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ARTICLE

MORM syndrome (mental retardation, truncal obesity, retinal dystrophy and micropenis), a new autosomal recessive disorder, links to 9q34

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A consanguineous pedigree is described where 14 individuals are affected with a novel autosomal recessive disorder, which causes static moderate mental retardation, truncal obesity, a congenital nonprogressive retinal dystrophy and micropenis in males. We have tentatively named this condition MORM syndrome. It shows similarities to Bardet–Biedl syndrome and Cohen syndrome, but can be distinguished by clinical features; the age of onset and nonprogressive nature of the visual impairment, the lack of characteristic facies, skin or gingival infection, microcephaly, 'mottled retina', polydactyly and small penis without testicular anomalies. Furthermore, linkage to the known Bardet–Biedl (BBS1–8) and Cohen syndrome loci was excluded. Autozygosity mapping identified a single homozygous subtelomeric region shared by all affecteds on chromosome 9q34.3, with a maximum LOD score of 5.64. We believe this to be the first example of the identification of a subtelomeric recessive locus by autozygosity mapping.
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Introduction

A Northern Pakistani pedigree of nine sib-ships containing 14 affected individuals was ascertained. A simplified family pedigree is shown (Figure 1). The family shows complex consanguinity. The inheritance pattern of the disorder is consistent with autosomal recessive, with both sexes similarly affected and no parent affected. Cytogenetic analysis was normal at 450–850 band quality in two parents and two affected individuals.

The disorder manifests as static moderate mental retardation, truncal obesity, a congenital nonprogressive retinal dystrophy and micropenis in males. This phenotype shows similarities to two autosomal recessive disorders, Bardet–Biedl syndrome and Cohen syndrome. Bardet–Biedl syndrome is characterised primarily by learning difficulties, obesity, retinal dystrophy, genital abnormalities, hypogonadism, polydactyly and renal abnormalities (both structural and functional); also patients with Bardet–Biedl syndrome have been reported to have congenital heart defects, diabetes and hypertension.[1] There is a great deal of phenotypic variation and genetic heterogeneity

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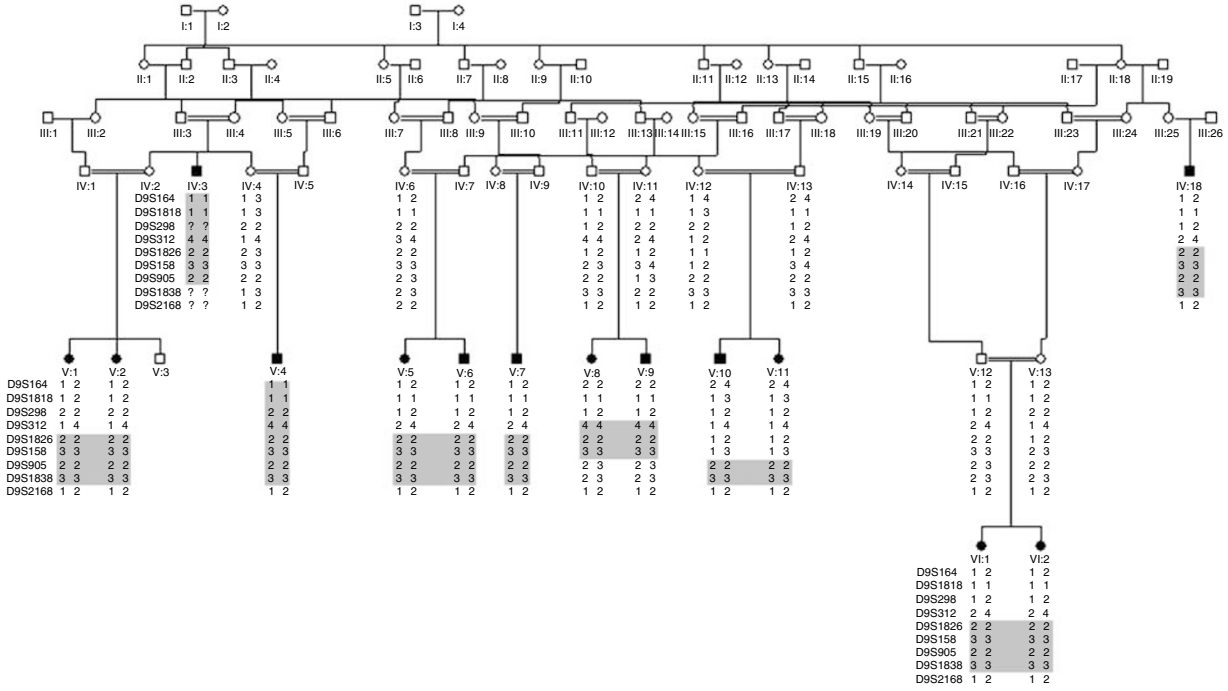

Figure 1 Pedigree of the reported family showing closest family links between parents of affected individuals. The majority of unaffected individuals have been omitted and only the closest consanguineous links between parents are shown. Disease phenotype for individuals with MORM syndrome is shown as filled in shapes. Shaded boxes denote regions of homozygosity in affected individuals subsequent to fine mapping.

within Bardet–Biedl syndrome. To date, eight loci have been identified (BBS1–8) and for five of these the disease causing gene has been isolated.[2–10] Recently, functional studies have shown that the BBS8 gene localises to the base of cilia, with the BBS8 mouse homologue and the BBS1, BBS2, BBS7 and BBS8 *Caenorhabditis elegans* homologues showing a similar pattern of expression.[10] It has therefore been hypothesised that the BBS proteins could have a role in ciliary function.[1,10] There is also recent evidence to suggest that Bardet–Biedl syndrome can undergo triallelic inheritance, where mutations at two different Bardet–Biedl loci genetically interact, and it has been hypothesised that this could account fot the phenotypic variation observed in Bardet–Biedl syndrome.[11] The primary features of Cohen syndrome include mental retardation, retinal dystrophy, hypotonia, microcephaly and characteristic facial features.[12] However, while not a primary feature, obesity has also been reported in some Cohen syndrome patients.[12] There is only one reported locus for Cohen syndrome on chromosome 8,[13] but it is hypothesised that Cohen syndrome may be genetically heterogeneous.[12]

The kindred live in a valley in the foothills of the Salt Range, in the Punjab of Northern Pakistan. Family lore has it that 16 Arabs settled in the valley 1000 years ago, and that since that time descendants have always married within the 'family'. The village of the descendants has moved twice since its origins to other locations within the same valley, most recently to its present location 300 years ago to offer greater protection from the marauding of Sikhs in the area at that time. The village currently numbers about 5000 with five different clans. The pedigree with affected individuals is a subgroup of one of the clans. The condition is only found in this subgroup and all the affected individuals can be traced back to one founding couple. There is a strong tradition of inter-marriage in all the clans and their subgroups.

Materials and methods

Patients

Ethical approval for this project was obtained in both the UK and Pakistan. The clinical details were based on the information gathered through multiple sources: (1) Interviews with the affected individuals and members of their families. (2) Reports from schools, with informed consent from individuals and families. (3) Historical information about the development gathered from more than one resource, to minimise the chances of recall bias. (4) Intellectual ability assessed by MA who is a trained Learning Disability Psychiatrist and has experience of working both in the UK and Pakistan, speaks the local language, and has a good knowledge of the local cultural norms. (5) Physical development assessed by referring to the norms developed for Pakistan and available in the local clinics.

DNA analysis

After obtaining informed consent and Ethical Committee permission, blood samples were collected from 14 affected individuals and eight of their parents from which DNA was extracted using standard methods. To test for linkage to one of the eight BBS or Cohen syndrome loci, polymorphic microsatellite markers were selected using published gene/loci data, the Human Genome Browser and the Genome Database.[2–10,13] Seven of the affected individuals were genotyped with these markers using standard methods: no evidence of linkage was found (data not shown). Further examination of the eight BBS loci revealed no evidence of triallelic inheritance (data not shown).[11]

A genome wide search for regions of shared homozygosity was performed on the family using the Weber Human Screening Set version 8 (Research Genetics), which contains 385 autosomal microsatellite repeat markers spaced at approximately 10 cM intervals with an average heterozygosity of 0.76. PCR amplification of all markers was performed according to the manufacturer's specifications using a Roboseq 4200 thermal cycler (MWG Biotech Ltd). Amplified markers were pooled and electrophoresed on an ABI Prism 377 gene sequencer (Applied Biosystems) using 4.2% polyacrylamide gels at 3000 V and 51 C for 2.5 h. Fragment length analysis was conducted using Genescan 3.1.2 and Genotyper 2.5 analysis packages (Applied Biosystems).

Fine mapping to reduce the candidate region was conducted by selection of known microsatellite repeats from the ABI Linkage Mapping Panel version I (Applied Biosystems), the Todd Panel[14] and the Marshfield Linkage Maps. Further refinement was achieved by analysing novel microsatellites and single-nucleotide polymorphisms (SNPs). Novel microsatellites were designed using the Human Genome Browser and Primer3 program.[15] Designed microsatellite markers were given a designation (human BAC accession number) (microsatellite repeat unit) (number of repeat units in the reference BAC), for example AL449425TA15. SNPs were identified using the Human Genome Browser and sequenced using an ABI3730xl 96 capillary DNA Analyser (Applied Biosystems) with the ABI BigDye Terminator v3.1 (Applied Biosytems), to the manufacturer's specifications. Sequence was analysed using SeqMan II v5.06 software (DNASTAR Inc.) and BLAST 2.[16]

Results

Patients

The pregnancy and birth of affected individuals was normal, there was no hypotonia and polydactyly or other congenital anomalies were not observed. Poor night vision was evident within the first year of life. By 3 years of age reduced visual acuity was apparent; however, thereafter no further visual loss occurred. Photophobia and nystagmus did not occur. Individuals were able to travel around the village using surroundings and familiarity, and were able to see large shapes and colour. Ophthalmic electrophysiological examination was not possible due to the remoteness of the family's village. The occurrence of cataracts in most affected individuals in the second or third decade of life lead to a further reduction in visual acuity. Examination showed a mildly atrophic retina with thinned blood vessels but without increased pigmentation or optic atrophy. No affected individual was hypersensitive nor had diabetes. All had delayed language acquisition and by 4 years static mild to moderate mental retardation was apparent. Motor milestones were normal and hypotonia and spasticity were not observed. By 5 years minor truncal obesity was noticeable in affected children, which became more marked in the second decade but neither acanthosis nigricans nor diabetes developed. Males did progress through puberty with appropriate growth gain, development of male hair pattern and testicular growth, but the penis remained at the prepubescent size. Affected males had not been treated with testosterone; therefore, it was unclear whether there was an end organ resistance to testosterone. Affected individuals enjoyed good health and had normal growth parameters; however, none were known to have born or fathered offspring (Figure 2). Three reputed affected individuals had died of natural causes (infection, accident and heart attack) in the preceding decade at 16, 25 and 40 years, respectively.

It was difficult to administer any formal IQ test because the individuals had visual impairment and also there was a limited choice of tests with normative data for the population. The assessment took into consideration the adaptive functioning, the impairment caused by visual impairment and socio-cultural issues. The criteria specified in The Diagnostic and Statistical Manual of Mental Disorders (DSM IV)[17] was used as a guide for specifying the level of mental retardation. Based on the experience of mental retardation as it presents in that population, these individuals had mild to moderate mental retardation.

DNA analysis

A region of potential homozygosity was identified in all affected individuals on chromosome 9q34.2–34.3 between markers D9S164 and D9S2168. Further refinement suggested a common overlap at band q34.3 between markers D9S158 and D9S905 (Figure 1). Information regarding marker order was derived from analysis of the May 2004 freeze (HG17 assembly) of the Human Genome Browser.

Multipoint analysis provided further indication of linkage to this region. The entire family was analysed, so the multipoint calculations took into account the marker inheritance and genotypes for all the affected individuals. A fully penetrant autosomal recessive mode of inheritance and a disease gene frequency of 0.0001 were assumed. Due to the complexity of the family structure equal allele

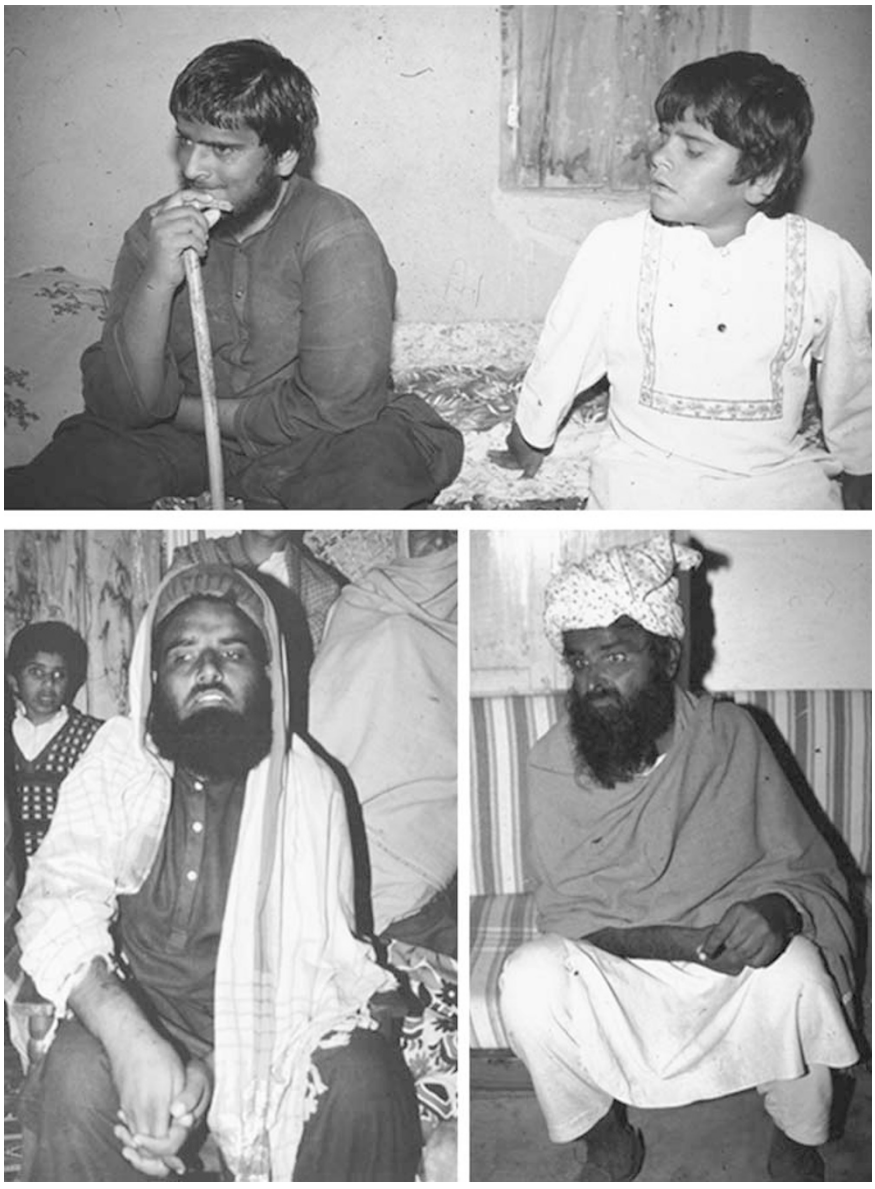

Figure 2 The figure shows three of the members of the pedigree affected with MORM syndrome, with mental retardation and reduced visual acuity. All are obese, especially in the context of other people within the village and family. The photographs were taken with informed consent from individuals and families.

frequencies were assumed for each marker when calculating LOD scores. Pedigree allele inconsistencies were identified using PedCheck.[18] Multipoint analysis was performed using the GENEHUNTER v2.1 program[19] for markers in the region, with the highest LOD score of 5.64 between markers D9S158 and D9S905 (Figure 3). Despite the occurrence of recombination events at both markers D9S158 and D9S905 (ie in affecteds V:8-V:11; Table 1), a positive LOD score could still be obtained due to the multipoint data available for the other affected individuals.

Haplotype inspection after analysis of novel microsatellites and SNPs within this critical region also confirmed linkage and provided further refinement. The minimum critical region as defined by meiotic crossover events is bounded by rs3812547 at 136.5 Mb and rs2811742 at 137.0 Mb, a genetic distance of approximately 1 cM (Table 1).

Discussion

We have studied 14 individuals from a single consanguineous pedigree. The number and age range of affected individuals within the family allow delineation of the clinical features and natural history of a new disorder,

which we have termed MORM syndrome. The clinical diagnoses of mental retardation were based on assessment of adaptive functioning as it was not possible to conduct a formal IQ test. However, the assessment was conducted by a clinician experienced in the field of mental retardation that had a sound knowledge and understanding of the socio-cultural issues of the local population and was fluent in the local language. While it is possible that the learning difficulties that presented in the patients could have been due solely to their visual impairment, the assessment of their IQ did take this possibility into account. The name is derived from the principle findings: mental retardation, truncal obesity, retinal dystrophy and micropenis.

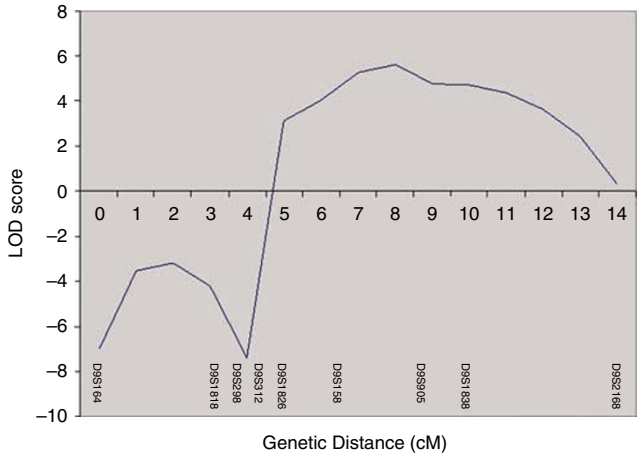

Figure 3 Multipoint analysis of the MORM family across the 9q34.2–34.3 region showing LOD score results for markers D9S164, D9S1818, D9S298, D9S312, D9S1826, D9S158, D9S905, D9S1838, and D9S2168.

MORM syndrome appears to be phenotypically and genetically distinct from the Bardet–Biedl and Cohen syndromes. There are similarities to Cohen syndrome, but the lack of characteristic facies, skin or gingival infection, microcephaly and 'mottled retina' in the family allow clinical differentiation.[12] Bardet–Biedl syndrome is primarily characterised by a rod-cone dystrophy with visual problem onset at a mean age of 8.5 y (100% over 9 y), postaxial polydactyly (69%), central obesity (72% of post pubertal), mental retardation (62%), hypogonadism (97% of males), and renal dysfunction; the figures in brackets being from a British clinical survey of cases.[20] While the family could be diagnosable as Bardet–Biedl syndrome by use of Beales' modified diagnostic criteria (rod-cone dystrophy, learning difficulties, obesity and male hypogonadism), the age of onset and nonprogressive nature of the visual impairment, lack of polydactyly and small penis without testicular anomalies are atypical.[20] In Bardet–Biedl type BBS5, reported only in Newfoundland to date, a small penis is reported with no polydactyly; however, we have no evidence of linkage to this or to the other known BBS loci.[21] Conversely, there is no evidence of

Table 1 Genotypes of individuals with MORM syndrome for markers within 9q34

	Genotype of affected individual												
Marker	*IV:18*	*V:1*	*V:2*	*V:4*	*V:5*	*V:6*	*V:8*	*V:9*	*V:10*	*V:11*	*VI:1*	*VI:2*	*Marker location (Mb)*
D9S158	3–3	3–3	3–3	3–3	3–3	3–3	3–3	3–3	**1–3**	**1–3**	3–3	3–3	136.3
rs12684650	1–1	1–1	1–1	1–1	1–1	1–1	1–1	1–1	1–1	1–1	1–1	1–1	136.3
rs3812547	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	**1–2**	**1–2**	2–2	2–2	136.5
AL592301GCT9	1–1	1–1	1–1	1–1	1–1	1–1	1–1	1–1	1–1	1–1	1–1	1–1	136.6
rs5901103	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	136.6
rs2229974	2–2	2–2	2–2	2–2	2–2	2–2	—	2–2	2–2	2–2	2–2	2–2	136.7
rs11574908	2–2	2–2	2–2	2–2	2–2	2–2	—	2–2	2–2	2–2	2–2	2–2	136.7
rs10521	2–2	2–2	2–2	2–2	2–2	2–2	—	2–2	2–2	2–2	2–2	2–2	136.7
rs3125000	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	136.7
rs3124598	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	136.7
rs3125004	2–2	2–2	2–2	2–2	2–2	2–2	–	–	2–2	2–2	2–2	–	136.7
rs3124603	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	136.7
rs3125006	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	136.7
rs9411208	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	136.7
rs4489420	2–2	2–2	2–2	2–2	2–2	2–2	–	2–2	2–2	2–2	2–2	2–2	136.7
rs2811748	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	137.0
rs2811744	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	137.0
rs2811742	2–2	2–2	2–2	2–2	2–2	**1–2**	—	2–2	2–2	2–2	2–2	2–2	137.0
rs4448378	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	137.1
AL449425TA15	2–2	2–2	2–2	2–2	2–2	2–2	2–2	2–2	**2–3**	**2–3**	2–2	2–2	137.1
D9S905	2–2	2–2	2–2	2–2	2–2	2–2	**2–3**	**2–3**	2–2	2–2	2–2	2–2	137.2

Novel microsatellite markers and SNPs used in the study to confirm linkage to chromosome 9q34.3. The markers are listed centromere to qter in descending order. The affected individuals are referenced to Figure 1, showing the family pedigree (affected individuals IV:3 and V:7 were not typed due to a lack of DNA). The genotype is given for each affected person for each marker. The meiotic crossovers in each affected are shown in bold/underlined. A dash signifies no result. The final column shows the physical location of each marker as derived from the Human Genome Browser.

any Bardet–Biedl syndrome or Cohen syndrome families linked to 9q34.

We have shown linkage to this condition and a ~1 cM region of 9q34, with a maximum LOD score of 5.64. The minimum critical region contains a number of potential candidate genes that may cause MORM syndrome. These include the cell surface neurogenic receptor NOTCH1, the gene KIAA0310 (which is highly expressed in brain and testis) and the G-protein modulator GPSM1. This latter gene is a strong candidate for causing MORM syndrome as recent evidence suggests its involvement in cell fate decisions and neurogenesis.[22] Another point of interest is that one of the protein variants of GPSM1 contains several tetratricopeptide repeat (TPR) domains, which are also to be found in the BBS4 and BBS8 proteins.[5,10] The region also contains several members of the lipocalin family and there is evidence that at least one of these could have a role in male fertility.[23]

We experienced difficulties in identifying this locus. This was due to both the eventual small size of the critical region (which was to be expected given the number of affected individuals in the family) and the 9q34 subtelomeric location (with an increased ratio of genetic to physical distance and a paucity of informative polymorphic markers). Probably, for the latter reasons, we believe this to be the first recessive subtelomeric locus identified by autozygosity mapping. MORM syndrome is a further condition exhibiting obesity/mental retardation with which the clinician has to struggle!

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Competing interests: *The authors declare that they have no competing financial interests.*

Electronic-Database Information: *Online Mendelian Inheritance of Man (OMIM) for clinical and genetic information on Bardet–Biedl syndrome (209900) and Cohen syndrome (216550):*
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
Genome Database (GDB) for polymorphic microsatellite data:
http://gdbwww.gdb.org/gdb/
Human Genome Browser for loci, gene, polymorphic marker and SNP physical location data for the human genome:
http://genome.cse.ucsc.edu/cgi-bin/hgGateway?org=human
Center for Medical Genetics for the Marshfield Linkage Maps:
http://research.marshfieldclinic.org/genetics/Map_Markers/maps/IndexMapFrames.html
Primer3 for design of novel microsatellite repeat markers:
http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi BLAST 2 for SNP sequence analysis:
http://www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html

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