Hum Genet (2007) 121:43–48
DOI 10.1007/s00439-006-0292-0

ORIGINAL INVESTIGATION

Homozygosity mapping in consanguineous families reveals extreme heterogeneity of non-syndromic autosomal recessive mental retardation and identifies 8 novel gene loci

Hossein Najmabadi · Mohammad Mahdi Motazacker · Masoud Garshasbi · Kimia Kahrizi · Andreas Tzschach · Wei Chen · Farkhondeh Behjati · Valeh Hadavi · Sahar Esmaeeli Nieh · Seyedeh Sedigheh Abedini · Reza Vazifehmand · Saghar Ghasemi Firouzabadi · Payman Jamali · Masoumeh Falah · Seyed Morteza Seifati · Annette Grüters · Steffen Lenzner · Lars R. Jensen · Franz Rüschendorf · Andreas W. Kuss · H. Hilger Ropers

Received: 15 September 2006 / Accepted: 25 October 2006 / Published online: 21 November 2006

Abstract Autosomal recessive gene defects are arguably the most important, but least studied genetic causes of severe cognitive dysfunction. Homozygosity mapping in 78 consanguineous Iranian families with nonsyndromic autosomal recessive mental retardation (NS-ARMR) has enabled us to determine the chromosomal localization of at least 8 novel gene loci for this condition. Our data suggest that in the Iranian population NS-ARMR is very heterogeneous, and they argue against the existence of frequent gene defects that account for more than a few percent of the cases.

Mohammad Mahdi Motazacker and Masoud Garshasbi have contributed equally to this work.

H. Najmabadi · K. Kahrizi · F. Behjati · S. E. Nieh · S. S. Abedini · R. Vazifehmand · S. G. Firouzabadi · P. Jamali · M. Falah · S. M. Seifati
Genetics Research Centre,
University of Social Welfare and Rehabilitation Sciences,
Tehran, Iran

M. M. Motazacker · M. Garshasbi · A. Tzschach · W. Chen · S. Lenzner · L. R. Jensen · A. W. Kuss · H. H. Ropers (✉)
Department Human Molecular Genetics,
Max Planck Institute for Molecular Genetics,
Ihnestr. 73, 14195 Berlin, Germany
e-mail: ropers@molgen.mpg.de

V. Hadavi
Genetic and Pathology Laboratory, Tehran, Iran

A. Grüters
Department of Paediatric Endocrinology,
Otto Heubner Centre for Paediatrics, Berlin, Germany

F. Rüschendorf
Gene Mapping Centre,
Max Delbrück Centre for Molecular Medicine,
Berlin, Germany

Introduction

With a prevalence of about 2%, mental retardation (MR) is the most common reason for referral to clinical genetic centres and one of the most important unsolved problems in health care. Mild forms of MR (intelligence quotient (IQ) 70–50) are thought to represent the lower end of the normal IQ distribution and to result from the interaction of many genes and non-genetic factors. In contrast, severe forms (IQ < 50, incidence –0.4%) may be due to catastrophic events such as prenatal hypoxia or, much more often, specific genetic factors, including chromosomal aberrations or defects of single genes. Down syndrome (trisomy 21) and other cytogenetically visible chromosomal aberrations are found in approximately 15% of all cases (Leonard and Wen 2002), and recent studies suggest that another 15% may be due to submicroscopic deletions and duplications spanning 100,000 to several million DNA base pairs (de Vries et al. 2005; Kirchhoff et al. 2005; Shaw-Smith et al. 2004; R. Ullmann; M. Kirchhoff et al., unpublished observations). Because in males, MR is significantly more frequent than in females, it had been assumed that defects of X-linked genes account for up to 25% of severe cases, but recent empirical data suggest that the contribution of X-linked defects is much lower, probably in the range of 10% (reviewed by Ropers and Hamel 2005).

So far, very little is known about the role of autosomal genes in MR. The reason for this is that severe autosomal dominant forms of MR (ADMR) manifest nearly always as sporadic cases because affected patients rarely reproduce, and in developed countries, due to small family sizes, most patients with autosomal recessive forms (ARMR) appear as isolated cases, too.

Functional considerations argue for ARMR to be more common than ADMR, and there is reason to believe that most of the patients with 'idiopathic' MR carry autosomal recessive gene defects (Bartley and Hall 1978; Priest et al. 1961; Wright et al. 1959). However, no more than three gene defects causing non-syndromic ARMR have been identified so far, each in a single large consanguineous family or clan (Basel-Vanagaite et al. 2006; Higgins et al. 2004; Molinari et al. 2002), and none seems to be a common cause of ARMR.

This may not be surprising, since about half of the estimated 25,000 human genes are expressed in the brain, and thus, the total number of gene defects causing ARMR could run into the thousands, (see Inlow and Restifo 2004). On the other hand, mutations in a single gene, *FMR1*, account for up to one fourth of all cases with X-linked MR (Fishburn et al. 1983), and in almost half of the patients with non-syndromic autosomal recessive deafness, mutations in the *GJB2* gene have been found (reviewed by Willems 2000).

Three years ago, we have joined forces to elucidate the molecular causes of ARMR in Iran. In this population, almost 40% of all children are born to consanguineous parents, and family sizes are much larger than in Western societies. As our first objective, we have tried to identify, or rule out, common forms of ARMR by performing large-scale homozygosity mapping in consanguineous families.

Patients and methods

Families with a minimum of two mentally retarded children were identified through collaboration with local genetic counsellors in several provinces. A subset of 107 families whose pedigree patterns and clinical data seemed to be compatible with moderate to severe non-syndromic ARMR were selected and visited by experienced clinical geneticists, or invited to the Genetics Research Centre in Tehran. Patients and unaffected relatives were examined in a standardized way using a questionnaire, and photographs were taken to document physical findings. The clinical geneticists assessed the mental status of the probands by monitoring their verbal and motor abilities, by interviewing the parents about developmental milestones and, in a minority of cases, by using more sophisticated tests such as a modified version of the Wechsler Intelligence Tests for children or adults. After obtaining written consent from the parents, blood was taken and DNA was isolated from all mentally retarded individuals, the parents and many of the unaffected sibs, particularly in small families with closely related (first cousin) parents. For one patient of each nuclear family, Fragile X testing was carried out by PCR and Southern blot analysis if X-linkage could not be excluded. Filter-dried blood of one patient per family was screened by tandem mass spectrometry to exclude disorders of the amino acid, fatty acid (e.g. phenylketonuria) or organic acid metabolism (Chace et al. 2003; Wilcken et al. 2003). We also performed standard 450 G-band karyotyping in order to exclude cytogenetically visible chromosomal aberrations.

Genotyping was performed with the Human Mapping 10k Array, Version 2 (Affymetrix, Kennedy et al. 2003). Details of data quality controls and linkage analysis have been published elsewhere (Garshasbi et al. 2006).

Results and discussion

Because of unclear parenthood, sample mix-ups that could not be resolved, or the absence of identifiable regions of homozygosity, 14 out of 107 families had to be excluded from further analysis. Mass spectrometry did not show abnormalities in any of the investigated patients, which is in keeping with earlier reports that in familial MR, errors of metabolism are rare (Opitz 1977). Apart from MR, 15 families showed consistent additional features such as microcephaly, skeletal abnormalities or cataracts. In these syndromic cases, SNP typing and homozygosity mapping revealed several disease specific single autosomal linkage intervals with LOD scores above 3. In one of these families with mild to moderate microcephaly, a deletion was shown to encompass a major portion of the *MCPH1* gene (Garshasbi et al. 2006), which had been implicated previously in primary microcephaly (Jackson et al. 2002). Results from other syndromic families will be reported separately.

In the remaining 78 families with non-syndromic ARMR (NSARMR), we have performed haplotype analysis to identify flanking SNP markers and to determine the size of the relevant linkage intervals. This led to the identification of 4 additional deletions of different size, but at least two of these, a 5.5 kb deletion situated between the *GPC3* and *GPC4* genes on Xq, and a 12.8 kb deletion in intron 2 of the *HTR2A* gene on chromosome 13, respectively, turned out to represent hitherto unknown polymorphisms (Motazacker, Garshasbi et al., unpublished observations).

In 12 of the families with NSARMR (pedigrees and severity of MR are shown in Fig. 1), single autosomal linkage intervals were found (Table 1). Neither of these encompassed previously reported ARMR genes,

nor loci for autosomal recessive primary microcephaly and MR (Woods et al. 2005) (see Fig. 2). 8 of these linkage intervals, with LOD scores above 3 (Morton 1998), represent novel gene loci for NS-ARMR. Therefore, these loci (Table 1 a) were named 'Mental Retardation 4 to 11' (MRT4–11), respectively, in accordance with the nomenclature used for previously mapped NS-ARMR loci (OMIM #249500, #607417, #608443) (Basel-Vanagaite et al. 2006; Higgins et al. 2004; Molinari et al. 2002). In 4 families with single linkage intervals, the LOD scores (between 2 and 3) were too low to formally prove that these sites represent additional MRT loci, but for some of these, this is still likely (Fig. 2 and Table 1). In all other families, whole-genome SNP typing revealed several different linkage peaks, reflecting the limited size of most of these families and/or high degree of inbreeding. In first-cousin marriages with two affected children, the minimum size of the families considered in this study, parametric linkage analysis typically resulted in 5–6 intervals, each with the maximally attainable LOD score of 1.8, but in many of them, the number of peaks could be reduced to about 3 by including all available unaffected sibs or other family members. Intervals were considered as potential sites of the causative mutation if the corresponding LOD scores were less than one unit lower than the highest peak observed in the respective family ('one LOD down method'), and if all affected family members were homozygous carriers of the same SNP haplotype. All families with single linkage intervals and LOD scores above 2 were clinically re-evaluated, in order to confirm the absence of additional features and thus their nonsyndromic status.

To study the genomic distribution of these intervals, and to get a first impression on the number and localization of genetic defects causing ARMR in the Iranian population, we have used a method that was previously employed to show that genetic defects underlying nonsyndromic X-linked MR cluster on proximal Xp (Ropers et al. 2003). After adding up the lengths of all physical genome intervals co-segregating with ARMR in a given family (as defined by the distance between the closest SNP markers flanking the respective haplotypes), their total 'weight' was normalized in accordance with the fact that each family represents one single mutation. Thereafter, the weighted linkage intervals from all families were graphically superposed. The resulting curve reflects the genomic distribution of the mutations causing ARMR in these families (see Fig. 2). The surface under the curve is a parameter for the proportion of gene defects mapping to the relevant genome segment. Single intervals from individual large families appear as bars of different height, but all with identical surfaces under the curve, as exemplified by the interval at the distal end of chromosome 8q. Given the considerable number and length of linkage intervals in small families, it is not surprising that some of these overlap. Still, it appears that their co-localization on some chromosomes is not completely random. For example, there is apparent clustering of linkage intervals on chromosomes 16 and 19, which may indicate that these regions carry genes that are mutated in more than one family. In contrast, none of the previously identified MRT genes map to these mutation hotspots, which suggests that they do not have a major role in NSARMR. The same seems to be true for the published loci for primary microcephaly: mutations in these genes do not seem to contribute significantly to NSARMR, although we and others have shown that they do not necessarily lead to massive head size reduction (Garshasbi et al. 2006; Trimborn et al. 2005). The fact that none of the three previously identified MRT genes nor the 8 (plus 4) linkage intervals defined by this study coincide, strongly argues against the existence of a single frequent genetic cause of ARMR in the Iranian population.

Thus, our findings suggest that ARMR is at least as heterogeneous as NSXLMR, for which 24 different etiologically relevant genes are known to date (Ropers 2006), and that many additional families will have to be investigated before firm statements can be made about the frequency and genomic distribution of ARMR genes.

To shed light on the nature and function of the novel MRT genes that we have localized so far, mutation screening of functional and positional candidate genes is in progress.

According to the ENSEMBL database, the 8 plus 4 newly identified MRT intervals contain 1027 brain-expressed genes with a wide spectrum of different functions. Several of these belong to gene families that have been previously implicated in cognition or mental handicaps, such as *ARHGEF1* in the MRT11 interval on chromosome 19. *ARHGEF1* encodes a Rho guanine nucleotide exchange factor and is closely related to *ARHGEF6* and *ARHGEF9*, two previously described genes for NSXLMR (Harvey et al. 2004; Kutsche et al. 2000). Glutamate receptors are known to play a central role in the brain, and it is therefore of interest that no less than three members of the *GRIK* family (glutamate receptor, inotrophic, kainate) map to novel MRT intervals: *GRIK2* to MRT 6, *GRIK3* to MRT 4 and *GRIK5* to MRT 11 on chromosomes 6, 1 and 19, respectively. However, the number of functionally interesting candidate genes mapping to these intervals is still very large, and the search for the causative mutations is complicated by the fact that they are not necessarily

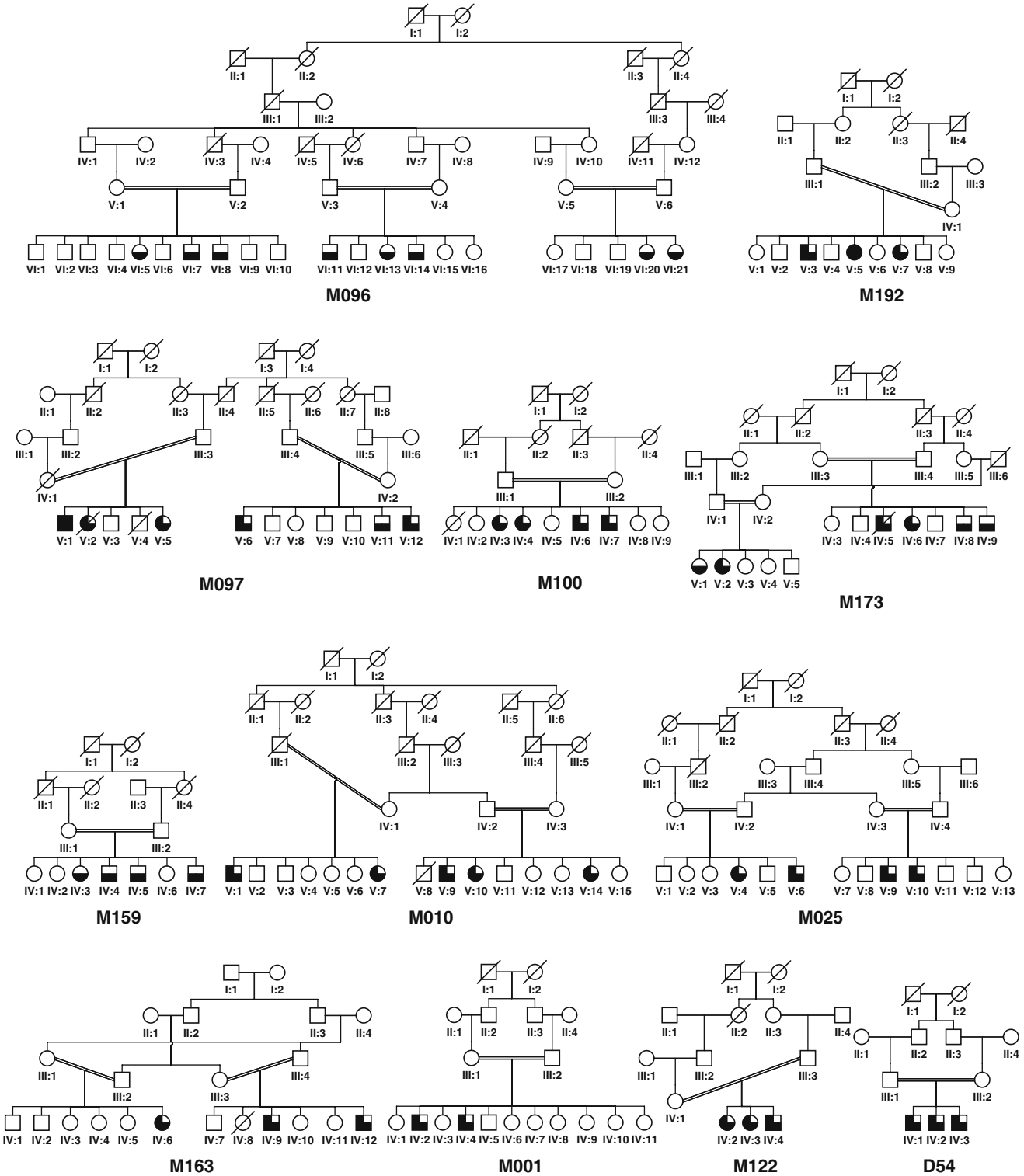

Fig. 1 Pedigrees and degrees of mental retardation for MRT and putative MRT families. The shown pedigrees are simplified but show the complete number of family members in the patient generation at the time of sample collection. Filled symbols indicate severe MR, three-quarter-filled symbols depict moderate MR and half-filled symbols mild MR

Table 1 Genome intervals harbouring novel genes for ARMR Regions of homozygosity in families with single linkage intervals, as defined by the closest flanking recombinant SNP markers

Chromosome	Start SNP (position in bp)[a]		End SNP (position in bp)[a]		Interval [Mbp]	LOD score	Family	MRT No.
a								
1	rs514262	(36955568)	rs953070	(46403641)	9.4	7.2[b]	M096	4
5	rs1824938	(5145028)	rs60701	(10786776)	5.6	3.0	M192	5
6	rs2246786	(95965958)	rs720225	(105949023)	10.0	4.3	M097	6
8	rs1113990	(28758718)	rs1534587	(35262498)	6.5	3.3	M100	7
10	rs1599711	(71041135)	rs942793	(80718164)	9.7	5.0[b]	M173	8
14	rs1998463	(26578858)	rs243286	(32780538)	6.2	3.2	M159	9
16	rs724466	(22705353)	rs3901517	(48948887)	26.25	5.2[b]	M010	10
19	rs2109075	(46844069)	rs8101149	(52292281)	5.4	4.0	M025	11
b								
5	pter	(1)	rs2115289	(5019895)	5.0	2.8	M163	
8	rs968652	(139482128)	qter	(146274826)	6.8	2.0	M001	
11	rs939038	(33932629)	rs644361	(75130040)	41.2	2.7	M122	
20	rs756529	(47444415)	rs728504	(55768572)	8.3	2.4	D54	

[a] Markers flanking regions of homozygosity that are shared by all patients of families with a single linkage interval. Physical positions are based on the May 2004 genome assembly

[b] Result of split-pedigree-analysis (GeneHunter software)

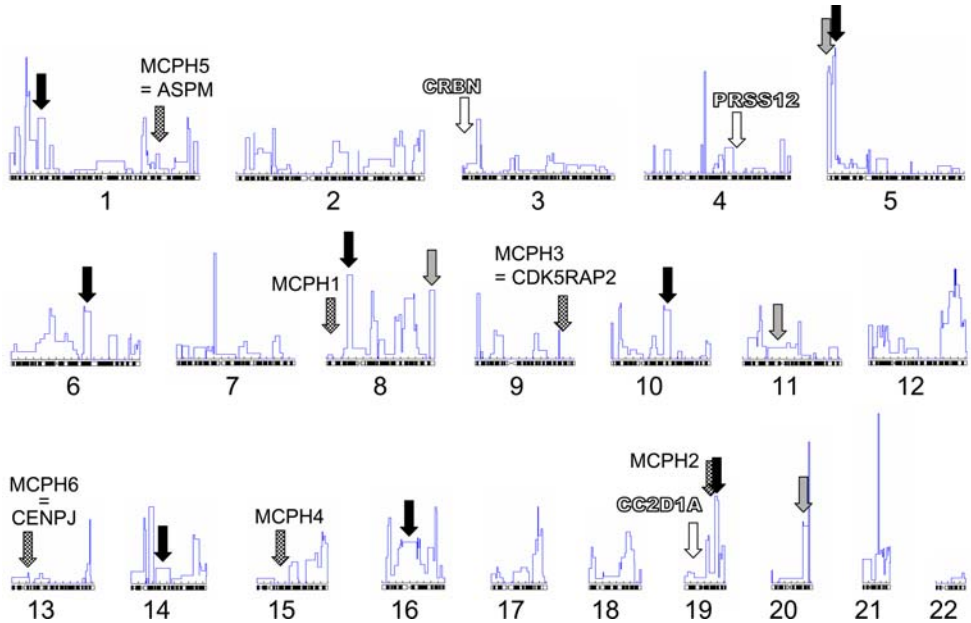

Fig. 2 Distribution of linkage intervals reveals heterogeneity of NSARMR and defines novel MRT loci. The curve results from superposition of weighted linkage intervals from 78 consanguineous families with NS-ARMR, as previously described for families with X-linked MR (Ropers et al. 2003). Large ARMR families with single co-segregating intervals are represented by rectangles of different shape, but identical surface, or 'weight'. *Black arrows*: single intervals with LOD scores >3 (= novel MRT loci), *grey arrows*: single intervals with LOD scores between 2 and 3. In small families with several co-segregating haplotypes, their cumulative length was used to normalize their weight. For this calculation, linkage intervals were disregarded if their LOD scores were more than 1 unit lower than those of the highest LOD score observed in that family. The surface under the curve is a parameter for the proportion of gene defects mapping to the relevant genome segment. Empty arrows: cloned MRT genes, checkered arrows: loci for ARMR with microcephaly

located in coding regions of these genes. Indeed, this seems to apply to some of the smaller intervals, where mutation screening of all coding sequences and exon-intron boundaries is already approaching completion, and where the search may have to be extended to evolutionarily conserved non-coding sequences.

Finally, after having excluded the existence of common genetic causes of ARMR, focusing on particularly

large families with small linkage intervals is another plausible next step to increase the chances of finding the underlying gene defects. These studies are already underway.

Acknowledgments We thank Jürg Ott for his advice concerning the significance levels of LOD scores in whole genome linkage studies, and Hannelore Markert for assisting us with the preparation of the manuscript. We are grateful to all patients, parents and other relatives for their active participation in this study, and to numerous genetic counselors and other health care professionals for their assistance. This study would not have been possible without support from the Deputy of Research, Iranian Ministry of Health and Medical Education (to HN) and a grant of the Max Planck Innovation Funds (to HHR).

References

Bartley JA, Hall BD (1978) Mental retardation and multiple congenital anomalies of unknown etiology: frequency of occurrence in similarly affected sibs of the proband. Birth Defects Orig Artic Ser 14:127–137

Basel-Vanagaite L, Attia R, Yahav M, Ferland RJ, Anteki L, Walsh CA, Olender T, Straussberg R, Magal N, Taub E, Drasinover V, Alkelai A, Bercovich D, Rechavi G, Simon AJ, Shohat M (2006) The CC2D1A, a member of a new gene family with C2 domains, is involved in autosomal recessive non-syndromic mental retardation. J Med Genet 43:203–210

Chace DH, Kalas TA, Naylor EW (2003) Use of tandem mass spectrometry for multianalyte screening of dried blood specimens from newborns. Clin Chem 49:1797–1817

de Vries BB, Pfundt R, Leisink M, Koolen DA, Vissers LE, Janssen IM, Reijmersdal S, Nillesen WM, Huys EH, Leeuw N, Smeets D, Sistermans EA, Feuth T, van Ravenswaaij-Arts CM, van Kessel AG, Schoenmakers EF, Brunner HG, Veltman JA (2005) Diagnostic genome profiling in mental retardation. Am J Hum Genet 77:606–616

Fishburn J, Turner G, Daniel A, Brookwell R (1983) The diagnosis and frequency of X-linked conditions in a cohort of moderately retarded males with affected brothers. Am J Med Genet 14:713–724

Garshasbi M, Motazacker MM, Kahrizi K, Behjati F, Abedini SS, Nieh SE, Firouzabadi SG, Becker C, Ruschendorf F, Nurnberg P, Tzschach A, Vazifehmand R, Erdogan F, Ullmann R, Lenzner S, Kuss AW, Ropers HH, Najmabadi H (2006) SNP array-based homozygosity mapping reveals MCPH1 deletion in family with autosomal recessive mental retardation and mild microcephaly. Hum Genet 118:708–715

Harvey K, Duguid IC, Alldred MJ, Beatty SE, Ward H, Keep NH, Lingenfelter SE, Pearce BR, Lundgren J, Owen MJ, Smart TG, Luscher B, Rees MI, Harvey RJ (2004) The GDP-GTP exchange factor collybistin: an essential determinant of neuronal gephyrin clustering. J Neurosci 24:5816–5826

Higgins JJ, Pucilowska J, Lombardi RQ, Rooney JP (2004) A mutation in a novel ATP-dependent Lon protease gene in a kindred with mild mental retardation. Neurology 63:1927–1931

Inlow JK, Restifo LL (2004) Molecular and comparative genetics of mental retardation. Genetics 166:835–881

Jackson AP, Eastwood H, Bell SM, Adu J, Toomes C, Carr IM, Roberts E, Hampshire DJ, Crow YJ, Mighell AJ, Karbani G, Jafri H, Rashid Y, Mueller RF, Markham AF, Woods CG (2002) Identification of microcephalin, a protein implicated in determining the size of the human brain. Am J Hum Genet 71:136–142

Kennedy GC, Matsuzaki H, Dong S, Liu WM, Huang J, Liu G, Su X, Cao M, Chen W, Zhang J, Liu W, Yang G, Di X, Ryder T, He Z, Surti U, Phillips MS, Boyce-Jacino MT, Fodor SP, Jones KW (2003) Large-scale genotyping of complex DNA. Nat Biotechnol 21:1233–1237

Kirchhoff M, Gerdes T, Brunebjerg S, Bryndorf T (2005) Investigation of patients with mental retardation and dysmorphic features using comparative genomic hybridization and subtelomeric multiplex ligation dependent probe amplification. Am J Med Genet A 139:231–233

Kutsche K, Glauner E, Knauf S, Pomarino A, Schmidt M, Schroder B, Nothwang H, Schuler H, Goecke T, Kersten A, Althaus C, Gal A (2000) Cloning and characterization of the breakpoint regions of a chromosome 11;18 translocation in a patient with hamartoma of the retinal pigment epithelium. Cytogenet Cell Genet 91:141–147

Leonard H, Wen X (2002) The epidemiology of mental retardation: challenges and opportunities in the new millennium. Ment Retard Dev Disabil Res Rev 8:117–134

Molinari F, Rio M, Meskenaite V, Encha-Razavi F, Auge J, Bacq D, Briault S, Vekemans M, Munnich A, Attie-Bitach T, Sonderegger P, Colleaux L (2002) Truncating neurotrypsin mutation in autosomal recessive nonsyndromic mental retardation. Science 298:1779–1781

Morton NE (1998) Significance levels in complex inheritance. Am J Hum Genet 62:690–697

Opitz J (1977) Diagnostic/genetic studies in severe mental retardation. In: Lubs HA FdlC (eds) Genetic Counseling. Raven, New York

Priest JH, Thuline HC, Laveck GD, Jarvis DB (1961) An approach to genetic factors in mental retardation. Studies of families containing at least two siblings admitted to a state institution for the retarded. Am J Ment Defic 66:42–50

Ropers HH (2006) X-linked mental retardation: many genes for a complex disorder. Curr Opin Genet Dev 16:260–269

Ropers HH, Hamel BC (2005) X-linked mental retardation. Nat Rev Genet 6:46–57

Ropers HH, Hoeltzenbein M, Kalscheuer V, Yntema H, Hamel B, Fryns JP, Chelly J, Partington M, Gecz J, Moraine C (2003) Nonsyndromic X-linked mental retardation: where are the missing mutations? Trends Genet 19:316–320

Shaw-Smith C, Redon R, Rickman L, Rio M, Willatt L, Fiegler H, Firth H, Sanlaville D, Winter R, Colleaux L, Bobrow M, Carter NP (2004) Microarray based comparative genomic hybridisation (array-CGH) detects submicroscopic chromosomal deletions and duplications in patients with learning disability/mental retardation and dysmorphic features. J Med Genet 41:241–248

Trimborn M, Richter R, Sternberg N, Gavvovidis I, Schindler D, Jackson AP, Prott EC, Sperling K, Gillessen-Kaesbach G, Neitzel H (2005) The first missense alteration in the MCPH1 gene causes autosomal recessive microcephaly with an extremely mild cellular and clinical phenotype. Hum Mutat 26:496

Wilcken B, Wiley V, Hammond J, Carpenter K (2003) Screening newborns for inborn errors of metabolism by tandem mass spectrometry. N Engl J Med 348(23):2304–2312

Willems PJ (2000) Genetic causes of hearing loss. N Engl J Med 342:1101–1109

Woods CG, Bond J, Enard W (2005) Autosomal recessive primary microcephaly (MCPH): a review of clinical, molecular, and evolutionary findings. Am J Hum Genet 76:717–728

Wright SW, Tarjan G, Eyer L (1959) Investigation of families with two or more mentally defective siblings; clinical observations. AMA J Dis Child 97:445–463