Genetic heterogeneity of Meckel syndrome

J Roume, H W Ma, M Le Merrer, V Cormier-Daire, D Girlich, E Genin, A Munnich

Abstract

Meckel syndrome (MKS) is a lethal, autosomal recessive condition characterised by an occipital meningoencephalocele, enlarged kidneys with multicystic dysplasia, fibrotic changes of the liver in the portal area with ductal proliferation, and postaxial polydactyly. Recently, a MKS gene has been mapped to chromosome 17q21-q24 in Finnish families, with no evidence of locus heterogeneity in this population. Here, we report the exclusion of chromosome 17q21-q24 in eight typical MKS families of North African and Middle Eastern ancestry and provide evidence for genetic heterogeneity of this condition. (*J Med Genet* 1997;34:1003-1006)

Keywords : Meckel syndrome; genetic heterogeneity; fetopathology

Patients and methods

A total of eight families of North African and Middle Eastern ancestry with 12 affected fetuses and 33 healthy relatives were included in the study. Among them, 7/8 were consanguineous and 5/8 had more than one affected fetus (fig 2, table 1). Ten fetuses were aborted in the second trimester after prenatal ultrasound diagnosis of MKS and 2/12 died shortly after birth. A postmortem examination was performed in all cases, except in the second case of family 8, according to a previously defined protocol.⁷ Obstetricians, fetopathologists, and geneticists provided us with ultrasonographic records, karyotype results, pedigrees, necropsy records with photographs, and histological specimens. All findings were reviewed by the authors (table 1). Criteria for inclusion in the study were (1) cystic dysplasia of the kidneys, (2) fibrotic changes of the liver, (3) occipital encephalocele or other malformations of the central nervous system, and (4) normal blood or cultured skin fibroblast karyotypes.⁸

DNA was extracted from either frozen tissue or cultured skin fibroblasts of the 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 using 0.5 U *Taq* polymerase (Life Technologies) in a buffer containing 1.5 mmol/l MgCl₂, 20 µmol/l of each deoxynucleotide, 1 µmol/l of primers, and 200 ng genomic DNA in a final volume of 20 µl. After an initial denaturation step at 94°C for 10 minutes, PCR was performed for 30 cycles with a denaturation step at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30

Unité de Recherches sur les Handicaps Génétiques de l'Enfant, INSERM U-393, Hôpital des Enfants-Malades, 149 rue de Sèvres, 75743 Paris Cedex 15, France
J Roume
H W Ma
M Le Merrer
V Cormier-Daire
D Girlich
A Munnich

Unité d'Embryofetopathologie, Hôpital St Antoine, 184 rue du Faubourg St Antoine, 75012 Paris, France
J Roume

Unité de Recherches d'Epidémiologie Génétique, INSERM U-155, Château de Lonchamp, Bois de Boulogne, 75016 Paris, France
E Genin

Correspondence to: Dr Roume.

Received 1 April 1997
Revised version accepted for publication 17 June 1997

Meckel syndrome (MKS, MIM 249000¹) is a lethal, autosomal recessive malformation of unknown origin characterised by (1) an occipital meningoencephalocele, (2) enlarged kidneys with multicystic dysplasia, (3) fibrotic changes of the liver in the portal area with ductal proliferation, and (4) postaxial polydactyly (fig 1).² The incidence of the disease ranges from 1/13 250 to 1/140 000 births, but in Finland the prevalence averages 1/9000 births.³ Recently, a MKS gene has been mapped to chromosome 17q21-q24 in Finnish families with no evidence of locus heterogeneity in this population.⁴⁻⁶ Here, we report the exclusion of chromosome 17q in eight typical MKS families of North African and Middle Eastern ancestry and provide evidence for clinical and genetic heterogeneity of this condition.

Table 1 Clinical and histopathological findings in 12 fetuses with Meckel syndrome

Family	1	1	2	2	3	4	5	6	7	8	8	8
Consanguinity	?	?	+	+	+	+	+	+	+	+	+	+
Origin	Morocco	Morocco	Tunisia	Tunisia	Pakistan	Senegal	Tunisia	Tunisia	Algeria	Morocco	Morocco	Morocco
Patients	II.2	II.3	V.3	V.4	III.3	IV.9	V.3	V.1	III.6	III.2	III.3	III.5
Gestation (wk)	32	18	21	14	19	17	24	23	21	25	37	24
Issue of pregnancy	Sb	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Sb	Ab
US prenatal diagnosis	+	+	+	+	+	+	+	+	+	+	+	+
Fetopathological evaluation	+	+	+	+	+	+	+	+	+	+	-	+
Sex	F	F	M	F	M	F	M	M	F	F	F	M
Enlarged polycystic kidneys	+	+	+	+	+	+	+	+	+	+	+	+
Fibrotic changes of liver	+	+	+	+	+	+	+	+	+	+	+	+/-
Occipital exencephalocele	0	+	+	+	+	+	+	+	+	0	+	0
Fossa posterior abnormalities	+	+	+	+	+	?	+	+	+	+	+	+
Ahdab-Barmada's triad ¹⁰	0	?	+	+	+	+	+	+	+	0	?	0
Microphthalmia	?	0	+	+	+	?	+	+	0	0	+	?
Cleft lip or palate	+	0	0	0	0	0	+	+	+	0	0	0
Heart defect	0	0	0	0	0	0	0	+	+	0	?	0
Genital hypoplasia	0	0	+	0	+	—	+	+	—	—	—	0
Pancreatic dysplasia	+	+	+	?	0	?	+	+	0	?	?	+/-
Hand polydactyly	0/0	0/0	+/+	+/+	0/0	+/+	+/+	+/+	0/0	0/0	+/+	+/+
Foot polydactyly	0/0	0/0	+/+	+/+	0/0	+/+	+/+	+/+	0/0	0/0	0/0	0/0

+, present; 0, absent; ?, not obtained; F, female; M, male; Sb, stillbirth; Ab, aborted; US, ultrasonographic.

Table 2 Pairwise linkage between the MKS locus and three microsatellite DNA markers of chromosome 17q

Locus	Families	Recombination fraction (θ)				
		0.00	0.01	0.05	0.10	0.20
D17S1607 AFMa046zg1	Total	−∞	−10.47	−4.69	−2.60	−1.04
	1	−0.23	−0.22	−0.18	−0.14	−0.07
	2	−∞	−1.00	−0.37	−0.17	−0.03
	3	−3.77	−1.16	−0.51	−0.27	−0.09
	4	−3.52	−0.67	−0.11	0.02	0.02
	5	−2.60	−1.09	−0.47	−0.24	−0.08
	6	−3.90	−0.94	−0.33	−0.13	−0.02
	7	−3.90	−1.28	−0.61	−0.36	−0.14
D17S1606 AFM338xg5	Total	−∞	−11.54	−5.19	−2.92	−1.20
	1	−∞	−1.04	−0.39	−0.16	−0.01
	2	−2.84	−1.08	−0.45	−0.22	−0.07
	3	−3.77	−1.16	−0.51	−0.27	−0.09
	4	−3.86	−1.01	−0.44	−0.28	−0.16
	5	−∞	−0.91	−0.33	−0.15	−0.05
	6	−3.70	−1.20	−0.54	−0.30	−0.11
	7	−3.60	−1.29	−0.64	−0.40	−0.21
D17S1604 AFMa163tf9	Total	−∞	−11.94	−4.98	−2.52	−0.15
	1	0.07	0.07	0.06	0.05	0.01
	2	−∞	−2.08	−0.81	−0.36	0.02
	3	−3.77	−1.16	−0.53	−0.30	−0.04
	4	−3.86	−1.01	−0.44	−0.28	−0.08
	5	−∞	−2.38	−1.09	−0.60	−0.08
	6	−3.90	−0.94	−0.33	−0.13	−0.00
	7	0.95	0.92	0.83	0.71	0.27
	8	−∞	−5.37	−2.67	−1.60	−0.25

Figure 1 Clinical and histopathological features in a typical case of Meckel syndrome. (A) Phenotype of a 21 week old male fetus. Note occipital meningoencephalocele, enlarged cystic kidneys, and postaxial polydactyly. (B) The malformation triad of the central nervous system with prosencephalic dysgenesis, occipital meningoencephalocele, and rhombic roof dysgenesis. (C) Typical multicystic renal dysplasia (HES). (D) Fibrotic changes of the portal area with ductular proliferation (HES).

seconds. The reaction was completed with an elongation step at 72°C for 10 minutes. Amplified products were separated on 6% polyacrylamide gels run under denaturing conditions and transferred onto charged nylon membranes (Hybond N+ Amersham). Membranes were hybridised overnight at 42°C with a poly-AC probe, labelled by chemiluminescence (ECL, direct nucleic acid labelling and detection system, Amersham Life Science), and exposed to x ray films for 10 minutes. Linkage analyses were performed using the MLINK and LINKMAP options of the 5.1 version of the LINKAGE program.⁹ The MKS gene was assumed to have complete penetrance and a gene frequency of 0.001.

Results

The eight families reported here fulfilled the criteria of typical MKS.^{2,8} Interestingly, associated malformations were often observed in the fetuses, namely polydactyly and occasionally cleft lip/palate, microphthalmia, congenital heart defect, pancreatic dysplasia, and genital hypoplasia. Moreover, 7/12 patients harboured the distinctive malformation triad of the central nervous system reported by Ahdab-Barmada *et al*¹⁰ and others,^{11,12} namely prosencephalic dysgenesis, occipital encephalocele, and rhombic roof dysgenesis (table 1). It is worth noting that intrafamilial clinical heterogeneity was noted in our series as reported in previous studies.¹³ Clinical heterogeneity was found in occipito-encephalocele (families 1 and 8) and hand polydactyly (family 8, table 1).

The following order and genetic distances between loci have been previously shown in CEPH reference families¹⁴: cen-D17S1607-(0.05)-(D17S1606, MKS)-(0.01)-D17S1604-tel.

Pairwise linkage analyses using microsatellite DNA markers of chromosome 17q excluded the disease gene from the genetic interval encompassing the Finnish locus in each and the combined MKS pedigrees (table 2). Haplotype reconstruction also excluded the MKS gene from chromosome 17q21-q24 in our families (fig 2).

Discussion

Recently, a MKS gene has been mapped to chromosome 17q21 in Finnish families with no evidence of locus heterogeneity in this population. Here, we report the exclusion of the Finnish locus on chromosome 17q21-q24 in eight typical MKS families of North African and Middle Eastern ancestry and show evidence of genetic heterogeneity of Meckel syndrome. Interestingly, Paavola *et al*⁶ have previously excluded the disease gene from chromosome 17q in a Bangladeshi family with atypical Meckel syndrome (the patient survived several days after birth). The present study therefore provides the first evidence of genetic heterogeneity in a typical form of Meckel syndrome.

This genetic heterogeneity will certainly hinder the mapping of MKS loci in our series, especially as more than one locus could be involved, and various related

• DNA not available

Figure 2 Pedigrees and haplotype analyses of chromosome 17q21-q24 in Meckel syndrome. Haplotypes are given for loci D17S1607, D17S1616, and D17S1604.

cerebroacrovisceral syndromes might overlap with MKS.¹⁵⁻¹⁸ For this reason, the goal of gene identification will be probably easier to achieve in defined ethnic groups (Finnish or North African families) or in clinical subclasses of the disease. Along these lines, we suggest giving consideration to the subgroup of patients harbouring the Ahdab-Barmada triad (prosen-

cephalic dysgenesis, occipital meningoencephalocele, and rhombic roof dysgenesis) as a potentially important clinical subgroup when investigating the genetic heterogeneity of MKS.¹⁰⁻¹²

We are grateful to the families for their cooperation. We wish to thank the Société Française de Foetopathologie and the Departments of Obstetrics, Genetics, and

- Embryofetopathology of Hôpital des Enfants Malades (A L Delezoide, N Morichon), Hôpital St Antoine (N Mulliez, M Gonzales, F Encha-Razavi, G Migne, J Martinovic, C Bignozzi, N Joye, J L Taillémite, Ch Roux), Hôpital Cochin-Port-Royal (Y Dumez, M Dommergues, F Narcy, C Fallet-Bianco), Hôpital Jean Verdier (M Bucourt), Paris, Hôpital Bretonneau, Tours (A Toutain, Cl Moraine), Maternité A Pinard, Nancy (B Le Heup), and Hôpital E Herriot, Lyon (R Bouvier, A Buenerd, M P Cordier) for their invaluable cooperation. JR is recipient of a grant from the Assistance Publique-Hôpitaux de Paris/CNRS (FPA 95001-FPA 96001).
- 1 Meckel JF. Beschreibung Zweier, durch sehr aehnliche Bildungsabweichungen entseller erchwister. *Dtsch Arch Physiol* 1822;7:99-172.
  - 2 Salonen R. The Meckel syndrome: clinicopathological findings in 67 patients. *Am J Med Genet* 1984;18:671-89.
  - 3 Salonen R, Norio R. The Meckel syndrome in Finland: epidemiologic and genetic aspects. *Am J Med Genet* 1984;18:691-8.
  - 4 Paavola P, Salonen R, Weissenbach J, Peltonen L. The locus for Meckel syndrome with multiple congenital anomalies maps to chromosome 17q21-q24. *Nat Genet* 1995;11:213-15.
  - 5 Paavola P, Salonen R, Weissenbach J, Peltonen L. Physical mapping of the Meckel syndrome at 17q21-q24. *Eur J Hum Genet* 1996;4(suppl 1):66A.
  - 6 Paavola P, Salonen R, Weissenbach J, Peltonen L. Towards the molecular pathogenesis of Meckel syndrome, a lethal malformation syndrome of the fetus; physical mapping and analysis of candidate genes. In: *Proceedings of the Seventh Manchester Birth Defects Conference, 8-11 October 1996* (abstract).
  - 7 Imbert MC. L'examen foeto-pathologique. Technique et apport de l'examen du placenta et de l'autopsie périnatale. *Ann Pathol* 1991;11:292-8.
  - 8 Blakenberg TA, Ruebner BH, Ellis WG, Bernstein J, Dimmick JE. Pathology of renal and hepatic anomalies in Meckel syndrome. *Am J Med Genet Suppl* 1987;3:395-410.
  - 9 Lathrop CM, Lalouel JM, Julier C, Ott J. Strategies for multilocus linkage analysis in humans. *Proc Natl Acad Sci USA* 1984;81:3443-6.
  - 10 Ahdab-Barmada M, Claasen D. A distinctive triad of malformations of the central nervous system in the Meckel-Gruber syndrome. *J Neuropathol Exp Neurol* 1990;49:610-20.
  - 11 Hori A, Orthner H, Kohlschütter A, et al. CNS dysplasia in dysencephalia splanchnocystica (Gruber's syndrome). *Acta Neuropathol* 1980;51:93-7.
  - 12 Aleksic S, Budzilovich G, Greco MA, et al. Cerebellocele and associated central nervous system anomalies in the Meckel syndrome. *Childs Brain* 1984;11:99-111.
  - 13 Fraser FC, Lytwyn A. Spectrum of anomalies in the Meckel syndrome or: "Maybe there is a malformation syndrome with at least one constant anomaly". *Am J Med Genet* 1981;9:67-73.
  - 14 Dib C, Fauré S, Fizames C, et al. A comprehensive genetic map of the human genome based on 5264 microsatellites. *Nature* 1996;380:152-4.
  - 15 Breuton LA, Dillon MJ, Winter RM. Ellis-van Creveld syndrome, Jeune syndrome, and renal-hepatic-dysplasia: separate entities or a disease spectrum? *J Med Genet* 1990;27:252-5.
  - 16 Lurie IW, Lazjuk GI, Korotkova IA, Cherstvoy ED. The cerebro-reno-digital syndromes: a new community. *Clin Genet* 1991;39:104-13.
  - 17 Walpole IR, Goldblatt J, Hockey A, Knowles A. Dandy-Walker malformation (variant), cystic dysplastic kidneys and hepatic fibrosis: a distinct entity or Meckel syndrome? *Am J Med Genet* 1991;39:294-8.
  - 18 Verloes A, Gillerot Y, Langhendries JP, Fryns JP, Koulischer I. 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* 1991;43:669-77.