

REVIEW

Diagnostic investigations in individuals with mental retardation: a systematic literature review of their usefulness

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There are no guidelines available for diagnostic studies in patients with mental retardation (MR) established in an evidence-based manner. Here we report such study, based on information from original studies on the results with respect to detected significant anomalies (yield) of six major diagnostic investigations, and evaluate whether the yield differs depending on setting, MR severity, and gender. Results for *cytogenetic studies* showed the mean yield of chromosome aberrations in classical cytogenetics to be 9.5% (variation: 5.4% in school populations to 13.3% in institute populations; 4.1% in borderline-mild MR to 13.3% in moderate-profound MR; more frequent structural anomalies in females). The median yield of subtelomeric studies was 4.4% (also showing female predominance). For *fragile X screening*, yields were 5.4% (cytogenetic studies) and 2.0% (molecular studies) (higher yield in moderate-profound MR; checklist use useful). In *metabolic investigations*, the mean yield of all studies was 1.0% (results depending on neonatal screening programmes; in individual populations higher yield for specific metabolic disorders). Studies on *neurological examination* all showed a high yield (mean 42.9%; irrespective of setting, degree of MR, and gender). The yield of *neuroimaging studies* for abnormalities was 30.0% (higher yield if performed on an indicated basis) and the yield for finding a diagnosis based on neuroradiological studies only was 1.3% (no data available on value of negative findings). A very high yield was found for *dysmorphic examination* (variation 39–81%). The data from this review allow conclusions for most types of diagnostic investigations in MR patients. Recommendations for further studies are provided.

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Introduction

Background

Mental retardation (MR) is a frequently occurring disorder with a major impact on the life of the affected person, the

family, and society. Establishing an aetiologic diagnosis is usually a challenge for every specialist involved, as the spectrum of possible underlying disorders is enormous and the range of available additional investigations extensive. Still the sheer knowing of the cause, the recurrence risk, the short-term and long-term prognosis, treatment options, availability of special services, contacts with other parents of children, and other issues are of great importance to parents, and often also forms the first step towards acceptance of the disability. Furthermore, the costs of a

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complete diagnostic work-up in a child with MR are considerable, and can be a major burden to many health care systems. This obliges clinicians to reconsider the usefulness of every diagnostic investigation.

The ability to determine a cause of MR is based largely on the use of specific diagnostic tools. In a given diagnostic setting, the physician depends on their availability and guidelines for application. Such guidelines should be established in an evidence-based manner, that is, based on information from original empirical studies on quality, yield, and usefulness of the diagnostic investigations. Until now, available guidelines have been based foremost on expert opinion,¹ with one recent exception, which became available after the present study was completed.²

Aims of the review

We initiated a systematic search for and analysis of all papers published in peer-reviewed journals in seven different languages during the last 35 years, reporting the application of one or more of the following major diagnostic investigations: dysmorphic examination, neurologic examination, neuroimaging, cytogenetic investigations (routine karyotyping and subtelomeric FISH analysis), fragile X screening, metabolic investigations. An additional goal was to investigate whether the yield differed depending on (1) setting (institution, outpatient clinic, school, population survey), (2) severity of MR, and (3) gender.

We have chosen these six investigations because (1) these are the most frequently applied, (2) these investigations have been applied in numerous studies in various populations and in various settings, and (3) each of the investigations may yield information sufficient for establishing an aetiologic diagnosis. This is rarely the case in other investigations such as ophthalmologic or electrophysiologic investigations, which we have excluded from this review.

Presentation of results

The different parts of this systematic literature review use the same methodology, independent of the diagnostic technique under study. This includes definitions, search strategy, selection criteria, the yield of the search strategy, selection procedure of studies using the Quorum flow diagram,³ data extraction and analyses, study quality assessment, and statistical analyses.

When reading and interpreting this review, it is important to note that inclusion or exclusion was dependent first and foremost on the availability in the paper of quantitative data on the accuracy and yield of diagnostic techniques in patient groups with MR. We are aware of the fact that potentially valuable information on other aspects of MR aetiology and management is not included in the present review due to our focused study aims. Furthermore,

the choice of diagnostic techniques does not indicate that other investigations in persons with MR are useless. An example may be that in our opinion every retarded child needs to be regularly checked for visual and auditive abilities, as disturbances may have a significant impact on the total functioning of a child.

Methods

This systematic review was designed according to the Cochrane Reviewers Handbook version 4.1.4.⁴ All consecutive steps and phases of the review are depicted in Figure 1.

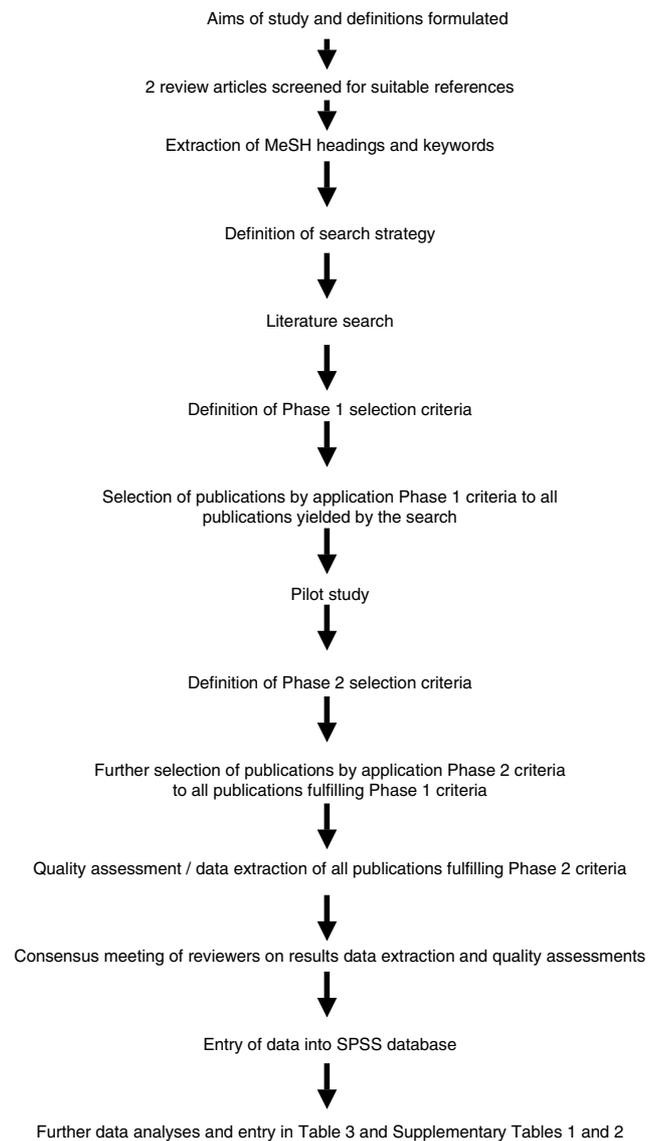


Figure 1 Flow chart of consecutive methodologic steps of systematic review. All steps were performed by two independent reviewers.

Definitions

Prior to designing the search strategy, the following definitions were formulated:

MR: The definition of MR of the American Association on Mental Retardation was used:⁵ MR refers to substantial limitations in present functioning. It is characterised by significantly subaverage intellectual functioning, existing concurrently with related limitations in two or more of the following applicable adaptive skills: communication, self-care, home living, social skills, community use, self-direction, health and safety, functional academics, leisure, and work. MR manifests before the age of 18.

Severity of MR: This was categorised according to the World Health Organization classification⁶ and DSM-IV criteria:⁷ profound (IQ = 0–20); severe (IQ = 21–35); moderate (IQ = 36–50); mild (IQ = 51–70); borderline (IQ = 71–85).

Investigations: Diagnostic investigations used to discern the aetiology of MR were: (1) dysmorphic exam: physical examination focused on the detection of dysmorphic features, minor anomalies, and malformations; (2) neurologic exam: physical exam focused on detection of neurologic abnormalities; (3) metabolic studies: standard 24 h urinary screenings of amino acids, organic acids, oligosaccharides, acid mucopolysaccharides, and uric acid; (4) cytogenetics: high-resolution G-banded karyogram screening for numerical and structural chromosome anomalies (minimal banding quality 350–400 bands),⁸ and FISH analysis screening for subtelomeric rearrangements;⁹ (5) fragile X screening: cytogenetic screening on chromosomes prepared using medium 199 for fragile sites in region Xq27.3,¹⁰ or molecular screening of the *FMR-1* gene for CGG expansions;¹¹ (6) Neuroradiologic studies: screening for intracranial abnormalities by magnetic resonance imaging (MRI) scan, computer tomography (CT) scan, and echo cerebrum.

Aetiologic diagnosis: A disorder was considered an aetiologic diagnosis if there was sufficient literature evidence external to this review to make a causal relationship of the disorder with MR likely, and if it met the Schaefer–Bodensteiner standard ('a specific diagnosis that can be translated into useful clinical information for the family, including providing information about prognosis, recurrence risk, and preferred modes of available therapy').¹²

Search strategy

The search strategy was based on two recent reviews of the diagnostic process in individuals with MR.^{1,13} Two investigators (CDMvK, RCMH) independently screened the bibliographies of these two reports for references of articles possibly suitable for this review. These articles were then retrieved and their MeSH headings and textual keywords were subsequently used to set up the search strategy by two independent reviewers (AGEL, CDMvK). Publications were retrieved by a computerised search (using OVID) of MED-

LINE (1966–June 2002), EMBASE (1983–June, 2002), Cochrane Database of Systematic Reviews and Controlled Clinical Trials (issue of the first quarter, 2002), Best Evidence Database (1991–June, 2002), using the following keywords: mental retardation, learning disorders, developmental disabilities, mass screening, cohort studies, case-control, retrospective studies, prospective studies. For the specific diagnostic investigations, the following keywords were used: neurologic abnormalities, chromosome abnormalities, metabolic diseases, tomography, mutations. For dysmorphic examination, two different search strategies were performed: one, using as keywords 'syndromes' and 'multiple congenital anomalies', and a second using as keywords the terms of the 17 most frequent syndromes:¹⁴ Down syndrome, trisomy chromosome 8, trisomy chromosome 13, trisomy chromosome 18, deletion chromosome 18p, Angelman syndrome, Bardet–Biedl syndrome, Cohen syndrome, Cornelia de Lange syndrome, Cri-du-Chat syndrome, fetal alcohol syndrome, fragile X syndrome, Prader–Willi syndrome, Smith–Lemli–Opitz syndrome, Sotos syndrome, Williams syndrome, Wolf–Hirschhorn syndrome.

The references of all identified relevant studies were hand searched for additional potentially relevant publications (CDMvK).

Selection criteria

The selection was performed by two independent reviewers (CDMvK, RCMH) in two consecutive phases. The selection criteria listed in Table 2 were applied to the titles and abstracts of publications. After a pilot study, more strict criteria were formulated and were applied during a second phase to articles fulfilling the first-phase criteria. Reasons for exclusion of articles during phases 1 and 2 are listed in Table 3. Only for population surveys, less strict criteria regarding the description of the severity of MR and of MR assessment methods were applied, as the large numbers of patients in the study groups hampered an exact description of all these items. For inclusion in the review, a reasonable certainty was needed that all included patients were indeed mentally delayed, next to the general criteria.

Studies describing comprehensive diagnostic evaluations of patients potentially have great value, but also have the drawback that the description of the number of patients in whom a specific investigation technique is performed is often lacking. Although it seemed often likely that each technique was performed in all patients, it cannot be derived from most publications with certainty. This prohibits accurate calculation of frequency of anomalies found with each of the diagnostic techniques. Therefore, such comprehensive studies were not included in the present review, unless reliable figures regarding the number of patients who underwent the individual studies were available.

Table 1 Overview of the databases and MeSH headings used in the computerised literature searches, and the strategy and yield of the each search

Cochrane Database of Systematic Reviews (first quarter 2002)	
1. mental retardation.mp	n = 35
2. developmental disabilities.mp	n = 5
3. learning disorders.mp	n = 2
4. 1 or 2 or 3 or 4	n = 37
Best Evidence (1991–June 2002)	
1. mental retardation.mp	n = 4
2. developmental disabilities.mp	n = 0
3. learning disorders.mp	n = 0
4. 1 or 2 or 3 or 4	n = 4
MEDLINE (1966–June 2002):	
1. exp mental retardation/or 'mental retardation'.mp	n = 35 069
2. exp learning disorders/or 'learning disorders'.mp or	n = 10 827
3. exp developmental disabilities/'developmental disabilities'.mp	n = 6 117
4. 1 or 2 or 3	n = 49 890
5. exp mass screening/or 'screening'.mp	n = 120 654
6. exp cohort studies/or 'cohort study'.mp	n = 373 570
7. exp case-control studies/or 'case-control study'.mp	n = 175 086
8. retrospective studies/or 'retrospective study'.mp	n = 145 323
9. prospective studies/or 'prospective study'.mp	n = 127 180
10. 5 or 6 or 7 or 8 or 9	n = 625 417
Metabolic investigational techniques	
11. exp metabolic diseases/or 'metabolic diseases'.mp	n = 367 910
12. 4 and 10 and 11	n = 277
Cytogenetic investigational techniques	
13. exp chromosome abnormalities/or 'chromosomal abnormalities'.mp	n = 43 400
14. 4 and 10 and 13	n = 293
Molecular investigational techniques	
15. exp mutation/or 'mutations'.mp	n = 221 274
16. 4 and 10 and 15	n = 116
Neuroradiologic investigational techniques	
17. exp tomography/or 'tomography'.mp	n = 218 829
18. 4 and 10 and 17	n = 103
Neurologic investigational techniques	
19. exp neurologic examination/or 'neurologic examination'.mp	n = 56 976
20. 4 and 10 and 19	n = 70
Dysmorphologic investigational techniques (MR/MCA search)	
21. exp abnormalities, multiple/or 'multiple abnormalities'.mp	n = 45 824
22. 'syndromes.mp	n = 33 588
23. 21 or 22	n = 77 643
24. 4 and 10 and 21	n = 412
Dysmorphologic investigational techniques (syndrome search)	
25. exp Down syndrome/or 'Down syndrome'.mp	n = 12 829
26. exp fetal alcohol syndrome/or 'fetal alcohol syndrome'	n = 2305
27. exp fragile x syndrome/or 'fragile x syndrome'.mp	n = 2312
28. exp de lange syndrome/or 'cornelia de lange syndrome'.mp	n = 390
29. exp chromosomes, human, pair 8/or exp trisomy/or 'trisomy 8'.mp	n = 9846
30. exp chromosomes, human, pair 13/or exp trisomy/or 'trisomy 13'.mp	n = 9241
31. exp chromosomes, human, pair 18/or exp trisomy/or 'trisomy 18'.mp	n = 9466
32. exp angelman syndrome/or 'angelman syndrome'.mp	n = 470
33. exp prader-willi syndrome/or 'prader-willi syndrome'.mp	n = 1265
34. exp williams syndrome/or 'williams syndrome'.mp	n = 497
35. exp bardet-biedl syndrome/or 'bardet-biedl syndrome'.mp	n = 226
36. exp cri-du-chat syndrome/or 'cri-du-chat syndrome'.mp	n = 493
37. 'wolf.hirschhorn'.mp	n = 163
38. 'smith-lemli-opitz'.mp	n = 337
39. 'sotos syndrome'.mp	n = 145
40. 'cohen syndrome'.mp	n = 67

Table 1 (Continued)

41. (chromosomes 18 and deletion).mp	n = 10
42. 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41	n = 456
43. exp prenatal diagnosis/	n = 33 480
44. 42 not 43	n = 412
EMBASE (1988–June 2002):	
1. exp mental retardation/or 'mental retardation'.mp	n = 25 121
2. exp learning disorders/or 'learning disorders'.mp or	n = 3835
3. exp developmental disabilities/'developmental disabilities'.mp	n = 4494
4. 1 or 2 or 3	n = 31 993
5. exp mass screening/or 'screening'.mp	n = 84 082
6. exp cohort studies/or 'cohort study'.mp	n = 14 264
7. exp case-control studies/or 'case-control study'.mp	n = 13 351
8. exp retrospective studies/or 'retrospective study'.mp	n = 24 207
9. exp prospective studies/or 'prospective study'.mp	n = 23 072
10. 5 or 6 or 7 or 8 or 9	n = 179 135
Metabolic investigational techniques	
11. exp metabolic diseases/or 'metabolic diseases'.mp	n = 324 456
12. 4 and 10 and 11	n = 341
Cytogenetic investigational techniques	
13. exp chromosome abnormalities/or 'chromosome abnormalities'.mp	n = 36 519
14. 4 and 10 and 13	n = 529
Molecular investigational techniques	
15. exp mutation/or 'mutations'.mp	n = 168 049
16. 4 and 10 and 15	n = 208
Neuroradiologic investigational techniques	
17. exp tomography/or 'tomography'.mp	n = 125 232
18. 4 and 10 and 17	n = 136
Neurologic investigational techniques	
19. exp neurologic examination/or 'neurologic examination'.mp	n = 36 471
20. 4 and 10 and 19	n = 19
Dysmorphologic investigational techniques (MR/MCA search)	
21. exp abnormalities, multiple/or 'multiple abnormalities'.mp	n = 7427
22. 'syndromes'.mp	n = 21 454
23. 21 or 22	n = 28 308
24. 4 and 10 and 21	n = 125
Dysmorphologic investigational techniques (syndromes search)	
25. exp Down syndrome/or 'Down syndrome'.mp	n = 5707
26. exp fetal alcohol syndrome/or 'fetal alcohol syndrome'	n = 1187
27. exp fragile x syndrome/or 'fragile x syndrome'.mp	n = 1882
28. exp de lange syndrome/or 'cornelia de lange syndrome'.mp	n = 148
29. exp chromosomes, human, pair 8/or exp trisomy/or 'trisomy 8'.mp	n = 4962
30. exp chromosomes, human, pair 13/or exp trisomy/or 'trisomy 13'.mp	n = 4809
31. exp chromosomes, human, pair 18/or exp trisomy/or 'trisomy 18'.mp	n = 4853
32. exp angelman syndrome/or 'angelman syndrome'.mp	n = 503
33. exp prader-willi syndrome/or 'prader-willi syndrome'.mp	n = 1036
34. exp williams syndrome/or 'williams syndrome'.mp	n = 570
35. exp bardet-biedl syndrome/or 'bardet-biedl syndrome'.mp	n = 200
36. exp cri-du-chat syndrome/or 'cri-du-chat syndrome'.mp	n = 92
37. 'wolf.hirschhorn'.mp	n = 122
38. 'smith-lemli-opitz'.mp	n = 237
39. 'sotos syndrome'.mp	n = 99
40. 'cohen syndrome'.mp	n = 43
41. (chromosomes 18 and deletion).mp	n = 8
42. 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41	n = 1530
43. exp prenatal diagnosis/	n = 20 067
44. 42 not 43	n = 825

Table 2 Criteria applied for the selection of articles for this review*Phase 1*

Articles must be published in peer-reviewed medical journals

Articles must be written in Dutch, English, French, German, Italian, Portuguese, or Spanish

Articles must report the application and yield of one (or more) of the diagnostic investigations (as defined in Methods)

The study group that was investigated comprised minimally 25 well-defined individuals with previously unexplained MR

The study group was examined personally by one of the authors or, for the purpose of this study, by a clinician who is not a co-author

Phase 2

The major goal of the study must include the detection of the aetiology of MR in patients in the study group

The study group should be an unselected series of cases derived from the general population, school, outpatient clinic, hospital, or institute

The study group may be selected from the same settings only based on criteria detectable by history taking or physical examination

The number and nature of abnormalities detected should be reported in detail

Table 3 Number and reasons for exclusion from the systematic review of publications on diagnostic investigations in patients with MR*Phase 1 (total excluded = 4412+400)*

I	Cohort <25	<i>n</i> = 373
II	Letter/review only/technique description only	<i>n</i> = 1104
III	No major diagnostic technique involved	<i>n</i> = 425
IV	Mass screening/individuals with a normal IQ included	<i>n</i> = 681
V	Number of patients with MR in study group unclear	<i>n</i> = 353
VI	Studies of individuals with a known cause of MR	<i>n</i> = 1175
VII	Language (other than the six listed)	<i>n</i> = 58
VIII	Patients not examined personally	<i>n</i> = 274
IX	Other	<i>n</i> = 369

Phase 2

	<i>Dysm exam</i>	<i>Cyto genet</i>	<i>Neur exam</i>	<i>Neur imag</i>	<i>Metab</i>	<i>FraX</i>	
I	Insufficient description selection	<i>n</i> = 21	<i>n</i> = 7	<i>n</i> = 0	<i>n</i> = 2	<i>n</i> = 4	<i>n</i> = 6
II	Insufficient description study group	<i>n</i> = 15	<i>n</i> = 17	<i>n</i> = 1	<i>n</i> = 2	<i>n</i> = 4	<i>n</i> = 7
III	I+II	<i>n</i> = 39	<i>n</i> = 48	<i>n</i> = 8	<i>n</i> = 1	<i>n</i> = 11	<i>n</i> = 8
IV	Selection bias	<i>n</i> = 1	<i>n</i> = 0	<i>n</i> = 0	<i>n</i> = 1	<i>n</i> = 0	<i>n</i> = 1
V	Insufficient data on technique	<i>n</i> = 15	<i>n</i> = 45	<i>n</i> = 33	<i>n</i> = 10	<i>n</i> = 22	<i>n</i> = 13

Only FISH studies screening for subtelomeric rearrangements were included. Information yielded by studies applying FISH to screen for specific interstitial microdeletions, such as a multi-FISH study for five different microdeletion syndromes,¹⁵ are not reported here. The various microdeletion syndromes each provide a distinct phenotype and this phenotype will urge the specific FISH study. These FISH studies will not be used for screening in an unselected group of patients with MR. Conversely, publications that report screening for subtelomeric rearrangements by other techniques than FISH analysis (eg automated fluorescent genotyping¹⁶ or comparative genomic hybridisation¹⁷) were also excluded. The yield of these different, only recently developed techniques is not yet well comparable and results of different studies cannot be pooled.

Molecular screening was narrowed down to *FraX* mutation screening. As a result, useful data on the yield of screening for other syndromes, such as methylation analysis of the Prader-Willi/Angelman region in patients with a suggestive phenotype,¹⁸ are not reported here. As

stated above for specific FISH studies, it was our primary goal to assess the yield of diagnostic investigations in individuals with unexplained MR, and not in those with phenotypic features suggestive of a particular underlying disorder. As the phenotype in fragile X may be unspecific, and results of searches for fragile X syndrome are available from a number of studies of persons with idiopathic MR,¹⁹ we have chosen to allow inclusion of this specific molecular investigation technique.

Pilot study

To test the applicability of the standard forms for data extraction and quality assessment (see below), a pilot study using eight articles was performed.^{20–27} The sample was selected so that it encompassed studies in different settings, applied (a combination of) different diagnostic investigations, and comprised articles published in different periods of time between 1966 and 2002. Three reviewers (CDMvK, MCEJ, RCMH) independently evaluated the papers using standard forms. In a subsequent

consensus meeting, points of disagreement were discussed and, if needed, the form was adapted to comments.

Data extraction

For each article, a standard form was used to extract details on study design, study population, applied diagnostic investigations, and outcome (yield of techniques; number of established a etiological diagnoses). If important data in a publication were lacking, but in the publication the reader was referred to another paper for these data, then this reference was retrieved and used in evaluating the selection criteria for this review. In evaluating the methodological qualities however, only the original paper was scored as if this additional information was not available.

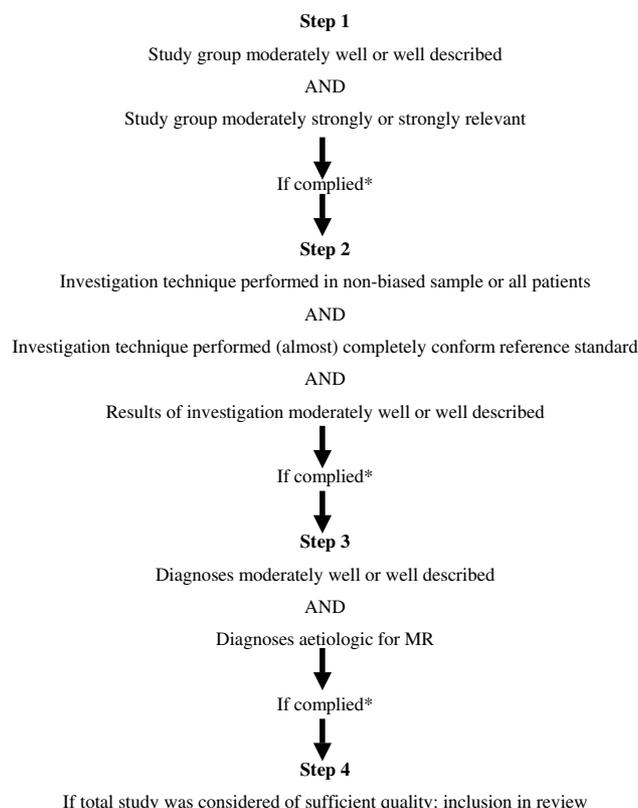
Data in articles from earlier investigations, that is, not performed for the purpose of the empirical study, were only included if they had sufficient details and were part of a larger series of results of investigations, performed indeed for the purpose of the study.

Correct categorisation of the grade of MR reported for a study group in an article was sometimes hampered by the use of classification systems that did not coincide with the DSM-IV system used throughout this review. A given study may define 'mild MR' as IQ 55–69 and 'moderate MR' as IQ 40–54, while the DMS-IV definitions use 50–70 and 35–50, respectively. This made it impossible to determine how many patients in fact did have mild and moderate IQ conforming to the DSM-IV definitions, and prevented determining any meaningful relation between the grade of MR and other variables. As studies may otherwise be useful, and loss of the results only on this ground was not desirable, we reinterpreted the data through the best-possible estimate. Possibly, some classification errors were introduced in this way, but one should realise that IQs derived from formal psychological tests often show intraindividual variations of 5 or more IQ points as well.

Finally, for some anomalies, the causal relation with MR remains uncertain. For chromosome anomalies, this holds for marker chromosomes, inversions, apparently balanced translocations, and some sex chromosome aneuploidies (eg 47,XXX); for dysmorphic examinations, the likelihood that, for instance, Ehlers–Danlos syndrome or a Poland anomaly represents the cause of MR is unlikely. For the other investigation techniques, similar examples can be given. Some authors clearly acknowledged this.²⁸ Therefore, such diagnoses were scored as having an uncertain causal relation with MR.

Quality assessment

Two reviewers (two of MCEJ, CDMvK, RCMH) independently assessed the quality of each study, conforming to previously published assessment models,^{4,29–31} scoring each of the following items: study design, study group, selection of the study group, clinical relevance of the study



* If the study did not comply these criteria, it was excluded from the review

Figure 2 Flow chart of steps in assessing the methodologic quality of a study on an investigation technique.

group, diagnostic technique applied, description of yield, and diagnoses established.

In consensus meetings, differences in the grading of the methodological quality were assessed for each study, conforming to the steps depicted in the flow chart in Figure 2. The quality was divided in items (Supplementary material Table 1; URL address at the end of the manuscript). Each item could be scored from 'poor' to 'good'. If a study scored 'poor' on one of the items Description and Clinical Relevance, it was excluded from the review. If the study scored 'moderate' or 'good' for each of these two items, then the quality of the technique reported in the study was assessed.

Only if the diagnostic investigation was applied in all patients, a random sample of the study group, or a nonrandom sample selected based on clearly described criteria, and if it was performed in part or completely, conforming to the reference standard in the year it was performed, and if the results were moderately to well described, the study was included in the next review step. Thus, studies with a good score for Description and Clinical Relevance of the study group might still be excluded due to

a poor quality of application of the technique or poor description of the results. For example, for the cytogenetic studies, we used the number of chromosome bands to decide whether a study was sufficiently reliable to report on only numerical chromosome anomalies (<350–400 bands) or also on structural anomalies (>350–400). Around the year 1983, high-resolution banding became widely available.⁸ We reasoned that a cytogenetic study performed after 1983 would most likely have applied this level of banding, and was therefore sufficiently sensitive to reliably pick-up structural anomalies. Finally, a 'moderate' to 'good' description of established diagnoses was necessary to allow inclusion in the final review. Although the presence of a list of diagnoses that are indeed (likely to be) aetiologic of MR did improve the quality score of a study, the absence of such a list would not lead to exclusion as long as diagnoses were provided in such a way that reinterpretation was possible.

For inclusion, a study had to score at least 'moderate' for six of the seven items. The total quality of the study was appraised as 'good', if 5–7 items were scored as 'good' and the remaining items as 'moderate'. In all other cases, the study was classified as 'moderate'.

Results

Search strategy

The flow diagram in Figure 3 summarises the number of articles accepted and rejected during the selection procedure. The search of the computerised databases identified a total of 4934 citations (Table 1).

Screening all titles of all publications allowed exclusion of 4412 studies clearly not related to the objective of the present study (Table 3). Of the remaining 522 papers, abstracts were considered in the selection procedure applying the phase 1 inclusion criteria, which were met by a total of 122 publications.

Screening of all references ($n = 3887$) of the 122 relevant papers in a similar manner resulted in an additional 97 studies. Thus, the total number of articles that entered the phase 2 selection procedure and form the basis of this review was 219. Table 3 indicates for each of the different investigation techniques the reasons for exclusion using phase 2 selection criteria.

Pilot study

Based on the phase 2 selection criteria, three of the total of eight publications in the pilot study were excluded from the review due to the following reasons: major goal was not elucidation of aetiology of MR;²⁴ patient selection criteria unclear;²⁵ number of patients with MR <25.²⁶ One publication²⁰ was included only for reporting numerical anomalies in MR patients, as the G-banding at that time (1971) was not of sufficient quality to detect small structural anomalies.

Selected articles

Supplementary material Tables 1 and 2 (available only on the Internet; URL address at the end of the manuscript) list the qualitative and quantitative data of all articles included in this review. Supplementary material Table 1 lists the quality of each individual article regarding the description of the patient group under study, the investigation technique, the description of the results of the investigation, the accuracy of the diagnoses, and the clinical relevance of the study. Supplementary material Table 2 provides detailed information of each individual study, grouped by investigation technique used. The major data provided from each study are year of study, study design, setting, selection of study group, characteristics of study groups including the degree of MR, details about investigation technique, number and percentages of detected anomalies, and number and percentages of detected diagnoses. If needed, specific remarks on each study were added. In the different sections of Supplementary material Table 2, the number of 'abnormalities detected' may differ from the numbers 'diagnoses made', as not all detected abnormalities are of aetiologic diagnostic significance (see also Data extraction).

Relation between diagnostic yield and setting, MR severity, gender

Table 4 shows the relation between the frequency of anomalies detected by each of the techniques on the one hand and the setting, MR severity, and gender on the other hand. The latter is listed for the totals only, as the number of studies in the separate categories was too small to allow analysis. Determination of the sex ratios of the abnormality frequencies was only possible for part of the studies, because some studies did not specify the gender distribution in relation to cytogenetic investigations results.

For the investigation technique Dymorphologic examination, no such crosstables could be gathered, as the different studies either performed the examinations in such a different way or the data were presented in such a different manner that pooling of results was not feasible or useful. Only for two specific items within a general dymorphologic evaluation a limited number of similar results became available. The first is the presence within families of other family members with MR. This was reported in 10 studies.^{59,63,65,80,92,99,106,113,114} The median number of families in which relatives with MR were present was 15.0% (range 7.5–46%). However, it was generally not stated to which extent the family was surveyed for MR nor to which extent it was reported in the publication. The population based study of Hou *et al*⁸⁰ reported that 15% of the families in a large population have a positive history of MR; the degree of familial relationship was not indicated. More or less the same holds for the second item, consanguinity. This was reported in eight studies.^{23,65,74,96,105,113–115} The median percentage of

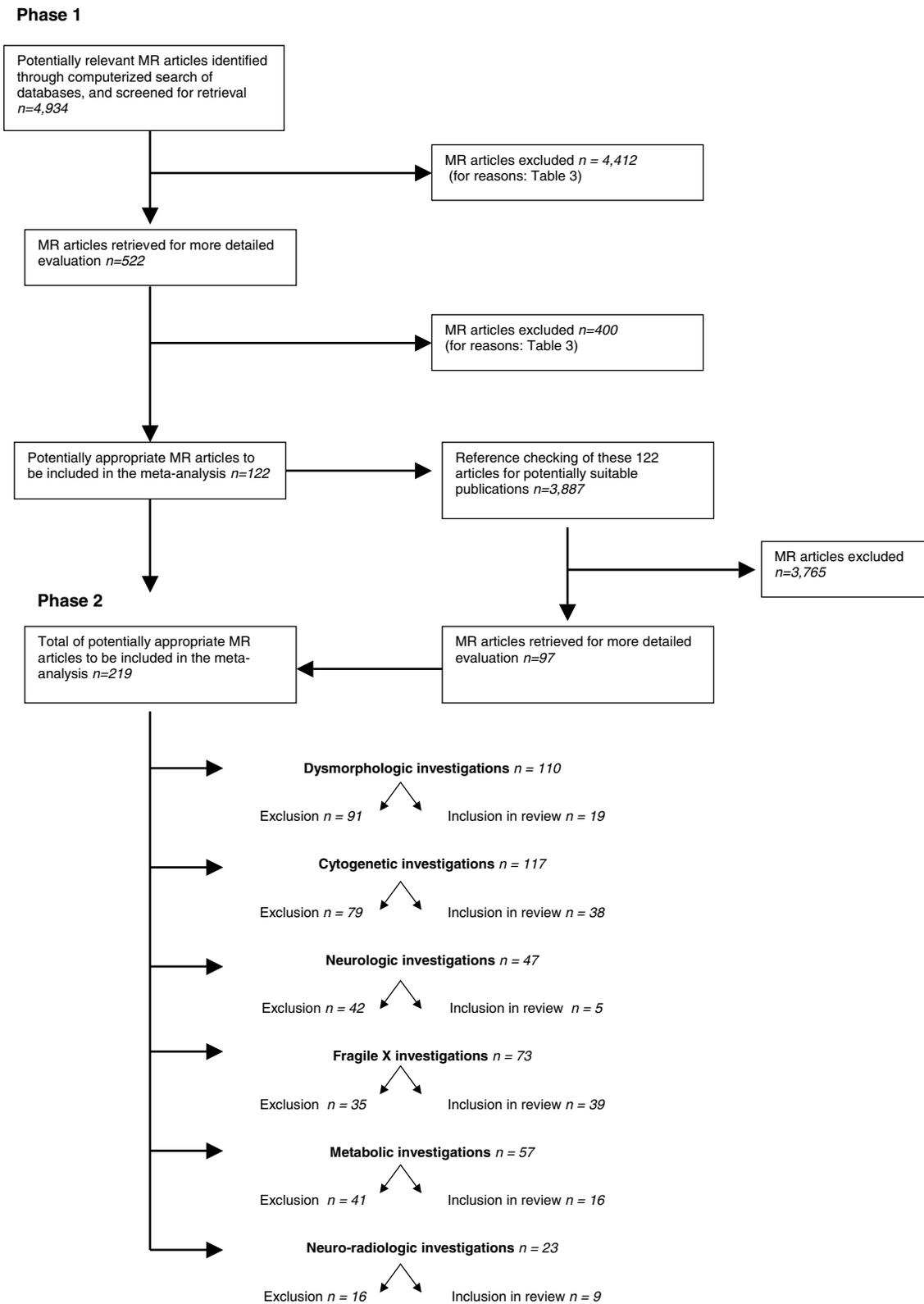


Figure 3 QUORUM flow diagram of publications included and excluded by reviewers during the selection procedure.

Table 4 Crosstables summarising diagnostic yield of the specific investigations per degree of MR and per type of setting

	<i>Borderline–mild</i>	<i>Moderate–profound</i>	<i>Unknown</i>	<i>Total</i>	<i>Gender</i>
(A) Yield of cytogenetic investigations					
<i>(A1) Frequency of detected numerical anomalies^a</i>					
Institution	<i>n</i> = 2 ^{20,53} 2.4%/1.8–2.9%	<i>n</i> = 7 ^{20,32,46,48,52,60,62} 9.5%/5.0–32.7%	<i>n</i> = 12 ^{28,33–39,42,45,51,55,61} 8.8%/0–42.9%	<i>n</i> = 20 8.8%/0–42.9%	<i>n</i> = 10 m:f = 7.5%:8.0%
Outpatient clinic	<i>n</i> = 2 ^{57,65} 2.6%/1.0–4.1%	<i>n</i> = 2 ^{57,65} 1.8%/1.7–1.8%	<i>n</i> = 4 ^{41,56,58,64} 6.7%/1.4–38.7%	<i>n</i> = 6 2.8%/1.0–38.7%	<i>n</i> = 2 m:f = 18.7%:23.7%
School	<i>n</i> = 1 ⁴⁷ 3.0%	<i>n</i> = 1 ⁵⁰ 0%	<i>n</i> = 2 ^{44,54} 9.5%/5.6–13.3%	<i>n</i> = 4 3.8%/0–13.3%	<i>n</i> = 2 m:f = 8.7%:7.7%
Population survey	<i>n</i> = 1 ⁵⁹ 4.1%	<i>n</i> = 1 ⁵⁹ 22.1%	<i>n</i> = 2 ^{43,63} 12.7%/11.5–13.8%	<i>n</i> = 3 13.3%/4.1–22.1%	m:f = no data
Mixed	<i>n</i> = 0 3%/1–4.1%	<i>n</i> = 0 7.8%/0–32.7%	<i>n</i> = 1 ⁴⁰ 2.7%	<i>n</i> = 1 2.7%	<i>n</i> = 1 m:f = 0:5.6%
Total	<i>n</i> = 6 3%/1–4.1%	<i>n</i> = 11 7.8%/0–32.7%	<i>n</i> = 21 8.8%/0–42.9%	<i>n</i> = 34 8.3/0–42.9%	<i>n</i> = 15 m:f = 7.2%:5.6%
<i>(A2) Frequency of detected numerical and structural chromosome anomalies^b</i>					
Institution	<i>n</i> = 1 ⁵³ 1.8%	<i>n</i> = 5 ^{46,48,52,60,62} 17.5%/9.5–37.6%	<i>n</i> = 5 ^{45,49,51,55,61} 8.3%/3.9–33.3%	<i>n</i> = 11 13.3%/1.8–37.6%	<i>n</i> = 5 m:f = 7.5%:4%
Outpatient clinic	<i>n</i> = 2 ^{57,65} 5.4%/2.0–8.8%	<i>n</i> = 2 ^{57,65} 7.9%/6.8–9.0%	<i>n</i> = 3 ^{56,58,64} 10%/8.2–48.5%	<i>n</i> = 5 8.2%/2.0–48.5%	<i>n</i> = 2 m:f = 24.4%:33.8%
School	<i>n</i> = 1 ⁴⁷ 5.4%	<i>n</i> = 1 ⁵⁰ 2.3%	<i>n</i> = 1 ⁵⁴ 8.8%	<i>n</i> = 3 5.4%/2.3–8.8%	<i>n</i> = 1 m:f = 5.2%:5.8%
Population survey	<i>n</i> = 1 ⁵⁹ 4.1%	<i>n</i> = 1 ⁵⁹ 26.0%	<i>n</i> = 1 ⁶³ 12.7%	<i>n</i> = 2 14%/4.1–26.0%	m:f = no data
Mixed	<i>n</i> = 0 4.1%/1.8–8.8%	<i>n</i> = 0 13.3%/2.3–37.6%	<i>n</i> = 0 9.4%/3.9–48.5%	<i>n</i> = 0 9.5%/1.8–48.5%	m:f = no data
Total	<i>n</i> = 5 4.1%/1.8–8.8%	<i>n</i> = 9 13.3%/2.3–37.6%	<i>n</i> = 10 9.4%/3.9–48.5%	<i>n</i> = 21 9.5%/1.8–48.5%	<i>n</i> = 8 m:f = 6.7%:8.3%
<i>(A3) Frequency of detected structural anomalies^b</i>					
Institution	<i>n</i> = 1 ⁵³ 0%	<i>n</i> = 5 ⁴⁸ 2.4%/0.7–4.9%	<i>n</i> = 5 ⁵¹ 3.2%/0.3–4.5%	<i>n</i> = 11 2.4%/0–4.9%	<i>n</i> = 5 m:f = 1.1%:3.4%
Outpatient clinic	<i>n</i> = 2 ⁵⁷ 2.9%/1.0–4.7%	<i>n</i> = 2 ⁵⁷ 6.2%/5.1–7.2%	<i>n</i> = 3 ⁶⁴ 7.5%/6.8–9.8%	<i>n</i> = 5 6.8%/1.0–9.8%	<i>n</i> = 2 m:f = 5.7%:10.2%
School	<i>n</i> = 1 ⁴⁷ 2.4%	<i>n</i> = 1 ⁵⁰ 2.3%	<i>n</i> = 1 ⁵⁴ 3.2%	<i>n</i> = 3 2.4%/2.3–3.2%	<i>n</i> = 1 m:f = 2.2%:2.9%
Population survey	<i>n</i> = 1 ⁵⁹ 0%	<i>n</i> = 1 ⁵⁹ 3.9%	<i>n</i> = 1 ⁶³ 1.2%	<i>n</i> = 2 1.6%/0–3.9%	m:f = no data
Mixed	<i>n</i> = 0 1%/0–4.7%	<i>n</i> = 0 3.8%/0.7–7.2%	<i>n</i> = 0 3.3%/0.3–9.8%	<i>n</i> = 0 3.2%/0–9.8%	m:f = no data
Total	<i>n</i> = 5 1%/0–4.7%	<i>n</i> = 9 3.8%/0.7–7.2%	<i>n</i> = 10 3.3%/0.3–9.8%	<i>n</i> = 21 3.2%/0–9.8%	<i>n</i> = 8 m:f = 1.8%:3.7%
<i>(A4) Frequency of subtelomeric FISH investigations</i>					
Institution	<i>n</i> = 0 0%/0–1.0%	<i>n</i> = 0 3.6%/0–10.3%	<i>n</i> = 0 3.3%/2.6–4.0%	<i>n</i> = 0 2.7%/0–10.3%	m:f = no data
Outpatient clinic	<i>n</i> = 3 ^{65–67} 1.0%	<i>n</i> = 1 ⁵⁷ 0%	<i>n</i> = 1 ⁷⁹ 28.6%	<i>n</i> = 2 14.6%/1.0–28.6%	<i>n</i> = 1 m:f = 34.1%:0%
School	<i>n</i> = 0 5.7%/5.4–5.9%	<i>n</i> = 0 2.6%	<i>n</i> = 0 4.8%/3.9–7.8%	<i>n</i> = 0 4.8%/2.6–7.8%	m:f = no data
Survey	<i>n</i> = 1 ⁶⁸ 10.3%	<i>n</i> = 1 ⁶⁸ 9.8%	<i>n</i> = 0 0%	<i>n</i> = 0 12.4%/0–20.7%	m:f = no data
Mixed	<i>n</i> = 1 3.0%	<i>n</i> = 4 12.6%/4.5–20.7%	<i>n</i> = 2 3.3/2.6–4.0%	<i>n</i> = 1 4.4%/0–10.3%	<i>n</i> = 1 m:f = 5.3%:14.5%
Total	<i>n</i> = 4 0.5%/0–10.3%	<i>n</i> = 4 6.7%/0–10.3%	<i>n</i> = 2 3.3/2.6–4.0%	<i>n</i> = 5 4.4%/0–10.3%	<i>n</i> = 3 m:f = 2.5%:3.6%
(B) Yield of fragile X investigations frequency of cytogenetically detected anomalies^c					
Institution	<i>n</i> = 3 ^{53,72,75} 0%/0–0%	<i>n</i> = 5 ^{60,62,72,75,78} 7.3%/2–13%	<i>n</i> = 4 ^{45,49,55,72} 4.7%/0–7.5%	<i>n</i> = 9 5.4%/0–13.0%	<i>n</i> = 9 m:f = 5.3%:0%
Outpatient clinic	<i>n</i> = 1 ⁵⁷ 1.0%	<i>n</i> = 1 ⁵⁷ 0%	<i>n</i> = 1 ⁷⁹ 28.6%	<i>n</i> = 2 14.6%/1.0–28.6%	<i>n</i> = 1 m:f = 34.1%:0%
School	<i>n</i> = 2 ^{47,74} 1.8%/0.6–3.0%	<i>n</i> = 3 ^{47,50,74} 5.5%/0–11.8%	<i>n</i> = 1 ⁴⁴ 0%	<i>n</i> = 4 4.8%/0–11.8%	<i>n</i> = 4 m:f = 4.5%:6.6%
Population survey	<i>n</i> = 2 ^{59,70} 5.7%/5.4–5.9%	<i>n</i> = 1 ⁵⁹ 2.6%	<i>n</i> = 3 ^{76,77,80} 4.8%/3.9–7.8%	<i>n</i> = 5 4.8%/2.6–7.8%	<i>n</i> = 3 m:f = 8.5%:3.4%
Mixed	<i>n</i> = 1 ⁷³ 3.0%	<i>n</i> = 2 ^{71,73} 12.6%/4.5–20.7%	<i>n</i> = 1 ⁷³ 0%	<i>n</i> = 2 12.4%/0–20.7%	<i>n</i> = 2 m:f = 12.4%:—
Total	<i>n</i> = 9 1%/0–5.9%	<i>n</i> = 12 5.6%/0–20.7%	<i>n</i> = 6 4.8%/0–28.6%	<i>n</i> = 22 5.4%/0–28.6%	<i>n</i> = 19 m:f = 5.4%:1.4%

Table 4 (Continued)

(B2) Frequency of molecularly detected mutations^d

Institution	<i>n</i> = 0	<i>n</i> = 1 ⁸⁴ 0.7%	<i>n</i> = 0	<i>n</i> = 1 0.7%	<i>n</i> = 1 m:f = 0.7%:—
Outpatient clinic	<i>n</i> = 5 ^{64,65,83,89,93} 2.8%/0–9.0%	<i>n</i> = 4 ^{64,65,83,93} 2.7%/0–4.9%	<i>n</i> = 1 ⁹¹ 3.1%	<i>n</i> = 6 3.4%/0.4–9.1%	<i>n</i> = 5 m:f = 2.4%:0.4%
School	<i>n</i> = 3 ^{86,87,92} 1%/0.8–1.1%	<i>n</i> = 2 ^{86,92} 2.3%/0.5–4.1%	<i>n</i> = 2 ^{85,90} 3.5%/2–5%	<i>n</i> = 5 2.2%/0.5–5.0%	<i>n</i> = 5 m:f = 2.5%:0.2%
Population survey	<i>n</i> = 0	<i>n</i> = 0	<i>n</i> = 1 ⁸² 13.6%	<i>n</i> = 1 13.6%	<i>n</i> = 1 m:f = 13.6%:—
Mixed	<i>n</i> = 0	<i>n</i> = 0	<i>n</i> = 3 ^{81,88,94} 1.6%/1.3–4.1%	<i>n</i> = 3 1.6%/1.3–4.1%	<i>n</i> = 3 m:f = 1.9%:0.9%
Total	<i>n</i> = 8 1.0%/0–9.0%	<i>n</i> = 7 2.7%/0–4.9%	<i>n</i> = 7 3.1%/1.3–13.6%	<i>n</i> = 16 2.0%/0–13.6%	<i>n</i> = 15 m:f = 2.0%:0.2%

(B3) Frequency of all detected anomalies^{c,d}

Institution	<i>n</i> = 3 ^{53,72,75} 0%/0%	<i>n</i> = 6 ^{60,62,72,75,78,84} 6.5%/0.8–13.0%	<i>n</i> = 4 ^{45,49,55,72} 4.7%/0–7.5%	<i>n</i> = 10 5.4%/0–13.0%	<i>n</i> = 10 m:f = 4.6%:0%
Outpatient clinic	<i>n</i> = 6 ^{57,64,65,83,89,93} 1.0%/0–9.0%	<i>n</i> = 5 ^{57,64,65,83,93} 2.7%/0–4.9%	<i>n</i> = 2 ^{79,91} 15.9%/3.1–28.6%	<i>n</i> = 8 2.5%/0–28.6%	<i>n</i> = 6 m:f = 3.2%:0%
School	<i>n</i> = 5 ^{47,74,86,87,92} 1.0%/0.6–3.0%	<i>n</i> = 5 ^{47,50,74,86,92} 4.1%/0–11.8%	<i>n</i> = 3 ^{44,85,91} 2.0%/0–5.0%	<i>n</i> = 9 2.4%/0–11.8%	<i>n</i> = 9 m:f = 2.0%:0.9%
Population survey	<i>n</i> = 2 ^{59,70} 5.7%/5.4–5.9%	<i>n</i> = 1 ⁵⁹ 2.6%	<i>n</i> = 4 ^{76,77,80,82} 6.3%/3.9–13.6%	<i>n</i> = 6 5.4%/2.6–13.6%	<i>n</i> = 4 m:f = 9.3%:3.4%
Mixed	<i>n</i> = 1 ⁷³ 3.0%	<i>n</i> = 2 ^{73,75} 12.6%/4.5–20.7%	<i>n</i> = 4 ^{73,81,88,94} 1.5%/0–4.1%	<i>n</i> = 5 4.0%/0–20.7%	<i>n</i> = 5 m:f = 4.0%:0.9%
Total	<i>n</i> = 17 1.0%/0–9.0%	<i>n</i> = 19 4.1%/0–20.7%	<i>n</i> = 17 3.9%/0–28.6%	<i>n</i> = 38 4.1%/0–28.6%	<i>n</i> = 34 m:f = 3.9%:0.2%

(C) Yield of PKU investigations^e

Institution	<i>n</i> = 3 ^{95,98,102} 0%	<i>n</i> = 4 ^{95,98,102,105} 0.1%/0–4.2%	<i>n</i> = 2 ^{62,101} 0.4%/0.3–0.5%	<i>n</i> = 6 0.3%/0–4.2%	
Outpatient clinic	<i>n</i> = 1 ⁶⁴ 0%	<i>n</i> = 2 ^{96,103} 0.3%/0–0.6%	<i>n</i> = 2 ^{99,104} 0.3%/0.2–0.4%	<i>n</i> = 4 0.3%/0–0.6%	
School	<i>n</i> = 1 ¹⁰⁰ 0.8%	<i>n</i> = 0	<i>n</i> = 0	<i>n</i> = 1 0.8%	
Survey	<i>n</i> = 0	<i>n</i> = 0	<i>n</i> = 1 ⁹⁷ 1.0%	<i>n</i> = 1 1.0%	
Mixed	<i>n</i> = 0	<i>n</i> = 0	<i>n</i> = 0	<i>n</i> = 0	
Total	<i>n</i> = 5 0%/0–0.8%	<i>n</i> = 6 0.1%/0–4.2%	<i>n</i> = 5 0.4%/0.2–1.0%	<i>n</i> = 12 n = 0.4%/0–4.2%	<i>n</i> = 5 m:f = 1:1

(D) Yield of neurologic investigations

Institution	<i>n</i> = 0	<i>n</i> = 0	<i>n</i> = 0	<i>n</i> = 0	
Outpatient clinic	<i>n</i> = 1 ⁶⁵ 47.3%	<i>n</i> = 1 ⁶⁵ 68.0%	<i>n</i> = 1 ⁶⁴ 7.3%	<i>n</i> = 2 32.5%/7.3–68.0%	
School	<i>n</i> = 0	<i>n</i> = 0	<i>n</i> = 0	<i>n</i> = 0	
Survey	<i>n</i> = 2 ^{63,107} 35%/27.0–42.9%	<i>n</i> = 2 ^{63,106} 45.1%/31.2–59.0%	<i>n</i> = 0	<i>n</i> = 3 42.9%/27.0–59.0%	
Mixed	<i>n</i> = 0	<i>n</i> = 0	<i>n</i> = 0	<i>n</i> = 0	
Total	<i>n</i> = 3 42.9%/27.0–47.3%	<i>n</i> = 3 59.0%/31.2–68.0%	<i>n</i> = 1 7.3%	<i>n</i> = 5 42.9%/7.3–68.0%	<i>n</i> = 1 m:f = 53.7%:62.7%

(E) Yield of neuroimaging investigations^f

Institution	<i>n</i> = 0	<i>n</i> = 0	<i>n</i> = 0	<i>n</i> = 0	
Outpatient clinic	<i>n</i> = 1 ⁶⁵ 35.2%	<i>n</i> = 1 ⁶⁵ 37.7%	<i>n</i> = 7 ^{58,64,108–112} 29.8%/6.2–48.7%	<i>n</i> = 8 31%/6.2–48.7%	
School	<i>n</i> = 0	<i>n</i> = 0	<i>n</i> = 0	<i>n</i> = 0	
Survey	<i>n</i> = 1 ⁶³ 7.1%	<i>n</i> = 1 ⁶³ 8.9%	<i>n</i> = 0	<i>n</i> = 1 9.8%	
Mixed	<i>n</i> = 0	<i>n</i> = 0	<i>n</i> = 0	<i>n</i> = 0	
Total	<i>n</i> = 2 21.2%/7.1–35.2%	<i>n</i> = 2 30.0%/8.9–37.7%	<i>n</i> = 7 29.8%/6.2–48.7%	<i>n</i> = 9 30.0%/6.2–48.7%	<i>n</i> = 2 m:f = 21.8%:27.4%

n: the number of studies yielding evidence for a particular frequency; (x%/x–x%): (median frequency in %/range in frequency in %); (m:f): median frequency in investigated males (%) vs females (%).

^aAll studies reporting karyotype investigations regardless of banding level or year of publication (*n* = 34).

^bOnly those studies reporting karyotype investigations applying a banding level of 350–400 bands or more, and/or published after 1983 (see Methods) (*n* = 21).

^cAll studies reporting cytogenetic fragile X investigations in lymphocyte cultures prepared in medium 199, scored for fragile sites at Xp27.3.

^dAll studies reporting molecular fragile X investigations of genomic DNA screening for mutations in the *FMR-1* gene.

^eOf 16 metabolic investigations included in this review, for the crosstables summarising yield of PKU screening per setting and grade of MR, only 12 were included as the screening had to have been performed in a study group who had (most likely) not previously undergone standard postnatal screening for PKU.

^fCT scan and MRI scan.

families with consanguinity was 9.1% (range 0.7–85.5%). As, again, it was usually not stated to which extent consanguinity was surveyed or reported, and also because of the different ethnic backgrounds of the study populations, it is not possible to draw meaningful general conclusions from these figures.

There was a limited number of studies that reported on the percentage of patients with abnormal physical features in their population: Ohdo *et al*⁵⁶ reported the presence in 55.0%, Majnemer *et al*⁵⁸ in 44.5%, Hunter⁶⁴ in 39.4%, and Van Karnebeek *et al*⁶⁵ in 81.9%.

Discussion

Limitations and merits of the review

The inclusion or exclusion of a given publication in this review does not in a simple way relate to the scientific value of an individual study: we had to exclude many valuable publications as they did not meet the specific goals of the present study. One example is the publication by Flint and co-workers in *Nature Genetics*, which added fundamental new knowledge on the detection of subtelomeric rearrangements in patients with idiopathic MR.¹¹⁶ Although this study is of significant importance, we were unable to include it in this review as the description of selection of patients was insufficient to provide reliable data on frequency of subtelomeric anomalies in unselected MR patients. Nor was it possible to determine the relation between yield of subtelomeric screening and clinical setting or specific characteristics of the studied group, such as MR severity and gender. Another example is the group of comprehensive studies that report a complete diagnostic work-up of an MR study group. Studies from this group of publications often had to be excluded because it was impossible to determine the exact yield of the individual additional investigations, in part because it was not specified in how many patients each of the investigations was performed.^{117,118}

A second limitation of our study is the fact that we are able to report only the positive findings of an investigation; none of the studies described the presence and value of 'negative findings' in the diagnostic process. In clinical practice, the exclusion of a specific anomaly (a 'negative finding') can be as useful as a positive one in establishing an aetiological diagnosis. It will require a separate study designed specifically for establishing the value of 'negative findings' to investigate this further.

A positive quality of the study is the broad character of the search strategy and the minute study selection process, minimising the chance of overlooking important publications. We allowed the inclusion of studies published in languages other than English; articles published during a long time interval, which allowed the inclusion of valuable studies dating back to more than 30 years; publications indexed in different databases, so that articles not listed in one electronic database but only in another were not

overlooked; and publications with unusual MeSH headings not retrieved from the databases but found through screening of bibliographies of potentially useful articles. This resulted in the 'detection' of several publications of unusual quality,^{57,80} which seem to have been overseen by many authors of earlier reviews and texts.

The present study also tries to provide an estimate of the quality of studies. Quality is a multidimensional concept, which relates to the design, conduct, and analysis of a study, its clinical relevance, and quality of reporting.³⁰ Assessment of quality is necessary to limit bias in the results of this systematic review, to gain insight into potential comparisons, and guide the interpretations of the findings.⁴ As there are only a small number of studies that have tried to measure quality,⁴ knowledge and experience in this area are limited. As quality assessments will always include some degree of clinical and methodological judgement, its results should be interpreted with some caution.

The similarities and differences with the only other comprehensive assessment in this area, the review of the American Academy of Neurology² merits a more detailed discussion. In this extraordinary paper, an evidence-based evaluation of the literature on diagnostic studies in individuals with MR is described. There are a number of limitations in this article however. One is the restriction to only papers in the English language. In the present studies, 197 of the 219 were English, the other 22 were non-English. A major issue – in our opinion – is the absence of details on the criteria used to select papers for the review. The authors mention all keywords of their search but do not mention how they selected papers out of the retrieved citations. The use of one of their keywords (mental retardation) in one literature database (Pubmed) gives 57 641 hits, indicating the importance of these inclusion and exclusion criteria. It was not reported whether the selection of papers was performed by a single investigator or by different investigators, and whether these worked independently or not.

These issues are important to prevent selection bias in the field; such bias can lead to both overestimation and underestimation of the virtues of a given diagnostic test. A last point of concern is that in their presentation of the results of individual studies, the difference in setting of the study or the degree of MR was not consistently reported. We have been unsuccessful in our efforts to gather this information from the authors of this article, and therefore cannot meaningfully compare their results with the present review. Despite these comments, the paper is of considerable value, and the results are in many ways similar to our results.

Number of available studies

The total number of articles reporting the results of one or more diagnostic investigations in MR patients that seem

appropriate for inclusion in this review was considerable. Yet, if one considers the number of citations that had to be screened in order to obtain publications truly relevant to our aim, the yield of our searches seems low. The low 'hit' rate of the computerised searches may be explained in part by the broad character of our search, which in turn was due to lack of accepted terms and MeSH headings exactly fitting the aims of this review. For example, articles reporting prenatal screening for well-known causes of MR such as Down syndrome or articles on the developmental follow-up of neonates who had suffered hypoxic insults also appeared in our search results. Further, there exist few other observational systematic reviews focused on disease aetiology, thus offering little information for optimising the effectiveness and efficiency of our search strategies. The high number of useful publications found through reference checking (and not computerised searches) suggests that either our search strategies or the quality of indexation of articles on this subject is suboptimal. We conclude that, although many articles did not meet the criteria for our review, the final number of publications of sufficient quality included in this review is considerable, and should allow for reliable conclusions.

Yield of investigation techniques

Chromosome anomalies A first conclusion that can be drawn from this review is that chromosome anomalies have been detected in all MR study groups. On average, the frequency of detected aberrations was around one in every 10 investigated patients. In general, cytogenetic studies therefore are a valuable diagnostic technique in studying individuals with MR. There is a considerable variation, however, in the frequency of detected aberrations, especially for numerical anomalies.

Numerical anomalies. Analysis of the relationship between yield of chromosome studies and clinical setting was hampered by the uneven distribution of studies among the different settings. Most studies were performed in institutions, which on average will comprise more severely retarded patients than schools and population surveys. The median frequency of anomalies was higher in individuals with moderate to severe MR than those with borderline or mild MR, and differences herein could well explain the variation in the frequency of detected chromosome anomalies in different settings. However, also in the group of borderline to mild MR, the number of cytogenetic anomalies is still considerably high, and allows the recommendation of routine karyotyping.

The relationship between gender and detection frequency of numerical anomalies is reported by a total of 15 studies on 6601 persons, which provides sufficient data to draw conclusions. In total, a slight male predominance is reported (M:F = 7.2%:5.6%). The difference is too small, however, to imply an increased likelihood of detecting numerical anomalies in males; we recommend that

cytogenetic investigations should be performed regardless of gender.

Obviously, the inclusion or exclusion of individuals with only clinically suspected Down syndrome in a study group influenced the reported yield of numerical anomalies. Whether or not the presence of other dysmorphic features influenced detection frequency was not systematically assessed in this review, as differences in the applied nomenclature and classification of such features between studies precluded reliable analyses. A recent study in a group of MR children from our group did provide evidence of the hypothesis frequently brought forward by Dr John Opitz, that individuals with aneuploidy show more and more widespread minor anomalies.⁶⁵ As chromosomal aberrations have been found in persons with a very mild phenotype,¹⁴ karyotyping should still be performed in all MR patients without other obvious causes, regardless of the number of abnormal physical findings.

Finally, numerical anomalies affected autosomes considerably more often than sex chromosomes, with a median frequency of 6.5 vs 0.4%. The most obvious explanation lies in the high number of persons with Down syndrome. The nine studies reporting on the relation between the severity of MR in patient and the type of chromosome affected, all show that numerical anomalies of the sex chromosomes occur foremost in borderline to mild MR, while numerical anomalies of the autosomes are detected mostly in patients with more severe MR. This distribution concurs with previous reports.

Structural anomalies. There is less variation in detection frequency of structural anomalies than of numerical anomalies. The pick-up rate of the former anomalies depends largely on the resolution of the cytogenetic investigation: more anomalies will be missed using 350–400 bands compared to higher band numbers. Although studies reporting FISH investigations screening for (sub-microscopic) structural rearrangements involving the chromosome ends were included in the present review, those involving interstitial regions were not. Only the advent of molecular karyotyping will provide reliable data on the frequency of structural rearrangements in MR individuals.

As for setting, the numbers per category are small, warranting caution when interpreting the data. The highest average of median frequency was reported by the five studies performed in an outpatient clinic, while the frequency reported by 11 institutional studies was almost three-fold lower, similar to studies performed in schools and population setting. Similar to the detection frequency of numerical anomalies, structural anomalies were reported more often in patients with moderate to profound MR than in those with a more mild MR grade. Again, frequencies in both groups are sufficient to advocate cytogenetic investigations regardless of MR severity. The relation between anomaly frequency and gender in all

studies reliably reporting on gender distribution showed a female predominance ($P < 0.05$). Structural anomalies more often affected the autosomes than the sex chromosomes in all studies except one, which reported an equal ratio of affected chromosome type. This female predominance in structural anomalies was also found for the subtelomeric FISH studies (see below). There is no good explanation for this finding. Theoretically, one can speculate that structural anomalies occur more often in females than in males due to (1) genes on the sex chromosomes which regulate the three-dimensional DNA structure, thereby influencing crossover frequencies or other *trans*-actions between chromosomes; (2) the decreased degree of condensation during meiosis in females,¹¹⁹ resulting in more frequent abnormal pairing of chromosome regions, for instance in those harbouring olfactory receptor-gene clusters.^{120,121} (3) Structural anomalies occur in both genders with equal frequency, but female predominance is explained by an increased prenatal or early postnatal survival of females with such an aberration. Empirical motivation of an increased prenatal survival in females is difficult because the sex ratio of spontaneous abortions remains largely unclear; one study reported a slightly higher frequency in males (XY/XX ratio = 1.03).¹²² As for the postnatal survival of preterm and/or low birth-weight infants, a male disadvantage has been reported in several studies.^{123,124} The mechanisms underlying the increased mortality (and morbidity) rate in these newborn males remain largely unknown, but are in support of the latter explanation. (4) Finally, the presently found female predominance may be the result of chance alone, as the number of studies on which this conclusion is based is still limited. The sex distribution of structural autosomal anomalies deserves more attention in future research.

Numerical and structural anomalies. The variation in the total number of chromosome anomalies is of course the sum of the separate variations in numerical and in structural anomalies. The above conclusions also apply.

FISH analysis of subtelomeric regions. For subtelomeric FISH studies, the results of the review are based on a relatively small number of studies. We found that the frequency of subtelomeric rearrangements in unselected MR patients may be lower than previously reported. As suggested earlier,⁶⁵ this investigation should be selectively applied until more efficient, less-expensive techniques become available. There was a marked variation between positive test results of studies. Explanations for differences in detection frequency are extensively discussed elsewhere.⁶⁵ In the present review, the number of studies per category of setting and MR severity is too small to allow firm conclusions about the relation of detection frequency with these two variables. As for gender, all three studies reporting reliable data on the frequency of rearrangements in males vs females again suggest a female predominance ($P < 0.05$).

Fragile X studies Studies for the fragile X syndrome were performed using cytogenetic techniques in former times, but after detection of the gene defect almost all studies used molecular techniques. The number of studies comparing the yield of both types of investigation techniques is small²⁴ and indicated that the yield is comparable. In the present review, we found that the yield of cytogenetic techniques to detect fragile X syndrome yielded a mean result of 5.4%, while molecular studies yielded only 2.0%. The cytogenetic studies were performed in a different period, during which still many patients with fragile X had to be detected. This may have created a bias during patient selections. Furthermore, patients with other fragile sites at the distal long arm of the X chromosome may be mistaken for patients with fragile X syndrome.

The most reliable study that screened an unselected group of patients is the study by De Vries *et al.*^{19,86} They reported on a large group of institution population and found fragile X by molecular techniques in 0.7%. Most other studies did not use unselected study groups, but groups selected on either X-linked MR, the presence (or absence) of macro-orchidism or other phenotypical features of fragile X syndrome, the absence of Down syndrome, or the absence of any known cause for MR. The reader is referred to Supplementary material Table 2 for further detailed information on studies of each selected study.

Several studies used phenotype checklists to increase their yield.^{19,77,81,82,84,91,125} Despite many differences in the nature of the checklists, this was usually successful, although not always.⁹¹ Checklists may ask for several items regarding the face (ear size, mandible size), brain growth (skull circumference), testicle size, skin constitution, degree of MR, behaviour, and family history for MR. Especially, the presence of normocephaly or macrocephaly, and a positive family history seem to be the most valuable criteria (Supplementary material Table 2). If a checklist becomes too long, it becomes cumbersome in general practice and its use decreases. This demands a limited set of criteria for future lists.

The yield of studies in study groups with a more pronounced degree of MR is reported to be, in general, larger (4.1%) compared to those in the borderline–mild MR group (1.0%). This is to be expected as, in general, fragile X gives rise to a more pronounced degree of MR in males.

There is a number of studies for fragile X syndrome in females.^{19,44,47,49,50,53,70,76,77,79,81,83,84,88,89,91,93,94,126} Many of the studies have selected their study group (see individual studies in Supplementary material Table 2), and even after such selection the yield in females was often low. The most reliable study is again the study by De Vries *et al.*^{19,86} who reported a yield of 0.3% in girls. This yield increased with use of clinical preselection criteria, a positive family history being the most obvious one.

Metabolic investigation The results of this systematic review on metabolic investigations in persons with MR are limited. One reason is the relatively small number of relevant publications that were available for this study. A second reason is that the nature of the various studies differed considerably: the metabolic pathways that were investigated in one study could completely differ from those in other studies. This lack of a standardised screening protocol precludes any comparison. Only for screening studies for phenylketonuria (PKU) sufficient studies were available to allow a crosstabulation (Table 4).

In general, the yield of metabolic studies is low. If one considers the results of the different studies for metabolic disorders in general, results varied from 0.2 to 8.4% (median 1.0%). The higher figures are from countries where specific entities are more common, such as aspartylglycosaminuria in Finland,⁵⁹ or the figures were reached by applying checklist criteria,^{64,65,105} sometimes in a highly inbred population.¹⁰⁵ In such specific study populations, the yield may be high. There is one population study⁹⁷ studying all school children with MR in special schools and institutes for the mentally retarded that showed a metabolic abnormality in 1.0% of the study population; this figure must be an under-estimation of the true frequency as children below 4 years were under-represented in this study. In other populations, neonatal screening programmes for metabolic disorders identify children with often treatable causes of MR.

A preselection of cases using a stepwise or checklist (like in fragile X syndrome) approach has been reported infrequently. One study¹²⁶ using a stepwise approach showed an increase of yield to 13.6%. Items in such an approach may be dysmorphic symptoms, hepatosplenomegaly, and ophthalmologic and neurologic findings.

Neurologic investigation In the paper from the American Academy of Neurology,² the results of diagnostic yield of clinical neurological studies were not mentioned as such, but only the results of EEG studies and neuroimaging were reported. The diagnostic yield of EEG studies fell outside the scope of our review as EEG studies in itself are rarely if ever diagnostic. For diagnostic purposes, there are no clues that EEG studies are indicated.² This does not mean that EEG studies are not useful in some children with MR: Shevell *et al*² demonstrated that available literature data indicate a yield of 4.4% for epilepsy-related diagnoses, and suggested that an EEG should be obtained in children with a history or examination features suggesting the presence of epilepsy.

Most clinicians will agree that a careful neurological investigation of every child with MR is obligatory. In our literature search, it appeared difficult to obtain evidence for the utility of this, as many papers did not differentiate their results according to the results of clinical neurological

examination. There is however a limited number of papers available (Table 4 and Supplementary material Table 2). As expected, all show a remarkably high yield in the settings for which data are available, that is, in outpatient clinic studies and population-based surveys, irrespective of the degree of MR. The total yield of aetiological diagnoses in all studies is 42.9%. This figure does not yet include the value of neurological exams in providing indications for other diagnostic studies such as neuroimaging or molecular analyses. Therefore, the true diagnostic yield of a neurological examination is probably higher. We conclude that every child with MR, irrespective of setting and degree of retardation, should undergo a basic, clinical neurological exam.

Neuroradiological studies The different neuroimaging modalities that entered the present study included cranial CT scanning and brain MRI studies. The results in Table 4 refer to the number of abnormalities found in such studies. In 30.0% of all studies, brain abnormalities were reported. None of the studies reported on the value of the absence of any neuroradiological abnormality for a diagnostic work-up. Therefore, the true value for finding abnormalities or the absence of abnormalities must be higher. MRI studies were reported to be more sensitive compared to CT scanning, as expected.^{65,111,127}

If the neuroimaging was performed 'on indication', that is, in cases with abnormal brain size or a focal neurological finding, the results increased. Shevell *et al*² reported that in an earlier study by their group¹²⁸ the percentage of abnormalities was 13.9% if performed on a 'screening basis', but increased to 41.2% if performed on an 'indicated basis'; the authors discussed other studies on smaller number of patients that showed similar results.

If one allows only completely diagnostic results to be tabulated, the results are much less impressive: Kjos *et al*¹¹⁰ reported 3.9% diagnoses in patients who had no known cause for their MR and followed no progressive or degenerative course, Bouhadiba *et al*¹¹² found 0.9% diagnoses in patients with neurological symptoms, and four other studies^{64,108,109,111} found no diagnosis on the basis of the neuroimaging alone. Three studies reported the results of unselected patients: Majnemer and Shevell⁵⁸ found a diagnosis by this type of investigation in 0.2% of patients, Stromme⁶³ in 1.4%, and Van Karnebeek *et al*⁶⁵ in 2.2% of patients.

For all of the above studies, the yields are somewhat lower in the patients with a borderline-mild MR compared to a moderate–profound retardation, although the differences are often small.

Neuroimaging is at present possible only in patients who remain immobile for a longer period of time. Many patients with MR, especially the younger ones, will not be able to accomplish this, and will need some type of sedation for such studies. This makes the burden to the

patient for performing neuroimaging studies much higher as compared to the physical exam, urine sampling, and blood sampling that is needed for the other diagnostic tests. This influences the desired diagnostic yield needed before one will perform a neuroradiological study. The increase in diagnostic yield in neuroimaging on an indicated basis (ie brain size abnormality, focal neurological finding, symptoms at history taking or physical exam indicating a higher chance for a brain anomaly such as phacomatose) is important in this respect. With the advent of new and faster imaging modalities, the burden to patients may decrease and the indication for neuroimaging may change. At present, we recommend neuroimaging on an indicated basis only.

Dysmorphological investigations The utility of a dysmorphological examination was difficult to evaluate. The main reason was that a very limited number of studies reported on the specific results of such examinations, while probably most if not all studies did make use of the presence or absence of unusual features at physical surface investigation, in part to steer further diagnostic studies. Numerical results on the resulting diagnostic yield cannot be derived from these studies. In larger groups of patients, the number of two or more dysmorphic features found was from high (39.4%,⁶⁴ 44.5%⁵⁸) to very high (55.0%,⁵⁶ 81.9%⁶⁵). Obviously, these figures depended heavily on the definition of dysmorphic features, and the way the dysmorphological exam was performed.⁶⁵ As in neurological exams, the figures on dysmorphic features do not represent the number of aetiological diagnoses made using this investigation technique, and also do not indicate how often the finding of dysmorphic features formed the reason to initiate further metabolic, cytogenetic, molecular, neuroradiological, or other investigations. One study reported about this in detail: Van Karnebeek *et al*⁶⁵ reported on the importance of physical examination, including paediatric neurological, and dysmorphological examinations, and found this to have been essential for the diagnosis in 62% and contributory in 79%, respectively. No distinction between the three parts of physical exam was made. Shevell *et al*¹²⁸ reported similar findings. In conclusion, taking a good clinical history and performing a detailed physical examination by a trained specialist remain the basis of every aetiological study in children with MR.

In performing literature reviews, one often encounters differences in the way the results of investigations are reported. In the present study, the largest differences were found in the way dysmorphological exams were reported: the definitions used, the extensiveness of the physical exam itself, and the terms used in descriptions varied widely. We conclude that there is definitely a need for more standardisation of reporting dysmorphological examinations.

Recommendations from this study

In both current clinical practice and in future empirical studies aimed at detecting the aetiological diagnosis in children with MR, the following guidelines can be formulated (each time it is indicated if the guideline is based on the present systematic literature search):

- In each child, a detailed clinical history should be taken and physical examination should be performed, irrespective of setting or degree of MR (basis: this study). This physical exam should also include both a detailed paediatric neurological exam and a dysmorphological exam (basis: this study). Therefore, a good clinical history and physical exam by a trained specialist remain the basis of aetiological studies in persons with MR.
- In each child, standard cytogenetic studies should be performed, irrespective of setting, degree of MR, and the presence (or not) of dysmorphic features (basis: this study). Only if noncytogenetic causes of MR are evident through history taking and physical exam, this can be omitted.
- FISH analysis for subtelomeric rearrangements should at present be used on stringent selection criteria, as can be found in the available checklists (basis: this study). If more efficient and less-expensive techniques become widely available, studies for subtelomeric (and possibly also interstitial) rearrangements may become indicated in each child.
- Molecular studies for fragile X syndrome have a lower yield than earlier expected, but may still be performed in all boys with MR (basis: this study). The chances for finding fragile X syndrome are higher in children with a more expressed degree of MR, and in an outward clinic setting (basis: this study). The use of checklists or simple criteria may increase the yield considerably; the two most powerful criteria seem to be the presence of a positive family history for MR and the absence of microcephaly (basis: this study). Studies in girls should not be performed routinely, but only if positive clues are present, of which a positive family history for MR is the most important.
- Metabolic studies should not be performed as the first diagnostic study in each child, but in the absence of clues for other causes, the yield is still of a sufficiently high level to allow testing (basis: this study). The use of checklists improves the yield considerably. Further development of such checklists is desirable. The nature of metabolic pathways studied in children with MR should become more standardised internationally.
- Neuroradiological studies have a high yield for brain abnormalities, but a low yield for establishing aetiological diagnoses (basis: this study). Neuroradiology should not be performed in each child, also because of the burden of neuroimaging for the retarded child. If specific symptoms are present (abnormal brain size, focal

neurological finding, symptoms at clinical history taking or physical exam that indicate a higher chance for a brain anomaly, such as a phacomatosis), neuroimaging is indicated. The yield is higher in patients with a more expressed degree of MR, and MRI scanning has a higher yield compared to CT scanning (basis: this study). The development of new and faster modalities may change this recommendation.

- The yield of specific neurological and dysmorphological studies should be studied in more detail (basis: this study). Investigation techniques and reporting of results of dysmorphological studies should become more standardised internationally.
- The cause for the higher yield in both structural and numerical chromosome anomalies in females needs further evaluation.
- The value for the diagnostic process of the absence of abnormalities, for instance, in neuroimaging should be further studied.
- If algorithms are developed using the above recommendations, they should be evaluated in clinical practice using various settings and study populations with various degrees of MR.

Final remark

If more and more reliable empirical data become available on diagnostic studies in children with MR, this should allow clinicians to weigh the *benefits* of performing specific investigations like the resolution of diagnostic uncertainty, prevention of further investigations, improved possibilities for genetic counselling, clinical management, and ultimately prevention and treatment, against the *disadvantages*, such as discomfort for the individual subjected to the testing procedures, anxiety for parents awaiting test results, and increase in costs. Studies dedicated to these subjects should be encouraged.

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