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.

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.21 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, non-syndromic mental retardation; IQ, intelligence quotient; PCR, polymerase chain reaction

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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 SUPERLINK™ 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.
(family 4, II-2) who had progressive microcephaly (OFC = 45 cm (−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.

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**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, 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 neurotrypsin 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, alpha 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, and spinocerebellar ataxia-6. It has also been shown to be associated with idiopathic generalised epilepsy.

Another potential candidate gene is the catalytic subunit C- alpha 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. 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. 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.marshallclinic.org/genetics/
- UCSC Genome Bioinformatics. http://genome.ucsc.edu/

*The first two authors, L Basel-Vanagaite and A Alkelai, contributed equally to this work.

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REFERENCES