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A Gene for Meckel Syndrome Maps to Chromosome 11q13

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Summary

Meckel syndrome (MKS) is a rare autosomal recessive lethal condition of unknown origin, characterized by (i) an occipital meningo-encephalocele with (ii) enlarged kidneys, with multicystic dysplasia and fibrotic changes in the portal area of the liver and with ductal proliferation, and (iii) postaxial polydactyly. A gene responsible for MKS in Finland has been mapped to chromosome 17q21-q24. Studying a subset of Middle Eastern and northern African MKS families, we have recently excluded the chromosome 17 region and have suggested a genetic heterogeneity. In the present study, we report on the mapping of a second MKS locus (MKS2) to chromosome 11q13, by homozygosity mapping in seven families that do not show linkage to chromosome 17q21-q24 (maximum LOD score 4.41 at recombination fraction .01). Most interestingly, the affected fetuses of southern Tunisian ancestry shared a particular haplotype at loci D11S911 and D11S906, suggesting that a founder effect is involved. Our observation gives support to the clinical and genetic heterogeneity of MKS.

Introduction

Meckel syndrome (MKS [MIM 249000]; Meckel 1822) is a rare autosomal recessive lethal condition of unknown origin, characterized by (*i*) an occipital meningo-encephalocele with both (*ii*) enlarged kidneys, with multicystic dysplasia and fibrotic changes of the liver in the portal area and with ductal proliferation, and (*iii*) postaxial polydactyly (Salonen 1984; Blankenberg et al. 1987). Intra- and interfamilial clinical variability have long been recognized in this syndrome (Fraser and Lytwyn 1981; Plauchu et al. 1981; Seller 1981; Moerman et al. 1993; Wright et al. 1994). Other malformations frequently include microphthalmia, cleft lip and palate, bowing of long bones, situs inversus, heart defects, and genital anomalies.

MKS has a reported incidence of 1/13,250–1/140,000 births. In Finland, its prevalence averages 1/9,000 births (Salonen and Norio 1984). Recently, a gene responsible for MKS in Finland has been mapped to chromosome 17q21-q24, with no evidence of either locus heterogeneity or linkage disequilibrium in this population (Paavola et al. 1995, 1996*a*, 1996*b*). MKS is also relatively frequent in Maghreb and in the Middle East (Crawford et al. 1978; Zlotogora 1997).

Studying a subset of Middle Eastern and northern African MKS families, we have recently excluded the disease gene from close vicinity to the Finnish MKS gene (Roume et al. 1997). In the present report, we show that homozygosity mapping in affected fetuses harboring severe cerebral anomalies results in the mapping of an MKS gene to chromosome 11q13 in seven inbred families.

Patients and Methods

Patients

Criteria for inclusion in the study were (1) cystic dysplasia of the kidneys, (2) fibrotic changes of the liver, (3) a distinctive malformation triad of the CNS, and (4) normal blood or cultured skin fibroblast karyotype. The CNS malformation triad included (*i*) prosencephalic dysgenesis (arhinencephaly-holoprosencephaly and related midline anomalies), (*ii*) occipital exencephalocele, with extrusion of the diencephalic-rhombencephalic dilated roof through the posterior fontanelle, and (*iii*) rhombic roof dysgenesis with absent brain tectum and agenesis-dysgenesis of the cerebellar vermis, similar that observed in the Dandy-Walker anomaly (Hori et al. 1980; Aleksic et al. 1984; Ahdab-Barmada and Claasen 1990; also see table 1).

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Table 1

Neuropathological Features of Ahdab-Barmada and Claasen (1990) Malformation Triad in Eight MKS Fetuses

CNS Anomaly	Status (Term) in[a]							
	Family 2		Family 3:	Family 4:	Family 5:	Family 6:	Family 7:	Family 9:
	Case V-3 (21 wk)	Case V-4 (14 wk)	Case III-3 (19 wk)	Case IV-9 (17 wk)	Case V-3 (24 wk)	Case V-1 (23 wk)	Case III-6 (21 wk)	Case IV-1 (25 wk)
Prosencephalic abnormalities:	+	+	+	+	+	+	+	+
Anencephaly	−	+	−	−	−	−	−	−
Prosencephalic dysgenesis	+	0	+	+	+	+	+	+
Absence of olfactory bulbs	+	0	−	0	+	+	+	ND
Fused thalami /hypothalamic hamartoma	+	0	+	0	+		ND	ND
Agenesis corpus callosum	+	0	−	0	+	+	+	ND
Hypoplasia third ventricle	+	0	+	+	+	+	ND	ND
Small optic nerves, microphthalmia	+	ND	ND	+	ND	+	−	ND
Microcephaly	+	0	+	+	+	+	0	+
Occipital exencephalocele	+	+	+	+	+	+	+	+
Rhombic roof abnormalities:	+	0	+	+	+	+	+	+
Absent brain tectum	+	0	+	+	ND	−	+	+
Agenesis/dysgenesis of cerebellar vermis	+	0	+	ND	ND	+	+	+
Elongated brain stem	+	0	+	ND	ND	+	+	+
Other anomalies:	+	0	+	0	+	+	+	+
Aqueductal stenosis/dysgenesis	0	0	+	ND	+	+	+	ND
Migration anomalies	+	0	+	0	+	+	+	+
Absence/hypoplasia of pyramids	ND	0	+	0	ND	+	+	+
Medullar and/or bulbar dysgenesis	+	0	+	0	+	+	ND	+

[a] + = Affected, − = nonaffected, 0 = not conclusive, and ND = not determined.

A total of seven consanguineous families of northern African and Middle Eastern ancestry were included in the study: four families (families 2, 5, 6, and 9) were from Tunisia, one (family 7) was from Algeria, one (family 4) was from Senegal, and one (family 3) was from Pakistan (fig. 1). Pregnancies were terminated during the second trimester, after ultrasound diagnosis of MKS. Ultrasonographic data, karyotype, autopsies, photographs, and histological specimens were obtained, and a detailed postmortem examination with thorough neuropathological evaluation was performed (table 1; also see Encha-Razavi 1997; Roume et al. 1997). These studies were completed with the approval of the institutional board: Comité Consultatif pour la Protection des Personnes of Necker Enfants Malades Hospital.

DNA Preparation and Genotyping

DNA was extracted, from either frozen tissues or cultured skin fibroblasts of affected fetuses and from lymphocyte pellets of their relatives, by SDS lysis, proteinase K digestion, phenol/chloroform extraction, ethanol precipitation, and Tris-EDTA resuspension. For genotyping, microsatellite DNA markers were amplified by 0.5 U of *Taq* polymerase (Life Technologies) in a buffer containing 1.5 mM $MgCl_2$, 20 μM of each deoxynucleotide, 1 μM of each primer, and 200 ng of genomic DNA, in a final volume of 20 μl. After an initial denaturation step at 94°C for 10 min, PCR was conducted for 30 cycles, each with a denaturation step at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s. The reaction was completed with an elongation step at 72°C for 10 min. Amplified products were separated on 6% polyacrylamide gels run under denaturing conditions and were transferred onto charged nylon membranes (Hybond N+; Amersham). Membranes were hybridized overnight at 42°C with poly-AC probes, were labeled by chemiluminescence (ECL [a direct nucleic-acid labeling and detection system]; Amersham Life Science), and were exposed to x-ray films for 10 min.

Statistical Analyses

Because all MKS families in the present study were consanguineous, we performed a genomewide scan based on the homozygosity-mapping method (Lander and Botstein 1987). When affected fetuses were found to be homozygous, the corresponding region was further studied by first typing the parents and relatives for flanking markers and then computing the pairwise and multipoint LOD score (Z) values. Genetic distances between markers were derived from the Généthon database (Dib et al. 1996). Z values were computed by the program GENEHUNTER (Kruglyak et al. 1996), under the assumption of a fully penetrant autosomal recessive disease gene (frequency .001). Since the number of alleles and the allele frequencies were unknown in the various ancestries, we recoded the marker alleles, allowing for only four equifrequent alleles in each family. Linkage

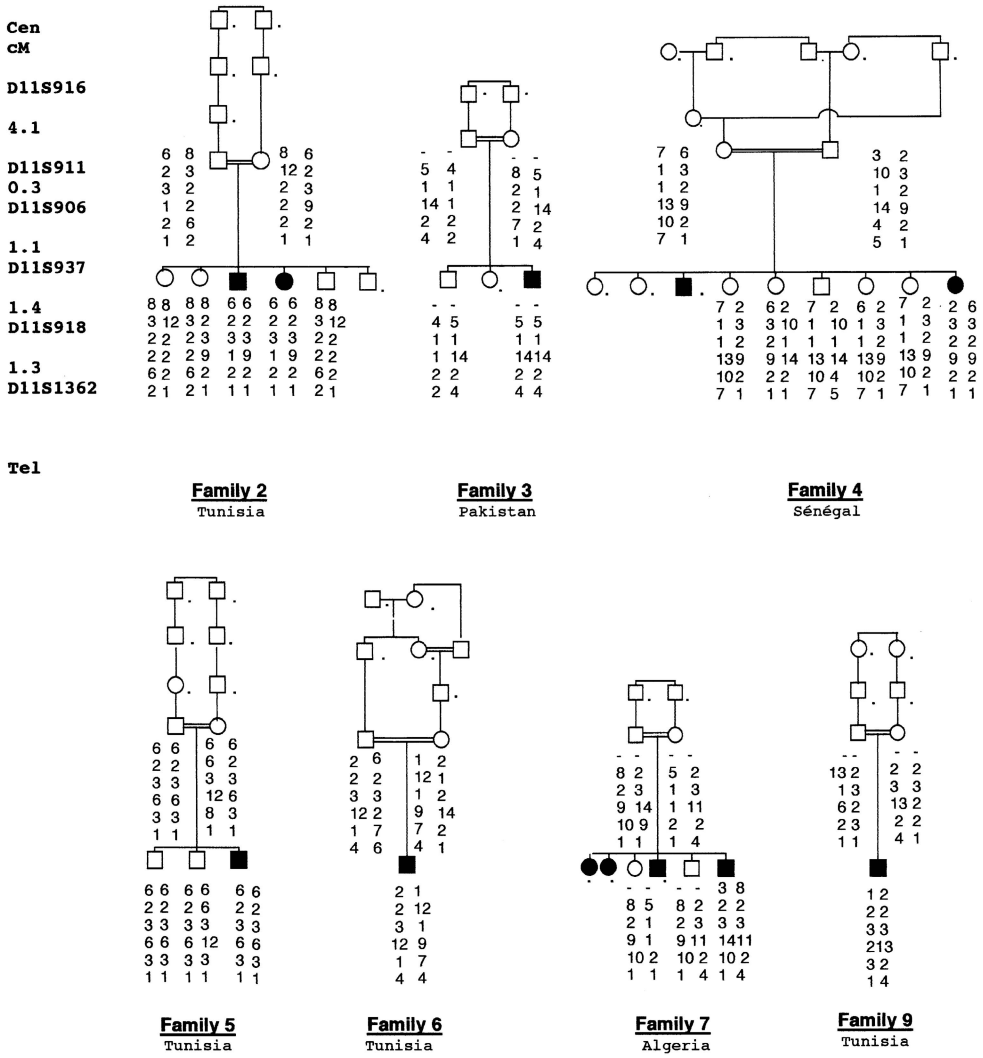

Figure 1 Pedigrees and haplotype analyses in seven nuclear inbred families with the distinctive Ahdab-Barmada and Claasen (1990) malformation triad of the CNS.

heterogeneity was tested by the admixture test (Smith 1963).

Results

The genomic DNA of eight MKS fetuses belonging to seven inbred families were studied by means of 250 microsatellite DNA markers spanning the whole genome at a genetic distance of ~10 cM (Dib et al. 1996). Among them, marker AFM 155xh10 at locus D11S911 on chromosome 11q13 revealed homozygosity in seven of the eight MKS fetuses. Subsequently, pairwise linkage between the disease gene and microsatellite DNA markers of chromosome 11q13 was tested in the seven MKS families. Genetic distances (in parentheses) in the following sequence of markers markers have been established elsewhere: cen–D11S916 (4.1 cM) D11S911 (0.3 cM) D11S906 (1.1 cM) D11S 937 (1.4 cM) D11S918 (1.3 cM) D11S1362–tel (Dib et al. 1996).

The highest pairwise Z (Z_{max}) values were obtained for markers AFM 155xh10 and AFM 107xc7, at, respectively, the D11S911 locus (Z_{max} = 4.41 at recombination fraction [θ] .01) and the D11S906 locus Z_{max} = 4.13 at θ = .01; see table 2). Multipoint linkage analysis showed that the maximum location score of the disease gene was at locus D11S911 (location score 4.03; see fig. 2).

Haplotype reconstruction revealed that all MKS fetuses but one (i.e., V-1 in family 6) were homozygotes at loci D11S906 and D11S911. Interestingly, the affected fetuses of northern African origin (families 2, 5, 7 and 9) shared the same haplotype at loci D11S911 and

Table 2

Pairwise *Z* Values between MKS and Six Microsatellite DNA Markers of Chromosome 11q

	Z AT θ =[a]					
LOCUS AND FAMILY	.00	.01	.05	.10	.20	.30
D11S916 (AFM 185ya1):						
2	1.53	1.49	1.33	1.13	.75	.41
3	.72	.70	.60	.48	.27	.13
4	−∞	−2.26	−.98	−.46	−.06	.06
5	.51	.49	.40	.29	.14	.06
6	−2.69	−.96	−.33	−.10	.05	.07
7	.00	.00	.00	.00	.00	.00
9	−1.26	−.89	−.43	−.23	−.08	−.03
Total	−∞	−1.53	.60	1.12	1.08	.70
D11S911 (AFM 155xh10):						
2	1.54	1.50	1.32	1.11	.70	.36
3	.72	.70	.60	.48	.27	.13
4	1.43	1.38	1.21	1.00	.61	.31
5	.47	.45	.35	.24	.10	.03
6	−2.71	−.98	−.34	−.12	.04	.06
7	.85	.82	.72	.59	.37	.19
9	.56	.53	.44	.34	.18	.08
Total	2.87	4.41	4.30	3.65	2.27	1.17
D11S906 (AFM 107xc7):						
2	1.53	1.49	1.31	1.10	.69	.35
3	.33	.31	.26	.21	.12	.06
4	1.51	1.47	1.29	1.08	.69	.36
5	.51	.49	.40	.29	.14	.06
6	−2.71	−.98	−.34	−.12	.04	.06
7	.85	.82	.72	.59	.37	.19
9	.56	.53	.44	.34	.18	.08
Total	2.58	4.13	4.08	3.50	2.23	1.16
D11S937 (AFM 256zb5):						
2	.02	.19	.44	.50	.41	.23
3	.72	.70	.60	.48	.27	.13
4	1.43	1.38	1.21	1.00	.61	.31
5	.47	.45	.35	.24	.10	.03
6	−2.69	−.96	−.33	−.10	.05	.07
7	−1.65	−.95	−.39	−.18	−.04	−.00
9	−1.28	−.84	−.36	−.17	−.05	−.01
Total	−2.98	−.04	1.51	1.77	1.35	.76
D11S918 (AFM 203vg1):						
2	1.23	1.19	1.04	.87	.55	.28
3	.33	.31	.26	.21	.12	.06
4	−∞	−.31	.24	.35	.31	.20
5	.17	.15	.09	.03	.01	−.01
6	−3.21	−.94	−.31	−.09	.05	.07
7	−2.25	−1.52	−.86	−.54	−.23	−.09
9	−1.28	−.86	−.38	−.18	−.05	−.01
Total	−∞	−1.97	.09	.64	.64	.49
D11S1362 (AFM 132xh9):						
2	1.23	1.19	1.04	.87	.55	.28
3	.72	.70	.60	.48	.27	.13
4	−∞	−.31	.24	.35	.31	.20
5	.51	.49	.40	.29	.14	.06
6	.75	.73	.64	.53	.34	.18
7	−2.02	−1.19	−.59	−.35	−.15	−.08
9	−1.28	−.86	−.38	−.18	−.05	−.01
Total	− ∞	.75	1.95	1.99	1.47	.76

[a] Because of rounding error, the sums of the entries in some columns do not exactly match the totals shown.

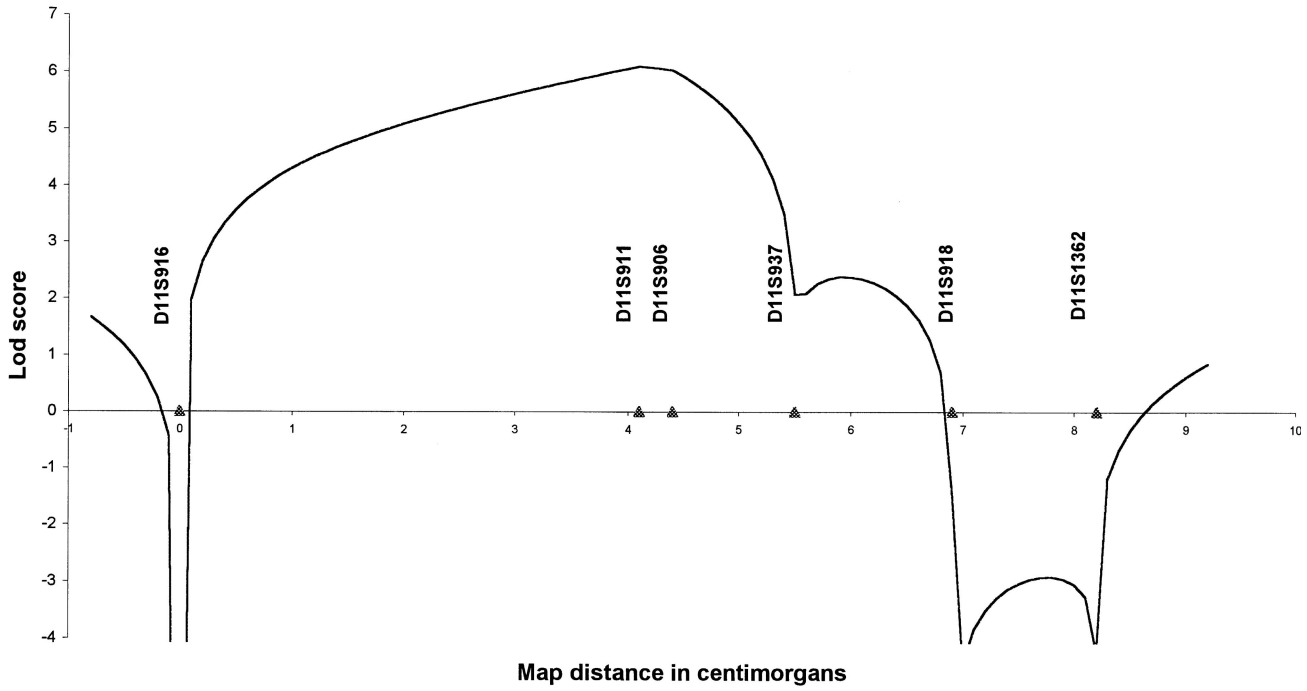

Figure 2 Multipoint-*Z* analysis of MKS2. Family 6 has been excluded.

D11S906, and the two families originating from Tataouine in southern Tunisia (families 2 and 5) shared a larger common region, encompassing loci D11S916, D11S911, and D11S906 (fig. 1).

A heterogeneity test was performed at each locus (increment distance 0.1 cM) in the interval defined by loci D11S916 and D11S1362 and was found to be significantly positive ($P = .0135$, with the test's one-sided nature being taken into account; Ott 1985, p. 116). The estimated proportion of families with linkage was .76. Rejection of homogeneity was mainly contributed by family 6, since, in this family, $Z < -2$ for the whole set of markers, which excludes linkage to the region. In this family, the affected fetus was not homozygous for any of the markers of chromosome 11q13. Exclusion of family 6 maximized the location score at locus D11S911 ($Z_{max} = 6.09$; see fig. 2).

Discussion

Here we have reported the mapping of a second MKS locus, MKS2, to chromosome 11q13, by homozygosity mapping in consanguineous families of northern African and Middle Eastern origin—families that do not show linkage to chromosome 17q21-q24 and whose affected fetuses share a common malformation of the CNS. Most interestingly, the affected fetuses of northern African ancestry shared a particular haplotype at loci D11S911 and D11S906, suggesting that a founder effect is involved. Since, in a previous investigation (Paavola et al. 1995), MKS1 had been mapped to chromosome 17, our observation in the present study gives support to the clinical and genetic heterogeneity of MKS (Roume et al. 1997). In addition, exclusion of chromosomes 11q13 and 17q21-q24 in family 6 suggests the existence of at least one other disease-causing gene in MKS. Studying families of Middle Eastern and European ancestry, Paavola et al. (1997) have also provided evidence of genetic heterogeneity of MKS. However, in that study, one case survived (which is unusual in MKS), two fetuses were not autopsied, and, apart from occipital encephalocele, no information regarding brain malformations was available on the other three affected fetuses.

The clinical delineation of MKS has long been confusing, and many authors have previously called attention to the number of ambiguous and overlapping cerebro-acro-visceral syndromes (Mecke and Passarge 1971; Seller 1981; Hunter et al. 1991; Lurie et al. 1991; Walpole et al. 1991; Verloes et al. 1991; Genuardi et al. 1993). Thus, it is possible that quantitative phenotypic differences in either cerebral changes or other clinical features could account for the genetic heterogeneity of MKS (Paavola et al. 1997; Roume et al. 1997). The mapping of a new disease locus, MKS2, to chromosome 11q13 in a subset of fetuses harboring the severe cerebral phenotype first described by Ahdab-Barmada and Claa-

sen (1990) gives strong support to this view. It is interesting to note that the D11S911-D11S906 interval encompassing the disease gene also encompasses Phox2a (Merscher et al. 1997), a gene strongly expressed in the hindbrain mouse (Pattyn et al. 1997). Ongoing research directed toward the goal of identifying the disease-causing genes will, it is hoped, help to resolve the complexity of MKS phenotypes.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Généthon, http://www.genethon.fr
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/omim (for MKS)

References

Ahdab-Barmada M, Claasen D (1990) A distinctive triad malformations of the central nervous system in the Meckel-Grüber syndrome. J Neuropathol Exp Neurol 49:610–620

Aleksic S, Budzilovich G, Greco MA, Reuben R, Feiginn I, Pearson J, Epstein F (1984) Cerebellocele and associated central nervous system anomalies in the Meckel syndrome. Childs Brain 11:99–111

Blankenberg TA, Ruebner BH, Ellis WG, Bernstein J, Dimmick JE (1987) Pathology of renal and hepatic anomalies in Meckel syndrome . Am J Med Genet Suppl 3:395–410

Crawford A, Jackson P, Kolher GH (1978) Meckel's syndrome (dysencephalia slanchnocystica) in two Pakistani sibs. J Med Genet 15:242–245

Dib C, Fauré S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, et al (1996) A comprehensive genetic map of the human genome based on 5264 microsatellites. Nature 380:152–154

Encha-Razavi F (1997) Neuropathologie foetale. Ann Pathol 17:281–286

Fraser FC, Lytwyn A (1981) Spectrum of anomalies in the Meckel syndrome; or, "Maybe there is a malformation syndrome with at least one constant anomaly?" Am J Med Genet 9:67–73

Genuardi M, Dionisi-Vici C, Sabetta G, Mignozzi M, Rizzonni G, Cotugno G, Neri MEM (1993) Cerebro-reno-digital (Meckel-like) syndrome with Dandy-Walker malformation, cystic kidneys, hepatic fibrosis, and polydactyly. Am J Med Genet 47:50–53

Hori A, Orthner H, Kohlschutter A, Schott KM, Hirabayashi K, Shimokawa K (1980) CNS dysplasia in dysencephalia splanchnocystica (Gruber's syndrome). Acta Neuropathol 51:93–97

Hunter AG, Jimenez C, Tawagi FG (1991) Familial renal-hepatic-pancreatic dysplasia and Dandy-Walker cyst: a distinct syndrome? Am J Med Genet 4:201–207

Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES (1996) Parametric and nonparametric linkage analysis: a unified multipoint approach. Am J Hum Genet 58:1347–1363

Lander ES, Botstein D (1987) Homozygosity mapping: a new way to map recessive trait with the DNA of inbred children. Science 236:1567–1570

Lurie IW, Lazjuk GI, Korotkova IA, Cherstvoy ED (1991) The cerebro-reno-digital syndromes: a new community. Clin Genet 39:104–113

Mecke S, Passarge E (1971) Encephalocele, polycystic kidneys and polydactyly as an autosomal recessive trait simulating certain other disorders: the Meckel syndrome. Ann Genet 14:97–103

Meckel JF (1822) Beschreibung zweier, durch sehr aehnliche Bildungsabweichungen entstellter Geschwister. Dtsch Arch Physiol 7:99–172

Merscher S, Bekri S, de Leeuw B, Pedeutour F, Grosgeorge J, Show STD, Müllenbach R, et al (1997) A 5,5 megabase high resolution integrated map of distal 11q13. Genomics 39: 340–347

Moerman PH, Pauwels P, Van den Berghe K, Lauweryns MS, Fryns JP (1993) Goldston syndrome reconsidered. Genet Couns 4:97–102

Ott J (1985) Analysis of human genetic linkage. John Hopkins University Press, Baltimore and London

Paavola P, Salonen R, Baumer A, Schinzel A, Boyd PA, Gould S, Meusburger H, et al (1997) Clinical and genetic heterogeneity in Meckel syndrome. Hum Genet 101:88–92

Paavola P, Salonen R, Weissenbach J, Peltonen L (1995) The locus for Meckel syndrome with multiple congenital anomalies maps to chromosome 17q21-q24. Nat Genet 11: 213–215

——— (1996*a*) Physical mapping of the Meckel syndrome at 17q21-q24. Eur J Hum Genet 4, Suppl 1:66

——— (1996*b*) Towards the molecular pathogenesis of Meckel syndrome, a lethal malformation syndrome of the fetus: physical mapping and analysis of candidate genes. Paper presented at the seventh Birth Defects Manchester Conference, Manchester, UK, October 8–11

Pattyn A, Morin X, Goridis C, Brunet JF (1997) Expression and interaction of the two closely related homeobox genes PHOX2a and PHOX2b during neurogenesis. Development 124:4065–4075

Plauchu H, Kemlin I, Bouvier R, Robert JM (1981) Le syndrome de Meckel: sa variabilité d'expression peut faire obstacle au diagnostic prénatal. J Genet Hum 29:431–440

Roume J, Ma HW, Le Merrer M, Cormier-Daire V, Girlich D, Genin E, Munnich A (1997) Genetic heterogeneity in Meckel syndrome. J Med Genet 34:1003–1006

Salonen R (1984) The Meckel syndrome: clinicopathological findings in 67 patients. Am J Med Genet 18:671–689

Salonen R, Norio R (1984) The Meckel syndrome in Finland: epidemiologic and genetic aspects. Am J Med Genet 18: 691–698

Seller MJ (1981) Phenotypic variation in Meckel syndrome. Clin Genet 20:74–77

Smith CAB (1963) Testing for heterogeneity of recombination values in human genetics. Ann Hum Genet 27:175–182

Verloes A, Gillerot Y, Langhendries JP, Fryns JP, Koulischer I (1992) Variability versus heterogeneity in syndromal hypothalamic hamartoblastoma and related disorders: review and delineation of the cerebro-acro-visceral early lethality (CAVE) multiplex syndrome. Am J Med Genet 43:669–677

Walpole IR, Goldblatt J, Hockey A, Knowles A (1991) Dandy-Walker malformation (variant), cystic dysplastic kidneys and hepatic fibrosis: a distinct entity or Meckel syndrome? Am J Med Genet 39:294–298

Wright C, Healicon R, English C, Burn J (1994) Meckel syndrome: what are the minimum diagnostic criteria? J Med Genet 31:482–485

Zlotogora J (1997) Genetic disorders among Palestinian Arabs. II. Hydrocephalus and neural tube defects. Am J Med Genet 71:33–35