ORIGINAL ARTICLE

Mapping of a new locus for autosomal recessive non-syndromic mental retardation in the chromosomal region 19p13.12-p13.2: further genetic heterogeneity

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Objective: To identify and clinically evaluate four consanguineous families of Israeli Arab origin with non-syndromic mental retardation (NSMR), comprising a total of 10 affected and 24 unaffected individuals. **Participants and methods:** All the families originated from the same small village and had the same family name. Association of the condition in these families with the two known autosomal recessive NSMR loci on chromosomes 3p25-pter and 4q24 (neurotrypsin gene) was excluded. **Results:** Linkage of the disease gene to chromosome 19p13.12-p13.2 ($Z_{\max} = 7.06$ at $\theta = 0.00$) for the marker D19S840 was established. All the affected individuals were found to be homozygous for a common haplotype for the markers cen-RFX1-D19S840-D19S558-D19S221-tel. **Conclusions:** The results suggest that the disease is caused by a single mutation derived from a single ancestral founder in all the families. Recombination events and a common disease bearing haplotype defined a critical region of 2.4 Mb, between the loci D19S547 proximally and D19S1165 distally.

Mental retardation (MR) affects approximately 1–3% of the general population.^{1,2} A defining feature of MR is an intelligence quotient (IQ) of less than 70. The aetiologies of MR are diverse and include chromosomal anomalies, recognisable malformation syndromes, monogenic syndromes, structural brain abnormalities, and environmental factors. Genetic aetiologies are found in approximately two thirds of cases.³ Non-syndromic mental retardation (NSMR) is the diagnosis of exclusion in mentally retarded individuals who do not have major physical abnormalities, dysmorphism, or neurological abnormalities. Our knowledge of the monogenic causes of MR has increased markedly in recent years. Biological processes entailed in signal-transduction pathways involved in neuronal maturation, establishment, stabilisation, and remodelling of connections between neuronal cells are important in the causation of mental retardation.⁴

A number of X linked genes associated with non-specific mental retardation have been identified, such as *FMRI*,⁵ *FMR2*,⁶ *RSK2*,⁷ *PAK3*,⁸ *GDII*,⁹ *ARHGEF6*,¹⁰ *IL1RAPL*,¹¹ *MECP2*,¹² *OPHN1*,¹³ *TM4SF2*,¹⁴ *FACL4*,¹⁵ and *ARX*.¹⁶ An autosomal recessive mode of inheritance may account for nearly a quarter of all individuals with NSMR.^{17,18,19} Searching for the genes responsible for NSMR is difficult owing to heterogeneity and the absence of clinical criteria for grouping the NSMR families for linkage analysis. Currently only one autosomal gene, the neurotrypsin gene on chromosome 4q24, is known to cause autosomal recessive NSMR (MIM #249500).²⁰ Neurotrypsin belongs to the subfamily of trypsin-like serine proteases and is highly expressed in brain structures involved in learning and memory.²⁰ A second locus for NSMR has been found on chromosome 3p25-pter in a large Italian family,²¹ but the gene causing NSMR in this family is still unknown.

Within the past two years, we evaluated clinically four consanguineous families comprising 10 affected and 24 unaffected individuals. We confirmed the presence of further genetic heterogeneity in these families by excluding association of the disease with loci on chromosomes 3p25-pter and

4q24. Linkage of the disease gene to chromosome 19p13.12-13.2 was established.

METHODS

Participants

The research study was reviewed and approved by the Human Subjects Committee of the Rabin Medical Center. We clinically evaluated four consanguineous families (two of them interrelated) comprising a total of 10 affected and 24 unaffected individuals; the evaluation included a comprehensive dysmorphological examination. The exact relationship of the third and fourth families to the other two families was not known, although they bore the same family name and lived in the same village. Family trees were constructed (fig 1).

In addition to the comprehensive dysmorphological examination, some of the subjects (those who agreed to cooperate) also underwent neurological examination and mental status assessment. The neurological examination included a detailed evaluation of the cranial nerves, deep tendon reflexes, and motor and sensory systems. Performance was assessed according to the Leiter International Performance Scale.²²

Microsatellite marker analysis

DNA was isolated from the blood samples by standard methods.²³ Four hundred microsatellite markers, spaced at 10-cM intervals, from ABI PRISM linkage-mapping set version 2.5 (Applied Biosystems; see *Data access* at end of article) were amplified by multiplex polymerase chain reaction (PCR), using standard protocols. Amplified markers were electrophoresed on an ABI 3700 DNA capillary sequencer and were analysed with GENESCAN and GENOTYPER software (Applied Biosystems).

Abbreviations: Abbreviations: MR, mental retardation; NSMR, nonsyndromic mental retardation; IQ, intelligence quotient; PCR, polymerase chain reaction

Figure 1 Haplotypes of 15 markers on chromosome 19p13.12-p13.2 studied in four Israeli Arab sibships with NSMR. The order of markers is depicted. The boxed regions indicate the homozygous regions in each affected individual. The arrows show recombination events.

For the additional microsatellite markers required, primer sequences, and marker order and distances were obtained from the Center for Medical Genetics, Marshfield Medical Research Foundation, Genome Database, UCSC Genome Bioinformatics and Ensembl databases (see *Data access*). Markers were amplified from genomic DNA, according to methods specified by the manufacturers. PCR products were separated on polyacrylamide gels and viewed using silver staining.

Linkage analysis

For the initial screening of linkage to the disease gene, genotyping of 20 members from families 1, 2, and 3 (7 affected and 13 unaffected persons) was carried out. For each marker, we undertook visual comparison of the degree of homozygosity and genotype sharing in affected and unaffected individuals. For missing parental marker data (individual II-3, family 2 and individual I-1, family 3), alleles were inferred from the genotypes of their offspring.

In addition, genotyping of all family members and linkage analysis were performed for each marker that showed increased homozygosity or genotype sharing in the affected individuals. The results of the two point linkage analysis using the program SUPERLINK²⁴ with these markers are given in table 1. We assumed a susceptibility allele with frequency 0.05 and a recessive mode of inheritance with

penetrance 0.99. Marker-allele frequencies were not available in this specific Arab population and equal allele frequencies were assumed. For lod score calculations, the number of alleles was set as the number observed in the pedigree, rather than the number observed elsewhere, in order to provide a conservative estimate of the lod score.

Sequencing of the candidate genes

All exons of the relaxin gene, including exon-intron junctions, were amplified by PCR with primers (Sigma-Aldrich) based on the genomic sequences available from GenBank. Both strands of the PCR products were sequenced with BigDye Terminators (Applied Biosystems) on an ABI 3100 sequencer. Sequence chromatograms were analysed using SeqScape software version 1.1 (Applied Biosystems).

RESULTS

Clinical evaluation

The initial clinical presentation in all 10 affected participants was mild psychomotor developmental delay during early childhood. All affected individuals had no or only single words and were severely mentally retarded, whereas their general physical examination was normal. There were no autistic features or seizures. The general appearance was normal, with no dysmorphic features: height, weight, and head circumference were normal in all except one subject

Table 1 Two point lod scores for chromosome 19p markers

Recombination fraction (θ)											
Locus	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	$\theta_{\max}$	$Z_{\max}$
D19S216	∞	-3.85	-2.75	-2.11	-1.66	-1.33	-1.06	-0.85	-0.67	0.08	-0.67
D19S884	2.74	3.64	3.79	3.83	3.81	3.77	3.72	3.65	3.57	0.03	3.83
D19S865	1.56	2.52	2.72	2.8	2.83	2.83	2.81	2.78	2.74	0.05	2.83
D19S403	2.63	2.59	2.55	2.51	2.46	2.41	2.36	2.31	2.25	0.00	2.63
D19S535	4.43	4.34	4.25	4.15	4.05	3.95	3.85	3.75	3.64	0.00	4.43
D19S394	6.46	6.33	6.2	6.06	5.92	5.77	5.63	5.48	5.33	0.00	6.46
D19S906	4.2	4.11	4.01	3.91	3.81	3.71	3.61	3.5	3.4	0.00	4.2
D19S1165	5.13	5.15	5.12	5.05	4.97	4.88	4.77	4.66	4.54	0.01	5.15
D19S221	6.25	6.11	5.97	5.82	5.68	5.53	5.38	5.22	5.07	0.00	6.25
D19S558	3.68	3.62	3.56	3.49	3.41	3.34	3.26	3.18	3.1	0.00	3.68
D19S840	7.06	6.88	6.7	6.52	6.34	6.16	5.98	5.79	5.61	0.00	7.06
RFX1	3.21	3.13	3.04	2.96	2.88	2.8	2.72	2.64	2.56	0.00	3.21
D19S547	1.49	1.48	1.46	1.44	1.42	1.39	1.36	1.33	1.3	0.00	1.49
D19S226	4.96	4.87	4.77	4.66	4.55	4.44	4.33	4.21	4.09	0.00	4.96
D19S414	∞	-6.08	-4.36	-3.33	-2.62	-2.08	-1.66	-1.32	-1.03	0.08	-1.03

(family 4, II-2) who had progressive microcephaly (OFC = 45 cms (-4 SD) at the age of 2½ years. Each of the parents of this person had a normal head circumference.

On neurological examination, the patients exhibited a dull facial expression. The cranial nerves were intact and eye movements were full. Muscle tone and strength in the upper and lower extremities were normal, with no muscle atrophy and no signs of cerebellar involvement. Gait, fine motor movement of the fingers, and superficial sensory perception were normal, as were deep tendon reflexes and the plantar reflex.

Formal psychological evaluation was carried out on patient II-1 from family 4 at the age of 8 years. During the examination his hyperactive behaviour was notable. His attention span was very short and cooperation partial; he was constantly moving during the examination and refused to obey the commands given during the test. The most prominent feature was his attempt to initiate verbal contact and to try to convey his wishes. It was impossible to understand his speech and consequently he became frustrated. His verbal comprehension was limited to understanding concrete tasks and commands. He was assessed by the Leiter International Performance Scale and was found to function at the 2½ year level—that is, in the severely mentally retarded range.

A number of laboratory tests were carried out on several of the patients. These showed that standard karyotype was normal, and the presence of CGG repeat expansion in the FMR1 gene was excluded. Plasma amino acids and urine organic acids, urine examination for mucopolysaccharides, full blood count, liver function tests, urea, creatinine, thyroid function tests, creatine kinase, pyruvate, lactate, and ammonia were all normal. Complete skeletal x ray was normal. CT scan of the brain of individual II-1 (family 4) was normal, as was the brain MRI of individual II-2 (family 4). Analysis of the family pedigrees (fig 1) was compatible with autosomal recessive inheritance.

Linkage analysis

Initially, linkage analysis to the two currently known loci causing NSMR on chromosomes 3p25-pter and chromosome 4q24 (neurotrophin gene) was performed (data not shown). Both loci were excluded as candidate loci for the gene causing NSMR in the families reported here.

At the initial genome-wide screen, seven affected children from families 1, 2, and 3 were found to be homozygous for the same allele for marker D19S221. On the basis of these results, we also genotyped all the family members for additional microsatellite markers (fig 1). Statistical analysis

provided strong evidence for linkage of NSMR to chromosome 19p13.12-p13.2 with the maximal lod score of 7.06 at $\theta = 0.00$ for the marker D19S840. The results of the two point linkage analysis with these markers are given in table 1.

Several recombination events were observed in the families, narrowing the candidate region to the chromosomal region between D19S414 (centromere) and D19S403 (telomere) (fig 1), spanning 21 cM (23 Mb). Haplotype-sharing studies revealed complete homozygosity in all affected individuals with markers cen-RFX1-D19S840-D19S558-D19S221-tel (fig 1). This finding strongly suggests the existence of linkage disequilibrium and a single founder mutation in all the families. Recombination events and a common disease-bearing haplotype defined a critical region of 2.4 Mb, between the loci D19S547 proximally and D19S1165 distally.

DISCUSSION

In this study we evaluated clinically and genetically four related consanguineous Israeli Arab families with severe NSMR. The affected individuals had no dysmorphic features, neurological symptoms, or epilepsy, and head circumference was normal in all but one patient. The presence of progressive microcephaly in only one of 10 affected individuals carrying the disease haplotype indicates that this patient may have a concomitant unrelated condition. Another more remote possibility is that other genetic/environmental factors are responsible for the variable disease phenotype caused by the same mutation.

We confirmed the presence of genetic heterogeneity in NSMR by excluding both the chromosome 3p25-pter and chromosome 4q24 loci as the cause of the NSMR in our families. We have identified a new gene locus for NSMR in the chromosomal region 19p13.12-p13.2. These data suggest that the extent of genetic heterogeneity in autosomal recessive NSMR may be significant. We conclude, from the data reported here and in other studies,^{20,21} that there are at least three genetic loci that can cause autosomal recessive NSMR. Additional autosomal loci are expected to be involved in the aetiology of NSMR. Because of the increasing number of possible autosomal recessive NSMR loci, we suggest that formal classification of these genes should be introduced. We suggest the nomenclature ARNSMR1 for the NSMR associated with the locus on chromosome 3p25-pter, ARNSMR2 for that associated with the neurotrophin gene, and ARNSMR3 for that associated with the locus on chromosome 19p13.12-p13.2.

Although we did not know the exact consanguineous links between the families, the research data supported linkage to

the same locus in all the families. This is based on the fact that the family name was the same in all the families, the fact that all the marriages were within the same extended family, and the finding of the same founder disease haplotype in all four families.

Recombination events and a common disease bearing haplotype defined a critical region of 2.4 Mb between the loci D19S547 proximally and D19S1165 distally. The minimal interval contains 56 known or hypothetical genes. In searching for the disease gene, the focus will be on those genes that are ubiquitously expressed in the brain. Some of the genes in the candidate region are interesting possibilities for the causative gene for the NSMR in our patients.

The calcium channel, voltage dependent, P/Q type, α 1A subunit (CACNA1A) gene belongs to the family of voltage sensitive Ca^{2+} channels that mediate the entry of calcium ions into excitable cells. These proteins are also involved in a variety of Ca^{2+} dependent processes, including hormone or neurotransmitter release, muscle contraction, and gene expression. The CACNA1A gene is known to cause episodic ataxia-2, familial hemiplegic migraine,²⁵ and spinocerebellar ataxia-6.²⁶ It has also been shown to be associated with idiopathic generalised epilepsy.²⁷

Another potential candidate gene is the catalytic subunit C- α of cAMP-dependent protein kinase (PRKACA). The inactive cAMP-dependent protein kinase is a tetramer composed of two regulatory and two catalytic subunits; cAMP dependent protein kinase A is required for long term potentiation in neonatal tissue.²⁸ It was suggested that developmental changes in synapse morphology, including a shift from dendritic shafts to dendritic spines and compartmentalisation of calcium, might underlie the changes in kinase activity.

The gene relaxin 3 shows homology to relaxins and is highly expressed in the pons/medulla, hippocampus, and olfactory regions in mouse brain.²⁹ We sequenced the whole coding region of this gene and did not find any mutations.

Research is under way to find the gene that causes the disease. Sequence homologies and functional similarities with the currently known non-specific genes that cause mental retardation will actively be sought.

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Data access

Accession numbers and URLs for data presented herein are as follows:

- Genome Database, <http://www.gdb.org/>
- Center for Medical Genetics, Marshfield Medical Research Foundation, <http://research.marshfieldclinic.org/genetics/>
- Ensembl, <http://www.ensembl.org/>
- UCSC Genome Bioinformatics, <http://genome.cse.ucsc.edu/>
- Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/>

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