), and a group of cases which cannot be classified or where information is untraceable (3.5). Patients with micro- or macrocephaly and neurological symptoms preferably are assigned to 3.2 (MR + micro-/macrocephaly) unless microcephaly developed over time secondary to severe neurological problems. If a patient with MR of unknown origin presents concomitantly with neurological and psychiatric signs, the dominant feature is assigned.

Diagnoses of "acquired MR" (4.1-4.3) are subdivided according to the timing of defect into prenatal (4.1), perinatal (4.2) and postnatal (4.3) acquired causes. In agreement with Wilska and Kaski [2001], the perinatal period comprises one week before till 4 weeks after birth. Code 4.1 I would be assigned to a patient with

fetal alcohol syndrome (FAS) and known alcohol intake of the mother, or in the case of documented exposition to other teratogenes, or in the case of serologically proven toxoplasmosis; code 4.1 II to a child with normal high-resolution banded chromosomes and phenotypically FAS but no (known) alcohol intake of the mother, or to a probable mercury embryopathy; code 4.2 I to a patient with evidence of intrapartum metabolic acidosis, early onset of severe neonatal encephalopathy, later spastic quadriplegic cerebral palsy and anomalies on MRI brain consistent with perinatal distress, or to a patient with kernicterus; code 4.2 II to a patient with cerebral palsy and normal results on investigation whose medical records refer to "severe perinatal problems and asphyxia", or to an individual with cerebral palsy and incompletely documented intracerebral bleeding due to intrapartum injury; code 4.3 I to patients with documented traumatic brain damage, or to an individual with proven lead intoxication; and code 4.3 II to a child with encephalitis after smallpox vaccination by history, or with hypernatremic dehydration at two months of age.

The proposed classification method is a basic approach open to refinement by further splitting of categories for individual users. For practical reasons, splitting of category II (provisional diagnoses) into probable and possible diagnoses can be considered. Splitting of group 3.2 into MR with microcephaly and MR with macrocephaly, or subdivision of other groups may likewise be considered. The achieved diagnostic code can be documented on an individual basis in the patient's charts or, more importantly, in the patient registration database. Databases for specific patient (sub)populations are widely used, and include registrations of institutionalized mentally retarded patients. Addition of the diagnostic code allows ready identification of those patients who have a provisional diagnosis and who thus need follow-up and reconsideration of their cases. The method also facilitates classification of the causes of MR for research and epidemiological purposes, as definite and provisional genetic or acquired diagnoses, pathomorphological, pathophysiological and causal diagnoses, can be distinguished and designated differently.

In conclusion, the method thus serves two major goals: determination of follow-up and classification with a more transparent use of the term diagnosis.

ACKNOWLEDGEMENTS

I am very grateful to Bryan Hall for his critical thoughts and helpful suggestions.

Table I: Classification of the causes of mental retardation

	1.1	1.2	1.3	1.4	2.1	2.2	2.3	3.1	3.2	3.3	3.4	3.5	4.1	4.2	4.3
Category	Genetic														
	CH	MG	KS	MF	Malformations		CNS malf.	Unknown							Postnatal
					CNS+ MCA	MR/ MCA		Pure MR	MR + micro/ macro	MR + N	MR + P/B	NC	Prenatal	Perinatal	
I Definite															
II Provisional															

Legends:

- 1.1 CH: chromosomal disorders
- 1.2 MG: monogenic disorders, including nonsyndromic XLMR
- 1.3 KS: known syndromes, including microdeletion syndromes⁷
- 1.4 MF: multifactorial, including cultural-familial MR
- 2.1 CNS malf.: CNS malformations of unknown origin
- 2.2 CNS+MCA: CNS malformation and multiple congenital anomalies
- 2.3 MR/MCA: MR + multiple congenital anomalies and/or dysmorphism
- 3.2 MR + micro/macro: MR + microcephaly or macrocephaly
- 3.3 MR + N: MR + neurological symptoms
- 3.4 MR + P/B: MR + psychiatric illness and/or serious behavioral problems
- 3.5 NC: unclassified/untraceable

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3 CLINICAL STUDIES

- 3.1 Clinical evaluation 30
 - Appendix A, B, C 37
- 3.2 Example of syndrome delineation:
Oculocerebrocutaneous syndrome: the brain malformation
defines a core phenotype
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3.1 CLINICAL EVALUATION

In 1997, the American Consensus Conference has established guidelines for the evaluation of a person with mental retardation (MR) (Curry et al., 1997). Very recently, the NVK (Nederlandse Vereniging voor Kindergeneeskunde, Dutch Association of Pediatrics) has agreed upon an evidence based guideline for the evaluation of children with developmental delay (DD) (to be published in 2005). Based on both and underlying references, in particular the study by Shevell et al. (2003), as well as the author's own experience, the process of evaluation will be described in this and following chapters. The diagnostic process can be divided into three major parts: assessment of the history, physical examination and diagnostic tests, and in general, follows this order.

Assessment of the history takes into account the pre- and postnatal, medical and developmental history, including the development of behavioral features, as well as a pedigree of the family. The pedigree assessment focuses on the occurrence of MR, congenital malformations, stillbirths and recurrent miscarriages, assesses consanguinity, and ideally encompasses at least three generations. Although time consuming, it is of utmost importance that the history of children with DD, or of adult or elderly persons with MR, is thoroughly assessed together with the parent(s) or with a close family member, and/or a personal care provider who knows the person in question well. In the case of institutionalized mentally retarded persons, it belongs to the tasks of the Physician for Persons with Intellectual Disabilities (Arts voor Verstandelijk Gehandicapten, AVG) to bring the family and care providers into contact with the clinical geneticist. It can be helpful to give a questionnaire to the family prior to the conversation (Appendix A). Medical data and results of neuropsychological tests regarding the degree of MR, the developmental profile and behavioral characteristics, have to be assembled and completed. In the Dutch system, these data are most often registered by the GP (general practitioner), in the charts of the institution or, for children, by the coordinating pediatrician. Additional information deduced from photographs taken in infancy, childhood and adolescence are useful to evaluate the changing phenotype with age. This approach is of particular value for the recognition of syndromes which are better recognizable at a younger age, for estimating the onset of microcephaly, and/or to distinguish primary dysmorphism from secondary deformation. When photographs of the family are also available, family resemblance can be taken into consideration.

The physical examination mainly consists of observation, inspection of the body surface, and a basic neurological examination. Contrary to what might be expected by the family, the examination is not a heavy demand on the patient.

Although it is advisable to use a documentation form (Appendix B) to facilitate a structured examination, the examination itself has to be flexible, and adapted as far as possible to the individual patient's needs and habits. For example, the time of examination of a person with an outstanding need for regular structures, e.g. autistic disorders, has to follow his/her schedule; and the examination of an individual with a fear of unknown surroundings should preferably take place in a familiar room. The various steps of the examination itself must be adapted to what the patient admits ("look first at what the patient lets you see"). Apart from the moral and ethical reasons, only a patient-centred procedure allows a realistic impression of his/her behavior, capacities and needs.

Observation aims at a general impression of the personality, of the degree of MR and of the behavior. Of particular interest are posture, movement, walking, mimic, and specific behavioral features such as tics or other stereotypies.

Surface inspection focuses on the recognition of congenital abnormalities and minor anomalies. According to their known or suspected causes, congenital abnormalities are subdivided into three categories (Aase, 1990):

- Malformations. These result from defects of embryogenesis arising during blastogenesis (days 1-28) or organogenesis (days 29-56). If the malformation has little or no effect on normal life or functioning, it is classified as 'mild', and otherwise as 'severe' or 'major' (Opitz, 1985; Opitz, 2000).
- Disruptions and deformations. These both interfere with normal development, the first by various disruptive events (e.g. vascular events, infections), and the second by mechanical disturbances.
- Dysplasias: these are produced by abnormal histogenesis.

In contrast to mild or major malformations, minor anomalies are disturbances of phenogenesis, and arise during fetal (days 57-266) or early postnatal life (Opitz, 2000). They differ from common variants by their prevalence: minor anomalies occur in up to 4% of the general population, and common variants exceed this percentage; together they form the group of 'minor variants' (Aase, 1990). Comprehensive reviews of the extensive discussions of minor congenital anomalies as opposed to mild malformations or normal variants, have been given by Frias & Carey (1996) and Merks et al. (2003). Merks et al. (2003) classify the phenotypic abnormalities found on physical examination conscientiously into common variants, minor anomalies, malformations, and other congenital abnormalities arising as the result of abnormal morphogenesis or the abnormal function of other structures. The use of the classification list in this paper is recommended for the classification of every phenotypic abnormality. It facilitates the determination of the importance of the abnormality, the pathogenetic mechanism, and in syndrome recognition. Beginning with the work by D.W. Smith, several studies have shown that the presence of multiple minor anomalies increases the risk for a major malformation, may predict an aneuploidy or another constitutional

disorder, or may be the clue to the recognition of a specific syndrome (Smith, 1971; for reviews see Frias & Carey, 1996, and Merks et al., 2003). The number and nature of minor anomalies therefore has to be documented even when no particular dysmorphism is recognized.

Subjective impressions have to be replaced by objective measurements whenever possible. Reference tables can be found in the texts of Hall, Froster-Iskenius & Allanson (1989) and of Gerver (2001). As many parameters are influenced by the ethnic background, specific growth charts have been established for different ethnic groups, and these should be used. Of all measurements, head circumference is of particular importance.

Photographs are taken for documentation of the status, and of changes in phenotype over time when serial examinations are possible. They form the basis of (interdisciplinary) consultations. They should include a front and side view of the face (without glasses), an overview with a minimum of clothing, and photographs of both hands and feet. Documentation of a characteristic behavioral trait requires the recording of a video.

Diagnostic tests include

- chromosome analysis at a band level of at least 500–550 bands per haploid set in every person, child or adult with unexplained MR.
- most often a DNA test for fragile X syndrome as it should be strongly considered in all males and females with unexplained MR.
- further DNA tests when clinical examination gives rise to the suspicion of a particular disorder that can be proven by molecular studies.
- targeted metabolic screening, preferably in combination with ophthalmologic evaluation including funduscopy.

These issues will be further discussed in chapters 4–6. The order of testing and whether they are done syn- or metachronously, depend on the clinical presentation. In many situations, a first step of diagnostic tests will include chromosome analysis and fragile X testing, and will proceed to e.g. metabolic testing when the patient shows one or more of the features possibly indicating a metabolic disorder (see chapter 6). It is, however, also possible that a suspicion on clinical grounds directly prompts a particular DNA test, e.g. for Angelman or Rett syndromes, or metabolic testing.

In agreement with the recommendations of the aforementioned Consensus Conference (Curry et al., 1997), a neurological evaluation, including neuroimaging studies (most often MRI), is indicated when the MR is associated with macro- or microcephaly, epilepsy, loss of acquired skills, or either focal neurological sign. However, diagnostic evaluation of a mentally retarded individual is frequently dictated by considerations of patient care rather than purposes of etiological evaluation. In most cases, MRI studies require transport to a hospital, and

general anesthesia. In each individual case, these disadvantages have to be balanced against the possible advantages, which are preventive management for the patient, and genetic counselling for family members in the case of an established diagnosis. Associated brain abnormalities have been described for an increasing number of syndromes, and special MR techniques (e.g. proton MR spectroscopy) have proven useful in the determination of inborn errors of metabolism (Battaglia, 2003). Furthermore, the specific genetic defects which lead to CNS malformations are becoming increasingly well known (Sarnat, 2004; Sarnat & Flores-Sarnat, 2004). Neuro-imaging studies thus contribute to etiological evaluation and subsequent application of medical guidelines and genetic counselling. However, the likelihood of establishing a diagnosis by neuro-imaging studies has not been established in general, but depends on the particular clinical features.

Evaluation by a variety of other specialists is occasionally indicated; most often, ophthalmological, psychiatric, psychological, and/or dermatologic evaluations are needed.

MULTI-DISCIPLINARY APPROACH

It is evident that the evaluation of the genetic etiology of MR interfaces to the work of several other specialists, the most important of which are the Physician for Persons with Intellectual Disabilities, pediatrician, (pediatric) neurologist, a metabolic clinical specialist, psychiatrist, psychologist and ophthalmologist. In this, the clinical geneticist often has a central and coordinating role. It is often useful to see the patient in a multidisciplinary setting, e.g. together with the Physician for Persons with Intellectual Disabilities in Institutions for the Mentally Retarded; in a team consisting of a pediatrician, pediatric neurologist, and clinical geneticist for the evaluation and follow-up of children with DD; or together with a psychologist or psychiatrist when behavioral problems or psychiatric illness, are addressed.

With regard to institutionalized patients, a good cooperation with the Physician for Persons with Intellectual Disabilities is of particular importance. It has been proven useful for the Physician for Persons with Intellectual Disabilities and clinical geneticist to agree upon the distribution of their respective tasks. This agreement may look as follows (adapted from Moog & Dijkstra, 2002):

The Physician for Persons with Intellectual Disabilities has to take an inventory of the population of the institution in order to know who has previously been seen for clinical genetic examination, and when. The admission of a new patient is an excellent moment to check for previous examinations, but a systematic screening of the records will often detect omissions. Often the Physi-

cian for Persons with Intellectual Disabilities is the link between the family, care providers, and the clinical geneticist. It is his/her responsibility to propose a genetic investigation, and to explain basically its different steps as outlined above, and its benefit (see chapter 7.1). In preparing the multidisciplinary consultation, the Physician for Persons with Intellectual Disabilities has to check the charts of the patients to be seen, and to prepare a summary of their medical history, and of previous investigations. A specific documentation form facilitates this work (Appendix C). The Physician for Persons with Intellectual Disabilities takes care of possible referrals to other specialists, and communicates the results to the clinical geneticist. Finally, he/she coordinates the change in care and preventive management based on a particular diagnosis (e.g. Healthwatch).

The clinical geneticist has a central role in coordinating the physical examination and laboratory tests. A pragmatic procedure which minimizes traumatic experiences is crucial for the patient. In addition, the clinical geneticist has to assure a good documentation, and for the communication of the results to the family and the Physician for Persons with Intellectual Disabilities.

Thus, the Physician for Persons with Intellectual Disabilities focuses on the preparation of the examination and any subsequent steps resulting from it, whereas the clinical geneticist's work focuses on the physical examination itself, as well as additional laboratory tests. Together, they take into consideration regular follow-ups and revisions of patients with provisional diagnoses, or with MR due to unknown causes.

SIGNIFICANCE OF CLINICAL EXAMINATION

The above comments on the clinical examination illustrate the importance of a thorough assessment of the history of the patient, and of physical examinations which can involve additional specialists. Syndrome recognition, the need for laboratory tests, and further etiological approaches all depend on the results and conclusions of clinical examinations. An increasing number of genetically determined MR syndromes can be confirmed by cytogenetic or molecular studies. However, primarily the diagnosis is a clinical one. For example, Rett syndrome is based on the history and physical and behavioral findings; Smith-Lemli-Opitz syndrome on the overall phenotype; 4p- syndrome in addition on photographs of infancy and childhood; a cryptic familial chromosome aberration on the family history; and *LIS1* related lissencephaly on a MRI scan of the brain.

Solid and well-documented clinical examinations are also essential for developmental research, to delineate arising genotype-phenotype correlations, and to determine specific genetic defects underlying a clinically known syndrome. Unfortunately, research in this field is often hampered by scarce published clinical

data. The paper by Judith Hall (2003) gives a summary of the clinical information that should be available.

The article on the brain malformations of patients with a rare MR/multiple congenital anomalies syndrome (oculocerebrocutaneous syndrome) which follows in chapter 3.2, illustrates the importance of clinical investigations.

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Appendix (in Dutch)

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APPENDIX A

Ponsplaatje

Naam en voornaam van uw kind:

Geboortedatum:

Thuisadres:

Telefoon:

Wanneer hebt U voor het eerst “iets” opgemerkt bij Uw kind en hoe?

Wat is volgens U de oorzaak?

.....

FAMILIE

Moeder

Naam:

Geboortedatum:

Welk onderwijstype hebt U gevolgd?

.....

Wat is Uw beroep?

Aantal vorige zwangerschappen (voor dit kind):

Aantal volgende zwangerschappen (na dit kind):

Aantal miskramen:

Vader

Naam:

Geboortedatum:

Welk onderwijstype hebt U gevolgd?

Wat is Uw beroep?

Familie (ook overleden familieleden met jaar van overlijden vermelden)

Broers en zussen van de moeder (voornamen en geboortejaar)

.....

Ouders van de moeder (naam + voornaam en geboortejaar)

.....

Broers en zussen van de vader (voornamen en geboortejaar)

.....

Ouders van de vader (naam + voornaam en geboortejaar)

.....

ZWANGERSCHAP

1. Waren er problemen tijdens de zwangerschap ja/nee/onbekend*

Zo ja, welke?

.....

2. Ziekten tijdens de zwangerschap? ja/nee/onbekend*

Zo ja, welke?

.....

3. Nam U medicatie tijdens de zwangerschap? ja/nee/onbekend*

Zo ja, welke?

.....

4. Had U een Röntgenonderzoek tijdens de zwangerschap?

ja/nee/onbekend*

5. Rookte U tijdens de zwangerschap? ja/nee*

6. Gebruikte U alcohol tijdens de zwangerschap? ja/nee*

Zo ja, hoeveel glazen per dag?

BEVALLING

1. Waren er problemen tijdens de bevalling? ja/nee/onbekend*

Zo ja, welke?

2. Duur van de zwangerschap: weken

3. Had U een keizersnede? ja/nee*

4. Hoeveel woog het kind bij de geboorte?
5. Hoe lang was het kind bij de geboorte?
6. Wat was de schedelomtrek van het kind bij de geboorte?

PERIODE NA DE BEVALLING

1. Waren er moeilijkheden in de periode na de bevalling? ja/nee/onbekend*
 Zo ja, welke?
2. Had de baby geelzucht na de bevalling? ja/nee/onbekend*
3. Was de baby Rhesus-factor negatief? ja/nee/onbekend*
4. Welke kleur had de baby na de bevalling? blauw/wit/rose*
5. Hilde de baby onmiddellijk na de bevalling? direct/niet direct*
5. Hoeveel dagen lag de baby in de couveuse?
6. Zoog de baby sterk/zwak*?
7. Waren er afwijkingen bij de geboorte?
8. Was langer verblijf in het ziekenhuis nodig?
- hoelang:
 waarom:
9. Wie was de dokter die Uw kind heeft gevolgd in de eerste levensjaren?
 Naam en plaats:

ONTWIKKELINGSMIJLPALEN

Hoe ontwikkelde Uw kind zich op de volgende punten: vroeg, normaal, laat of nooit?

Vermeld eventueel de leeftijd.

- zitten zonder steun leeftijd: vroeg/normaal/laat/nooit/onbekend*
- kruipen leeftijd: vroeg/normaal/laat/nooit/onbekend*
- lopen zonder hulp leeftijd: vroeg/normaal/laat/nooit/onbekend*

- eerste woordjes leeftijd: vroeg/normaal/laat/nooit/onbekend*
(buiten mama-papa)

MEDISCHE GESCHIEDENIS VAN HET KIND VAN GEBOORTE TOT NU TOE?

1. Is het kind ooit ernstig ziek geweest? ja/nee*
 Zo ja, waardoor
 op welke leeftijd(en)
2. Is het kind ooit opgenomen in een ziekenhuis? ja/nee*
 Zo ja, wanneer en wat was de reden?

3. Had het kind ooit stuipen? ja/nee*
 Zo ja, was dit met of zonder koorts? met koorts/zonder koorts*
 op welke leeftijd?
 hoe vaak?
4. Had het kind ooit oogproblemen? ja/nee*
 Zo ja, welke?

5. Had het kind ooit neus/keel/oorproblemen? ja/nee*
 Zo ja, welke?

* doorhalen wat niet van toepassing is

APPENDIX B

Datum: Huize: Arts:

Naam: Geb.Dat:

VRAAG

Voorgeschiedenis:
.....
.....Familie:
.....
.....

ALGEMEEN

Gedrag:
.....
.....
.....
.....

Sprak: Gehoor:

Nivo: Visus:

Zindelijk: Oogheelkundig ond.: ja/nee

Medicatie:

NEUROLOGISCH

Neurologisch onderzoek: ja/nee jaar:

Lopen: Epilepsie: ja/nee

Type:
.....Tonus:
.....

MATEN

Lengte: Span: Gewicht:
 Habitus: Properties:
 SO: (P...) ICD: (P...) OCD: (P...)
 Oren: (P...)
 Handen: R (P...) Dig 3: R (P...)
 L (P...) Dig 3: L (P...)
 Tepelafstand: (P...)

GELAAT

Dysmorphie: ja/nee

Schedelvorm: Gelaatsvorm:
 Haargrenzen: Wenkbrauwen/wimpers:
 Ogen: Oren:
 Neus: Philtrum: Lippen:
 Maxilla: Manbibula:
 Palatum: Tanden: Nek:

THORAX/RUG

Scoliose: ja/nee

EXTREMITÄTEN

Handen: syndactylie: clinodactylie:
handlijnen:

Voeten:

GENITALIA

Menstruatieanamnese:

HUID

Beharing:
Nagels:

INTERNE AFW

.....

.....

.....

ONDERZOEK

Chromosomen: ja/nee uitslag: jaar: te:

.....

DNA naar ja/nee uitslag: jaar: te:

Metabool: ja/nee uitslag: jaar: te:

CT/MRI-scan: ja/nee uitslag: jaar: te:

Röntgen:.....

SAMENVATTING & CONCLUSIE

.....

.....

.....

ADVIES & BELEID

.....

.....

.....

APPENDIX C

STANDAARDFORMULIER KLINISCHE GENETICA

Naam:

Geboortedatum:

Instelling:

.....

Specifieke vraagstelling:

.....

Mate verstandelijke handicap:

(indien getest, testuitslag vermelden)

Neurologische problemen:

epilepsie

spasticiteit

mobiliteit

overigen

Psychiatrische problemen/ gedragsproblemen:

Algemene voorgeschiedenis:

(partus, begin retardatie, evt. aangeboren afwijkingen, comorbiditeit)

.....

.....

Contact familie/klinische genetica:

(familie bij onderzoek aanwezig - familie komt op polikliniek klinische genetica - telefonisch

contact met familie opnemen - geen familie - anders)

.....

Familie/ curator akkoord?

Zo ja naam en relatie tot client:

.....

Familieanamnese:

(m.n. andere personen met MR of ontwikkelingsachterstand)

.....

Eerder genetisch onderzoek:

(chromosomenonderzoek en ander onderzoek, datum en uitkomst)

.....

Algemeen diagnostisch onderzoek door neuroloog:

(datum, naam en uitkomst)

.....

Beeldvorming hersenen:

(datum, soort onderzoek, uitkomst)

.....

Algemeen diagnostisch onderzoek oogarts:

(datum, naam en uitkomst)

.....

Ander onderzoek:

.....

Actuele medicatie:

..... (dosering niet nodig)

Datum consult:

Gemaakte afspraken:

.....

.....

3.2 Oculocerebrocutaneous syndrome: the brain malformation defines a core phenotype

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ABSTRACT

Background: Oculocerebrocutaneous syndrome (OCCS) is characterised by the presence of orbital cysts and anophthalmia or microphthalmia, focal aplastic or hypoplastic skin defects, skin appendages and brain malformations. The eye and skin abnormalities are well described but the neuropathological features less so. To date, 28 patients with an unequivocal diagnosis of OCCS have been reported, with a preponderance of males.

Objective: To evaluate the brain imaging studies, clinical records, photographs and pathological material of two new and nine previously reported cases of OCCS.

Results: There was a consistent pattern of malformations in eight of the 11 cases, consisting of frontal predominant polymicrogyria and periventricular nodular heterotopia, enlarged lateral ventricles or hydrocephalus, agenesis of the corpus callosum sometimes associated with interhemispheric cysts, and a novel mid-hindbrain malformation. The latter consisted of a giant and dysplastic tectum, absent cerebellar vermis, small cerebellar hemispheres in most cases, and a large posterior fossa fluid collection.

Conclusions: The mid-hindbrain malformation appears pathognomonic for OCCS. The eye and skin features of OCCS show considerable overlap with several other syndromes, such as encephalocraniocutaneous lipomatosis, oculo-auriculo-vertebral spectrum, and focal dermal hypoplasia, none of which has a comparable pattern of brain malformations. In particular the unique mid-hindbrain malformation also distinguishes OCCS from related syndromes with comparable forebrain anomalies. The pattern of malformation described thus helps in differentiating OCCS from other entities. The mid-hindbrain malformation points to a defect of the mid-hindbrain organiser as the underlying pathogenic mechanism.

Keywords: oculocerebrocutaneous syndrome; polymicrogyria; giant tectum; absent vermis

INTRODUCTION

Oculocerebrocutaneous syndrome (OCCS), also known as Delleman syndrome, is a rare multiple congenital anomaly-mental retardation syndrome characterised by the triad of eye, brain and skin malformations, sometimes associated with other features such as craniofacial clefts, skull or rib defects, and urogenital anomalies. We have found reports of 28 patients with unequivocal OCCS, with a preponderance of affected males.[1][2] These reports exclude patients with a possible diagnosis of OCCS or who have features of overlapping syndromes, in particular encephalocraniocutaneous lipomatosis (ECCL) and oculoauriculovertebral spectrum (OAVS). The cause of OCCS is still unknown.

The eye malformations consist mainly of cystic anophthalmia or microphthalmia and colobomata, while the skin abnormalities consist of skin appendages and focal aplasia or hypoplasia. Both have been well described.[1] Many reports mention cerebral cysts, but this might refer to several different anomalies such as arachnoid, porencephalic or posterior fossa cysts, ventricular enlargement, or any other abnormal fluid collection. Several patients have had agenesis of the corpus callosum. However, many of the reports predate modern brain imaging modalities and even recent reports rarely give a detailed description. A systematic description of the brain malformation in OCCS is not available. We therefore reviewed the brain abnormalities in a series of 11 patients with OCCS, using a variety of brain imaging studies and one necropsy report. While some of the images were old and incomplete, we were still able to document a remarkably consistent pattern of malformations that includes a novel mid-hindbrain malformation.

METHODS

We obtained all available data on the brain in 11 patients with OCCS, including medical records with reports of brain imaging studies, clinical photographs (Fig. 1), and all or portions of the original brain imaging studies (Fig. 2-3 and Supplementary Figures). The brain imaging studies included brain magnetic resonance imaging (MRI) in seven patients, cranial computed tomography (CT) in four, and pneumoencephalography (PEG) in one; one patient had both MRI and CT. We also reviewed a necropsy report and limited pathological tissue from the child who had the PEG.

Figure 1. Clinical features of patients 2 and 3. Note focal aplastic defects above the ears and blister-like lesions in patient 2 (A-B). Patient 3 (C) shows left microphthalmia, and a cleft and mass of tissue with a skin appendage in the left lower lid near the outer canthus.

RESULTS

Clinical reports and review of brain abnormalities

Patient 1

This boy was born with a large cystic left eye, right microphthalmia, eyelid colobomas and other ocular anomalies, and typical skin appendages.[3] When re-evaluated at six years, he had severe mental retardation, intractable epilepsy, severe right hemiparesis, and decreased tactile sensation.

Brain MRI (Fig. 2 and 3A-D) showed severe polymicrogyria (PMG) with very thick cortex in the left frontal, temporal and perisylvian regions, at least one underlying periventricular nodular heterotopia (PNH), enlarged lateral ventricles greater on the left, and agenesis of the corpus callosum (ACC). The midbrain was angled forward, resulting in a nearly horizontal aqueduct that abruptly enlarged into the fourth ventricle about half way down the midbrain. The midbrain tectum was massively enlarged (to around 3 cm in length), rotated far upwards of the normal position behind the midbrain tegmentum, and indented inferiorly by the enlarging aqueduct into an arched shape. The superior cerebellar peduncles were long, thick and straight, extending almost vertically downward to join the cerebellar hemispheres, which were almost normal in size. The cerebellar vermis was completely absent, although a small and unusual white matter tract connected the two hemispheres. The fourth ventricle was continuous with a large posterior fossa fluid collection, suggesting cystic enlargement. Similar images from a normal MRI study are shown for comparison in Supplementary Figure S1.

Figure 2. The classical brain malformation of the oculocerebrocutaneous syndrome (OCCS). Brain MRI from patient 1 providing details of the OCCS brain malformation. Images through the cerebral hemispheres show an irregular surface, reduced sulcation, thick 10-15 mm cortex, and reduced white matter typical of polymicrogyria involving the left frontal, temporal and parietal lobes (F-H, J-P and white arrows in G, K and L). The lateral ventricles are mildly enlarged, especially on the left, and the corpus callosum is absent (E, I and J, black arrows in I and J). Images through the posterior fossa show a massively enlarged tectum and absent cerebellar vermis. The midbrain tegmentum is flexed forward but otherwise normal. The aqueduct is short and nearly horizontal (horizontal white arrow in E), and enlarges into the fourth ventricle behind the upper midbrain. The fourth ventricle is continuous with a large posterior fossa fluid collection. The midbrain tectum is greatly enlarged (black arrows in E, H and O) and rotated upward, and appears to form an arch over the enlarged aqueduct (black arrows in G, L and N). The cerebellar vermis is completely absent (B-G and L-P, black arrows in B, F, K and P). The cerebellar hemispheres are nearly normal in size, and seen in the midline because of the missing vermis (white arrow in E). The superior cerebellar peduncles are thick and dysplastic, descending vertically from the dysplastic midbrain to the cerebellar hemispheres (horizontal black arrow in K). The lower brainstem and spinal cord appear normal.

Patient 2

This boy was born to Mexican parents who were second cousins. His birthweight was 3.66 kg, length 51 cm, and head circumference 36 cm. He had bilateral orbital cysts, a cleft left nostril with denuded skin, left macrostomia, multiple skin lesions, and right cryptorchidism. The skin lesions included a fleshy mass with tags and pits on the left cheek; tags in the middle of his forehead on top of a hemangioma, on his scalp to the left of the anterior fontanelle, and at the tip of the coccyx; a focal aplastic defect above the right and a crescent shaped one above the left ear (Fig. 1A-B), another focal aplastic skin lesion just below the xyphoid process, and three deflated blister-like lesions on the right neck (Fig. 1A). The left orbital cyst enlarged and was removed. Pathological examination showed malformed retinal and glial tissue with dystrophic calcifications. The mass on the cheek was also removed together with a maxillary exostosis beneath it. Pathological examination confirmed a hamartoma. At one year, he had seizures and developmental delay.

Brain MRI at eight months (Fig. 3E-H) showed asymmetric PMG involving the frontal and perisylvian region, several PNH along the anterior body of the left lateral ventricle, mildly enlarged ventricles, and ACC. The PMG, PNH and ventricular enlargement were all more severe on the left. The upper midbrain was angled forward, resulting in a nearly horizontal aqueduct. The aqueduct was short, and abruptly enlarged into an extra ventricle just above the fourth ventricle. The tectum was again massively enlarged (to around 4 cm in length), rotated upward and curved, providing the roof of the extra ventricle. The cerebellar vermis was absent and the hemispheres were probably small from the limited images available. The fourth ventricle communicated with a large posterior fossa fluid collection.

Patient 3

This girl was born to a 33 year old Hispanic woman and her 32 year old unrelated husband. Her birth weight was 3.77 kg, length 50 cm, and head circumference 35 cm. She had multiple anomalies including left microphthalmia, a disorganised mass of tissue with a skin appendage and cleft in the left lower lid near the outer canthus (Fig. 1C), and multiple skin lesions consisting of tags in the left nares, the periumbilical region, the right groin and posterior to the anus; punched out aplastic defects in the left scalp and right heel, and multiple circumscribed hypoplastic defects with depression of subcutaneous tissue and lack of hair over the left leg. On pathological examination, the swelling near the left eye was a benign hamartoma with 46,XX chromosome constitution, while the hypoplastic skin defects were neuroectodermal developmental dysplasias. An omphalomesenteric duct remnant was found in the umbilical cord. Her heart was structurally normal but displaced in the chest by an anterior diaphragmatic eventration

Figure 3. The classical brain malformation of the oculocerebrocutaneous syndrome (OCCS). MRI from patients 1 to 4 also demonstrate the typical OCCS brain malformation. Views of the cortex show polymicrogyria (horizontal white arrows in B-D, G-H, K-L and N-P), which is asymmetric in all four patients with more severe changes on the left (the right side of the images) in patients 1-3 (A-L) and on the right in patient 4 (M-P). Several periventricular nodular heterotopia are seen adjacent to the frontal horns and anterior bodies of the lateral ventricles (black arrows inside the ventricles in B, H and P). The white matter is poorly myelinated in patients 2-4 (E-P) (patient 1 is older). The corpus callosum is absent in patients 1, 2 and 4 (A, E and M), and dysplastic in patient 3 (black arrow in I). Images through the posterior fossa show a massively enlarged and upwardly rotated tectum (long white arrows in A, E, I, and M) and absent vermis (vertical arrows in J and N) in all four patients. In all, the midbrain is angled more forward than normal leading to a short, horizontal aqueduct, enlarging prematurely into the fourth ventricle (A, I, M) or appearing to form an abnormal extra ventricle behind the midbrain (E); the fourth ventricle is continuous with a large posterior fossa fluid collection. The cerebellar hemispheres are small and have a dysplastic foliar pattern in patients 3 and 4 (J and N), and are seen in the midline because of the absent vermis (short white arrows in A, E, I and M).

Figure S1. Normal brain MRI images comparable to Figures 2A-H demonstrate a normal Sylvian fissure (arrow in A), normal cerebellar vermis (arrows in B and D, and bottom arrow in E), normal superior cerebellar peduncles (arrow in C), normal midbrain tectum (top arrow in E and arrow in F), and normal cortex (arrows in G).

that was repaired. A few new skin lesions were seen during the first year, but not thereafter. Serial neurological examinations showed dysarthria, hypotonia and a mild right hemiparesis. Cognitive functioning at three years was normal. An electroencephalogram was normal. Otoacoustic emission and sound field testing documented normal hearing bilaterally.

Brain MRI at one day (Fig. 3I-L) showed asymmetric PMG in the left frontal and perisylvian regions, possible PMG in the right frontal lobe, and mildly enlarged left lateral ventricle. No heterotopia were seen but the resolution was low. The corpus callosum was dysmorphic with a thin body and absent splenium. The midbrain was angled slightly forward and the lower aqueduct was mildly enlarged. The midbrain tectum was enlarged (to around 1.5-2 cm) but not rotated upward. The cerebellar vermis was absent and both hemispheres small, especially the left. The fourth ventricle was large and communicated with a large posterior fossa fluid collection.

Patient 4

This boy was blind because of bilateral cystic anophthalmia, and had typical skin appendages. His case has been published in detail previously.[1] [4] At eight years, he had severe mental retardation and epilepsy.

Brain MRI (Fig. 3M-P) showed frontal PMG extending to the perisylvian, parietal and temporal regions, PNH along both frontal horns and lateral bodies of the lateral ventricles, mildly enlarged lateral ventricles, and complete ACC. The

cortical malformation was more severe on the right. The midbrain was anteverted resulting in a nearly horizontal orientation of the aqueduct, which was mildly enlarged in its inferior portion. The tectum was moderately enlarged (1.5–2 cm) and round. The cerebellar vermis was absent and the hemispheres both very small. The fourth ventricle was continuous with a large posterior fossa fluid collection.

Patient 5

This boy had left cystic microphthalmia and eyelid coloboma, and typical skin lesions of OCCS.[1] [4] At 26 months, he had moderate mental retardation and epilepsy.

Brain MRI (Fig. S2C–D) at seven months and later CT (Fig. S2E–H) showed frontal PMG extending an uncertain distance posteriorly, more severe on the right. The lateral ventricles were enlarged, left more than right and separated by several interhemispheric cysts posterior to the third ventricle – some with increased signal indicating lack of communication with the ventricles – suggesting total ACC. Lower images showed an enlarged tectum with prominent and probably horizontal aqueduct, absent cerebellar vermis, moderate hypoplasia of the right and severe hypoplasia of the left cerebellar hemispheres, and a large posterior fossa fluid collection.

Patient 6

This boy was one of the original patients from the first report by Delleman et al., and was reported again at age 17 years.[5][6][7] He presented with right cystic microphthalmia, bilateral eyelid coloboma, bilateral focal hypoplastic skin defects including a typical crescent-shaped defect behind the ear, and skin appendages. He also had severe mental retardation.

Cranial CT (Fig. S2I–L) done at ages four and seven years showed a thick and irregular cortex typical of PMG; this was present diffusely and was most severe in the smaller right frontal lobe. The lateral ventricles were asymmetrically enlarged with the right much larger than left, and separated by a midline cyst that appeared to be an extension of the third ventricle. The latter suggests partial ACC. The midbrain was dysplastic with an enlarged aqueduct on one image and an unusual round mass of tissue on the highest image. The pons appeared mildly small, the vermis absent, and the cerebellar hemispheres small especially on the right. A large fluid collection was located just behind the cerebellum that probably communicated with a low occipital skull defect, consistent with a meningocele.

Patient 7

This boy was patient 2 in the original paper reporting OCCS.[5][6] He had bilateral cystic microphthalmia and eyelid colobomas, typical skin lesions, severe

Figure S2. The classical OCCS brain malformation. Lower resolution brain imaging studies in four other patients showed similar changes. These consisted of a pneumoencephalogram in patient 8 (A-B), two images from an MRI in patient 5 (C-D), and cranial CT scans in patients 5 (E-H), 6 (I-L) and 7 (M-P). Images through the cerebral hemispheres in patients 5 and 6 show loss of the normal gyral pattern and moderately thick cortex (white arrows in D and L) that probably represent polymicrogyria. The resolution is too low to assess the cortex in patient 7 (M-P). The lateral ventricles are mildly enlarged and widely separated in all four patients (asterisk in A, arrows in B and P; also seen in F-H and J-L) indicating agenesis of the corpus callosum, with large interhemispheric cysts in patients 5 and 6 (asterisks in D and J). Images through the posterior fossa in patients 5-7 show a large and dysplastic midbrain tectum (black arrows in C, J-K and O), absent vermian (angled arrows in C, E, I, M and vertical arrows in F-G), and small, asymmetric cerebellar hemispheres (C, E-G, I-K, M-O with vertical arrows in I and N). All four patients have large posterior fossa fluid collections (A-I and L).

mental retardation and a seizure disorder. He died at two years from complications of hydrocephalus.

Only a low resolution CT (Fig. S2M-P) from 1977 was available for review, and the gyral pattern was too indistinct to assess. The lateral ventricles were mildly enlarged, more on the right, and widely separated, suggesting complete ACC. The tectum was large and dysplastic, the vermis absent, and the cerebellar hemispheres were hypoplastic, the right side being more severely affected. The midbrain appeared to connect to the cerebellar hemispheres directly. Serial scans were reported to show progressive hydrocephalus.

Patient 8

This German boy presented in the neonatal period with bilateral cystic anophthalmia, skin appendages in the periorbital region and on his trunk and the scalp, and numerous focal skin defects. He died at age one year.[7]

PEG done years ago (Fig. S2A-B) showed asymmetrical, enlarged, and widely separated lateral ventricles typical of ACC, probable hydrocephalus, a large posterior fossa fluid collection, and a skull defect in the occipital midline suggesting a meningocele. Necropsy showed PMG, hydrocephalus, an interhemispheric cyst and a malformation of the midbrain tectum, which was 5 cm in length and extended beyond the cerebellum. We were able to obtain a single block of tissue from the cortex, most probably from the occipital lobe, which confirmed a severe cortical dysplasia, although preservation was too poor to classify the type. Several intraabdominal neurofibromas were found along the sympathetic chain.

Patient 9

This three year old Belgian boy had eyelid and iris colobomas but no other eye anomalies, and atypical skin abnormalities consisting of small periorbital nodules, a small skin appendage on the thumb and linear skin defects on the trunk and arms.[8]

A suboptimal and incomplete MRI (Fig. S3A-B) showed a normal or possibly mildly dysplastic gyral pattern, enlarged and asymmetric lateral ventricles, larger on the left, and partial ACC with a small frontal remnant near the genu. Several loculated cysts with high signal (protein content) were seen within the lateral ventricles. The brainstem and cerebellum appeared grossly normal.

Patient 10

This Dutch boy had unilateral cystic microphthalmia, characteristic skin lesions and mild mental retardation. He was also reported previously.[9]

The report of his MRI noted enlarged ventricles, but none of these images were available for review. A single midsagittal MRI (Fig. S3C) showed a normal gyral pattern along the medial surface and total ACC. The pons appeared moderately

Figure S3. Incomplete OCCS brain malformation. In contrast, brain imaging studies in three OCCS patients were also suboptimal and incomplete, but showed less severe malformations. These consisted of an old MRI in patient 9 (A-B), a single MRI mid-sagittal image in patient 10 (C), and a few CT images through the posterior fossa (D) in patient 11. The gyral pattern appears normal on limited views in patients 9 and 10 (B and C). The lateral ventricles are mildly enlarged and separated with several intraventricular cysts in patient 9 (asterisks in B), but cannot be assessed in patients 10 or 11. The corpus callosum is absent save for a small anterior remnant in patient 9 and completely absent in patient 10 (black arrows in A and C). The brainstem including the tectum, and the cerebellum appear normal in all three patients (A, C and D). The left eye is cystically enlarged in patient 11 (D).

narrow or flat, but the midbrain including the aqueduct and tectum, and cerebellar vermis appeared normal.

Patient 11

This Russian baby was born with a huge anophthalmic orbital cyst on the left, which had already been diagnosed prenatally by ultrasound. He had typical skin lesions located on the left side or the midline. In addition, he had bilateral cryptorchidism and mild anomalies of the ribs. Apart from mild hypotonia, his psychomotor development was reported to be normal at two years. His case has been published in abstract.[10] In addition, clinical data and photographs were available.

Cranial CT scan was reported as normal except for the eye. A single suboptimal image was available for review (Fig. S3D). It showed a mildly enlarged space anterior to the left temporal pole, normal brain stem and cerebellum, and left cystic anophthalmia.

DISCUSSION

OCCS is a rare malformation syndrome that hitherto has been diagnosed on the basis of the typical eye and skin abnormalities. We reviewed the brain abnormalities of 11 patients with OCCS which are summarised in Table 1. Owing to the rarity of OCCS, we included patients ascertained over many years and as a result, many of the available brain imaging studies were suboptimal and incomplete. We were able to review five patients in detail (patients 1 to 5), one adequately (patient 6), and five to a limited extent (patients 7 to 11). Despite this, we were able to document a remarkably consistent malformation in eight of the 11 patients and unexpectedly found a novel mid-hindbrain malformation. The three remaining patients had similar but less severe forebrain abnormalities, but lacked the mid-hindbrain malformation, which we suspect to be pathognomonic for OCCS.

Typical OCCS brain malformation

In the eight patients with the most typical malformation complex, the forebrain malformation consisted of: frontal predominant PMG; PNH always located beneath the PMG; complete or partial ACC, sometimes associated with interhemispheric cysts; and enlarged third and lateral ventricles complicated by hydrocephalus in four patients.

The novel mid-hindbrain malformation was found in all eight patients, and consisted of a giant tectum, absent vermis and large posterior fossa fluid collection. The midbrain tegmentum was flexed forward, making the aqueduct nearly horizontal in four of the five patients with complete MRI studies. The giant and dysplastic tectum was rotated upward well above the normal position, and appeared to form an arch over the enlarged lower aqueduct in several patients. The cerebellar hemispheres were missing or hypoplastic with a dysplastic foliar pattern in seven of eight patients. The fourth ventricle communicated widely with a large posterior fossa fluid collection, sometimes associated with an occipital meningocele. These anomalies, excluding the midline malformations, were asymmetric in all eight patients. The more severe PMG, more numerous PNH, larger lateral ventricles, and smaller cerebellar hemispheres were always on the same side when we could assess this.

The key forebrain malformations of PMG, PNH, enlarged ventricles, and ACC are relatively common individually, but occur together in only a few syndromes, as we will review. Thus this pattern should be very helpful for diagnosis. The mid-hindbrain malformation has previously been confused with typical Dandy-Walker malformation (DWM), which should not be surprising. The usual diagnostic criteria for DWM include hypoplasia of the cerebellar vermis and widely open fourth ventricular outflow tract communicating with a large posterior fossa

Table 1. Brain abnormalities in patients with the oculocerebrocutaneous syndrome

Patient	Sex	Study	MCD	R/L asym	PMG location	PNH	Large	ACC	IHEM cyst	MIDB	Cerebellum	absent vermis	HEM hypo	PF cyst	Figure No.	Database No.
1	M	MRI	L>R		F>PS-P-T	+	L>R	+	-	+	+	+	-	+	2, 3A-D	LR01-191
2	M	MRI	L>R		F>PS-P	+	L>R	+	-	+	+	+	+	+	3E-H	LR04-005
3	F	MRI	L>R		F>PS-P	...	L>R	+	-	+	+	+	L>R	+	3I-L	LR04-010
4	M	MRI	R>L		F>PS-P-T	+	R>L	+	-	+	+	+	R>L	+	3M-P	LR01-135
5	M	CT, MRI†	L>R		F>PS-P-T	-	L>R‡	+	+	+	+	+	L>R	+	S2C-H**	LR01-031
6	M	CT†	R>L		F>PS-P-T	-	R>L‡	+	+	+	+	+	R>L	+§	S2I-L**	LR01-136
7	M	CT†	...		...	...	R>L‡	+	-	+	+	+	R>L	+	S2M-P**	LR01-221
8	M	PEG¶	...		+	...	R>L‡	+	...	+	+	+	...	+§	S2A-B**	LR01-211
9	M	MRI†	...		...	...	L>R	+	-	-	-	-	-	-	S3A-B**	LR01-253
10	M	MRI†	...		...	...	...	+	...	-	-	-	-	-	S3C**	LR01-222
11	M	CT†	...		...	...	...	...	...	...	-	-	-	-	S3D**	LR01-307

Legend: ACC: agenesis of the corpus callosum; F: female, frontal; IHEM hypo: cerebellar hemisphere hypoplasia; IHEM: interhemispheric; LV: lateral ventricles; M: male; MCD: malformations of cortical development; MIDB: midbrain; R/L asym: right-left asymmetry; L>R: left side more severe; R>L: right side more severe; P: parietal; PF: posterior fossa; PMG: polymicrogyria; PNH: periventricular nodular heterotopia; PS: perisylvian; T: temporal; +: present; -: absent; ...: data not available. *Partial ACC, †Suboptimal or incomplete study; ‡Hydrocephalus; §Cephalocoele (meningocele); ¶Necropsy report; **Supplementary material

fluid collection, or so called cystic enlargement of the fourth ventricle.[11][12] While the typical OCCS mid-hindbrain malformation described here includes each of these criteria and so could be labelled as DWM, we think this would lead to diagnostic confusion. The OCCS mid-hindbrain malformation includes many other anomalies and is clearly much more severe and complex than DWM. Despite considerable experience in evaluating brain malformations in many different disorders, we have not seen this mid-hindbrain malformation in any other context. We hypothesise that all or most patients with the giant tectum-absent vermis malformation have OCCS, and that most but not all patients with OCCS have the mid-hindbrain malformation.

Incomplete OCCS brain malformations

Only suboptimal imaging studies were available for the remaining three OCCS patients, but at least two had ACC and none had the mid-hindbrain malformation. The gyral pattern and cortex were either normal or mildly dysplastic in patient 9, but resolution of the MRI was too low to be sure. This could not be assessed in the other two patients. Patient 9 also had mildly enlarged lateral ventricles with several cysts within them that appeared quite different from the midline cysts seen in two patients from the more typical OCCS group. Two of the three (Nos 10 and 11) had typical eye and skin changes of OCCS, although patient 10 was less severely affected than any of the others. In contrast, patient 9 had atypical linear skin defects with unusual localisation. However, one patient with less typical eye and skin lesions was also found in the group with the typical brain phenotype. We do not yet have enough evidence to determine whether OCCS in these three patients results from the same cause as OCCS in more typical patients.

The differential diagnosis of OCCS

Our results have implications for the differential diagnosis of several related syndromes. Some have overlapping eye and skin changes such as ECCL, OAVS, and focal dermal hypoplasia (FDH or Goltz syndrome). Others have similar brain malformations, especially Aicardi syndrome and a recently recognised group of syndromes with PNH and PMG.

ECCL and OAVS have considerable overlap with OCCS with regard to the skin, eye and other associated abnormalities. Extensive lipomatosis of the brain and spinal cord are characteristic of ECCL.[13] In addition, asymmetries of the cerebral hemispheres, an abnormal gyral pattern, cortical calcifications, porencephalic or arachnoid cysts and dilated lateral ventricles have been described repeatedly.[14][15][16][17] However, mid-hindbrain malformation has never been

described in ECCL. In OAVS, only a minority of the patients have developmental delay. A few have hydrocephalus or various brain malformations, but no pattern has emerged except that patients with microphthalmia, clefts or other evidence of a severe phenotype are more likely to have developmental problems.[18] Several patients with (possible) OCCS and overlap with OAVS have been reported.[19][20][21][22] One boy had PMG over the frontal lobes, ACC and a midline cyst and so probably had OCCS.[20] Several other patients with overlapping features of OAVS and OCCS have had massive hydrocephalus without the brain anomalies characteristic of OCCS. In this group, the combination of severe hydrocephalus with anophthalmia or severe microphthalmia and clefts favours OAVS.

FDH is X-linked disorder mainly affecting females characterised by a combination of cutaneous, ocular, neurological, and skeletal features.[23] Focal hypoplastic skin lesions, often with herniation of fatty tissue, and linear pigmentation are the predominant cutaneous features. In contrast to OCCS, the skin lesions commonly follow the lines of Blaschko, indicating a mosaic defect. Skin tags are papillomas, in contrast to the (mostly periorbital) hamartomatous tags in OCCS. Ocular features are very diverse and mostly affect the anterior chamber; chorioretinal and iris colobomas are reported but orbital cysts have not been described. Skeletal defects of the hands and feet may occur. X-rays show striation of the bones in most patients. Whereas many patients are mentally retarded, brain malformations have rarely been described and do not correspond to the brain malformations seen in OCCS.

Aicardi syndrome

The typical OCCS brain malformation overlaps substantially with the brain malformation seen in Aicardi syndrome, which is thought to be X-linked with embryonic lethality in males. Although the typical eye anomalies (chorioretinal lacunae) are different and the skin generally is normal, other features of Aicardi syndrome overlap considerably with OCCS. For example, the brain malformations in Aicardi syndrome include PMG, rare PNH, enlarged ventricles (but not typically hydrocephalus), and ACC.[24][25] Interestingly, a few patients with Aicardi syndrome had DWM.[26] Other overlapping features include microphthalmia, coloboma of the optic nerve, scalp lipoma, cleft lip/palate and costovertebral defects.

PNH-PMG syndromes

We have recently delineated several syndromes with PMG, PNH, and other anomalies (Dobyns WB, unpublished data). The frontal-perisylvian subtype consists of PNH lining the lateral body and frontal horns of the lateral ventricles, overlying PMG that is most severe in the perisylvian area, and sometimes ACC

and mild cerebellar vermis hypoplasia. The malformation is usually symmetrical, and no other anomalies have been observed. A posterior subtype consists of PNH of the temporal horns, trigones and occipital horns of the lateral ventricles, overlying PMG most severe in the temporal, parietal and occipital lobes, and frequent ACC and hypoplasia of the entire cerebellum. This malformation complex is usually asymmetrical, but again no other anomalies have been found; in particular the mid-hindbrain malformation described in this paper has not been present.

Pathogenesis of OCCS

Analysis of available data regarding the pathogenesis of OCCS provides two important clues, both of which support a genetic etiology for OCCS despite the lack of familial recurrence. First, a striking preponderance of males has been reported, including 10 of the 11 patients reported here. The only female in our series had the typical brain malformation, which suggests that she has the same syndrome. Among the six other female patients reported, one did not undergo brain imaging,[6] one had a normal cranial CT,[27] and four had malformations that may fit the classical brain phenotype.[28][29][30][31] These data suggest that OCCS may be X-linked.

Second, the unusual mid-hindbrain malformation suggests an abnormality of the isthmus organiser, which is located between the embryonic midbrain and the first segment of the hindbrain (rhombomere 1 or Rh1) and controls development of the midbrain, upper pons and cerebellum. Recent experimental data in the mouse show that two transcription factors (the rostrally expressed *Otx2* gene and caudally expressed *Gbx2* gene) repress each others' expression, forming a sharp boundary between the midbrain and Rh1. This establishes the position of the developmental signaling center for this region and ultimately defines the posterior limit of the midbrain and the anterior limit of the cerebellum.[32][33][34] One possible interpretation of the giant tectum-absent vermis malformation in OCCS is that the boundary between the midbrain and hindbrain has been shifted caudally in the dorsal portion of the embryonic neural tube, which is less extensive than observed in *Gbx2* knockout mice and so appears provisionally unique.

CONCLUSION

The reported pattern of brain malformations in patients with OCCS extends the phenotype and should prove very useful in establishing the diagnosis and differentiating it from other entities, in particular from syndromes with overlapping skin and eye features. These observations should contribute to unravelling the underlying pathogenic mechanism. The novel mid-hindbrain malformation

points to a defect in early pattern formation at the stage when the isthmus organizing center is established, setting up the boundary of the midbrain and cerebellum.

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4 CYTOGENETIC STUDIES

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4.1 EVOLUTION OF TECHNIQUES

G-banding and high-resolution (HR) analysis

In 2005, we can look back at more than a half century of analysis of human chromosomes. Chromosomes are most often studied in a culture of peripheral T-lymphocytes using a heparinized blood sample after vena puncture. The mitotic cell division is first stimulated by phytohemagglutinin and then arrested in the metaphase. In 1952, the introduction of a hypotonic treatment of the lymphocyte culture allowed the distinction of each of the human chromosomes. However, it still took 4 years to fix the human chromosome number to 46 (Tjio & Levan, 1956); prior to that time, it had been believed that there were 48. In the beginning, unbanded equally coloured chromosomes were obtained which could be divided by length and localisation of the centromere into groups A-G (autosomes) with group C including the sex chromosomes X, and the sex chromosome Y. Only numerical and rough structural anomalies could be detected. In 1959, Down syndrome was found to be caused by trisomy of a chromosome of the G-group (Lejeune et al., 1959) which later was identified as chromosome 21. Around 1970, different banding techniques were discovered utilizing either fluorescent dyes or the Giemsa stain (G-banding) (Caspersson et al., 1970; Sumner et al., 1971). Each chromosome then could be distinguished by a particular banding pattern.

In the mid-1970s, analysis of chromosomes at a higher resolution became feasible by blocking the cell division in the prometaphase when chromosomes are longer (Yunis et al., 1976). This was the birth of the so-called high resolution (HR) chromosome analysis which subsequently was refined step-by-step. Nowadays, HR-G banding with at least 500–550 bands per haploid set is the gold standard of chromosome analysis. On microscopic examination, the chromosome band resolution can be accurately and simply established by the sum of bands on chromosome 1 and 2 multiplied by 6 (Welborn & Welborn, 1993). For a rough estimation and as a rule of thumb, at a level of 550 bands, chromosome 11p shows three distinct bands (p12; p14; p15.4) and band 22q13.2 is visible.

The analysis of well-spread chromosomes with a resolution of at least 550 bands per haploid set permits the detection of rather subtle aberrations; dependent on the chromosome region involved, small deletions and duplications of about 5 Mb can become visible. For example, in 60–70% of cases of Wolf-Hirschhorn syndrome (deletion 4p16), or in 90% of cases of Smith-Magenis syndrome (deletion 17p11.2) the deletions can be detected cytogenetically by HR analysis (Juyal et al., 1996; Battaglia et al., 2001). In the remaining cases, the deletion is too small for direct visualization, and additional FISH investigation is needed (see below). If a patient with MR has been subjected to chromosome analysis in the past, it is of

particular importance to check the quality and resolution of this analysis, as technical improvements during the last decades meanwhile allow the detection of many more subtle imbalances. If a resolution of merely 400 bands or less per haploid set of human chromosomes was achieved during the previous analysis, the chromosome investigation should be repeated following a HR standard method if the MR is still unexplained. Examples of the detection of chromosomal anomalies on re-evaluation of patients with MR are given in chapter 4.2.

Fluorescence in-situ hybridization (FISH)

In the late 1970s, a technique was introduced which revolutionized the cytogenetic field by linking it to molecular techniques: the Fluorescent In-Situ Hybridization (Rudkin & Stollar, 1977). The acronym FISH turned out to be a lucky choice as it became possible to fish and visualise each required DNA fragment. Basically, the method requires that a specific DNA fragment (a probe) is (1) labelled with a reporter molecule, (2) the probe is subsequently hybridized to denatured DNA of chromosomes or nuclei which have been immobilized on a microscope slide, and (3) the hybridized probe is subsequently detected with an antibody-fluorochrome complex directed against the reporter (Trask, 1991). The probe is chosen to bind to a complementary sequence, and thus to produce a signal at the site of hybridization and reveal its presence or absence. FISH thus depends in particular on the availability of appropriate DNA probes for hybridization. The FISH technique was further developed in the late 1980s (Lichter et al., 1988). For the analysis of chromosome aberrations, in particular three types of probes are used for numerous applications (for review see Xu & Chen, 2003; Salman et al., 2004):

- Whole chromosome painting (WCP) probes consist of a library of probes with affinities to a particular chromosome and stain ("paint") it over its entire length, with the exception of the centromeric and telomeric regions. WCP probes are mainly used for the detection of rearrangements involving two or more chromosomes, e.g. balanced and unbalanced translocations and insertions. In the cytogenetic evaluation of MR, the painting of all chromosomes with different colours (so-called 'total paint') has proven particularly useful for the work-up of familial cases of MR, as obligate carriers of subtle balanced translocations can be easily detected (see chapter 4.3). In recent years, two multicolor karyotyping techniques have arisen from the use of WCP probes: M-FISH and SKY (see below).
- Chromosome-specific repetitive sequence probes hybridize near the centromere and are in particular used for FISH on non-dividing (interphase) cells. Interphase FISH can be used to score a large number of cells or to detect abnormalities preferentially present in non-dividing cells (Xu & Chen,

2003). It is applied, among others, for the detection of mosaic chromosome aberrations, e.g. isochromosome 12p in mosaic state (Pallister-Killian syndrome) on direct buccal smear preparations (Manasse et al., 2000).

- Unique sequence probes are used to identify specific chromosome anomalies: subtle deletions/duplications, translocations, inversions, and marker chromosomes. The size of the probes varies considerably from 1 Kb to 1 Mb, dependent on the target size. They can be applied to both metaphase and interphase preparations. Because the chromatin of nuclei is much less condensed in the interphase than during cell division, interphase FISH is very applicable for the analysis of loci that are close together, e.g. for the characterization of microdeletions and duplications.

FISH with unique sequence probes is often used in the cytogenetic evaluation of MR: for the confirmation of a clinically diagnosed chromosomal abnormality, the elucidation of syndromes of possibly chromosomal origin, the characterization and refinement of abnormalities found by HR analysis, and for the study of genotype-phenotype correlations (for illustration see Moog et al., 1994; Moog et al., 1996; Moog et al., 2000; Moog et al., 2004).

FISH is particularly often used for the detection or confirmation of microdeletion syndromes. Selected examples are listed in Table 4.I, other examples are Angelman, Prader-Willi and Rubinstein-Taybi syndromes (see Table 5.I). Many of these syndromes are contiguous gene syndromes, i.e. syndromes with variable clinical manifestations depending on the size of the deletion and the involvement of contiguous genes. If reproduction is possible, microdeletion syndromes behave as autosomal dominant traits.

A particular subset of unique sequence probes are subtelomeric probes which are used to detect cryptic translocations or imbalances involving the subtelomeric region. In 1995, the era of systematic investigation for subtelomeric rearrangements began with the study of Flint (Flint et al., 1995), although the power of FISH techniques had been used earlier to detect cryptic and subtelomeric anomalies. The issue will be discussed in detail in chapter 4.3.

Microdissection

In 1986, microdissection, i.e. the physical dissection of a part of a G-banded chromosome with a micro-needle, became possible for human chromosomes (Bates et al., 1986), and subsequently could be combined with FISH in the so-called micro-FISH technique. Micro-FISH comprises four steps: the microdissection of chromosomal material, the amplification of the separated chromosomal fragment by PCR (polymerase chain reaction), its labeling with a fluorescent reporter molecule, and lastly its hybridization to a normal metaphase (Engelen, 1998).

Table 4.I: Selected well-known microdeletion syndromes

Chromosome region deleted	Condition	Major features	CGS
Del(4)p16	Wolf-Hirschhorn syndrome	Severe MR Pre/postnatal growth retardation Distinct facies Congenital anomalies	+
Del(5)p15	Cri-du-chat syndrome	Severe MR Cat-like cry Microcephaly, round face, typical face	+
Del(7)q11.2	Williams syndrome	MR Typical facies Characteristic behaviour Cardiac defects	+
Del(11)p13	WAGR syndrome	Wilms tumor Aniridia Gonadoblastoma MR	+
Del(11)q23.3	Jacobson syndrome	MR Growth retardation Craniostenosis Cardiac defects	—
Del(8)q24.1	Langer-Giedion syndrome	Multiple exostoses Bulbous nose, peculiar face Sparse scalp hair Mild MR, dependent on deletion size	+
Del(17)p11.2	Smith-Magenis syndrome	MR Characteristic behaviour Typical facies	+
Del(17)p13.3	Miller-Dieker syndrome	Profound MR, seizures Lissencephaly Microcephaly, typical facies	+
Del(22)q11.2	22q11 syndrome, includes: Velocardiofacial (VCF) s. DiGeorge syndrome	DD/learning problems Abnormalities of 3rd + 4th pharyngeal pouches Cardiac outflow defects Cleft palate/velopharyngeal insufficiency Hypocalcaemia Renal abnormality	—

Legend: CGS: contiguous gene syndrome

Micro-FISH can identify chromosome abnormalities when HR-karyotyping and FISH alone turn out to be insufficient. The most important applications are the identification of small, mosaic or complex marker chromosomes of any origin, the characterization of complex rearrangements, e.g. translocations, and the sophisticated delineation of deletions and duplications (Engelen et al., 1996a; Engelen et al., 1996b; Engelen et al., 1996c). Another advantage is that also long-term stored specimens can be used for microdissection.

Techniques arisen from FISH

During the last decade, a number of further techniques has been developed from FISH. The most important ones will be mentioned:

- Techniques of differential chromosome painting: M-FISH (multicolour FISH) (Speicher et al., 1996; Eils et al., 1998; Uhrig et al., 1999) and SKY (spectral karyotyping) (Schröck et al., 1996; Macville et al., 1997; Schröck et al., 1997) both emerged from whole chromosome painting (WCP). They essentially consist of simultaneous WCP painting of all chromosomes in a single experiment followed by subsequent computer-assisted spectrum analysis of the colour signals achieved; they differ in the manner of the imaging process. All WCP based analyses have two major limitations: they are inadequate for the detection of intrachromosomal rearrangements as deletions, duplications and inversions; and their resolution is restricted to 1–3 MB (Lee et al., 2001). They are most often used in combination with other techniques.
- Conventional or chromosome CGH (comparative chromosome hybridization) is a technique which screens all chromosomes for imbalances by comparing the DNA content of the cells under investigation with that of a normal reference genome. It is based on two-colour fluorescent labeling of the specimen and the reference DNA, and hybridization of both to normal metaphase chromosomes. Copy number changes (gains and losses) of DNA sequences in the specimen can be detected and localized by a shift of the fluorescence ratio to one of the colours (for review see Kirchhoff et al., 2001; Salman et al., 2004). For this technique, neither dividing cells nor parental samples are necessary. It can also be applied to formalin-fixed, paraffin-embedded, or frozen samples. The sensitivity however is usually considered to be low (4–10 Mb), and can be maximally improved to 3 Mb when using the high resolution CGH technique (Kirchhoff et al., 2001). Furthermore, balanced rearrangements cannot be detected, and, in particular, subtelomeric imbalances may be missed. These factors contribute substantially to the limitation of conventional CGH in the evaluation of MR (Joly et al., 2001; Ness et al., 2002; Kirchhoff et al., 2004).

Chromosome CGH is not a high-throughput technique. Meanwhile, this and other cytogenetic techniques have to compete with array-based formats (array CGH) and other new technologies which are faster, have a higher resolution, can be standardized more readily, and which are amenable for high-throughput analyses (Ried, 2004). These will be discussed in chapter 7.2.

Having found a normal karyotype by HR-banding, different pathways may be followed for further work-up depending on the clinical presentation (Xu & Chen, 2003). In patients with features of mosaicism such as body asymmetry and linear or patchy pigmentary changes, additional chromosome counts, or investigations in other tissues e.g. buccal smear or fibroblasts, are needed. When a microdeletion syndrome is suspected, additional FISH studies are required. Not infrequently, several techniques have to be combined to detect and further characterize a subtle anomaly or complex rearrangement. The following chapter (4.2) illustrates that a thorough application of (different) techniques of classical and molecular cytogenetics can enable the diagnosis of patients who on a first investigation were assumed to have a normal karyotype. This is followed in chapter 4.3 by a discussion of subtelomeric abnormalities and the ways to detect them. A synopsis of the prevalence of cytogenetic anomalies in MR as found by classical cytogenetic investigations and by subtelomeric studies, will close the part on Cytogenetic Studies.

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4.2 Benefit of combined techniques: Chromosome bands and ends revisited

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In this Journal, we read with great interest the editorial comment by Biesecker on “The end of the beginning of chromosome ends” (*Am J Med Genet* 2002;107:263–266). This editorial comments on three studies published in the same issue of the Journal and previous studies of subtelomeric chromosome aberrations as a cause of mental retardation. Biesecker speculated about the end of the subtelomeric era and future methodological advances, focusing hope on future universal (chip) technologies. However, there is no such technology in daily practice as yet. Working daily with patients with unexplained mental retardation (with or without dysmorphism or congenital anomalies) and making decisions on the necessary cytogenetic work-up, we sincerely appreciate Biesecker’s efforts to give a concise review on an extensive topic and are grateful that he addressed the general aspects of the issue. Nevertheless, we felt that the title (“The end of . . .”) and the seeming dissatisfaction with currently available techniques had something fatalistic, irreconcilable with the optimistic (perhaps compelling?) attitude of most clinical geneticists.

Thus, we looked back to specific chromosome aberrations diagnosed on revision or work-up at the Department of Clinical Genetics at Maastricht, The Netherlands. In the Table below, we give examples of chromosome aberrations found through different additional techniques. Previously, all these patients had a normal karyotype result or an aberration that could not be completely classified (patients 6 and 7) on a first investigation by GTG-banding. The benefit of these ultimately successful diagnostic studies is their immediate impact on genetic counseling.

We admit that in retrospect, the dysmorphic features of patient 10 (micro-brachycephaly, flat mid-face, prominent forehead, large anterior fontanelle, horizontal eyebrows, thin lower lip) were judged as characteristic for deletion 1p36, but had not been previously recognized as such at national and international meetings by several expert dysmorphologists. We also note that in retrospect, after having diagnosed Charcot-Marie-Tooth 1A, patient 3 did have a tiny, but cytogenetically visible, duplication of 17p. To most clinical geneticists, these are familiar aspects of etiological investigations.

The yield of subtelomeric screening cannot be translated into an unequivocal number of rearrangements. Several studies have shown that the proportion of abnormal findings strongly depends on selection criteria and previous cytogenetic work-up. The paper by Riegel et al. [2001] not only gives an excellent review on the development of subtelomeric screening but also clearly shows the influence of preselection: a better initial clinical evaluation and cytogenetic pre-investigation by higher quality GTG-banded karyotype and region specific FISH when the clinical examination raised the suspicion of a specific aberration, results in a lower incidence of anomalies detected by subtelomeric screening.

Table 4.II: Clinical and cytogenetic findings in patients with chromosome aberrations diagnosed on revision or work-up

Pat nr	Sex +age	1st analysis	Clinical features	Work-up by	Aberration
1	F, 22 y	46,XX (1976)	Severe MR, short stature, microcephaly, facial dysmorphism	HR, confirmed by FISH	Dup(4) (q32q34)
2	F, 22 y	46,XX (1976)	Severe MR, short stature, postaxial polydactyly, facial dysmorphism	HR, confirmed by FISH	Del(3) (p25.3→pter)
3	M, 6 y	46,XY	Developmental delay, Charcot-Marie-Tooth 1A, autism spectrum disorder, subtle dysmorphism	FISH + revision of HR	Dup(17) (p11.2p12)
4	F, 4 y	46,XX in blood	Small stature, hemihypotrophy, skeletal asymmetry, retarded bone age	GTG and FISH in fibroblasts	Mos tris 22
5	M, 5 m	46,XY in blood	Developmental delay, facial features suggesting Pallister-Killian syndrome, hypospadia	GTG and FISH in buccal mucosa + fibroblasts	Iso(12)(p10)
6	M, 1 y	Variant 15p or der(15) ?	Severe growth retardation, MCA, facial dysmorphism	Microdissection	Der(15) (6pter→6p22.1:: 15p12→15qter)
7	M, 21 y	Dup (5p)	MR, behavioral problems	Microdissection	Der(5)t(5;13) (p15.3;q22)
8	M, 48 y	46,XY	Mild MR (uncle)	Total paint (carrier) followed by FISH (both patients)	Der(4)t(4;18) (q35;p11.3) in both
9	M, 27 y	+ uncle + nephew	Moderate MR, MCA (nephew)		
10	F, 1.5 y	46,XX	Severe delay, hypotonia, Subtelomere screening		Del(1)(p36)
11	M, 41 y	46, XY	Both: moderate MR, macrocephaly,	Subtelomere screening	Der(4)t(4;7) (q33;q34) in both
12	M, 45 y	+ brothers	iriscoloboma, behavioral problems		

Legend: F: female, M: male, y: year, m: months, MR: mental retardation, MCA: multiple congenital anomalies, HR: high resolution GTG-banding, FISH: fluorescent in-situ hybridisation

Having used the gold standard of high resolution-GTG banding with at least 500-550 bands per haploid set, we conclude that there is not a single technique that can be used in the cytogenetic diagnostic work-up of patients with unexplained mental retardation. In different clinical situations, with varying clinical expertise, and different clinical testing resources, it is reasonable to use any one of, or a

combination of, approaches to diagnose these patients. Up to now, we lack a single assay that is easy to use, reliable and inexpensive that detects balanced and unbalanced aberrations, in mosaic or non-mosaic form, at the beginning of chromosome ends and all bands in between. But we do have powerful techniques detecting a growing number of aberrations and should use them as conscientiously and effectively as possible – until there are better ones.

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4.3 Subtelomeric chromosome aberrations: still a lot to learn

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ABSTRACT

Cryptic subtelomeric chromosome aberrations are a significant cause of mental retardation (MR). More than 4,000 patients have been investigated, and the mean overall prevalence of subtelomeric rearrangements has been found to be 5.2%. In order to contribute to knowledge on the clinical presentation of subtelomeric rearrangements, we retrospectively studied patients with unexplained MR who had been evaluated for subtelomeric abnormalities by different fluorescence *in situ* hybridization (FISH) techniques. 102 patients had an unexplained combination of MR with dysmorphism, congenital anomalies and/or a positive family history, and were investigated by total subtelomeric FISH (TS) (89/102), or by total painting (TP) in an obligate carrier in the case of familial MR (13/102). In 59 additional patients, a sequence-specific FISH was performed on clinical indication. In the 102 patients studied by TS or TP, 6 pathogenic aberrations (5.9%) were found in addition to one polymorphism. In total, 8 clinically significant subtelomeric aberrations were found in the 161 index patients; 4 of these 8 aberrations were familial. We report on the clinical presentation of all patients with an aberration and review the relevant literature. Factors complicating the interpretation of subtelomeric rearrangements are discussed, such as the occurrence of variants, clinical variability, and limited knowledge of the phenotype.

Keywords: cryptic chromosome abnormalities, FISH, monosomy, subtelomeric rearrangements, trisomy

INTRODUCTION

Cryptic subtelomeric chromosome aberrations are undetectable by conventional banding techniques either because of their small size or the similarity of segments involved and have been recognized as a major cause of mental retardation (MR) in 1993 (1). They can be screened for by different methods: fluorescent *in situ* hybridization (FISH) with a complete set of subtelomeric probes; techniques derived from FISH such as multiple amplifiable probe hybridization (MAPH), multiplex ligation-dependent probe amplification (MLPA), and primed *in situ* labeling (PRINS); and by genotyping with genetic markers [for review see (2-4)]. Differential chromosome painting techniques such as spectral karyotyping (SKY) and multiplex FISH have been shown to be inadequate to detect small rearrangements (3,5). Data on the sensitivity of chromosome comparative genomic hybridization (CGH) for the detection of small subtelomeric rearrangements are controversial (6-8). Microarray-based CGH is a new, promising high-throughput method for whole genome analysis; one application is the detection of subtelomeric imbalances (9,10). To date, however, FISH with a commercially available set of probes is the most often applied technique established in most laboratories. Subtelomeric studies by FISH and its variants, genotyping, or array CGH has been reported in at least 36 studies on more than 4000 patients [for review see (4), and in addition (10-27)]. The overall frequency of disease-related subtelomeric chromosome aberrations in these 36 studies summarized is about 5.2% ranging overall from 0-23%, but in larger studies ($n > 50$) from 0-16%. It depends on the patient ascertainment criteria, cytogenetic pre-screening, and the expertise of the examining clinician (2,28). A more sophisticated pre-screening at a high banding level or the clinical recognition of a particular phenotype which can be confirmed, e.g. by targeted FISH, lowers the yield of total subtelomeric screening (TS). Despite this large body of reported subtelomeric aneusomies, the literature on the clinical presentation and the pathogenicity of detected rearrangements is still limited.

In order to expand the knowledge on genotype-phenotype correlation, we retrospectively investigated the group of patients with unexplained MR studied at the Department of Clinical Genetics at Maastricht (Netherlands) for subtelomeric aberrations by TS FISH, total painting (TP) FISH and by targeted subtelomeric FISH. We report on the clinical presentation of detected anomalies, review the literature of these, and evaluate existing scoring lists for their detection.

PATIENTS AND METHODS

161 index patients with unexplained developmental delay (DD)/ MR referred to the Department of Clinical Genetics at Maastricht, the Netherlands, were evaluated for subtelomeric abnormalities. All patients had normal results on previous analysis of GTG-banded chromosomes at a 550 band level and were examined by a clinical geneticist trained in dysmorphology. 102/161 patients had unexplained MR with physical anomalies (dysmorphism or congenital malformations). In 13 cases of familial MR, TP in an obligate carrier was primarily performed. 89 patients were evaluated by TS FISH. In 59/161 patients, a specific subtelomeric aberration was suspected on clinical grounds, and a targeted subtelomeric FISH was done.

Chromosomes were prepared from peripheral blood lymphocyte cultures following standard procedures and GTG-banded by treatment with trypsin and staining with Giemsa. From each patient, 10–15 metaphases were analyzed with a resolution of at least 550 bands per haploid set of chromosomes. TP was performed with the Chromoprobe Multiprobe system (Cytocell, Adderbury, UK) following the manufacturer's specifications. Total telomere analysis with FISH probes specific for the 41 subtelomere regions was performed with ToTelVision probes (Vysis, Downers Grove, IL) following the manufacturer's specifications. FISH with band-specific probes was performed as described by Lichter et al. (29). To estimate the respective sizes of two recurrent subtelomeric deletions, additional FISH analysis was performed with publically available probes from a 1-Mb clone set (Sanger institute, Cambridge, UK): 635E18, 575L21, 510D11, 484P7, 338N10, 438L4, 37J18, 785P20, 465B22, and 14E10 for chromosome region 1p36.22–36.33; and 546M8, 463B12, and 556H17 for chromosome region 2q37.3 and in addition the subtelomeric probe 3K23 (courtesy of Dr. J. Hoovers, Amsterdam).

RESULTS

Results are summarized in Table 1. With total subtelomere analysis (TS), 5 of 89 index cases (5.6%) were found to have a subtelomeric imbalance; two patients had a *de novo* deletion 1p36; and one patient had a deletion 2q37, which should be considered to be a polymorphism, as his father was a healthy carrier of the same aberration. Furthermore, two sib pairs with an unbalanced translocation deriving from familial translocations t(4;7)(q33;q34) and t(16;18)(q24.3;q23) were detected. In both cases, initial testing of both parents by TP was impossible. With TP, familial translocations were found in 2/13 cases (15%): t(4;18)(q35;p11.31) and t(13;20)(q34;p13). In the family with t(4;18), patients

with imbalances due to both derivative chromosomes were observed (patients 8-10), and in the family with t(13;20), the index patient was shown to have inherited the derivative chromosome 13 (patient 11).

In 2/59 patients (3.4%) analyzed with only one subtelomeric FISH probe [targeted subtelomeric FISH (TF)], deletions were found: one deletion 10q26, and one deletion 2q37.3.

CLINICAL REPORTS AND LITERATURE REVIEW

De novo rearrangements

Deletion 1p36

The development of patient 1 was severely delayed from the beginning, and she was hypotonic. During the first years of life, she had feeding problems and gastroesophageal reflux and was susceptible to upper airway infections. Severe sensorineural hearing loss was diagnosed, and at age three years, she developed generalized epileptic seizures which were successfully treated with valproic acid. Around four years of age, she began to bite her hands, strike herself on the head, and scream. She developed an obsession to tense her abdominal muscles; lying on her back, she compulsively and simultaneously raised her stretched legs and her head and could stay for a long time in this position. On examination at five years, she could raise her head and roll over but could not sit stably unsupported and had no speech. She showed axial hypotonia and was free of seizures. Increasing behavioral and sleeping problems were major points of concern. However, she was a pleasant girl able to make good contact with her family. She showed brachymicrocephaly, a flat face, up-slanting palpebral fissures, and straight eyebrows (Fig.1). Cardiologic and ophthalmologic evaluations showed normal results. A magnetic resonance imaging (MRI) scan of the brain revealed delayed myelination. FISH with 10 probes covering chromosome region 1p36.22-36.33, showed that 510D11 was the most proximal probe to be deleted and that the deleted region was distal of probe 575L21, indicating that the size of the deletion was at least 9 Mb. The deletion was however undetectable on analysis of GTG-banded chromosomes at a 550 band level.

Patient 2 showed DD and abnormal contact behavior from the beginning. As an infant, he also had feeding problems. He had a large, late-closing anterior fontanelle. At 16 months, generalized hypotonia was noticed, and he developed behavioral problems with autistic features, later also hyperactivity and tantrums. On examination at 13 years, he was a moderately to severely retarded friendly boy, whose behavior was dominated by autistic signs. He was able to walk, and to

Table 1: Subtelomeric abnormalities detected by different FISH techniques

Pat.	Aberration	T	Gender, Age**	Clinical features	Family	Fig
1	ish del(1)(p36.22)(CEB108-.510D11-.575L21+)	TS	Female, 2 y	Severe DD + hypotonia, BP, microcephaly, D	normal	1
2	ish del(1)(p36.3)(CEB108-.465B22-.785P20+)	TS	Male, 17 y	Moderate to severe MR, BP	normal	1
3	ish del(2)(q37.3)(1011017-.3K23-.556H17+)	TF	Male, 4 y	Mild to moderate DD, truncal obesity, brachy-mesophalangism	n.a.	-
4	ish del(2)(q37.3)(1011017-.556H17+)	TS	Male, 3 y	Severe DD, hypotonia, oculomotor dyspraxia, microcephaly, hypogenitalism	F same anomaly	-
5	ish del(10)(q26)(2136A1-)	TF	Male, 45 y	Severe MR, BP, short stature, omphalocele, microcephaly, D	n.a.	2
I.6	ish der(4)t(4;7)(q33;q34) (dj963A6-.2000A5+)	TS	Male, 45 y	Moderate MR, BP, macrocephaly, coloboma	M normal F deceased	2
I.7	ish der(4)t(4;7)(q33;q34) (dj963A6-.2000A5+)	TS	Male, 41 y	As patient I.6		2
II.8	ish der(4)t(4;18)(q35;p11.31)(wcp18+)	TP*	Male, 27 y	Severe MR, BP, MCA, D	M t(4;18)	1
II.9	ish der(4)t(4;18)(q35;p11.31)(wcp18+)	TP*	Male, 47 y	Mild MR	S t(4;18)	1
aII.10	ish der(18)t(4;18)(q35;p11.31) (wcp4+)	TP*	Male, 54 y	Severe MR, ichthyosis, hypogenitalism	F t(4;18)	-
III.11	ish der(13)t(13;20)(q34;p13)(wcp20+)	TP*	Male, 13 y	severe MR, growth retardation, hypospadias, scoliosis, D	F t(13;20)	1
IV.12	ish der(18)t(16;18)(q24.3;q23) (dj964M9-.240G10+)	TS	Female, 15 y	Moderate MR, BP, SS, brachydactyly, CHD, lordosis, D	n.a.	2
IV.13	ish der(18)t(16;18)(q24.3;q23) (dj964M9-.240G10+)	TS	Female, 11 y	Moderate MR, SS, CHD, CP, D	n.a.	1

Legends: Pat: patient number, for cases 6-13 preceded by family number (I-IV), T: technique, TF: targeted subtelomeric FISH, TP: total painting, TS: total subtelomeric screening, BP: behavioral problems, CHD: congenital heart defect, CP: left palate, D: dysmorphism, DD: developmental delay, MCA: multiple congenital anomalies, MR: mental retardation, SS: short stature, F: father, M: mother, S: sister, y: years, * performed in carrier with balanced translocation, ** age at diagnosis by telomere test, n.a.: not available

speak inarticulately. There were no neurological problems apart from generalized mild hypotonia. Height and weight were within the normal range, his head circumference was 52 cm (-1SD). He had deep-set eyes, ears with thickened helices (Fig. 1), a mild scoliosis, and an asymmetric thoracic flattening on the right side. His hands had broad distal phalanges, and the distal phalanx of his left thumb was short. Ophthalmologic and audiologic examinations and a CT scan of the brain, showed normal results. FISH with probes 438L4, 37J18, 785P20, 465B22, and 14E10, specific for chromosome region 1p36.32-36.33, showed that the deleted region was distal of probe 785P20, indicating that the size of the deletion was maximally 3.2 Mb.

Deletion 1p36 is the most common subtelomeric aneusomy, with a frequency of about 1/5,000 (30). The deletion is visible by high resolution banding techniques in the majority of cases (31-32). Interstitial deletions, derivative chromosomes and complex rearrangements together form 30% of 1p36 monosomy, but presumably terminal deletions (70%) may also be more complex on additional molecular study (11,32). 1p36 deletions are maternally derived in about 65% of cases (30). The phenotype is characterized by moderate to severe retardation, hypotonia, often postnatal growth delay, and craniofacial dysmorphism as seen in patient 1, with microbrachycephaly, a large anterior fontanelle, deep-set eyes, a flat nasal bridge, midface hypoplasia, ear anomalies and a pointed chin (31-34); the eyebrows may be strikingly straight and low-set (35). Seizures, eye or vision problems, and hearing deficit occur in >50%. Heart defects, cardiomyopathy, or orofacial clefting can be associated. Behavioral problems have been repeatedly reported in about half of the patients (31,33,36). These include self-injurious behavior with hand biting and head striking, aggressiveness with banging or throwing objects, striking people and tantrums, hyperactivity, low frustration, and autism. Both patient 1 and 2 showed behavioral problems, patient 1 in combination with a hitherto unreported compulsive posture, whereas their facial phenotype and degree of retardation differed remarkably. It is possible, although this has yet to be further examined, that serious behavioral problems are a key feature of deletion 1p36. The difference in phenotype may be related to the different sizes of the deletion. So far, the discussion is, however, controversial whether the 1p36 deletion syndrome is a true contiguous gene syndrome (32,34,37).

Deletion 2q

Patient 3 was born as the first child of healthy parents originating from Somalia. Delivery was at term, and growth parameters at birth were normal. When referred for pediatric evaluation because of DD at the age of 3 years 11 months, he was a mildly to moderately retarded boy with good general health. He had a normal height (0 to -1SD for age), and a weight above 2 SD with truncal obesity.

Head circumference was 53 cm (+ 1SD); he had a high and prominent forehead but showed no other facial peculiarities. The fourth metacarpals were shortened bilaterally. Acanthosis was noticed in the neck, fitting with his ethnic background. Eye examination showed no retinal abnormalities. Because of mild to moderate retardation in association with his stature and brachymesophalangism, a terminal 2q deletion was suggested. This was confirmed by FISH with probes 10I1017 and 3K23, specific for the subtelomeric region of chromosome 2q. FISH showed that the deleted region was distal of probe 556H17, indicating that the size of the deletion was at most 1.2 Mb. The parents were not available for testing. The deletion is considered causative as it is known that the described phenotype can be caused by deletion 2q37.

One further patient (patient 4) was shown to have a cryptic subtelomeric deletion 2q on TS screening. Additional FISH with probes 556H17, 463B12 and 546M8, specific for chromosome region 2q37.3 showed these probes to be non-deleted. As his healthy father showed the same FISH pattern, this abnormality was regarded as a variant.

Subtelomeric deletions of chromosome 2q may cause phenotypic abnormalities, or may be present as variants without affecting the phenotype. The latter has mainly been deduced from the fact that these subterminal deletions also occur in normal parents and other family members, sometimes in several generations. In subtelomeric screening studies, a cryptic deletion 2q37.3 has been found as a variant in 2–6 % of cases (11,16,17,19,24,38). The non-variant deletions are associated with heterogeneous phenotypes comparable to those caused by cytogenetically visible deletions 2q37. More than 60 patients have been reported with deletion 2q37 [for review see (39,40)]. So far, no convincing genotype-phenotype correlation has been found. All patients exhibit MR and facial dysmorphism. About 50% show Albright Hereditary Osteodystrophy (AHO)-like syndrome with brachymetaphalangism, short stature and a stocky build. Contrary to AHO, Gs-alpha activity levels are normal, and there are no cutaneous ossifications in AHO-like syndrome (41). A recognizable facial phenotype with a round face, frontal bossing, prominent columella, and a thin upper lip may be present (39,42). However, in deletion 2q37, a non-specific phenotype with moderate to severe MR, growth retardation and aspecific dysmorphism are also seen (43). Patients with monosomy 2q37 are prone to epilepsy, autism and hyperkinetic behavior. Congenital heart defects, Wilms tumor, and urogenital anomalies can be associated (39). Cryptic terminal deletions so far are more often associated with AHO-like syndrome (17,40,44–46) than with non-AHO phenotypes (17,24,28). A case of AHO-like syndrome with a cryptic interstitial deletion 2q37.1–37.3 has also been reported (47). In recent years, the minimal deleted interval has been narrowed to approximately 3 Mb, and *GPR35*, *GPC1* and

STK25 have been proposed as candidate genes for the AHO-like syndrome and for brachymetaphalangy in particular (39,40,43,48).

Deletion 10q26

Patient 5 was formerly described to have Noonan syndrome but was severely mentally retarded and had no speech. He was born with an omphalocele which had been surgically corrected. Behavioral problems became evident at an early age. Sensorineural hearing impairment was diagnosed at 16 years. Eye, vision, and kidney abnormalities were ruled out by clinical investigations. Chromosome analysis in 1974 revealed a normal karyotype. On examination at age 45 years, he was short statured (height 151 cm), microcephalic (head circumference 51 cm) and had short, broad hands. His behavior was friendly, but overactive and self-determining. Because of his phenotype (Fig.2) with low set posteriorly rotated dysplastic ears, a broad nose, down-slanting palpebral fissures, hypertelorism, webbed neck, widely spaced nipples, and bilateral cryptorchism, a terminal 10q deletion was suspected and confirmed by targeted FISH. On chromosome analysis at a 550-band level, the deletion was now also seen.

Recently, Scigliano et al. reviewed the clinical features of 32 cases with *de novo* terminal deletions 10q, and Irving et al. reported on 12 new cases with breakpoints in 10q26.1 to 10q26.3 (49,50). These deletions are often cytogenetically visible. The clinical findings consist of MR of various degrees, postnatal growth retardation and a recognizable craniofacial phenotype with microcephaly, a triangular face, hypertelorism, strabismus, a broad nasal bridge, ear anomalies, and a short neck, as seen in patient 5. About 25% of the patients develop behavioral problems such as hyperactivity, reduced attention span, and aggressive, destructive and self-injurious behavior (51). Urogenital anomalies and cardiac defects are common and should be screened for (50). Patients with cryptic subtelomeric deletion 10q *sensu strictu* have been infrequently reported (50). There are several reports of patients with a subterminal deletion of 10q in combination with other imbalances and no consistent phenotype (35).

Familial translocations

Partial Deletion 4q, Partial Duplication 7q

Details of two brothers (patients 6 and 7) with MR, behavioral problems, macrocephaly and iris-coloboma due to monosomy 4q33-qter and trisomy 7q34-qter have been published previously (52) (Fig.2). No other comparable rearrangements or pure imbalances of the segments involved on chromosomes 4 and 7 have been reported.

Partial Deletion 4q, Partial Duplication 18p

Patient 8 was born with post-axial polydactyly of his right hand, and septal heart defects [atrial septal heart defect (ASD) and ventricular septal heart defect (VSD)] which necessitated later surgery. At 2 years and 9 months, he began to walk and was reported to be hypotonic and growth retarded. He had recurrent pneumonia and otitis. At 5 years, he developed a hyperkinetic, destructive and aggressive behavior. On examination at 27 years, he had moderate to severe MR, could speak words inarticulately, and had a nasal speech. He was cheerful, active, made good contact, and at that time, showed few behavioral problems. He was short statured (height 1.65 m, less than -2.5 SD), very slender (weight 48.5 kg) and had a head circumference of 54 cm. He had short palpebral fissures, synophris, anteverted nares, a short philtrum and small ears with thickened helices (Fig. 1).

Several members of his mother's family were also mentally retarded, including an uncle (patient 9). Two sibs of his mother were born small for gestational age, had multiple congenital anomalies with cleft palate, and died during the first month of life. No miscarriages were reported in the family. Patient 9 was borderline to mildly mentally retarded without formal testing. He had neither congenital anomalies, nor medical or behavioral problems. He lived independently, had been married for 22 years and worked at a sheltered work place. On examination at 47 years, he had normal growth and body habitus. He showed a small nose with anteverted nares and prominent ears, but no other dysmorphic signs (Fig. 1).

Both patient 8 and 9 had a normal karyotype on analysis of GTG-banded chromosomes at a 550 band level. A total paint in the mother of patient 8, respectively, sister of patient 9, revealed a cryptic reciprocal translocation (4;18)(q35;p11.31). Patient 8 and 9 were subsequently shown to have a 46,XY,ish der(4)t(4;18)(q35;p11.31)(wcp18+) karyotype with monosomy 4q35-qter and trisomy 18p11.31-pter. The discovery of the translocation led to re-examination and counseling of this large family. An unbalanced offspring with the derivative chromosome 18 and monosomy 18p11.31-pter and trisomy 4q35-qter was subsequently observed (patient 10). He had severe MR, ichthyosis, and hypogenitalism. Growth and posture were normal; he had no neurological problems. He was formerly supposed to have Rud syndrome (OMIM 308200).

The involved 4q segment is smaller than the monosomic segments of patients 6 and 7. De Vries (35) reviewed cases with submicroscopic 4q35-qter deletions and different partial trisomies of other chromosomes. An infant boy with deletion 4q34.3-qter and inverted duplication of the more proximal segment 4q has been described with DD, hypotonia, dysmorphism, and congenital heart disease. One pure deletion 4q35-qter has recently been reported in a boy with MR and schizoaffective disorder (53). The distal deletion 4q found in a baby boy with seizures, genital hypoplasia, and normal development was shown to be familial and

possibly represents a variant (54). To the best of our knowledge, pure subtelomeric duplication 4q35-qter has only been reported as polymorphism (55).

Partial monosomy of the terminal segment of 18p may be heritable and associated with normal or near normal phenotype even when the segment p11.2-pter is involved (56,57). It has been suggested that terminal deletions 18p are only associated with MR if the patient shows holoprosencephaly as a result for haploinsufficiency of the HPE4 locus (35). A roughly comparable imbalance as in patient 10 has been reported in a newborn with Wiedemann-Beckwith syndrome (58). A case of monosomy 18p11.23-pter in combination with partial trisomy 16q was interpreted as familial variant (47). We are not aware of reports of pure segmental trisomy 18p11.31-pter.

Partial Deletion 13q, Partial Duplication 20p

The clinical findings of this boy (patient 11) with der(13)t(13;20)(q34;p13) derived from a paternal translocation, have been published previously (59). In brief, he presented with severe developmental and growth retardation, hypospadias, scoliosis, and facial dysmorphism including unilateral ptosis (Fig. 1). Several translocation carriers, but no other family members with an unbalanced karyotype, were identified subsequently.

Partial Deletion 18q, Partial Duplication 16q

Patients 12 and 13, who are sisters, were referred by the pediatric cardiologist for evaluation. Patient 12 had a congenital anomaly of the atrioventricular and semilunar valves with valvular aortic stenosis. Her development was delayed from the beginning and she developed behavioral problems. On clinical examination at 14 years, she had moderate MR, a length of 154 cm (-2 SD), and a hypotonic, elongated face with a broad nasal bridge, and short palpebral fissures (Fig.2). She showed brachymesophalangism of fingers 4 and 5 bilaterally (Fig.2), and an extreme lordosis of the spine.

Her younger sister (patient 13) was also born with a congenital cardiac valve defect. Furthermore, she had a cleft palate which was surgically corrected. On examination at age 10 years, she had also moderate MR. Her behavior was optimistic and hyperactive. She had a length of 133 cm (-2 SD) and dysmorphic facial features resembling her sister (Fig.1). Her hands were normal. The family history was negative for MR and congenital anomalies.

Routine chromosome analysis revealed a normal 46,XX karyotype in both sisters, TP showed normal results in patient 12, and a 22q11.2 deletion was excluded. Subsequent subtelomeric chromosome analysis showed an unbalanced cryptic translocation between chromosomes 16 and 18 resulting in a partial deletion 18q23-qter and a partial duplication 16q24.3-qter in both sisters. The chro-

Figure 1: Facial phenotype of 6 patients, all with a subtelomeric aberration. The numbers refer to the number of the patient in Table 1.

Figure 2: Facial phenotype of 4 patients and hands of patients 5 and 12, all with a subtelomeric aberration. The numbers refer to the number of the patient in Table 1.

mosome abnormalities are presumably caused by a cryptic translocation (16;18)(q24.3;q23) in one of the parents who refused testing.

To the best of our knowledge, there are no other reports of this specific chromosome aberration. Microscopically visible deletions 18q23-qter have been frequently reported. MR, short stature, microcephaly, ear anomalies, hypertelorism, broad nasal bridge, cleft palate, long and tapering fingers, and congenital heart disease are frequent findings (60). Male individuals with 18q deletion frequently have external genital abnormalities. The cardiac defects most commonly reported are VSD, ASD, and pulmonic stenosis (61); in patients 12 and 13, septal defects and specific valvular cardiac anomalies were seen. Kline et al (62) reported hypotonia in all patients examined. Facial hypotonia was also a notable feature in patient 12. However, the overall clinical picture of terminal deletion 18q is variable, and a genotype-phenotype correlation could only be established with regard to the head circumference, ear length and thumb abnormalities (63).

Nine patients with trisomy 16q24-qter have been reported with a concomitant small deletion of other chromosome regions in all cases (47,64-67). Brisset et al. (67) concluded in their review that chromosome region 16q24 could be critical for low birth weight, generalized hypotonia, severe MR, feeding difficulties, distinctive facies with periorbital edema in the newborn period, a high forehead with bitemporal narrowing, multiple vertebral abnormalities, abnormal genitalia, and anal malposition. The only pure distal trisomy 16q, however extending to 16q23, was reported by Savary et al. (68). The clinical features at 4 months of age consisted of hypotonia, craniofacial dysmorphism differing from patients 12 and 13, thickened gingiva, finger pads and proximal implantation of the first toes.

DISCUSSION

We report on the clinical presentation of the subtelomeric aberrations found in a study of 161 patients with unexplained DD/MR evaluated by different FISH techniques. As we aimed at expanding the knowledge on the phenotype of subtelomeric imbalances and as it has been shown previously that a careful clinical examination and assessment of the family history may lower the yield by TS screening (2), we also included the aberrations found by TP and by TF.

Both the yield of six pathogenic aberrations in the group of 102 patients studied by TS and TP (5.9 %), and the yield of four pathogenic subtelomeric aberrations found in 89 patients studied by TS (4.5 %), are approximately consistent with the frequency reported in the literature. 50% of detected anomalies derived from familial translocations confirming that a family history of MR is a strong indicator for a subtelomeric chromosomal imbalance (2,20,69). In these situations, TP, first

performed in an obligate carrier, can be the initial method of choice. Its efficiency is confirmed by the detection of translocations in two out of 13 index cases evaluated by TP, although the sample size is too small for statistical conclusions. The sensitivity of TP for the detection of derivative rearrangements is, however, restricted, as has been shown by the evaluation of patient 12.

A checklist has been proposed to improve the yield of subtelomeric screening and is based on a family history of MR, growth anomalies, dysmorphism and other physical anomalies (70). Meanwhile, there has been criticism about its reliability (16,18,71). Recently, a similar checklist has been published which distinguishes different degrees of MR (27). In our study, two clinically relevant aberrations occurred in patients (patient 2 and 3) with a score beneath the respective cut-off of both checklists. In addition, patient 9 would not have been selected for subtelomeric testing according to both scoring lists, although other affected family members would have been selected. We therefore agree with Walter et al. (27) that a checklist, although of value for research purposes, constitutes only one aspect in the clinical setting. The overall phenotype, including neurological and behavioral aspects as well as the complete family history, has to be taken into consideration. It is intriguing that 50% of the 12 patients described with phenotypically relevant subtelomeric imbalances presented with serious behavioral problems.

The diagnosis of a subtelomeric chromosome aberration has immediate impact on counseling, in particular in case of familial rearrangements. Not infrequently, the detection of a cryptic chromosome anomaly leads to revision of a previous 'diagnosis', as has been shown by the history of patients 5, 6, 7 and 10. Several factors, however, may hamper counseling on prognosis or on the phenotype that can be expected. Subtelomeric imbalances may be associated with a very variable phenotype, as illustrated by patients 8 and 9. When a hitherto unreported translocation is found, it may be impossible to deduce the phenotype associated to unbalanced derivatives, from published reports on the respective partial monosomy and trisomy (see for example, patients 8–10). A particular problem is the finding of non-pathogenic variants which has been discussed here for terminal deletion 2q. Recently developed technologies as MAPH, MLPA, and microarray-based CGH are fast and high-throughput methods with high resolution, but the interpretation of results is complicated by a growing number of copy number polymorphisms (22,23,72). It has been suggested that the use of a 'molecular ruler' may give important information on telomeric imbalances by defining the size of the segment of imbalance, and better predicting the phenotype (73). This has been applied to patients 1–4, helping establishing further genotype-phenotype correlations. So far, an aberration, deletion or duplication, is considered pathogenic when it occurred *de novo* or was inherited from clinically similarly affected parents. However, the lack of accessibility of family members for

testing, the incomplete penetrance, and the clinical variability of chromosomal abnormalities are possibly complicating factors. In this context, further reports of the clinical presentation of subtle chromosomal imbalances will be of great value.

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4.4 PREVALENCE OF CYTOGENETIC ABERRATIONS IN MENTAL RETARDATION

A review of 11 studies, published between 1977 and 1996, indicated a prevalence of 4%–28% of clinically relevant chromosome aberrations in mentally retarded people (Curry et al., 1997). In 2003, Xu and Chen extended the review by including five further studies published between 1998–2000, and calculated a prevalence of 4%–34% with a mean of 16.1% (Xu and Chen, 2003). In the same year, Shevell et al. reviewed six studies performed in a pediatric population, five of which were not quoted in the review by Xu and Chen; they found a range of 2.9%–11.6% of chromosomal aberrations, with a mean prevalence of 3.7%. In an extensive literature review, Van Karnebeek et al. found a mean of 9.5% of chromosome aberrations in MR, varying from 4.1% in individuals with borderline or mild MR, to 13.3% in persons with moderate to profound MR (van Karnebeek et al., 2005). All these data addressed chromosome anomalies detectable by classical cytogenetic investigations; the most frequent of these chromosome anomalies is trisomy 21 (Down syndrome). The aforementioned mean frequency of 5.2% for subtelomeric rearrangements thus has to be added to the other figures. The wide ranges in the different surveys are due to differences in patient ascertainment criteria, to differences in the degree of MR and in the presence of physical anomalies, and to differences in cytogenetic facilities. However, (very) roughly it can be concluded that one in 10 people with MR has a cytogenetically visible chromosome anomaly, and that one in 20 individuals a cryptic subtelomeric aberration explaining the MR. Chromosome anomalies thus are the most frequent genetic cause of MR. Because of this, *every* individual with unexplained MR should have a chromosome investigation.

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5 MOLECULAR STUDIES

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5.1.1 FRAGILE X SYNDROME

Since a long time, it has been known that there is a male excess among patients with MR (Penrose, 1938; Lehrke, 1968). The overall male to female ratio is approximately 1.4:1 and is more pronounced for mild than for severe MR (Hamel, 1999). The predominance of males among mentally retarded individuals has stimulated the search for genes on the X-chromosome. These X-linked forms of MR could affect approximately 1 in 550–600 males, and about 1 in 400 females could be a carrier (Hamel & Poppelaars, 2000).

Fragile X syndrome was the first specific X-linked disorder causing MR that became widely known. Since the initial report by Martin and Bell (1943), followed by the report by Lubs of the “marker X” (1969), and the rediscovery of this syndrome in the 1970s, the characteristics of fragile X syndrome have been well and abundantly delineated. For a review, the reader is referred to the paper by de Vries et al. (1998). In brief, the phenotype of affected males is characterized by MR, a characteristic personality and shy behavior with lack of eye-contact, hyperactivity, hand-flapping, and echolalic speech; and physical findings which include relative macrocephaly, an elongated face, large protruding ears, smooth skin and macro-orchidism. All of these phenotype characteristics are variable and may be influenced by ethnic differences. The name “fragile X” derives from the observation of an abnormal X-chromosome with a fragile site at terminal Xq when using a folic acid depleted medium (Sutherland, 1977). This cytogenetic abnormality was used for Fragile X analysis until the discovery of the gene.

The gene responsible for fragile X syndrome is located at Xq27.3 and was identified in 1991 by different groups (Verkerk et al., 1991; Yu et al., 1991; Oberle et al., 1991) and called *FMR1* (Fragile X Mental Retardation 1) gene. Fragile X syndrome was the first disorder known to be caused by the expansion of a trinucleotide repeat (‘dynamic mutation’). Meanwhile, at least 13 other neurological, predominantly neurodegenerative disorders are known to be caused by the same mechanism, including Huntington’s disease, Myotonic Dystrophy, and several types of Spinocerebellar Ataxias (Costa Lima & Pimentel, 2004). The *FMR1* gene has a repeated CGG triplet in its first exon, which varies in the normal population from 6 to 54 repeats. Fragile X syndrome is associated with instability and subsequent expansion of repeats. On the basis of the number of repeats, a premutation (43 to 200 repeats) and a full mutation (>200 repeats) can be distinguished. Repeats between 43 and 54 are often referred to as intermediate alleles, and can be transmitted stably or behave as a premutation. Generally, a premutation is not associated with MR, but it may be the cause of premature ovarian failure in females or midlife movement disorders (FXTAS, Fragile X-associated Tremor/Ataxia Syndrome), particularly in male carriers (Sherman, 2000;

Hagerman & Hagerman, 2004a; Hagerman & Hagerman, 2004b). It may be transmitted to the next generation stably or, when transmitted by a woman, in an expanded state, e.g. as a full mutation. A full mutation leads to repression of transcription of the *FMR1* gene due to an abnormally methylated CGG stretch, and thus to the lack of the normally encoded protein FMRP (for review of the *FMR1* gene and its function see Oostra & Chiurazzi, 2001). Clinically, males with a full mutation have fragile X syndrome with MR as major feature. About 50 % of females carrying a full mutation are also mentally retarded, although to a lesser and more variable degree than males.

Fragile X syndrome is recognized as the most common monogenetic cause of MR. Prevalence estimates range between the initially presumed prevalence of about 1 in 1500 males and 1 in 2500 females (Warren & Nelson, 1994), to 1 in 6000 males established some years later in the Netherlands (de Vries et al., 1997). Most probably, the general prevalence for males is close to 1 in 4000 (Stevenson & Schwartz, 2002). Because of its high prevalence, and its implications for patient management and family counselling, given also the variability of the phenotype, in particular in female patients, *FMR1* testing should be strongly considered in all males and females with unexplained MR (Curry et al., 1997). DNA analysis of the *FMR1* gene is reliable, and indicated even in the absence of a positive family history. Microcephaly can be considered as exclusion criterium in male patients (de Vries et al., 1999). Checklists to evaluate which patients should be tested have been set up by Giangreco et al. (1996) for the pediatric and by de Vries et al. (1999) for both the pediatric and adult male population. These score for MR, (facial) phenotype, family history, and personality and behavior. Preselection with these checklists allows exclusion from testing in 60 % and 86 %, respectively. Future studies are yet needed to confirm the sensitivity of the scoring systems.

Following the discovery of fragile X syndrome, a growing list of X-linked MR syndromes has been identified, and also the number of autosomal forms of MR syndromes has increased steadily. In January 2005, the OMIM (Online Mendelian Inheritance in Man) catalog registered more than 1000 entries in which MR is a major or possibly associated feature. Most of these syndromes are rare, and/or only a (small) proportion of patients has MR. Some clinically relevant syndromes with MR as a major feature will be briefly presented here.

5.1.2 X-LINKED MR SYNDROMES OTHER THAN FRAGILE X SYNDROME

At present, in total 140 XLMR syndromes have been delineated, based on the clinical presentation. In 66, the responsible gene has been identified, and in addi-

tion, 50 X-chromosomal regions have been mapped but no pathogenic gene mutation has yet been identified (Ropers and Hamel, 2005; www.ggc.org/xlmr.htm; <http://XLMR.interfree.it>). For more clinical information on the syndromes listed below and on X-linked MR in general, the reader is referred in particular to the comprehensive treatise by Stevenson, Schwartz and Schroer (2000) and to the review written by Ropers and Hamel (2005).

Rett syndrome (OMIM #312750) is a severe neurodevelopmental disorder affecting mostly females. It is an important cause of severe MR in females and has an estimated prevalence of 1 in 10,000 girls at 14 years of age (Kerr, 1992). Diagnostic criteria were set up in the 1980s and revised in 2001 (Hagberg et al., 1985; The Rett Syndrome Diagnostic criteria Work Group, 1988; Hagberg et al., 2002). They focus on a characteristic course with normal initial development, followed by stagnation and regression of motor and mental abilities, loss of acquired skills, in particular purposeful hand-use, deceleration of head-growth, and autonomic failure. Classic and variant forms can be distinguished. The findings in particular in adolescent and adult females have been abundantly described by Smeets et al. (2003, 2005). In 1999, mutations in the *MECP2* gene have been found to cause Rett syndrome (Amir et al., 1999). They can be found in up to 90 % of females with classic Rett syndrome. The much rarer disorders in male patients associated with *MECP2* mutations will be discussed separately (see chapter 5.2).

Coffin-Lowry syndrome (OMIM #303600) has a well recognizable pattern of somatic features (Lowry et al., 1971; Gilgenkrantz et al., 1988; Hunter, 2002): short stature, hypotonia and joint hypermobility, soft hands with tapering fingers, and a distinctive facies evolving in childhood with hypertelorism, down-slanting palpebral fissures, anteverted nares and full lips (for review see Hanauer & Young, 2002). Female carriers may be normal, but often show less pronounced features. Coffin-Lowry syndrome is caused by mutations in the *RSK2* gene which maps to Xp22 (Trivier et al., 1996). Mutations are found in less than half of the patients and the condition is considered to be possibly heterogeneous (Zeniou et al., 2002).

XLMR-hypotonic facies syndrome (OMIM #301040) is caused by mutations in the *XNP* gene on Xq13 (Gibbons et al., 1995). They cause a spectrum of previously clinically delineated syndromes, including ATRX (α -thalassemia mental retardation) syndrome (Yntema et al., 2002; Abidi et al., 2003). The core phenotype of the whole spectrum consists of severe MR, hypotonia and in particular a hypotonic face possibly associated with growth impairment, genital anomalies and distinctive craniofacial features with microcephaly, hypertelorism, a small nose with anteverted nares, and a carpe-like, open mouth with prominent lips. DNA analysis is hampered by the great size and the complex organization of the

gene, and can be preceded by a screening test for hemoglobin inclusions in erythrocytes.

All three syndromes are chromatin remodelling disorders (for review see Ausio et al., 2003), meaning that mutations in these genes cause changes of the package of DNA into chromatin which lead to a general deregulation of transcription. Another chromatin remodelling disorder is Rubinstein-Taybi syndrome (see 5.1.4, Table II): the CREBBP protein is activated by and interacts with *RSK2*.

The *ARX* gene also contributes significantly to XLMR (Stromme et al., 2002; Stevenson and Schwartz, 2002; Mandel & Chelly, 2004). It directs neuronal proliferation, differentiation and migration within the forebrain. Different disorders are associated with this gene. X-linked lissencephaly with abnormal genitalia (XLAG) is caused by loss-of-function mutations (Kitamura et al., 2002), whereas a recurrent poly-A expansion (24 bp duplication), and rarer missense mutations, are associated with MR with dystonia, epilepsy/seizures and other neurological signs, and with X-linked West syndrome (ISSX) (Partington et al., 2004).

All four genes (*MECP2*, *RSK2*, *XNP* and *ARX*) are also associated with non-specific MR (see below).

5.1.3 SYNDROMIC AND NONSYNDROMIC X-LINKED MENTAL RETARDATION

In the early 1990s, the syndromic forms of X-linked MR (MRXS) discussed above were distinguished from non-syndromic forms (MRX) which have non-specific MR as the only sign (for review of both see Chelly & Mandel, 2001; Stevenson & Schwartz, 2002; and Ropers & Hamel, 2005). At present, at least 21 MRX genes have been identified. However, each accounts probably for less than 1% of MRX cases, with the exception of *ARX* mutations, which have a higher frequency in familial MRX (Mandel & Chelly, 2004). The prevalence of *JARID1C* mutations and of *PQPB1* mutations, which both cause non-syndromic and syndromic forms of MR, has not yet been established. According to first reports, these may also be high-prevalence contributors to XLMR (Kalscheuer et al., 2003; Lenski et al., 2004; Jensen et al., 2005).

In the pathogenesis of MR, aberrant Rho signaling plays an important role. Rho proteins (Rho GTPases) form a subgroup of the Ras superfamily of small GTP-binding proteins. They regulate the actin cytoskeleton in neurons, neurite outgrowth and branching, and synaptic connectivity (Ramakers, 2000; Ramakers, 2002). Several MRX genes have been shown to be involved in Rho-signaling, three of which (*OPHN1*, *PAK3*, *ARHGEF6*) participate directly in cellular signaling through RhoGTPases (Ramakers, 2002). It is intriguing to speculate that polymorphisms and mutations in Rho-linked genes may also

Table 5.1: X-linked genes causing syndromic (MRXS) and non-syndromic (MRX) MR

Gene (OMIM)	Locus	MRXS	Reference
<i>FGD1</i> (305400)	Xp11.21	Aarskog-Scott syndrome	Orrico et al. 2004
<i>JARID1C</i> (314690)	Xp11.23	MR and microcephaly, possibly with spastic paraplegia, seizures, hypotonic face a/o short stature	Jensen et al. 2005
<i>PQPB1</i> (300463)	Xp11.23	Renpenning syndrome	Stevenson et al. 2005
<i>RSK2</i> (300075)	Xp22.1-p22.2	Coffin-Lowry syndrome	Trivier et al. 1996 Zenou et al. 2002
<i>ARX</i> (300382)	Xp22.13	X-linked lissencephaly with abnormal genitalia; X-linked West syndrome; MR with dystonia a/o seizures	Stromme et al. 2002 Frints et al. 2002 Partington et al. 2004
<i>OPHN1</i> (300127)	Xq12	MR, cerebellar hypoplasia, hypotonia, seizures	Billuart et al. 1998 Philip et al. 2003
<i>XNP</i> (300032)	Xq13	XLMR-hypotonic facies syndrome	Gibbons et al. 1995
<i>AGTR</i> (300034)	Xq22-q23	MR, seizures	Vervoort et al. 2002 Bienvenu et al. 2003
<i>MECP2</i> (300005)	Xq28	Rett syndrome; different neurodevelopmental disorders in males*	Amir et al. 1999 Miltenberger-Miltenyi & Laccone 2003 Moog et al. 2003
<i>SLC6A8</i> (300036)	Xq28	Creatine deficiency syndrome	Salomons et al. 2003

*: see chapter 5.2

contribute to the natural variance of cognitive functioning in general, and to high cognitive functioning (Ramakers, 2002).

The distinction between MRX and MRXS has become less clear since it became known during the last years, that mutations in several X-linked genes can result in both MRX and MRXS. To date, at least 10 genes are known causing syndromal and nonsyndromal MR (see Table 5.1). The different expression may partly be explained by the different locations and/or type of mutations or different effects on splicing or gene function, as has been shown e.g. for the *FGD1* and *ARX* gene. These factors result in interfamilial variability. However, disorders related to

mutations in several genes, e.g. *XNP*, *RSK2*, *ARX*, show both inter- and intra-familial variability. The expression may also be influenced by an alternative mode of translation initiation, as recently shown for the *XNP* gene (Howard et al., 2004), or by other epigenetic, genetic or environmental modifiers (Stevenson & Schwartz, 2004). In order to illustrate these “vanishing boundaries between non-specific and syndromic forms of X-linked MR” (Frints et al., 2002), the phenotypic effects of *MECP2* mutations in male patients will be discussed in the chapter 5.2.

So far, it can be concluded that probably 100-150 genes for X-linked MR may exist which constitute 10-12 % of all genes involved in MR. Of these, about 50% have been identified. At least 10 genes may produce syndromic and nonsyndromic XLMR. Additional research is still needed, e.g. on genotype-phenotype correlation and in particular to identify other modifiers of gene expression.

5.1.4 AUTOSOMAL FORMS OF MENTAL RETARDATION DIAGNOSED BY MOLECULAR INVESTIGATION

Table 5.II is a selected list of well-known autosomal MR syndromes which can be confirmed by DNA analysis. Some other syndromes which primarily are identifiable by biochemical screening, will be mentioned in chapter 6, e.g. Smith-Lemli-Opitz syndrome, Sanfilippo syndrome and phenylketonuria. All of these, as for X-linked syndromes, have in common that, based on a suspicion on clinical grounds, molecular studies are specifically requested for the disorder in question for confirmation of the diagnosis and genetic counselling. This is in contrast to genome analysis by conventional chromosomal studies which are a screening method.

In contrast to the impressive number of syndromic autosomal forms of MR, only very few autosomal forms of non-specific MR have been identified so far. In 2002, the gene encoding neurotrypsin has been found to be involved in autosomal recessive non-specific MR (Molinari et al., 2002). Three further genetic loci have been found for autosomal recessive non-syndromic MR on chromosomes 3p, 4q and 19p. Several candidate genes for the chromosome 3p type, designated as *MRT2A* (OMIM 607417), have been analyzed recently but no mutation has been detected. This suggests that *MRT2A* is not related to homozygous mutations in one of the candidate genes, but to another, as yet unidentified, pathogenetic mechanism (Higgins et al., 2004). The involvement of autosomal genes in MR may be more complex than a model of monogenic inheritance. At present, molecular studies for non-syndromic autosomal forms of MR are irrelevant in daily practice.

Table 5.II: Selected autosomal dominant MR syndromes

Syndrome (s.) (OMIM)	Features in addition to MR	Locus	Gene	Molecular diagnosis	% of patients with MR
Angelman s. ^o (105830)	Microbrachycephaly, typical face Paroxysmal laughter Seizures, ataxia	15q11- q13	<i>UBE3A</i>	80%*	100%
Prader-Willi s. ^o (176270)	Neonatal hypotonia Short stature Hyperphagia Behavioral problems	15q11- q13	- - -	100%**	95%
Noonan s. (163950)	Short stature Heart defect Typical face	12q24.1	<i>PTPN11</i>	50 %	15-35%
Rubinstein- Taybi s. ^o (180849)	Growth retardation Broad thumbs + great toes Typical face Heart defect	16p13.3 22q13.2	<i>CREB</i> <i>EP300</i>	40%***	Almost 100%
Myotonic dystrophy (160900)	Infantile + congenital form: hypotonia, poor feeding, weakness	19q13.2- q13.3	<i>DMPK</i>	100%	Cong. form: 100%
Cornelia de Lange s. ^o (122470)	Growth retardation Upper limb anomalies Typical face	5p13.1	<i>NIPBL</i>	50%	Almost 100%
Tuberous sclerosis complex (191100)	Hamartomata Epilepsy	9q34 16p13	<i>TSC1</i> <i>TSC2</i>	80%	>50%
Sotos s. (117550)	High birthweight Overgrowth Macrocephaly, Typical face	5q35	<i>NSD1</i>	90%	variable

Legend:

^o virtually no transmission because of degree of MR and/or reduced fertility

* microdeletions, paternal UPD 15 and mutations

** microdeletions and maternal UPD 15

*** including microdeletions; most of mutations found in *CREB*

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5.2.1 Neurodevelopmental disorders in males related to the gene causing Rett syndrome in females (*MECP2*)

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ABSTRACT

Mutations in the *MECP2* (methyl-CpG-binding protein 2) gene are known to cause Rett syndrome, a well known and clinically defined neurodevelopmental disorder. Rett syndrome occurs almost exclusively in females and for a long time was thought to be an X-linked dominant condition lethal in hemizygous males. Since the discovery of the *MECP2* gene as the cause of Rett syndrome in 1999, *MECP2* mutations have, however, also been reported in males. These males phenotypically have classical Rett syndrome when the mutation arises as somatic mosaicism or when they have an extra X chromosome. In all other cases, males with *MECP2* mutations show diverse phenotypes different from classical Rett syndrome. The spectrum ranges from severe congenital encephalopathy, mental retardation with various neurological symptoms, occasionally in association with psychiatric illness, to mild mental retardation only. We present a 21-year-old male with severe mental retardation, spastic tetraplegia, dystonia, apraxia and neurogenic scoliosis. A history of early hypotonia evolving into severe spasticity, slowing of head growth, breathing irregularities and good visual interactive behaviour were highly suggestive of Rett syndrome. He has a *de novo* missense mutation in exon 3 of the *MECP2* gene (P225L). The clinical spectrum and molecular findings in males with *MECP2* mutations are reviewed.

Keywords: male; *MECP2*; missense mutation; Rett syndrome

INTRODUCTION

Rett syndrome (RTT, MIM *312750) is a progressive neurodevelopmental disorder occurring almost exclusively in females. After an apparently normal development until 6–18 months of age, girls with classical RTT show regression with deceleration of head growth and loss of speech and acquired motor skills, in particular purposeful hand use. They develop stereotypic hand movements, breathing irregularities, ataxia and seizures. After a period of pseudo-stabilisation and then further deterioration, the condition is mostly characterised by severe mental retardation with a relatively spared and often remarkable visual communicative ability, a progressive scoliosis and a variable degree of spasticity and rigidity often leading to wheelchair dependency. Clinical diagnosis is made in accordance with recognized criteria.¹ Apart from classical RTT, a *forme fruste*, a variant with preserved speech, cases with late onset of regression and congenital onset variants are known to occur in females.

In 1999, RTT was reported to be caused by mutations in the gene encoding methyl-CpG-binding protein 2 (*MECP2*) located at chromosome Xq28.² RTT had been thought to be an X-linked dominant condition leading to prenatal death in hemizygous males. In recent years however, *MECP2* mutations have also been reported in males. These males may present with a RTT phenotype when they have X-chromosome aneuploidy or somatic mosaicism for the mutation. However, when *MECP2* mutations occur in non-mosaic form and in chromosomally normal males, they lead to various neurological phenotypes different from RTT. We present a 21 year old male with severe mental retardation and a neurological disorder with features reminiscent of RTT in whom a *de novo* *MECP2* mutation was found, and review reported male patients with a *MECP2* mutation.

CASE REPORT

Patient report

The patient was born at term with a birthweight of 3900 g. Pregnancy and delivery had been uneventful. He had two older healthy brothers. The family history was negative for mental retardation and neurological disorders. There were no feeding problems. His very early infantile development was reportedly normal. Head circumference at birth is unknown but was 46.5 cm at age 11 months and 47.5 cm 3 months later (both slightly below the 50th centile). Around 3.5 months of age, his mother noticed 'a change' in making contact and in behaviour towards her. Shortly thereafter, he began to show axial hypotonia and a

marked head lag. His psychomotor development became severely retarded. He never learned to sit alone nor to walk and lost his ability to stand with support at the age of 2 years. However, he had some fine motor adaptive ability. From 2 years onwards, he showed motor restlessness and developed extrapyramidal symptoms. There was no speech development. He apparently had epilepsy from infancy to 7 years of age. A video taken at 11 years of age shows his posture in the wheelchair to be hypotonic with severe kyphoscoliosis and pelvic asymmetry. He could turn the pages of photo albums and booklets. His concentration was poor with little attention span. He developed severe spastic tetraplegia. At the age of 16 years, he lost purposeful hand use and became completely apraxic with flexion contractures of the wrists. There were periods of great anxiety, protest and over-agitation associated with dysregulation of body temperature.

On clinical examination at the age of 21 years, he was severely mentally retarded and confined to a wheelchair due to severe spastic tetraplegia. He was lean and small with a head circumference of 53 cm (3rd to 10th centile). He had a severe kyphoscoliosis, a hip dislocation on the right side, joint contractures of fingers, wrists, elbows and knees and had warm, sweaty hands and feet. He was abnormally prone to agitation and to repetitive daytime sleeping. Sometimes he became extremely pale and had shallow breathing interrupted by short apnoeic periods. He was able to eat mashed food, did not chew and had delayed swallowing. He had a good visual contact, recognizing his parents and caregivers both visually and vocally, but seemed to be very reserved towards strangers. His parents described him as having particularly strong feelings of sympathy or antipathy towards other people. He loved music and family soaps on television.

Chromosome analysis showed a normal 46,XY karyotype. A CT scan of the brain at 7 years of age and a MRI scan at 12 years of age, an EEG, SSER, BER and EMG were all reported as normal.

Materials and Methods

Genomic DNA was prepared from peripheral blood leukocytes using the Wizard Genomic DNA purification kit (Promega) according to manufacturers recommendations.

The *MECP2* coding region was polymerase chain reaction (PCR) amplified in eight, mostly overlapping, segments. The obtained PCR products obtained were screened for mutations utilising bi-directional sequencing utilising the Big Dye 2 Terminator Cycle Sequencing Kit (Perkin-Elmer Biosystems). After analysis on the ABI PRISM 3100 Genetic Analyzer (Perkin-Elmer Biosystems) the data were analysed using the Vector NTI DNA alignment software.

The presence of the identified putative mutation site in the *MECP2* segment ex3b was subsequently assessed using a single-strand conformational polymor-

phism (SSCP) analysis approach (GenePhor, Pharmacia Biotech) under the conditions showing an altered migration profile when the nucleotide substitution is present. As well as the DNA of the patient, his mother and brothers, an additional 96 control chromosomes (25 female and 46 male) were included in the SSCP study.

Results

DNA sequence analysis of the *MECP2* gene of our index patient revealed the presence of a cytosine to thymidine nucleotide substitution at position 674 of the coding region. This nucleotide substitution (C674T) leads to a proline to leucine amino acid change in the transcriptional repression domain (TRD) at position 225 of the MeCP2 protein (P225L). In contrast, subsequent DNA sequence analysis of the patient's mother and two elder brothers showed no base alteration in the *MECP2* gene. These results were confirmed by SSCP analysis. In comparison to the gel mobility profile of his family members, only the patient's amplified *MECP2* segment ex3b DNA showed an altered profile, indicative of the presence of a base change. Similarly, no changed mobility profiles were observed with the SSCP analysis of the 96 control chromosomes.

Consequently, our data indicate that the observed C674T substitution in our patient has arisen de novo and is not to be found in the general population.

CLINICAL CHARACTERISTICS OF *MECP2*-RELATED DISORDERS IN MALES

Since 1999, there has been a growing number of publications reporting male patients with a *MECP2* mutation. A summary of these cases, their clinical findings, family history and the particular mutation is given in Table 1.³⁻³⁰ The phenotypes cover a huge spectrum of neurodevelopmental disorders, ranging from classical RTT to mild mental retardation only and encompass congenital encephalopathy and mental retardation of different degrees in association with diverse neurological features and/or psychiatric illness.

Male patients who presented with a classical or almost classical RTT phenotype can be divided into two groups: those with aneuploidy of the X-chromosome (patients 1-4 in Table 1) and those in whom somatic mosaicism of the *MECP2* mutation was found (patients 5-7). In the former group, one mutation is reported as a de novo mutation,⁵ in the other reports this is not specified. The mutations in the latter group arose by definition due to a postzygotic event and thus occurred de novo. The RTT phenotype of both groups can be explained by the presence of a second X-chromosome containing the normal wild type allele as is the case in

female RTT. In both groups, patients are not consistently hemizygous for the mutation and thus differ considerably from cases 8–25 whom may be regarded as having true manifestations of *MECP2*-related disorders in males.

Five patients (cases numbers 8–12 in Table 1) presented with a severe congenital encephalopathy and died during the first two years of life.^{4, 15–19} They were all born in families with girls affected with typical RTT and/or a second boy with the same condition. Two of the five mothers were identified as asymptomatic mutation carriers with a skewed X-inactivation pattern,^{4–16} and another manifested mild learning disability and mild neurological symptoms.¹⁵ In the other two mothers, no mutation was found in blood; the recurrence of the mutation in a second child was most probably due to germline mosaicism.^{18–19}

Cases 13–18 all concerned families with several affected males.^{20–28} Most of them presented with a combination of neurological symptoms and mental retardation. The neurological features were very diverse and comprised pyramidal and extrapyramidal symptoms, ataxia, seizures and hypotonia. The degree of mental retardation was severe (Case 13 and 14) to mild (Case 15 and 18) and showed intrafamilial variability (Case 16). In case 18, no neurological symptoms were associated with mental retardation. The males described by Klauck et al. (Case 17) showed a combination of mental retardation, neurological symptoms (pyramidal signs), psychosis and macro-orchidism, the so called PPM-X syndrome.²⁷ In this family, the clinical features were variable as there were males with or without a psychiatric illness. One of the affected males of Case/Family 15 had severe behavioural problems. Regarding the carrier women, most were asymptomatic (Families 16–18, some carriers of Families 13 and 15) whereas others showed mild symptoms (borderline intelligence or mild retardation, mild hypotonia, gait disturbance, speech difficulty, tremor).

In Cases 19–22, only one male was affected due to a familial *MECP2* mutation detected also in at least the mother; these female mutation carriers were asymptomatic. In Cases 23 and 24, no DNA of the mother was available for mutation analysis. Again, clinical findings comprised mental retardation with (Case 19) or without neurological or other features (Cases 21–24); unfortunately, the clinical documentation of the latter cases was scant. Case 20 presented with a psychiatric and developmental receptive language disorder.³⁰ None of the male patients of Cases 13–22 showed deceleration of head growth which is an obligate feature in classical RTT.

When X-chromosome inactivation (XCI) was investigated, symptomatic carrier women had a random X chromosome inactivation (Families 13 and 14). In asymptomatic mutation carriers, however, both a skewed (Case 18) and a balanced X chromosome inactivation pattern (Cases 13, 19, 21, 22) were found. Thus the lack of symptoms in carrier women can not consistently be explained by a skewed XCI.

Compared with the whole group of male patients with a *MECP2*-related disorder, the present case shows some peculiarities. First, the mutation of our patient arose de novo which was not shown in any of the reported patients. However, the possibility can not be ruled out that the mutation is caused by germline mosaicism in his mother. Second, the clinical course and findings were dominated by features characteristic of RTT: a history of early hypotonia evolving into severe spastic tetraplegia and apraxia, slowing of head growth, neurogenic scoliosis and joint contractures, abnormal breathing pattern and a preserved visual interactive behaviour. The major difference with classical RTT is that postnatally he had only a short period of apparently normal development, that his hand use was relatively preserved for several years and that he did not develop the characteristic, stereotypic hand movements. The present case expands the described spectrum of MeCP2 related phenotypes in male variants of the Rett syndrome occurring in a chromosomally normal boy.

MOLECULAR GENETICS

MeCP2 protein and gene

MeCP2 is an ubiquitously expressed DNA-binding protein, which is particularly abundant in the brain. It contains a methyl-CpG binding domain (MBD), a transcriptional repression domain (TRD), and a final C-terminal segment. MeCP2 binds with its MBD to methylated DNA throughout the genome, is subsequently involved in deacetylation of core histones and chromatin condensation and thus leads to repression of transcription. Loss of function of MeCP2, especially in differentiated, postmitotic neurons, is predicted to lead to deregulated expression of putative target genes with a potential damaging effect during central nervous maturation.³¹

The gene encoding MeCP2 is located at Xq28 and undergoes X-inactivation. It comprises 4 exons, with the coding sequence in exons 2 to 4.³² Over 185 mutations have been reported in RTT patients. About two thirds are truncating and one third missense mutations, the latter occurring mostly in the MBD.³³⁻³⁴ 10% are intragenic deletions or complex rearrangements leading to frameshifts in the C-terminal segment. The mutation detection rate in sporadic female cases with classical RTT is 70-85% and lower in Rett variants.³⁴

MECP2 mutations in male patients

Most of the mutations found in Cases 1-12 (Table 1) who showed a RTT phenotype or had severe congenital encephalopathy, are known to occur in females

Table 1: Males with *McCP2* mutations

Case/ Family	Karyotype	Mutation	Region	Age	Clinical finding	Family history	Ref.
1	47,XXY/ 46,XY	T158M (C473T)	MBD	9 y	Classical R-TT phenotype		3 (pat 2)
2	47,XXY	T158M (C473T)	MBD	13 y	Classical R-TT phenotype		4
3	47,XXY	Y141X (C423G)	MBD	5.5 y	Classical R-TT phenotype	De novo mutation	5, 6
4	47,XXY/ 46,XY	R270X (C808T)	TRD	3.5 y	Classical R-TT phenotype	M 45,X/46,XX (low-grade mos)	7 (pat2)
5	46,XY	81F/S (241del2) Som mos	MBD	6 y	Atypical R-TT phenotype		8, 9
6	46,XY	R133H (G398A) Som mos	MBD	14 y	Classical R-TT phenotype		10
7	46,XY	R270X (C808T) Som mos	TRD	12 y	Classical R-TT phenotype		11
8	46,XY	268F/S (803del1)	TRD	Died at 12 m	Congenital encephalopathy	Maternal aunt and maternal half S: classical R-TT. M: mut. carrier, mild neurological symptoms, mild learning disability, SXCI	12, 13, 14, 15

Case/ Family	Karyotype	Mutation	Region	Age	Clinical finding	Family history	Ref.
9	46,XY	T158M (C473T)	MBD	Died at 11 m	Congenital encephalopathy	S: classical RTT. B: same clinical course, died at 9 m. M: asymptomatic mut. carrier, SXCI.	16
10	46,XY,inv (X)(q27q28)	384F/S (1154del32)	CTS	Died at 21 m	Congenital encephalopathy	B: similar clinical course, same inversion, died at 18 m. M: asymptomatic mut. carrier, SXCI.	4
11	nm	252F/S (754insC)	TRD	Died at 24 m	Congenital encephalopathy	S: classical RTT, same mutation. M: no mutation.	17 (A), 18
12	46,XY	163F/S (488del2)	Between MBD and TRD	Died at 13 m	Congenital encephalopathy	S: classical RTT, same mutation. M: no mutation.	19
13	a: nm b: 46,XY	Q406X (C1216T)	CTS	a: Died at 39 y from pneumonia b: 7 y	Severe MR + neurological features	Uncle (a) + nephew (b) affected 1 fem. mut. carrier: asymptomatic 1 fem. mut. carrier: borderline intelligence, mild facial hypotonia. Both random XCI.	20, 21
14		A140V (C419T)	MBD	27-40 y	Severe MR + neurological features	4 brothers affected M and S: mildly retarded, gait disturbance, speech difficulties, microcephaly. Both ± random XCI.	22, 23
15		R167W (C499T)	Between MBD and TRD	25-50 y	Mild MR + neurological features	4 males in 4 generations affected Obligate carrier women: at least 2 with essential tremor, normal intelligence	24 (A), 25
16		E137G (A410G)	MBD	21-56 y	MR of different degrees + neurological features	7 males in 2 generations affected All obligate carrier women of normal intelligence	25, 26

Case/ Family	Karyotype	Mutation	Region	Age	Clinical finding	Family history	Ref.
17	nm	A140V (C419T)	MBD	Different ages	MR, psychosis, pyramidal signs, macro-orchidism (PPM-X syndrome)	6 affected males of 1 family	27
18		del388-467 (1161del240)	CTS	nm	Mild MR	3 males in 2 generations affected Carrier woman: asymptomatic, SXCI	28
19	46,XY	G428S (G1282A)	CTS	3 y	Severe MR + neurological features	M and 2 maternal aunts: asympt mut. carriers, random XCI.	29
20	46,XY	A140V (C419T)	MBD	12 y	"developmental receptive language disorder", schizophrenia	M: asymptomatic mut. carrier	30
21	nm	A140V (C419T)	MBD	nm	Moderate MR (IQ 60-40) ^a	M: asymptomatic mut. carrier, balanced XCI	25
22	nm	A140V (C419T)	MBD	nm	Moderate MR (IQ 60-40) ^a	M: asymptomatic mut. carrier, balanced XCI	25
23	nm	P399L (C1196T)	CTS	nm	Moderate MR (IQ 60-40) ^a	M: normal intelligence. No DNA available.	25
24	nm	R453Q (G1358A)	CTS	nm	Moderate MR (IQ 60-40) ^a	M: normal intelligence. No DNA available.	25
25	46,XY	P225L (C674T)	TRD	21 y	Severe MR + neurologic features, male Rett variant	<i>De novo</i> mutation	Present case

Legends: MBD: methyl-CpG binding domain; TRD: transcriptional repression domain; CTS: C-terminal segment; nm: not mentioned; y: years; m: months; R TT: Rett syndrome; MR: mental retardation; a: insufficient clinical documentation; M: mother; S: sister; B: brother; mut.: mutation; XCI: X-chromosome inactivation; SXCI: skewed X-chromosome inactivation; A: abstract

with classical RTT. It is obvious that mutations leading to classical RTT in females do not necessarily result in prenatal death in males; they may be underdiagnosed because of the non-specific congenital onset encephalopathy.³⁵ It is also possible that the smaller number of males could relate to the paternal origin of the mutation in a large percentage of girls with classical RTT.

However, the relatively milder cases presenting with a spectrum of neurodevelopmental disorders (Cases 13–25) were associated with mutations not found in females with classical RTT. In contrast to the *MECP2* mutations reported in girls with classical RTT, truncating mutations (frameshift and stopcodon mutations) seem to be an exception in males with a normal karyotype: apart from one (Case 13) which is a very late truncating mutation, there are no truncating mutations reported in males. The mutation A140V is found in several male patients (Cases 14, 17, 20–22) and causes different phenotypes. It is intriguing that in both cases with psychiatric features (Case 17 and 20) this mutation was found. Kudo et al. studied the effect of this and the E137G mutation (Case 16) on MeCP2 function as compared with the protein function when a classical Rett mutation is present.³⁶ They found evidence for only mild impairment of MeCP2 function in both cases and argued that A140V is unlikely to be a polymorphism. That different mutations cause different functionality of the MeCP2 protein has certainly to be confirmed by future studies.

The described, newly identified missense mutation (P225L) observed in the boy currently reported is located in the TRD and is a highly conserved residue in mouse, chicken and *Xenopus* sequences. Since this particular mutation was shown to be de novo, it is highly probable that the leucine substitution is pathogenic. Moreover, a disease causing missense mutation has previously been identified in several female Rett patients at the same position (C674G) in the *MECP2* coding sequence, leading to a proline to arginine substitution.^{37–38} These two amino acid substitutions in the TRD may well have different effects on the function of the MeCP2 protein on basis of their chemical constitution and thus manifest distinct phenotypes.

CRITERIA FOR *MECP2* SCREENING IN MALES

On the basis of our current knowledge of MeCP2-related disorders, DNA analysis of the *MECP2* gene in combination with a chromosome investigation should seriously be considered in males with

- classical Rett syndrome,
- congenital encephalopathy,

- mental retardation and diverse neurological findings (including mental retardation and neurological findings reminiscent of Rett syndrome) when no other explanation has been found after a careful examination,
- a combination of mental retardation, pyramidal signs, psychosis and macroorchidism (PPM-X syndrome).

So far, it is debatable whether non-syndromic X-linked mental retardation (XLMR) should be added to the list of criteria for DNA testing. In the present series, Family 18 is the only example of non-specific mental retardation caused by a *MECP2* mutation. Unfortunately, the clinical findings of the four sporadic cases (Cases 21-24) are too poorly documented to exclude further neurological anomalies or features of RTT.²⁵ The mutation in family 18 was detected after the screening of 176 families with mental retardation of (possible) X-linked inheritance (material collected by the European XLMR consortium); in 13/176 families, the genetic defect could be linked to Xq28.²⁸ A subsequent study reported the occurrence of only one *MECP2* mutation in 500 males with unexplained mental retardation.³⁹ The low yield of these studies weaken the result of a previous study which suggested that *MECP2* might be highly mutated in X-linked mental retardation and account for $\pm 2\%$ of MR.²⁵ No *MECP2* mutations were detected in 42 autistic males with or without mental retardation.⁴⁰

In conclusion, *MECP2* mutations give rise to a spectrum of neurodevelopmental disorders in males which partially depend on the type of mutation and functionality of the protein.³⁶ Further studies will broaden our view on the clinical spectrum and possibly modify the criteria for testing.

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5.2.2 *MECP2* mutations are an infrequent cause of mental retardation associated with neurological problems in male patients

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ABSTRACT

Mutations in the methyl-CpG-binding protein 2 (*MECP2*) gene located on Xq28, cause Rett syndrome (RTT) in female patients. Meanwhile, non-mosaic *MECP2* mutations unknown in girls have been found in an increasing number of male patients with a normal 46,XY karyotype. They can cause a broad spectrum of neurodevelopmental disorders which often show a combination of mental retardation (MR) with neurological symptoms. We present the results of *MECP2* analysis in a group of 72 male patients with an unexplained combination of MR and neurological features, and review the mutational reports published on male patients since the discovery of the *MECP2* gene. Analysis included sequencing of exon 1 which thus far was mostly omitted from DNA screening. One pathogenic mutation has been found in a patient with Rett variant, in addition to an unclassified variant and a series of non-pathogenic changes. No changes have been found in exon 1. Criteria for testing of male patients are classic RTT, severe neonatal encephalopathy, and RTT variant which may be clinically underrecognized. Testing can also be considered in males with a combination of unexplained MR and (progressive) neurological manifestations although the yield of *MECP2* analysis is probably low in this situation. Based on the literature, *MECP2* testing in males with MR only is debatable.

Keywords: male patients, *MECP2*, Rett variant, unexplained mental retardation

INTRODUCTION

Rett syndrome (RTT, OMIM #312750) is a clinically defined neuro-developmental entity occurring mainly in females. Diagnostic criteria have been established for classic RTT leaning upon a characteristic developmental profile, and for variant forms of RTT [1]. RTT is thought to be the 2nd most common cause, after trisomy 21, of severe mental retardation (MR) in females [2]. In up to 90% of individuals with RTT, mutations in the *MECP2* (methyl-CpG-binding protein 2) gene can be found [3–5]. For further review of the clinical and genetic aspects, the reader is referred to a recent publication by Weaving et al. [6].

In the last few years, the phenotypic spectrum of *MECP2* mutations has expanded considerably and mutations have also been reported in an increasing number of male patients, reviewed by others and us [7,8]. Apart from males with RTT caused by a *MECP2* mutation and X-chromosome aneuploidy or somatic mosaicism of the mutation, and from male patients with severe neonatal encephalopathy due to a known *MECP2* mutation, reported cases consist of a group of patients with a broad spectrum of neurodevelopmental disorders. They have MR of various degrees, mostly in combination with neurological features, sometimes associated with psychiatric disorders, up to mild MR only [8]. These individuals often have familial mutations which are unknown to occur in females affected with RTT.

In order to study the phenotypic spectrum, the *MECP2* gene has been screened for mutations in various cohorts of mentally retarded males. The initial suggestion that *MECP2* mutations might be a significant cause of MR in males, comparable even to *FMR1* mutations [9], could not be confirmed by further studies as reviewed in detail recently [10]. In the latter study, no pathogenic mutation was found in a group of 103 males with unspecific MR. Interestingly however, the majority of published male patients with *MECP2* mutations presented with MR in combination with various neurological signs. In addition, *MECP2* studies in male patients did often not include analysis of exon 1. In this study, a group of 72 carefully examined male patients with an unexplained combination of MR and neurological symptoms was tested for *MECP2* mutations, including exon 1 analysis. Aim of the study was to further elucidate the phenotype of disorders caused by *MECP2* mutations in males and to reconsider the criteria for mutation analysis.

PATIENTS AND METHODS

Patients

A group of 72 male patients with MR and various neurological symptoms aged 2–83 years, with a mean age of 29.8 years, was tested for mutations in the *MECP2* gene. All patients had undergone a careful clinical evaluation by a pediatrician or a physician for persons with intellectual disabilities, and by a clinical geneticist trained in dysmorphology. Cytogenetic analysis was performed in all and additional investigations (e.g. *FMR1* analysis) in most cases. Patients with known diagnoses, as well as those with dysmorphism, an unexplained MR/multiple congenital anomalies syndrome or with indications for an external (e.g. perinatal) cause of their MR, were not included in the study group. The disorder was reported to be progressive in 12 patients including two with a clinical diagnosis of Rett variant. The neurological features were further unselected, and the patients were affected by one or more of the following features: different forms of epilepsy or seizures, spasticity, hypotonia, dystonia, choreoathetosis, ataxia or other cerebellar signs, tremor, rigidity, and other signs. The most frequent neurological features were epilepsy in 40/72 patients, including 5 males with West or Lennox Gastaut syndrome, and spasticity in 34/72 patients.

Methods

Total genomic DNA was extracted from blood samples using the Wizard Genomic DNA purification kit (Promega). All 4 exons of *MECP2*, including intron–exon boundaries, were amplified by PCR (in overlapping fragments for the exons 3 and 4) in each individual. Purified PCR fragments were screened for mutations by bi-directional sequencing, using the Big Dye Terminator cycle sequencing kit (Applied Biosystems) and analysis on an ABI 3100 DNA automated Sequencer.

The presence of an identified sequence change was tested in a control panel of 100 X-chromosomes which derived from 50 healthy male and 25 healthy female individuals.

RESULTS

In total, 7 sequence changes of the *MECP2* gene were identified (see Table 1). The c.674C>T mutation (p.P225L) occurred in a 22 year old male with RTT variant. He had an apparently normal early infantile development, showed hypotonia from 4 months of age emerging into severe spasticity, became severely

retarded and lost his initial motor abilities. At age 22 years, he had severe MR, spastic tetraplegia, dystonia, complete apraxia, neurogenic scoliosis, breathing irregularities and a good visual interactive behaviour highly suggestive for RTT. His data have been published in detail elsewhere [8]. This missense mutation has not been found in DNA of his mother and two brothers, nor in the control panel. The c.1214C>T mutation (p.P405L) was found in a 29 year old male with severe MR. His psychomotor development was retarded from the beginning, he had infantile convulsions and developed epilepsy from age 16 years on, autistic behaviour and characteristic, stereotypic hand movements. His head circumference was normal, he showed neither further neurological signs nor breathing irregularities; his visual interactive behaviour was poor. The family history was complicated by borderline intelligence in both parents and several other paternal and maternal family members. The sequence change was found also in the mother and the patient's sister who was of borderline intelligence and had epilepsy. It has not been detected in the control panel. Male family members were unavailable for testing.

The c.815C>T change (p.P272L) has been registered as polymorphism, unfortunately without reference [11], because it has been found in male and female relatives of a girl with RTT. Very probably, it thus is a nonpathogenic variant. In 4 other males, changes were classified as nonpathogenic variants because they were also found in the healthy maternal grandfather (c.602C>T and c.378-74C>T) or in our male control panel (2x c.378-17delT), respectively.

DISCUSSION

In this paper, we report on the results of *MECP2* analysis in 72 male patients with MR and associated neurological features. Adding to a previous review on *MECP2* related disorders in males, we want to update the knowledge on the phenotypic spectrum of *MECP2* mutations in males with MR, and we critically review clinical features thought to be suggestive for a *MECP2* mutation in males.

In the reported cohort of 72 male patients with MR and neurological features, 7 sequence changes have been found, of which 5 were probably nonpathogenic variants (see Table 1). The c.674C>T mutation is thought to be a pathogenic change, as it occurred *de novo*, is so far unreported in male control panels, and affects a highly conserved residue in the transcriptional repression domain (TRD) [8]. At the same position, C>G transitions have been reported repeatedly as pathogenic mutations. The c.1214C>T mutation is hitherto unreported and affects a conserved residue in the C-terminal segment (CTS). It occurred as a familial mutation, male family members were unavailable for testing and the

Table 1: Sequence changes identified in the *MECP2* gene in the present study

Nucleotide change	Aminoacid change	Domain	Control panel	Segregation in family	Conclusion	Clinical findings
c.674 C>T	p.P225L	TRD	neg	De novo mutation	Pathogenic	Male RTT variant
c.1214 C>T	p.P405L	CTS	neg	M & S carrier	Unclassified Variant	MR, epilepsy, autism
c.378-74 C>T	—	Intronic	—	Healthy M & GF carrier	Non-pathogenic	
c.378-17delT (2x)	—	Intronic	3 females 2 males	—	Non-pathogenic*	
c.602 C>T	p.A201V	IDR	—	Healthy GF carrier	Non-pathogenic*	
c.815 C>T	p.P272L	TRD	—	Healthy M carrier	Non-pathogenic*	

Legends: CTS: C-terminal region, IDR: Inter domain region, TDR: Transcription repression domain, GF: grandfather, M: mother, S: sister, *registered as non-pathogenic in the IRSA database [11]

family history made an unmistakable segregation study impossible. Up to now, it has to be regarded as an unclassified variant. The low yield of (possibly) pathogenic changes suggests that a *MECP2* mutation does not seem to be a common cause of neurodevelopmental disorders even in this clinically highly selected group.

In this study, we also analysed exon 1 and part of the promoter region. Previously, this part of the *MECP2* gene was considered noncoding. Recently, however, a new isoform has been discovered encoded by exons 1, 3 and 4, but not exon 2, and named MECP2B [12–14]. MECP2B was shown to be more abundant in human brain than the first described MECP2A isoform. In addition, the translation of the MECP2A transcript is severely impaired by translational interference with the upstream, in exon 1 initiated, open reading frame [12]. Considering these characteristics of MECP2B, it has been suggested that mutations in exon 1 could possibly be responsible for yet unexplained RTT patients. So far, they have however rarely been found in female patients with classic or atypical RTT testing negative for mutations in exons 2–4 [13–15]. A recent report indicates that exon 1 mutations are not a common cause of X-linked MR in male patients [16]. Previous *MECP2* studies in male patients however did not include exon 1 analysis. The present study does not indicate that the discussed neurodevelopmental disorders in males may be associated with exon 1 mutations. Including exon 1 analysis in routine screens will certainly give more insight in its significance. Because of the possibly crucial function of the MECP2B protein, it can in particular not be excluded that exon 1 mutations may be involved in more severe conditions such as neonatal encephalopathies in males.

Table 2 presents an overview of 8 published cases of neurodevelopmental disorders in males due to presumably pathogenic *MECP2* mutations. Three families were identified via the European Consortium for X-linked MR (families MRX16, T36, T44), two of which with nonsyndromic MR (MRX) and one with associated progressive neurological symptoms (MRXS). One further family also showed severe MR and a progressive neurological disorder. Two patients with *de novo* mutations, including the patient of the present study, had RTT variants. Finally, there were two sporadic patients with mutations in the CTS: one with a *de novo* mutation and clinical features unreported in all other patients (case 8), and one who clinically poorly documented (case 7). The recessive value of the mutations of these cases is difficult to explain by the type of the mutation and/or the location within the gene, mainly due to the small sample size. Compared to *MECP2* mutations causing RTT in females, there are more missense mutations, but nonsense mutations also occur. It is noteworthy that both *de novo* mutations leading to severe Rett variant, occurred in the TRD and resemble known recurrent mutations in females with RTT: the p.P225L mutation occurred at the same position as the recurrent p.P225R mutation; and the p.G273fsX mutation

Table 2: Overview of *MECP2* mutations in published male patients

Nr	Base change	Amino acid change	Domain	Clinical findings	Family history	Ref.
1	c.410A>G	p.E137G	MBD	MR of different degrees 7 ♂ in 2 G affected (MR-X16)	All obligate carrier ♀: normal intelligence	[9,39,40]
2	c.499C>T	p.R167W	MBD / TRD	Mild MR + neurological features 5 ♂ in 4 G affected (T36)	Obligate carrier ♀: at least 2 with essential tremor, normal intelligence; random XCI	[9,39]
3	c.674C>T	p.P225L	TRD	RTT variant	<i>De novo</i> mut.	Present study [8]
4	c.810_816dup7	p.G273fsX	TRD	RTT variant	<i>De novo</i> mut.	[41]
5	c.1161_1400del240	p.P388_P467 del80	CTS	Severe to mild MR 3 ♂ in 2 G affected (T44)	Carrier ♀: asymptomatic, SXCI	[20,39]
6	c.1216C>T	p.Q406X	CTS	Severe MR + neurological features Uncle (a) + nephew (b) affected	1 ♀ mut. carrier: asymptomatic; 1 ♀ mut. carrier: borderline intelligence, mild facial hypotonia; both random XCI.	[42,43]
7	c.1358G>A	p.R453Q	CTS	Moderate MR (IQ 60–40) ^a	M: normal intelligence. No DNA available.	[9]
8	c.1415_1416del2	p.E472fsX	CTS	Moderate MR, hypotonia, obesity, gynecomastia	<i>De novo</i> mut.	[19,44]

Legends: MBD: methyl-CpG binding domain; TRD: transcriptional repression domain; CTS: C-terminal segment; RTT: Rett syndrome; MR: mental retardation; ^a: insufficient clinical documentation; M: mother; G: generation; mut.: mutation; XCI: X-chromosome inactivation; SXCI: skewed X-chromosome inactivation; ♂: male(s); ♀: female(s)

encodes for a protein comparable to the product of mutations c.806delG (p.269fs) and c.808C>T (p.R270X) which occur in 3% and 6% of females with RTT, respectively. Mutations that occurred in the CTS (cases 5–8) all affect the functionally essential WW domain binding region which extends from residue 337 to the C terminus [17]. The recent report of a boy with severe retardation and features suggestive of RTT and a microduplication Xq28 involving the *MECP2* gene suggests that in some cases, also overexpression of *MECP2* might be pathogenetically involved [18].

Several clinically defined cohorts, including male individuals, have been screened for *MECP2* mutations. In cohorts of patients with MR only, collected by the XLMR consortium or testing negative for fragile X syndrome or clinically carefully selected as patients with unspecific MR [10,19,20], a low frequency of mutations has been found. The two patients identified in these studies to have a *MECP2* mutation, are included in Table 2 as patients 5 and 8. Based on our current knowledge, MR alone thus is a debatable criterion for *MECP2* analysis [10].

In view of the clinical overlap between RTT and Angelman syndrome (AS), one further group of interest have been male and female patients with presumed AS who tested negative for 15q11–q13 abnormalities [21–25]. In these studies, pathogenic *MECP2* mutations have been found only in a small percentage of female patients suspected to have AS [21–24]. Remarkably, they partly developed clinically a phenotype consistent with or suggestive of RTT [21–23]. No *MECP2* sequence changes of pathogenic value have been found in male patients with a clinical diagnosis of AS so far. Though two patients with somatic mosaicism of a known *MECP2* mutation have been ascertained by testing individuals negative for methylation defects in 15q11–q13, they clinically had RTT variant and classic RTT, respectively [21,24]. Altogether, *MECP2* mutations probably are much less frequently associated with a phenotype consistent with AS than presumed.

As the behaviour of patients with RTT might be mistaken for an autism spectrum disorder, and as RTT is classified as a form of autism in the DSM-IV [26], several studies have also been initiated to screen for *MECP2* mutations in a cohort of male and female individuals with autism, with or without MR. Most studies found no convincing evidence that *MECP2* mutations might contribute to the pathogenesis of autism [27–30], although in one study, pathogenic *MECP2* mutations have been found in 2 out of 69 autistic females [31]. However, the behavior of RTT patients most often is distinctly different from autistic behaviors, although low intensity in contact, odd behaviors, attention deficit and hyperactivity may predominate the disorder profile in infants for a long time. RTT is characterized at long term by the dissociation between diminishing neuromotor abilities, and a relatively good visual interactive behavior and preserved personality.

Combining the results of our study and the literature, *MECP2* analysis should be considered in particular in males with a history suggestive of R TT (R TT variant). As a detailed knowledge of the patient's history and awareness of the behavioral characteristics of R TT, in particular of the visual interactive abilities, is important for the diagnosis of a R TT variant, this disorder is possibly underdiagnosed in male patients. Preliminarily, testing may likewise be considered when MR is associated with a progressive neurological disorder, in particular in the lack of information on the patient's early history, although the yield of testing in this situation seems to be low.

In concurrence with other studies, the detected *MECP2* sequence changes comprised a high proportion of nonpathogenic polymorphisms or unclassified variants [7,10,19]. The validation of hitherto unreported nucleotide changes leading to amino acid changes, has to be done carefully and shows some general problems. A *de novo* change is a strong argument for pathogenicity. However, mutations are often familial. The change of a highly conserved amino acid or the rareness of a variant which remains undetected in a control panel, are helpful but insufficient arguments to assess pathogenicity. Testing of unaffected male family members is strongly advisable, but often they are inaccessible for investigation. In addition, there are, up to now, very few functional studies [32,33]. In general, the pathogenicity of *MECP2* mutations in males can hardly be confirmed and only rejected. We fully agree with Laccone and coworkers who reclassified two previously published cases of presumably pathogenic mutations as non-disease related variants and therefore appealed to "... proceed with caution" [34]. The p.A140V mutation has been reported in 6 other, familial cases with remarkably diverse phenotypes [9,35-38], but so far is of unknown value [11,34].

In conclusion, a synopsis of the current knowledge on *MECP2* related disorders in mentally retarded males has been given. For the time being, appropriate criteria for *MECP2* testing in male patients are: male R TT, classic or atypical, and severe neonatal encephalopathy negative on testing for other causes. A combination of unexplained MR and (progressive) neurological manifestations, in particular of extrapyramidal and/or pyramidal origin, seems to be a less certain criterion.

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6 METABOLIC STUDIES

To test or not to test? Metabolic testing in mentally retarded
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6. To test or not to test? Metabolic testing in mentally retarded adolescent and adult patients

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ABSTRACT

Metabolic disorders are responsible for only a small fraction of mental retardation (MR), in particular in adolescent and adult patients. Testing in a selected group of patients has therefore been strongly recommended, and criteria for testing have been formulated repeatedly. On the other hand, knowledge on in-born errors of metabolism associated with MR has increased remarkably, and has delineated a broad phenotypic spectrum including MR associated with neurologic signs, abnormalities on neuro-imaging studies, malformations and dysmorphism.

In order to evaluate the criteria for metabolic testing in adolescent and adult mentally retarded persons, we analysed retrospectively the outcome of metabolic investigations performed during a 10-year period in 256 mentally retarded persons aged 16 years or older from 245 families. All patients had been carefully clinically evaluated. Testing included in most cases screening for congenital disorders of glycosylation (CDG), and for defects in cholesterol biosynthesis (e.g. Smith-Lemli-Opitz syndrome, SLO) in 112 patients. In 13 patients from 10 families, a metabolic disorder explaining the MR was found: homocystinuria (N = 1), galactosemia (N = 1), phenylketonuria (N = 2, from the same family), Sanfilippo type A (N = 1), Sanfilippo type B (N = 3), SLO syndrome (N = 5, from 3 families). The respective diagnosis was suspected on clinical grounds in 4/10 index patients; 5 further index patients fulfilled one or several criteria for targeted metabolic testing. One diagnosis (homocystinuria) would have been missed if clinical selection had been performed. Regression, neurodegenerative signs, behavioral problems, or a sibling with the same condition and/or consanguinity, were important indicators for an underlying metabolic defect. We propose an adapted list of clinical findings which may indicate the need for metabolic testing in adolescent and adult mentally retarded persons.

Keywords: mental retardation; metabolic testing

INTRODUCTION

For several reasons, it is difficult to estimate the proportion of mental retardation (MR) which is due to inborn errors of metabolism. In different studies, there is no uniform set of detectable metabolic diseases as both the definition of metabolic diseases, as well as the screening protocols for their detection, differ. Studies on the etiology of MR do not always comprise a separate category for metabolic disorders, but include them in categories such as single gene or neurodegenerative disorders, or do not even mention them at all. In addition, disorders only possibly related to MR may or may not be included in the yield of metabolic testing.

Despite these limitations, Curry et al. [4] concluded in their review that 0-5% of MR are due to metabolic conditions; the study with the highest yield of 5% however, was based on a relatively small pediatric population (60 children) and included entities with uncertain relationship to MR [14]. Allen and Taylor [2] found in their large population based South Carolina study which included all ages, that metabolic disorders explain MR in 0.8% of cases. Van Karnebeek et al. [28] found a mean yield of 1% in a literature review of metabolic studies, and admitted that much higher figures can be found in specific, e.g. highly inbred, populations. Shevell et al. [22] evaluated metabolic studies involving pediatric populations with developmental delay, and found a similar yield of about 1% in the largest studies using non-selective screening protocols. The overall conclusion is that metabolic disorders constitute only a small fraction of the causes of MR. This proportion may even be less than 1% in adolescent and adult patients as inborn errors of metabolism most often present in infancy and childhood, and are associated with increased mortality early in life. Furthermore, neonatal screening programs for metabolic disorders increasingly led to the early detection of various conditions, and thus to a decrease of these diagnoses later in life.

Because of the low yield of metabolic testing and in addition, a high frequency of non-specific, non-diagnostic abnormalities necessitating further evaluation but not resulting in a clear diagnosis, testing in a selected group of patients has been strongly recommended for all ages [4]. This has been confirmed by Van Buggenhout et al. [26] for adult patients with MR; they screened 306 adult, institutionalized patients, and diagnosed metabolic disorders explaining the MR in four cases, but found in 40 patients metabolic abnormalities of unknown significance. Curry reinforced the need for focused testing on the basis of history, or on physical, neurologic and behavioral assessments, including a MRI of the brain, also in adolescents [5].

Despite a low yield, metabolic testing may however have large implications for the patients and their families [10]. Metabolic conditions may be potentially preventable, or amenable to treatment. As with other secure diagnoses, there is

also an immediate impact on genetic counselling. Another point of consideration is that the knowledge on inborn errors of metabolism associated with MR, has increased considerably. A surprisingly broad phenotypic spectrum has been delineated, including MR associated with neurologic signs, abnormalities on neuroimaging studies, congenital malformations, and dysmorphism [10]. The question arises as to whether a focused testing procedure relies on a sufficient number of signs which indicate a possible metabolic defect.

After some surprising metabolic diagnoses in adolescent and adult mentally retarded patients, we decided to analyse retrospectively the outcome of metabolic investigations performed over a 10-year period in mentally retarded persons aged 16 years or older. In particular, we were interested in the criteria which led to the tests, and in the clinical presentation at the time of diagnosis.

MATERIAL AND METHODS

Patients

Between 1994–2003, a metabolic testing was performed in 256 patients from 245 families with unexplained MR. The patients included in the study were 16 years or older, with a mean age of 43.5 years. 120 patients were females and 136 were males. 26 of the 245 index patients had a brother or sister with the same disorder, consanguinity was documented in three families. All patients lived in the south of the Netherlands, 92% of them in different institutions for the mentally retarded. The degree of MR was mild in 29, moderate in 61, severe or profound in 150, and not further specified in 16 patients. The history of 35 patients reported regression in childhood or later in life. 63 of 256 patients showed severe behavioral problems, 22 had a psychiatric disorder, and 20 patients were reported to have autism; in 10 of these 20 patients, MR and autism were the only features. 71 of 256 patients had seizures, eight showed ataxia and 12 other neurologic features possibly indicating a metabolic disorder, such as hypotonia or parkinsonism. The following eye features possibly related to metabolic disorders were found: cataracts in 10 of 256 patients, retinal abnormalities in five and ophthalmoplegia in one patient. Bone abnormalities in two patients and ichthyosis in one patient were further indicators for metabolic testing. To establish an etiological diagnosis, all patients had undergone a careful clinical investigation. This included physical examination by a clinical geneticist, assessment of the family history whenever possible, cytogenetic analysis, and *FMR1* (Fragile X Mental Retardation 1) gene analysis in most cases. In none of the patients, a metabolic disorder had been diagnosed previously. During the period under investigation, clinical criteria for testing had not been formally assessed. In general, metabolic screening had been

performed because of a clinical suspicion that a specific disorder existed, or was based on signs possibly indicating a metabolic disorder (targeted testing). A small subgroup of 38 patients had been tested without clinical preselection (unselected screening).

Methods

The programme for metabolic screening of patients with MR in the period 1994 – 2003 comprised investigation of random urine and plasma samples according to standard procedures (Table I). 229/256 patients were also investigated for congenital disorders of glycosylation (CDG) and 112 patients for abnormalities in cholesterol biosynthesis after introduction of the analysis of sterols in 1998.

Table I. Analytical programme for selective metabolic screening (1994–2003)

Urine	
Group screening tests	Method
Amino acids	Ion-exchange chromatography
Alditols/monosaccharides	GC-MS
Indoles/phenolic metabolites	RP-HPLC
Mucopolysaccharides	DMB assay; electrophoresis
Oligosaccharides	TLC
Organic acids	GC-MS
Purine/pyrimidine bases and nucleosides	RP-HPLC
Sialic acid	RP-HPLC
Plasma/serum	
Assay	Method
Amino acids	Ion-exchange chromatography
Total homocysteine	RP-HPLC
Sialic acid	RP-HPLC
Sterols	GC-MS
Very long chain fatty acids	GC-MS
Sialotransferrins	IEF

Legends: DMB: dimethyl-methylene blue spectrophotometric assay, GC-MS: gas-chromatography-mass spectroscopy, IEF: iso-electric focusing, RP-HPLC: reversed-phase high-performance liquid chromatography, TLC: thin-layer chromatography

RESULTS

In 13/256 patients from ten families, a metabolic disorder which caused their MR could be diagnosed: homocystinuria ($N = 1$), galactosemia ($N = 1$), phenylketonuria (PKU) ($N = 2$, both from the same family), Sanfilippo type A ($N = 1$), Sanfilippo type B ($N = 3$), and SLO syndrome ($N = 5$, from 3 families). Diagnoses of a metabolic disease unrelated to MR, e.g. hypercholesterolemia, have not been included. Numerous other anomalies have been found on a first metabolic investigation which however, could not be confirmed on further examination. In four of these cases, further analysis was not possible, and thus we cannot exclude that one or several of these patients also suffered from a metabolic disorder. These cases, however, have also been left out of consideration.

The clinical data of the ten index patients are summarized in Table II.

In patient 1, a first metabolic testing was performed elsewhere in infancy because of developmental delay, feeding problems and hepatic dysfunction. However, galactosemia was not identified, and only an unexplained increase of several amino acids was detected. As the exact screening protocol was unavailable, the reason for this negative result is unknown. On examination at age 16 years, Angelman syndrome was primarily suspected, based on the clinical signs. Methylation defects of chromosome region 15q11-13 were ruled out. A metabolic screening was initiated because of the combination of MR with epilepsy and unexplained liver disease, and showed increased excretion of galactose and galactitol. Subsequent enzyme investigation indicated galactose-1-phosphate uridyl-transferase (GALT) deficiency [27]. The diagnosis of classic galactosemia was confirmed by DNA analysis which showed compound heterozygosity for two *GALT* mutations. A galactose-restricted diet was started to ameliorate further progression. Ophthalmologic examination revealed no cataracts.

In patient 2, a metabolic testing was done because of an unexplained MR syndrome and a brother presenting with the same features. PKU was diagnosed in both. In retrospect, the clinical features of both brothers, i.e. MR, microcephaly, fair hair, blue eyes, were consistent with a PKU phenotype, but in the beginning had not been recognized as such. A low phenylalanine diet was initiated in both individuals.

Patient 3 did not show any sign indicative for a metabolic condition in general (Table III), or for homocystinuria in particular. She had moderate MR and hypermetropia without further eye features, a length of 163 cm and weight of 69 kg with truncal obesity, and was tested unselectedly. Homocystinuria was detected by increased urinary excretion of homocystine, strongly increased total homocysteine in plasma and slightly increased plasma methionine.

In patient 4, Sanfilippo syndrome (mucopolysaccharidosis type III, MPS III) was suspected because of severe behavioral problems, coarse facial features,

macrocephaly, short broad hands, and the patient's history (see Table II). Urinary mucopolysaccharides in this patient were strongly increased. On the electrophoretic pattern heparansulfate was present as predominant fraction. Sanfilippo A (MPS IIIA) was confirmed by a strongly decreased activity of heparan N-sulfatase in leucocytes and skin fibroblasts.

In patients 5-7, obvious regression in childhood or young adulthood, and the combination of MR with behavioral problems prompted a metabolic screening. In all three cases, the urinary excretion of mucopolysaccharides was increased, with increased heparansulfate as assessed by electrophoresis. The diagnosis Sanfilippo type B was confirmed by severely deficient activity of alpha-N-acetylglucosaminidase in plasma, leucocytes and cultured fibroblasts.

Patients 8-10 were all severely mentally retarded and showed clinical signs pointing to the diagnosis of SLO syndrome, e.g. 2-3 syndactyly of the toes, short stature, severe genital anomalies, microcephaly, facial dysmorphism and light-hypersensitivity of the skin. Patient 8 and his brother have been published previously [6, 7]. In all patients, severely decreased cholesterol levels and increase of the cholesterol precursors 7- and 8-dehydro-cholesterol were assessed. The diagnosis was confirmed by severely decreased activity of 7-dehydrocholesterol reductase in fibroblasts, and by DNA-analysis which showed compound heterozygosity for two mutations in the *DHCR7* gene in all three patients.

DISCUSSION

We evaluated the outcome of metabolic testing in 256 patients from 245 families (age = 16 years) with unexplained MR. In particular, we analysed the clinical pictures and the circumstances that had led to testing of the index patients.

Relatively few classic metabolic diseases can be associated with a clinically recognizable phenotype in adulthood, e.g. SLO syndrome, several types of mucopolysaccharidoses (MPS), or untreated PKU. The majority of metabolic diagnoses however, is the result of metabolic testing, initiated because of one or several signs indicating the general need of metabolic investigations. Criteria for such testing have been formulated by a Consensus Conference of the American College of Medical Genetics [4, Table VI]; they partly refer to the pediatric situation, e.g. the failure of appropriate growth. Van Buggenhout et al. [26] in their study of adult mentally retarded patients concluded that, in addition to these criteria, behavioral or neurodegenerative problems or specific dysmorphic signs should prompt a metabolic testing. Curry emphasized the importance of neurologic and psychiatric symptoms, white matter abnormalities on MRI, or specific physical signs, as possible indicators for a metabolic disorder in the adolescents [5].

Table II. Metabolic diagnoses among 256 mentally retarded patients and summary of clinical data

Pat Nr	Diagnosis (OMIM)	Sex, Age	Clinical features at time of examination	History	Family history	Ind. for test
1	Galactosemia (#230400)	M, 16 y	Severe MR, spasticity, epilepsy, microcephaly, happy behavior, frequent laughter	Developmental delay from birth, liver disease at 4 m, no galactosemia on testing, progressive spasticity, epilepsy from 13 y	P + P's sister epilepsy	TT
2	PKU (+261600)	M, 49 y	MR, microcephaly, fair hair, blue eyes		B (62 y) same disorder	TT
3	Homocystinuria (+236200)	F, 41 y	Moderate MR, hypermetropia			US
4	Sanfilippo A (#252900)	M, 34 y	Deep MR, psychosis, severe behavioral problems + unrest, macrocephaly, coarse facial features, short stature, brachydactyly	Pre- and postnatal growth retardation, slow development, recurrent infections, umbilical hernia, hydrocele, regression from 8 y, later behavioral problems + cyclic psychoses		CS
5	Sanfilippo B (+252920)	M, 28 y	Severe MR, no speech, unrest	Umbilical and inguinal hernia, often infections, behavioral problems from beginning, mental development delayed, regression from 18 y		TT
6	Sanfilippo B (+252920)	F, 45 y	Severe MR, behavioral problems, decreased mobility and balance	Developmental delay from 4y, regression from 25 y		TT
7	Sanfilippo B (+252920)	F, 63 y	Severe MR, dementia, poor motion and facial expression	Behavioral problems from beginning, slow regression from 10 y	Consanguinity	TT

Pat Nr	Diagnosis (OMIM)	Sex, Age	Clinical features at time of examination	History	Family history	Ind. for test
8	SLO syndrome (#270400)	M, 32 y	Severe MR, microcephaly, II/III syndactyly of toes, genital anomalies	Pre- and postnatal growth retardation, severe feeding problems and recurrent infections in early childhood, cleft palate repaired	Consanguinity B same disorder	CS
9	SLO syndrome (#270400)	M, 32 y	Severe MR, II/III syndactyly of toes, sex reversal, aspecific facial dysmorphism	Severe developmental delay from the beginning, raised as a girl but 46,XY karyotype	Sib died at young age due to MCA	CS
10	SLO syndrome (#270400)	F, 52 y	Severe MR, no speech, II/III syndactyly of toes, aspecific facial dysmorphism, severe behavioral problems		S same disorder	CS

Legends: PKU: phenylketonuria, SLO: Smith-Lemli-Opitz, F: female, M: male, m: months, y: years, MCA: multiple congenital anomalies, MR: mental retardation, B: brother, P: father, S: sister, CS: clinical suspicion, TT: targeted testing, US: unselected screening

In the present study, inborn errors of metabolism which explained the MR, were diagnosed in 13 patients from ten families. The respective diagnosis was suspected on clinical grounds in 4/10 index patients. Five further patients fulfilled one or several criteria for targeted metabolic testing as defined by the aforementioned Consensus Conference [4], or had a sibling with the same (unexplained) disorder. In concordance with Van Buggenhout et al. [26], in particular regression, neurodegenerative signs and behavioral problems were shown to be important indicators for underlying metabolic defects, furthermore a sibling with the same condition or consanguinity assessed in 2/10 families of our study. Based on the literature and our own experience, Table III proposes an adapted list of selected clinical findings urging the consideration of metabolic investigations in adolescent and adult patients with MR. One patient did not present any clinical sign indicating an inborn error of metabolism (patient 3 with homocystinuria, see below).

Table III. Selected clinical findings which may indicate the need of metabolic testing in adolescent and adult mentally retarded persons [adapted from Curry et al., 1997]

Loss of psychomotor skills, regression*, neurodegenerative signs*
Seizures, ataxia, hypotonia, recurrent somnolence/coma
Serious behavioral problems*, psychiatric signs*
Coarse appearance
Hepatosplenomegaly
Eye abnormalities (cataracts, ectopia lentis, ophthalmoplegia, corneal clouding, retinal abnormalities)
Skin abnormalities (angiokeratoma, “orange-peel” skin, ichthyosis)
Bone abnormalities (dysostosis, occipital horns)
Arachnodactyly
Structural hair abnormalities
Abnormal sexual differentiation
Unexplained deafness (with onset in childhood*)
Sibling with similar features and/or consanguineous parents*

* Feature added to the Table by Curry et al. [1997]

The diagnoses of this study included galactosemia, homocystinuria, phenylketonuria (PKU), Sanfilippo syndromes A and B, and Smith Lemli Opitz (SLO) syndrome; all of these are autosomal recessive conditions known to be frequently found among metabolic disorders associated with MR. Classic galactosemia normally presents in the neonatal period and if untreated, often leads to early death. Untreated survivors develop early cataracts, chronic liver disease, and MR [8]. Neonatal screening for galactosemia has been introduced in many countries in order to promote early treatment with galactose-

restriction. However, long-term complications such as infertility, ataxia, a subtle decline in IQ, and speech abnormalities, have been frequently recognized even among well-treated individuals [30, 21]. Discussions about the benefits of early diagnosis by newborn screening and on the cost efficiency of such a program is controversial [3, 19]. In the Netherlands, newborn screening does not yet include galactosemia.

PKU is the prototype of preventable, genetic MR. A low phenylalanine diet and strict metabolic control throughout life lead to a normal or near-normal development (for recommendations of management, see [18]). Newborn screening programs for the early detection of PKU have been established in most countries, in the US about 40 years ago and in Western Europe including the Netherlands in the 1970s. It has however to be born in mind that among adult and elderly mentally retarded persons, PKU may still be a relative frequent finding. Untreated individuals with PKU, in addition to MR as the clinical hallmark, can show seizures, behavioral problems and/or psychiatric pathology, microcephaly, eczema, a pale pigmentation, and a peculiar odor [20]. If PKU is diagnosed in previously untreated individuals, at least behavioral problems should prompt dietary treatment [18]. 30 to 40 years after the implementation of newborn screening, fetal damage due to maternal PKU may however become a more important issue of concern. Maternal PKU syndrome comprises MR, microcephaly, intra-uterine growth retardation and congenital heart malformation. For its prevention, careful metabolic monitoring before and during pregnancy is essential [18]. For untreated pregnancies, the risk of an affected child is greatly increased [12, 13].

The major clinical features of homocystinuria consist of MR, a characteristic Marfanoid habitus but with joint limitations and generalized osteoporosis, eye features such as ectopia lentis and myopia, and vascular problems [16, 17]. Psychiatric problems are seen in about 50 % of patients [1]. However, clinical variability is well recognized with IQ ranging from 10 to 135, and about one third of patients have normal intelligence [17]. Homocystinuria may also present as aspecific MR as has been illustrated by patient 3 [17]. Thus, this diagnosis may only be established on systematic metabolic screening and can be missed if only targeted metabolic testing is performed.

Sanfilippo syndromes are characterized by severe involvement of the central nervous system leading to neurologic degeneration, behavioral problems, and mental decline; somatic features are relatively mild, in particular in Sanfilippo B which is the most common subtype of MPS in the Netherlands [29]. Possible somatic problems in adulthood are epilepsy, cardiomyopathy, arthritis, bullous skin lesions, and swallowing difficulties requiring feeding by a percutaneous endoscopic gastrostomy (PEG) tube. Compared to the other MPSIII types, the course of Sanfilippo B is protracted. Developmental delay and behavioural prob-

lems are typically noted after 4–6 years, cognitive decline is evident at 10 to 30 years, and patients between 20 to 40 years are characterized by severe MR and increasing behavioral problems. Loss of mobility may be seen late in adulthood. Sanfilippo A is more frequent in North Europe, and is characterised by a much earlier onset and a more rapid deterioration [27]. Due to the rather aspecific phenotype of, in particular, MPS IIIB, and the variability in expression, Sanfilippo syndromes may be underdiagnosed in the mentally retarded, or diagnosed only late in life as illustrated by patients 5–7 [15].

SLO is characterized by recognizable craniofacial features in childhood, in addition to 2–3 syndactyly of the toes, genital anomalies in males, and mostly severe MR [11]. However, the facial appearance changes with age and is less characteristic in adulthood; a coarse face with broad alveolar ridges, large crowded teeth, and a bulbous nasal tip may be present. Patients are usually short statured and show a sunlight hypersensitivity. The genital anomalies in males may be less prominent or even disappear in adulthood [6]. Behavioral problems with self-injurious behavior are common [25]. Trials with a combination of a high cholesterol diet with statin treatment so far gave no convincing results [23].

During the past decade, a rapidly growing number of congenital disorders of glycosylation (CDG) have been identified. CDG cause a broad range of phenotypes, but usually present in childhood [9]. In the present cohort of patients aged 16 years or older, screening for CDG syndromes has been done on a large scale by isoelectric focusing of serum transferrin, but no patient was diagnosed with this disorder.

The discussed diagnoses have been assessed over a 10 year period during which the clinical criteria for testing have been inconsistent. It seems thus inappropriate to use the data to establish the prevalence of metabolic causes of MR in adolescent and adults. It should also be emphasized that metabolic diagnoses in adolescent or adult patients with MR who did not meet the general criteria for this study, e.g. who had not undergone an examination by a clinical geneticist, were not included. In general, neonatal screening programs will further decrease the number of adolescents and adults who present with unexplained MR due to an inborn error of metabolism. The advent of new analytical methods, in particular of tandem spectrometry, has revolutionized newborn screening and a wide range of disorders, in particular aminoacidemias and organic acidemias, are now diagnosed early in life. However, a non-uniform range of diseases is screened for in the different countries, and several of the diagnoses of the present study group (SLO, MPSIII) are not amenable to screening on blood spots. The diagnosis of metabolic disorders in adolescent and adult mentally retarded individuals thus remains a challenge. Once a diagnosis established by biochemical investigation, patients can be further evaluated for enzyme function, genotype or other factors possibly influencing the phenotype. Knowledge of both the natural history and adult

phenotype of metabolic disorders, and of the underlying gene defect and pathophysiology will be necessary to promote the treatment of metabolic disorders.

In conclusion, most metabolic diagnoses, if not specifically suggested on clinical grounds, would have been established by screening in a selected group of patients. We therefore agree that metabolic testing should follow a targeted procedure. In view of the possible benefit of a diagnosis, however, we want to stimulate awareness of the broad spectrum of possible indicators, such as those listed in Table III, and strongly recommend to discuss patients with unexplained MR with a metabolic clinical specialist.

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7 GENERAL DISCUSSION

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7.1. BENEFIT OF GENETIC EVALUATION OF MENTAL RETARDATION

This thesis is dealing with a number of clinical and laboratory diagnostic investigations in MR, and their yield. A consideration of the benefits of evaluations of MR seems equally important.

The central issue of the genetic evaluation of MR is its causality. Once a causal diagnosis has been established, questions regarding its clinical presentation, pathogenesis, management, prognosis, recurrence risk, and possibly even its prevention can (partly) be answered. In other terms, answers to the most important questions of the parents, or other family members, and of care providers can be given appropriately. The enormous benefit of a causal diagnosis has frequently been addressed (among others by Curry et al., 1997, Wilska and Kaski, 2001, and Alexander, 2002). According to this literature and to this thesis, the most important aspects will be briefly addressed:

Establishing a **diagnosis** in the very first place means giving an answer to the most burning question: what does he/she have? Giving the „what“ a name means making things clear, means a release from uncertainty, and means having the power of knowledge (see below: what's in a name?). In general, a causal diagnosis also permits conclusions about **causation and pathogenesis**, and thus it permits answers to the second question: why did it happen? Not infrequently, wrong admissions, in particular those regarding possible teratogenic effects and traumatic events during pregnancy, and the effects of perinatal distress, can be corrected. Sometimes a guilt feeling may disappear.

Multiple questions regarding **management and prognosis** focus on: what does this diagnosis mean to my child (brother/sister)? In a growing number of genetic syndromes, management guidelines are available, often via the internet. They are edited for the most common syndromes as practical one-volume books (e.g. Wilson and Cooley, 2000; Cassidy and Allanson, 2005). They contain information on medical complications and their prevention, as well as schemes for regular follow-up; a summary of the natural history, and in addition, short-term and long-term prognoses are given. Furthermore, in an increasing number of cases, knowledge about developmental profiles and behavioral patterns can be translated into an adapted psycho-social care. Patient support groups provide further information. They facilitate the contact between families in order to let them share their experiences and knowledge, and to give the opportunity to address open questions. For the Dutch situation, the reader is referred to the websites of the Federation of Dutch organisations of parents of people with MR (Federatie voor Ouderverenigingen, www.fvo.nl), of the Erfocentrum, a national information center (www.erfelijkheid.nl), and of the Dutch alliance of patient support groups (Vereniging Samenwerkende Ouder- en Patientenorganisaties,

www.vsop.nl). In the absence of such a group, the clinical geneticist can help the family contact other families who have a member with the same condition. It also belongs to the tasks of the clinical geneticist to provide information on the syndrome in question, and on its management, to the care providers. For patients with specific, well-known syndromes, e.g. with Down, Prader-Willi or Rett syndromes, multidisciplinary teams offer their services in specialised out-patient clinics. There are thus numerous, non-exclusive ways to gain insight into a particular syndrome and to practice preventive management. The goal is to optimise care for a mentally retarded person, by appropriately responding to the special needs of a given diagnosis.

Questions regarding **heritability and recurrence risk** are addressed by the question: what does it mean to the family? Based on the diagnosis and its pattern of inheritance, a recurrence risk can be given, and the possibilities of prenatal diagnosis can be discussed. This is subject to genetic counselling, as well as alternative options of family planning. Investigation and counselling of family members can be initiated, e.g. in case of familial chromosome aberrations or X-linked forms of MR.

Issues of **treatment and cure** give rise to the question: is there anything one can do? So far, in most cases there is no cure, and treatment refers to symptoms as seizures or sleeping disorders, and not to the causes. The few exceptions are mainly metabolic disorders. As the efficiency of therapy crucially depends on a very early diagnosis, neonatal screening has been introduced in most countries for a varying number of metabolic disorders (see chapter 6); for future perspectives of the treatment of metabolic and other disorders see below.

What's in a name? Several of the above mentioned issues can only be addressed when a causal diagnosis with known etiology, impact on management and/or genetic background can be established; examples are Coffin-Lowry syndrome, velocardiofacial syndrome (deletion 22q11.2), and Smith-Lemli-Opitz syndrome. Sometimes however, information is very restricted as the diagnosis may be purely descriptive. This is true for a huge number of very rare syndromes with unknown inheritance which have been delineated on the basis of only a few cases. Surprisingly, it seems to be a general experience that even in these cases diagnosis has a beneficial effect. This effect can be observed even when the cause of a syndrome is unknown, when too few cases have been published to permit reliable information on management and prognosis, and when neither treatment nor prenatal diagnosis are available – even then, parents or family members feel relieved on hearing the diagnosis.

Obviously, giving a name to a disease or disorder has a beneficial effect which reaches far beyond its apparent consequences in terms of clinical management or family planning – an effect that in the author's opinion is universal and may be

archaic. It might relate to what in philosophy and religion has been widely discussed as the mystery of naming someone or something: knowing the name of something or someone means having power over the person or the thing to be named (RGG, 1960). A name thus is much more than just a designation. It represents the identity of the thing in question, but it also defines our relationship to it. Interestingly, etymologically the term “name” is one of the most archaic words of any language. The concept of the person’s name as *pars pro toto* has a related origin, and was strong in archaic times and particularly during the Middle Ages. Hence a thing becomes real for us when we have given it a name – a diagnosis thus contributes to relieving the handicap of a child or sibling of its nebulosity and uncertainty, and permits it to enter a manageable reality.

In contrast to the beneficial aspects of a diagnosis the effects of an incorrect diagnosis are detrimental. Only definite diagnoses should be communicated as certain, provisional ones should be communicated clearly as only “possible” or “probable” (see chapter 2). When it is less likely that a person has a particular disorder than not having it, the presumed „diagnosis“ in general should not be mentioned at all, but only be kept in mind. The clinicians’s urge to establish a diagnosis at the end of a tiresome period of “unknowns” is understandable, but this should underscore the need for extra alertness about the probability of a diagnosis.

In conclusion, the benefits of genetic evaluation are evident, both for the person with MR, and for the family and care providers. As, in addition, clinical genetic investigations on the etiology of MR are not a heavy demand on the patient, every individual with MR has the right to have a careful clinical genetic evaluation, at least once in her or his life.

7.2. FUTURE PERSPECTIVES

How will the genetic evaluation of MR evolve? What will be the chances and pitfalls of etiological studies on MR? Will growing possibilities present themselves in the advantage of the patient?

Clinical evaluation

In the clinical evaluation of a mentally retarded person, there will be both a greater need and chance for more systematic approaches. In chapter 3.1, a classification of an extensive list of physical abnormalities proposed by Merks et al. (2003) was recommended for systematic use in the examination of individuals with MR. In 2004, Shalev and Hall offered a tool for the standardized description of behavioral

profiles which can be integrated in the genetic work-up of any patient with MR. The proposed method of classification of the causes of MR (chapter 2) is meant to contribute to the general need of classification systems in the field. All these tools can be used to register patients with MR systematically in order to facilitate follow-up procedures, insight into pathogenesis and genotype-phenotype correlation studies. Registration databases should use the terminology of well established dysmorphology databases, e.g. the London Dysmorphology Database, permitting cross-link searches.

Elaborated data-bases in general are increasingly applied in the field of clinical evaluation of MR. They reflect the growing need of standardization, classification and cross-linking of results. Recently, it has been suggested to discontinue publication of case reports and to deposit them instead directly into databases (Biesecker, 2004). This would require the development of shared resources, agreements on confidentiality and, in particular, on standardization (Biesecker, 2004). The discussed problems of interpretation of subtle chromosome aberrations (see chapter 4.3 and 7.2, below) will partly be resolved by the further development of cytogenetic databases which include a thorough clinical description, as e.g. ECARUCA (European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations). Mutation databases facilitating genotype-phenotype correlation have been established since a long time, e.g. the IRSA database for *MECP2* mutations (see chapter 5.3).

Genome analysis: cytogenetic and molecular analysis

There is no doubt that new technologies that have been developed in recent years will be increasingly implemented in daily practice, and that these will probably change studies on the etiology of MR dramatically. The most important ones will be briefly discussed.

MAPH (multiple amplifiable probe hybridization) and **MLPA** (multiplex ligation-dependent probe amplification) are both techniques derived from FISH and PCR based (see chapter 4.1 and 4.3). They both use a set of specifically designed probes, each flanked by the same primer-binding sites, and hybridise it to the test DNA. Each probe can be subsequently identified and quantified in a single experiment (for review see Sellner & Taylor, 2004). MAPH and MLPA allow the screening of multiple loci simultaneously for deletions and duplications, e.g. for all chromosome ends and/or for loci of frequently occurring microdeletion syndromes, or for all exons of a gene. Both are fast and simple methods with a high resolution and high throughput. It has been shown that they are useful for detecting both interstitial and subtelomeric imbalances in MR (Koolen et al., 2004; Kriek et al., 2004). Very probably these methods will rapidly further evolve

as diagnostic tools, and will in particular be used to scan (simultaneously) for subtelomeric rearrangements and for known microdeletion/microduplication syndromes. They will increasingly replace FISH with a complete set of subtelomeric probes which is still the technique most often applied to screen for subtelomeric abnormalities (see chapter 4.3). Furthermore, targeted FISH with sequence specific probes, so far employed to confirm a particular clinical diagnosis, e.g. deletion 22q11.2, deletion 7q11.2 (Williams syndrome) or deletion 17p11.2 (Smith-Magenis syndrome), will increasingly be replaced by MLPA or MAPH screens for a whole series of microdeletions. HR-G banding will, however, still be the most widely used cytogenetic technique in routine and will remain the gold standard of initial chromosome analysis (Salman et al., 2004). It is well established in all laboratories, is able to screen all chromosomes entirely for both balanced and unbalanced abnormalities, and is able to detect mosaicism even at a low level (see chapter 4.1). Its disadvantages are the need for cell culture, its labour intensive methodology, its relatively long turn-around time, and its low resolution of 5–8 Mb.

Array-based technologies meanwhile have acquired major significance. Array CGH (Comparative Genomic Hybridization) allows genome-wide examination for copy number changes, i.e. even very small deletions and duplications, in a single experiment by comparing test (patient) DNA with reference (normal) DNA. The metaphase chromosome spread of conventional CGH (see chapter 4.1) has now been replaced by an array of DNA clones on a chip (Pinkel et al., 1998). The resolution is greatly increased and depends on the distance between the selected DNA clones. Array CGH is fast and allows a very high turnover. It has been applied extensively in cancer genomics and has multiple other applications in clinical genetics (for review, see Oostlander et al., 2004). It can be performed on any sample which provides DNA, and no cell culture is needed. It has been introduced as a promising “rapid and sensitive automated diagnostic procedure” in the evaluation of patients with MR (Veltman et al., 2002) and has proven useful in the detection of submicroscopic imbalances (Vissers et al., 2003; Schoumans et al., 2004; Shaw-Smith et al., 2004). This technique has been used to prove that *de novo*, apparently balanced, translocations are often unexpectedly much more complex rearrangements (Gribble et al., 2005). Its disadvantages are the very limited ability to detect mosaicism, and the difficulty to detect aberrations without copy number changes, e.g. *de novo* balanced translocations. For further comparison of array CGH, and old and new cytogenetic techniques, the reader is referred to the review by Salman et al. (2004). The design, setup and validation of such arrays are however expensive and major technical challenges. Alternatives are publicly available arrays (“chips”), which have meanwhile also been developed at the level of single nucleotide polymorphisms (SNPs). Array SNP has been

developed very recently. It allows both genotyping and copy number analysis. The era of clinical application of array SNP in the etiological investigation of MR has just begun. A study using the GeneChip Mapping 10K SNP array demonstrates that deletions and duplications of about 5 Mb can be reliably detected, but that smaller imbalances can be readily missed due to insufficient SNP density in the respective region (Rauch et al., 2004). The resolution so far is not much better than that of HR chromosome banding, but it is only a question of time before SNP arrays with much higher resolution are available for the evaluation of MR. Array SNP is a very promising technology for detecting deletions and duplications and probably has the future.

Array based technologies are applicable not only for molecular karyotyping, but also for the study of any monogenic or polygenic condition. MLPA/MPAH and array-based technologies illustrate that the boundary between cytogenetics and molecular genetics have vanished, and that genome analysis nowadays is the more appropriate term to use.

Interpretation of results. The methods discussed above, and in particular array-based methods, comprise a major challenge: they will detect a growing number of copy number changes, deletions or duplications, of unknown significance. The interpretation will be based on segregation studies and literature reviews, but will often be hampered by the inaccessibility of family members for testing, or the existence of few (if any) published reports. Other complicating factors are the possible clinical variability known e.g. for many subtle chromosome aberrations (see chapter 4.3), and/or reduced penetrance. In every individual case, geneticists will have to proceed with caution, and not conclude too rapidly that e.g. a subtle deletion is pathogenic only because it has occurred *de novo*. A yet unknown proportion of copy number changes will finally turn out to be polymorphisms.

In general, it can be predicted that data of possibly pathogenic significance will be created increasingly, but also that the subsequent interpretation will become a consuming demand upon geneticists. The correlation of subtle cytogenetic abnormalities with the phenotype will be very difficult, and often identifying a gene will be easier than determining the relationship between gene and disorder. The benefit of the evaluation of MR delineated above, however completely depends on the clinical applicability of the acquired information. Questions regarding management and prognosis can not be answered if they concern hitherto undescribed abnormalities, and neither genetic counselling of the family nor prenatal diagnosis can be reliably offered if there are doubts on the pathogenicity.

Metabolic investigations

Two developments are likely to influence the yield of metabolic investigations contrarily. On the one hand, neonatal screening programs will increasingly identify children with metabolic disorders associated with developmental delay. As discussed in chapter 6, this will lead to a decrease of the respective diagnoses later in life. Neonatal screening has evolved from the Guthrie test which tested for phenylketonuria (PKU) on blood spots on filter paper, to tandem mass spectroscopy which allows automated testing of a wide range of disorders, in particular of amino- and organic acid metabolism. Since the advent of tandem mass spectrometry and other analytical methods, a huge number of mostly very rare conditions is (theoretically) amenable to screening. In Western countries, PKU screening has been introduced throughout, apart from screening for congenital hypothyroidism (CH) which is done by conventional techniques. Screening for other diseases however, e.g. galactosemia, aminoacidemias and organic acidemias, has been introduced in the different countries non-uniformly because cost-benefit analysis did not show unequivocal financial benefit (Clague and Thomas, 2002). Overall however, PKU, galactosemia and other metabolic disorders will be the vanishing diagnoses in the evaluation of MR.

On the other hand, new technologies have led to the detection of a bunch of new, previously unknown disorders. Creatine transporter deficiency was recently identified as an X-linked disorder and is associated with MR, epilepsy and autism, but possibly also with aspecific MR (Salomons et al., 2003); analysis is by tandem mass spectroscopy. Other examples are Walker-Warburg syndrome (WWS), muscle-eye-brain disease (MEB) and Fukuyama muscular dystrophy (FCMD) which are related syndromes with developmental delay, severe neurological features affecting the brain and/or muscles, and eye abnormalities. They were all shown to be caused by disturbances of O-glycosylation, WWS by deficiency of O-mannosyltransferase encoded by *POMT1* (Beltran-Valero de Bernabe et al., 2002), MEB by deficiency of a glycosyltransferase encoded by *POMGnT1* (Yoshida et al., 2001), and FCMD by a defective phospholigand transferase encoded by the fukutin gene (Kobayashi et al., 1998). The confirming diagnosis, however, after neurological and metabolic work-up, is by DNA-analysis of the respective genes. This group of disorders illustrates that the link between metabolic investigations and genome analysis becomes increasingly important. They also urge the need for a close cooperation between clinical geneticists, molecular geneticists, metabolic clinical specialists and neurologists. Predictably, this development will still continue and open a new field; these will be the raising diagnoses in the evaluation of MR.

From care to cure

It is possible that some of the future (neuro)metabolic disorders will offer the possibility of treatment, as has already been shown for creatine synthesis defects which may show improvement by treatment with creatine monohydrate (van der Knaap et al., 2000). Recently, hypotheses on treatment have also emerged for some non-metabolic disorders. For fragile X syndrome, treatment with metabotropic glutamate receptor (mGluR) antagonists or lithium was shown to have positive effects in a *Drosophila* Fragile X model (McBride et al., 2005). For chromatin remodelling disorders, e.g. Rubinstein-Taybi syndrome, in vitro and in vivo studies on pharmacological histone deacetylases (HDAC) inhibition are subject to actual research (Langley et al., 2005). Both approaches have derived from research on the pathogenetic background, illustrating that research is needed for the longterm benefit of the patient. The clinical applicability, however, will probably not be shown in the near future.

Conclusion

It appears to the author that this thesis has been written at a turning-point of genetic evaluation of MR. In coming years, clinical geneticists working on the evaluation of MR will have to achieve a difficult balance between understanding and applying new technologies, translating the results of these into information which is relevant for a mentally retarded person, and implementing it in patient care. To the author, the piece which links research to a better care for individuals with MR, still seems the most challenging one.

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SUMMARY

Mental retardation (MR) originates before the age of 18, and is a lifelong disability characterised by significant limitations both in intellectual functioning (IQ <70) and in several areas of adaptive behavior. It has a major impact on the life of the affected individual and his/her social interaction with others.

Chapter 1 addresses general aspects of MR and gives an outline of this thesis. Worldwide, a prevalence of 0.38% has been established for severe MR (IQ <50) whereas the prevalence for mild MR (IQ 50–70) is up to 3% and varies widely. In the Netherlands, at least 0.7% of the population is mentally retarded. About half of them live in residential settings, with an increasing proportion of these living in small community-based groups. General practitioners and specialised Physicians for Persons with Intellectual Disabilities (Arts voor Verstandelijk Gehandicapten, AVG) are the most important medical care providers. Health problems are twice as frequent as in the general population and partly depend on the cause of the MR. This thesis provides an overview of the current state of the art of clinical genetic diagnostic investigations in MR. These comprise clinical evaluation, and cytogenetic, molecular and metabolic analyses. In addition to a general overview of each aspect, all of them will be illustrated by a representative study. The aim is both to promote diagnostic research in the field of the etiology of MR, and to provide a practical tool for care providers for mentally retarded persons.

In **chapter 2**, studies on the etiology of MR published since the beginning of the 1980s are reviewed. It is shown that the yield of the studies critically depends on the chosen methodology and classification, but even more on the use of the term diagnosis. A new, dynamic method of classification is proposed which includes the degree of certainty of a diagnosis. This novel method is meant to allow classification with a more transparent use of the term diagnosis, and to facilitate follow-up of patients.

Chapter 3 deals with the clinical evaluation of a mentally retarded patient. This includes assessment of the history, and a physical examination which focuses on observation, surface inspection, anthropometric measurements and a basic neurological examination. Phenotypic abnormalities such as malformations and minor anomalies are of particular importance. A multidisciplinary approach reflects the complex needs of a mentally retarded person. For institutionalised patients, the cooperation of the Physician for Persons with Intellectual Disabilities with the clinical geneticist is of particular importance. A distribution of their respective tasks is proposed. The clinical examination allows syndrome recognition, provides the basis for subsequent laboratory tests, and is essential for developmental research.

For illustration, the brain malformations of oculocerebrocutaneous syndrome (OCCS), a rare neurocutaneous disorder, are reviewed. A consistent pattern of malformations could be delineated in the majority of patients. It comprises a complex forebrain malformation, and a novel mid-hindbrain malformation which is thought to be pathognomonic for OCCS. The delineated pattern of brain malformations allows differentiation from other syndromes and points to a defect of the mid-hindbrain organizer as the underlying pathogenetic mechanism.

In **Chapter 4**, a summary of cytogenetic studies in MR is given. Over the past more than 50 years, techniques have evolved from non-banded to high resolution banded chromosome analysis: nowadays, the latter is the gold standard. FISH techniques and all techniques derived from it play a major role. The report of a series of patients illustrates that the combined application of different classical cytogenetic techniques can enable the diagnosis of patients who were assumed to have a normal karyotype after their first investigation. Cytogenetically visible aberrations are found in roughly 10% of patients with MR.

During the last decade, it has been shown that in addition, cryptic subtelomeric abnormalities contribute significantly to the etiology of MR. Both a review of previously published studies and a retrospective study of the patients evaluated at the Department of Clinical Genetics at Maastricht, reveals a prevalence of subtelomeric rearrangements of about 5%. The clinical presentation of all aberrations detected at Maastricht is reported, and the relevant literature reviewed. The interpretation of detected subtelomeric abnormalities may be complicated by different factors, such as the occurrence of variants and clinical variability.

Chapter 5 concerns forms of MR that can be diagnosed by molecular analysis. Fragile X syndrome and other, well-known X-linked MR syndromes are briefly reviewed. The distinction between non-syndromic X-linked MR (MRX) with non-specific MR only, and syndromic X-linked MR (MRXS) with associated features, has been softened by the detection of at least 10 genes involved in both forms. Autosomal MR syndromes and non-syndromic forms of MR are also shortly discussed; so far, the latter ones have no clinical significance.

Mutations in the *MECP2* gene cause Rett syndrome in female patients, and, much less frequently, MRXS and MRX in males. The phenotypic spectrum of neurodevelopmental disorders in males caused by *MECP2* mutations is critically reviewed, and a case of a 21 year old male with male Rett variant is described. *MECP2* analysis in a group of 72 males with unexplained combinations of MR and different neurological features shows that even in this clinically highly selected group, *MECP2* mutations are infrequently found. Rett variant may however be clinically underrecognised in males.

Chapter 6 addresses the question whether and when adult patients with MR should be recommended for metabolic testing. Metabolic disorders are responsible for only a small fraction of MR, probably for less than 1%. It has however been shown that they are associated with a remarkably broad phenotypic spectrum. A review of metabolic investigations performed over a 10-year period in adolescent and adult persons with MR confirms that targeted testing in a clinically selected group is appropriate. The spectrum of possible indicators however is broad, and a proposal is made for an adapted list of clinical findings which may indicate the need for metabolic testing.

Chapter 7 finally deals with the benefits of genetic evaluation of MR, and with future perspectives. The benefits of a causal diagnosis are numerous and comprise relief from uncertainty, optimisation of care procedures, genetic counselling and possibly prevention. Giving a name to the clinical problem seems to have an essential role. It is concluded that every person with MR has the right to have an etiological evaluation.

Future perspectives will be dominated by the need for systematic approaches requiring standardization and classification in different fields, and by new technologies such as MLPA/MAPH and array-based technologies. The interpretation of the resulting flood of data will be a major demand on clinical geneticists.

SAMENVATTING

Mentale retardatie (MR) wordt beschouwd als een levenslange handicap die begint voor de leeftijd van 18 jaar, en wordt gekenmerkt door significante beperkingen van intellectuele vermogens ($IQ < 70$) én van meerdere gebieden van adaptief gedrag. Het heeft een grote invloed op het leven van de aangedane persoon en op de sociale interacties met anderen.

Hoofdstuk 1 beschrijft de algemene aspecten van MR en geeft een overzicht van de inhoud van dit proefschrift.

Wereldwijd is een prevalentie van 0.38% voor ernstige MR ($IQ < 50$) aangetoond, terwijl de prevalentie voor milde MR ($IQ 50-70$) in de verschillende studies sterk varieert en kan oplopen tot 3%. In Nederland is minstens 0.7% van de populatie mentaal geretardeerd. De helft van deze personen verblijft in aangepaste instellingen, waarbij overigens het aandeel kleinschalige woonvormen steeds groter wordt. De meest belangrijke medische behandelaars zijn huisartsen en AVGs (Artsen voor Verstandelijk Gehandicapten). Medische problemen komen bij mensen met MR tweemaal zo vaak voor als in de algemene bevolking en houden deels verband met de oorzaak van de MR.

Dit proefschrift geeft een overzicht van de huidige stand van zaken van het klinisch genetisch onderzoek naar MR. Dit onderzoek omvat een klinische evaluatie, en cytogenetische, moleculaire en metabole laboratoriumtesten. Ieder onderdeel bevat een algemeen overzicht en wordt geïllustreerd door een representatieve studie.

Dit proefschrift beoogt het diagnostisch onderzoek op het gebied van de etiologie van MR te bevorderen en een hulpmiddel aan te reiken voor de praktijk van hulpverleners voor mentaal geretardeerden.

In **hoofdstuk 2** wordt een overzicht gegeven van studies naar de etiologie van MR die sinds het begin van de jaren '80 verschenen zijn. Daaruit wordt duidelijk dat de gerapporteerde resultaten niet alleen in sterke mate afhangen van de methode en classificatie waarvoor gekozen werd, maar meer nog van het gebruik van de term 'diagnose'. Daarom wordt een nieuwe, dynamische manier van classificatie, die rekening houdt met de waarschijnlijkheid van de diagnose, voorgesteld. Door toepassing van deze nieuwe methode wordt de term 'diagnose' transparanter en de follow-up van patiënten gemakkelijker.

Hoofdstuk 3 behandelt de klinische evaluatie van een patiënt met MR. Deze omvat het vastleggen van de voorgeschiedenis van de patiënt en het verrichten van lichamelijk onderzoek dat zich met name richt op observatie, inspectie, anthropometrische metingen en een basaal neurologische verkenning. Phenotypische afwijkingen zoals malformaties en kleine anomalieën zijn bijzonder belangrijk. Een multidisciplinaire aanpak is daarbij aangewezen en weerspiegelt

de complexe behoeften van mensen met MR. Voor patiënten in instellingen is de samenwerking tussen de AVG en de klinisch geneticus bijzonder belangrijk; een taakverdeling tussen hen wordt voorgesteld. Het klinisch onderzoek leidt tot syndroomherkenning, is de basis voor verder beleid en laboratorium onderzoek, en is tevens essentieel voor wetenschappelijk onderzoek. Ter illustratie worden de hersenafwijkingen van patiënten met het oculocerebrocutaan syndroom (OCCS), een zeldzaam neurocutaan syndroom, beschreven. Een consistent patroon van afwijkingen kon bij de meeste van deze patiënten worden aangetoond. Dit bestond uit een complexe afwijking van de voorhersenen en een unieke malformatie van het tectum en de kleine hersenen, die waarschijnlijk pathognomonisch is voor OCCS. De beschreven hersenafwijkingen onderscheiden OCCS van andere syndromen en wijzen naar een mogelijk pathogenetisch mechanisme.

In **hoofdstuk 4** wordt een samenvatting van cytogenetische studies naar MR gegeven. Tijdens de afgelopen 50 jaar hebben de technieken van chromosomenonderzoek zich ontwikkeld van niet-gebandeerde karyogrammen naar high-resolution gebandeerde chromosomenanalyse, die tegenwoordig de gouden standaard is. Het FISH-onderzoek en andere technieken die hieruit ontstaan zijn, spelen een belangrijke rol bij de opheldering van chromosomale bevindingen. De opgenomen publicatie van een serie patiënten illustreert dat de toepassing van gecombineerde cytogenetische methoden afwijkingen kan opsporen bij patiënten die bij een vroeger uitgevoerd onderzoek een normaal karyotype toegerekend hadden gekregen. Cytogenetisch zichtbare chromosoomafwijkingen worden bij grofweg 10% van patiënten met MR gevonden.

De afgelopen 10 jaar is bovendien aangetoond dat cryptische subtelomere chromosoomafwijkingen significant bijdragen aan de oorzaken van MR. Een overzicht van reeds gepubliceerde studies én een retrospectieve studie van patiënten die bij de afdeling Klinische Genetica Maastricht onderzocht zijn, laat een prevalentie van ongeveer 5% van subtelomere afwijkingen zien. De kliniek van alle Maastrichtse patiënten met een subtelomere afwijking wordt beschreven en een bijbehorend literatuuroverzicht gegeven. De interpretatie van de betekenis van subtelomere afwijkingen kan soms door verschillende factoren bemoeilijkt worden, zoals het voorkomen van varianten en klinische variabiliteit.

Hoofdstuk 5 betreft vormen van MR waarvan de oorzaak met moleculair onderzoek vastgesteld kan worden. Fragiele X syndroom en andere bekende X-gebonden MR syndromen worden kort beschreven. Het onderscheid tussen niet-syndromale X-gebonden MR (MRX) met aspecifieke MR als enig kenmerk, en syndromale X-gebonden MR (MRXS) met bijkomende kenmerken vervaagt door de ontdekking dat minstens 10 genen bij beide vormen

betrokken zijn. Autosomale MR syndromen en niet-syndromale vormen van MR worden eveneens kort besproken; tot nu toe hebben de laatst genoemde geen betekenis in de klinische praktijk.

Mutaties in het *MECP2* gen veroorzaken Rett syndroom bij vrouwen en, veel minder frequent, MRX en MRXS bij mannen. Het phenotypische spectrum van ontwikkelingsstoornissen bij mannen veroorzaakt door *MECP2* mutaties, wordt kritisch beschouwd. Daarnaast wordt de casus van een 21-jarige jongeman met Rett variant beschreven. *MECP2* genanalyse in een groep van 72 mannen met een onverklaarde combinatie van MR en diverse neurologische verschijnselen laat zien dat *MECP2* mutaties zelfs in deze klinisch goed geselecteerde groep maar zelden worden gevonden. Mogelijk is Rett variant wel ondergediagnostiseerd bij mannen.

Hoofdstuk 6 benadert de vraag of en wanneer volwassen mentaal geretardeerde patiënten in aanmerking voor metabool onderzoek komen. Metabole aandoeningen zijn de oorzaak van slechts een klein deel van alle gevallen van MR, mogelijk minder dan 1%. Deze tonen echter een opvallend breed phenotypisch spectrum. Een overzicht van metabole onderzoeken verricht in een periode van 10 jaar bij adolescenten en volwassen personen met MR bevestigt dat testen in een klinisch geselecteerde groep van patiënten aangewezen is. Er is echter een breed spectrum van klinische bevindingen die op een mogelijke metabole aandoening kunnen wijzen. Een aangepaste lijst met dergelijke bevindingen wordt voorgesteld.

Hoofdstuk 7 tenslotte bediscussieert het nut van een genetische evaluatie naar MR en het toekomstig perspectief. Het belang van een juiste causale diagnose heeft zowel sociale als klinische aspecten. Te denken valt daarbij onder meer aan verlossing van onzekerheid, optimalisatie van behandeling en begeleiding, erfelijkheidsvoorlichting en mogelijk preventie. De naamgeving speelt hierbij een cruciale rol. De conclusie is dat iedere persoon met MR recht op gedegen etiologisch onderzoek heeft.

Het toekomstig perspectief wordt bepaald door de behoefte aan systematische benaderingen die standaardisatie en classificatie op diverse gebieden vereisen, en door nieuwe technologieën zoals MLPA/MAPH en op micro-array-gebaseerde technieken. De interpretatie van de resulterende overvloed van data zal een behoorlijk beroep doen op de kennis en kundigheid van klinisch genetici.

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CURRICULUM VITAE

Ute Moog was born in Leverkusen, Germany, on October 26, 1954. She grew up in an atmosphere inspired by books and music which also shaped her schooling. Between 1973 and 1980 she studied medicine in Germany, first in Saarbrücken, and after 1975, in Aachen. Her studies were funded by the Studienstiftung des Deutschen Volkes. Fascinated by the possibility of direct visualisation of defects underlying diseases, she chose as the topic of her first thesis "Chromosomenuntersuchung bei epileptischen Kindern mit Phenytoin-Monotherapie" under the supervision of Prof.dr. M. Habedank. She defended her dissertation and was awarded her doctoral degree by the Rheinisch Westfälisch Technische Hochschule Aachen in 1982.

A home-based period followed in which she raised her three sons, Martin, Tobias and Florian to self-sufficiency. During this time, she dedicated herself to music, playing and teaching the oboe, and performing chamber music semi-professionally.

In 1993, she returned to medicine, taking up a position in the Department of Clinical Genetics at the University Hospital in Maastricht. Between 1995 and 1999 she was a trainee in clinical genetics, and became registered as a clinical geneticist in 1999. Since that time, she has been a staff member of this Department, as well as an extraordinary member of the medical staff in the Heerlen and Venlo hospitals. She has been happy to work and live more as a member of the European community than as a German or Dutch inhabitant, and to keep her sense of foreign languages vivid. The continuous close cooperation with the Center for Human Genetics in Leuven, Belgium, headed by Prof. dr. J.P. Fryns, has inspired her specialisation in genetic evaluation of mental retardation. This includes acting as an expert consultant for clinical genetics in various centers for the mentally retarded. Her research work which she regularly presents at international meetings, is summarised in this, her second thesis.

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