Original articles

Department of Human Genetics, University Hospital Nijmegen, PO Box 9101, 6500 HB Nijmegen, The Netherlands
H G Yntema
B C J Hamel
A P T Smits
H Kremer
H-H Ropers
D F C M Smeets
H van Bokhoven

Max Planck Institute for Molecular Genetics, Berlin, Germany
H-H Ropers

Department of Child Neurology, University Hospital Nijmegen, Nijmegen, The Netherlands
T van Roosmalen

Department of Neurology, University Hospital Nijmegen, Nijmegen, The Netherlands
B van den Helm

Correspondence to: Dr van Bokhoven.
Received 23 January 1998
Revised version accepted for publication 30 March 1998

Localisation of a gene for non-specific X linked mental retardation (MRX46) to Xq25-q26

Helger G Yntema, Ben C J Hamel, Arie P T Smits, Tanja van Roosmalen, Bellinda van den Helm, Hannie Kremer, Hans-Hilger Ropers, Dominique F C M Smeets, Hans van Bokhoven

Abstract
We report linkage data on a new large family with non-specific X linked mental retardation (MRX), using 24 polymorphic markers covering the entire X chromosome. We could assign the underlying disease gene, denoted MRX46, to the Xq25-q26 region. MRX46 is tightly linked to the markers DXS8072, HPRT, and DXS294 with a maximum lod score of 5.12 at $\theta=0$. Recombination events were observed with DXS425 in Xq25 and DXS984 at the Xq26-Xq27 boundary, which localises MRX46 to a 20.9 cM (12 Mb) interval. Several X linked mental retardation syndromes have been mapped to the same region of the X chromosome. In addition, the localisation of two MRX genes, MRX27 and MRX35,

partially overlaps with the linkage interval obtained for MRX46. Although an extension of the linkage analysis for MRX35 showed only a minimal overlap with MRX46, it cannot be excluded that the same gene is involved in several of these MRX disorders. On the other hand, given the considerable genetic heterogeneity in MRX, one should be extremely cautious in using interfamilial linkage data to narrow down the localisation of MRX genes. Therefore, unless the underlying gene(s) is characterised by the analysis of candidate genes, MRX46 can be considered a new independent MRX locus.
(J Med Genet 1998;35:801-805)

Keywords: non-specific mental retardation; linkage; X chromosome

Figure 1 Five generation family with MRX. Twenty-seven family members, including 10 affected males, were available for DNA study. Haplotypes of the linked markers in Xq25-q26 and the recombined markers delimiting the probable gene location are shown. Deduced haplotypes from subjects who had died are shown between brackets. The disease chromosome is indicated by a black bar.

Table 1 Summary of clinical measurements (centiles)

	Height (cm)	Weight (kg)	OFC (cm)	Ear length (mm)	OCD (mm)	ICD (mm)	THL (cm)	Testicular volume (cm ³)
II.18	160 (<3)	56 (<10)	56 (50)	75 (97)	95 (75-97)	38 (>97)	18 (50-75)	20 (50)
III.12	182 (50)	85 (90)	56 (50)	71 (75-97)	81 (3-25)	29 (25-50)	19.5 (97)	22 (50-90)
III.13	168 (<10)	83 (90)	56.5 (50-90)	60 (50)	83 (25)	31 (50)	19 (75-97)	23 (50-90)
III.15	182 (50)	92 (>90)	58.5 (>90)	65 (75)	95 (75-97)	35 (75-97)	18 (50-75)	25 (50-90)
III.16	182 (50)	—	59 (>90)	61 (50)	84 (25-50)	31 (50)	19 (75-97)	—
III.20	172 (3-10)	76 (50-90)	58 (90)	60 (50)	91 (50-75)	32 (50-75)	19 (75-97)	20 (50)
III.24	163 (<3)	75.5 (50-90)	—	—	—	—	—	—
III.26	170 (3-10)	60 (10)	54 (10-50)	60 (50)	105 (>97)	35 (75-97)	19 (75-97)	—
III.28	181 (10-50)	91 (>90)	58 (50-90)	65 (75)	105 (>97)	38 (>97)	19.5 (97)	—

OCD = outer canthal distance.
ICD = inner canthal distance.
THL = total hand length.
— = no data.

X linked mental retardation (XLMR) is considered to be the most frequent type of mental handicap in males. It has been estimated that mutations in X chromosomal genes account for 25 to 50% of all cases of mental retardation.¹ A small part of XLMR can be attributed to recognisable syndromes and to date more than 100 XLMR syndromes (MRXS) have been described.² More often, however, the mental handicap is not associated with consistent phenotypic characteristics. This is referred to as non-specific XLMR or MRX.

Linkage analyses in individual families with MRX have currently shown over 50 loci on the X chromosome which are clustered in eight non-overlapping regions.³⁻⁴ The inclusion of the FRAXE mental retardation gene⁵ and the RAB-GDI gene⁶ suggests a minimum of 10 X linked genes which are involved in non-specific mental retardation. In this report, we present a large family with non-specific X linked mental retardation and the mapping of the underlying gene to Xq25-q26, a region that has rarely been implicated in MRX.

Patients and methods

CLINICAL REPORT

The family (fig 1) was ascertained when IV.7 was referred for genetic counselling. The pedigree included 12 mentally retarded males in three generations, nine of whom were clinically examined. In order to determine the mental status of these patients, the following intelli-

gence tests, standardised for school aged children, were used: (1) the Wechsler Intelligence Scale for Children Revised (WISC-R), (2) the Coloured Progressive Matrices (CPM),⁹⁻¹⁰ (3) the Revised Amsterdam Child Intelligence Test (RAKIT),¹¹ and (4) McCarthy Scales of Children's Abilities.¹² The RAKIT and McCarthy Scales were applied to assess intellectual functioning below an equivalent of 6 years.¹³ Scores of all tests were transferred into age equivalents according to the classification of the American Association of Mental Deficiency (AAMD).¹⁴ Cytogenetic analysis and molecular study of the FMR-1 gene was performed in several patients. In III.28 the diagnostic tests included cerebral CT scan and metabolic screening. Informed consent was obtained in all instances.

DNA ANALYSIS

DNA from 26 relatives was isolated from peripheral blood lymphocytes, according to the procedure of Miller *et al.*¹⁵ In order to determine the most likely location of the gene, linkage analysis was performed with highly polymorphic markers distributed along the X chromosome. Analysis of these markers involved amplification by polymerase chain reaction (PCR) carried out in a 96 well Thermal Cycler (MJ Research Inc, Waterston, MA). Each reaction contained 100 ng genomic DNA, 30 ng of each of the primers, in 15 µl 1x Supertaq buffer (50 mmol/l KCl, 1.5 mmol/l MgCl₂, 10 mmol/l Tris-HCl, pH 9.0, 0.1% Triton X-100, 0.01% (w/v) gelatin) in the presence of ³²P-dCTP with 0.06 U Supertaq (HT Biotechnology Ltd, England). Amplification was achieved by 35 cycles of one minute at 94°C, two minutes at 55°C, and three minutes at 72°C with the locus specific primer pairs registered in the Genome Database (<http://gdbwww.gdb.org/gdb/>). The radiolabelled PCR products were mixed with 15 µl sample buffer (95% formamide, 20 mmol/l EDTA, 0.05% xylene cyanol, 0.05% bromophenol blue), heated to 95°C for two minutes, and 4 µl of this mixture was separated on a 6.6% denaturing polyacrylamide gel. Subsequently, the gel was dried and exposed overnight to Kodak X-OMAT film to visualise the separated allelic bands.

Two point linkage analyses of the 24 polymorphic markers and the disease locus were performed with the MLINK option of the

Table 2 Summary of psychometric data

Cases	Intelligence*	Non-verbal reasoning*	Visual/motor skills*	Adaptive functioning*	Academic achievement		
					Reading†	Writing†	Arithmetic†
II.18	—	+/-	+/-	+/-	+	+	—
III.12	—	—	—	+	+	+	+
III.13	—	+/-	+	—	—	—	—
III.15	—	+/-	+/-	+/-	—	—	+/-
III.16	+/-	+	—	+	+	+/-	+
III.20	—	+/-	‡	+/-	+	+	‡
III.24	—	—	—	—	—	—	—
III.26	---	Unable	Unable	---	—	—	—
III.28	--	Unable	--	--	—	—	—

*Levels of mental and adaptive functioning, transferred into age equivalents:
+ => 10-9 years (= borderline and above; IQ-range ± 70-84 and above);
+/- = 8-3 and 10-9 years (= mild; IQ range 50/55-70);
- = 5-7 to 8-2 years (= moderate; IQ range 35/40-50/55);
-- = 3-2 to 5-6 years (= severe; IQ range 20/25-35/40);
--- = < 3-2 years (= profound; IQ range <20/25).

†Levels of academic functioning, transferred into didactic age equivalents (dae):
+ => 30 months (= adequate);
+/- = 1-29 months (= inadequate);
-- = 0 months (= unable).

‡No actual information available.

Figure 2 Faces of affected males. (A) II.18 at 65 years, (B) III.12 at 57 years, (C) III.13 at 54 years, (D) III.15 at 47 years, (E) III.16 at 38 years, (F) III.20 at 44 years. (Photographs reproduced with informed consent.)

computer program LINKAGE (version 5.03)^{16,18} on the basis of X linked recessive inheritance with full penetrance. The relative order of marker loci was obtained from the

Genome Database and the Report of the Sixth International Workshop on X Chromosome Mapping.¹⁹

Results

CLINICAL REPORT

In all patients, pregnancy and delivery were uneventful. The mental retardation was noticed during early childhood and appeared non-progressive. None of them had convulsions. All patients were either institutionalised or living and working in a sheltered environment. Physically they were all healthy. Their behaviour was normal, except for the brothers III.24, III.26, and III.28 who were described as difficult to control, chaotic, and autistic-like, and III.16 who showed obsessive behaviour. III.11 died at the age of 11 during heart surgery. He was attending a special education unit. Obligate carriers were all normal. Results of the clinical measurements and psychometric studies are summarised in tables 1 and 2, respectively.

Only minor anomalies were noted in some of the affected males. II.18 (fig 2A) was seen at the age of 65 years. He was short statured. Hypertelorism, a fleshy nose, full lower lip, kypholordosis, and lower leg varices were noticed. III.12 (fig 2B) showed no abnormalities except for lower leg varices when seen at the age of 57 years. III.13 (fig 2C) was examined at the age of 54 years. Except for a left sided hydrocele no abnormalities were detected. III.15 (fig 2D) and III.16 (fig 2E) showed no abnormalities on examination at the ages of 47 and 38, respectively. III.20 (fig 2F) was examined at the age of 44 years. A left sided hydrocele and a large hyperpigmented spot on the back were seen. III.24 showed a highly arched palate, short and broad neck, and flat feet on examination at the age of 34 years. He was short statured. Ophthalmological examination of III.26 at the age of 29 years showed hypertelorism, myopia gravis ODS, and some lenticular opacities. In III.28 hypertelorism and myopia gravis OD, resulting in amblyopia and divergent strabismus, were seen on ophthalmological examination. The degree of mental retardation in the affected family members tested varied from mild to profound. It is concluded that pedigree structure and clinical data are fully compatible with a diagnosis of non-specific X linked mental retardation.

DNA ANALYSIS

For the initial analyses, 23 markers spread along the X chromosome were genotyped on 12 potentially informative family members. The family was informative for all tested markers. Once tentative linkage with HPRT and DXS294 was obtained, another marker in the region, DXS8072, was typed and more affected and unaffected family members were genotyped for the linked markers (26 relatives in total). Table 3 presents the results of the two point linkage analysis with the lod scores for the MRX locus and each marker locus. The proximal locus DXS425 at Xq25 and the more distal locus DXS984 at the Xq26-q27 bound-

Table 3 Lod scores between MRX46 and markers along the X chromosome (in order from Xpter to Xqter)

	θ					
	0.000	0.050	0.100	0.200	0.300	0.400
DXS1060	−∞	−1.93	−0.91	−0.08	0.19	0.19
GHGXG	−∞	−2.05	−1.01	−0.16	0.13	0.17
DXS443	−∞	−1.96	−1.14	−0.42	−0.11	0.01
DMD	−∞	−6.04	−4.01	−2.07	−1.03	−0.39
DXS538	−∞	0.02	0.40	0.52	−0.35	0.10
DXS7	−∞	−0.68	−0.29	−0.15	−0.26	−0.29
DXS1003	−∞	−6.34	−4.30	−2.34	−1.26	−0.53
ALAS2	−∞	−2.23	−1.19	−0.35	−0.04	0.02
DXS453	−∞	−5.46	−3.72	−2.05	−1.12	−0.49
DXS559	−∞	−2.07	−1.34	−0.77	−0.49	−0.23
DXS986	−∞	−2.07	−1.34	−0.77	−0.49	−0.23
DXS3	−∞	−0.79	−0.39	−0.23	−0.30	−0.28
DXS178	−∞	−2.07	−1.34	−0.77	−0.49	−0.23
Col4A5	−∞	−2.07	−1.34	−0.77	−0.48	−0.23
DXS424	−∞	−1.96	−1.14	−0.42	−0.11	0.01
DXS1001	−∞	−0.96	−0.25	0.21	0.27	0.15
DXS425	−∞	0.14	0.96	1.31	1.09	0.57
DXS8072	4.43	3.99	3.54	2.60	1.63	0.66
HPRT	5.12	4.70	4.26	3.32	2.28	1.12
DXS294	4.95	4.51	4.04	3.07	2.03	0.96
DXS984	−∞	2.14	2.34	2.10	1.53	0.81
FraXAc2	−∞	0.09	0.28	0.35	0.28	0.16
DXS1113	−∞	−0.06	0.15	0.25	0.22	0.13
DXS1108	−∞	−0.06	0.15	0.25	0.22	0.13

ary recombined with the disease in the family, delimiting the probable gene location. Significant lod scores were obtained with markers DXS8072, HPRT, and DXS294, indicating that the genetic defect responsible for the disease in this family is located in Xq25-q26, with a maximum lod score of 5.12 at $\theta=0$ (at HPRT). A reconstruction of the haplotypes of the markers in the Xq25-q26 region is shown in fig 1.

Discussion

We have presented a new family with recessive non-specific X linked mental retardation and assigned the causative genetic defect, MRX46, to Xq25-q26. This region is estimated to comprise a genetic distance of 20.9 cM²⁰ and a 12 Mb region on the physical map.¹⁹

Seven XLMR syndromes have been assigned to X chromosomal segments overlapping the linkage interval for MRX46 (fig 3). Huang *et*

*al*²¹ reported on a family with an X linked mental retardation disorder (MRXS5), including Dandy-Walker malformation, basal ganglia disease, and seizures. Börjeson-Forsman-Lehmann syndrome (BFLS)^{20 22 23} is characterised by severe mental retardation, microcephaly, hypogonadism, obesity, short stature and distinct facial anomalies including large ears, prominence of the supraorbital ridge, and ptosis. Malmgren *et al*²⁴ reported a family with severe X linked mental retardation syndrome linked to DXS294 at Xq26. This so-called Gustavson syndrome includes microcephaly, severe mental retardation, optic atrophy with decreased vision or blindness, severe hearing defect, characteristic facial features, spasticity, seizures, and restricted joint mobility. The patients die during infancy or early childhood.²⁵ Cowchock syndrome, an X linked motor-sensory neuropathy type II with deafness and mental retardation has been mapped between DXS425 and HPRT.^{26 27} Recently some of us described a family presenting with varying degrees of mental retardation in conjunction with isolated growth hormone deficiency.²⁸ The underlying genetic defect for this disorder could be assigned to the Xq24-q27.3 region. Interestingly, in a recent report an unrelated family with similar symptoms is described in which the genetic defect is localised to the same region.²⁹ In this family, X linked recessive panhypopituitarism (XPH) is associated with a duplication of the DXS1001 locus, suggesting that this phenotype is caused by a gene dosage effect. Lesch-Nyhan syndrome (OMIM 308000) is another well known syndrome with MR, caused by mutations in the HPRT1 gene. It cannot be ruled out that one of the genes involved in these MR syndromes is also causative in MRX46, but given the pronounced distinctive features of each syndrome, it is difficult to imagine how mutation in one such gene may cause (severe) non-specific MR.

Very recently, two novel MRX loci encompassing Xq26, MRX27, and MRX35 have been uncovered by linkage analysis in apparently unrelated families from the United Kingdom and Belgium, respectively.^{30 32} MRX27 has been localised to the Xq24-q26 region with flanking recombination events in affected males at DXS424 and DXS102, which spans all but the distal portion of the MRX46 linkage interval (fig 3).³¹ MRX35 maps to Xq22-q26 with flanking markers DXS178 and HPRT. DNA samples from this family (kindly provided by Professor J P Fryns and Dr R Raeymaekers) allowed us to assign DXS8072 as the distal flanking marker in MRX35. Hence, the overlap between MRX46 and MRX35 comprises a region of less than 2 cM at the Xq25-q26 boundary (fig 3). If the same gene is involved in the three MRX families that map to Xq26, the region to search for candidate genes would be significantly reduced. However, given the genetic heterogeneity of MRX one should be extremely cautious of using interfamilial linkage data to narrow down the localisation of MRX genes. This is especially true since MRX46 seems to differ

Figure 3 Localisation of XLMR conditions in the Xq25-q26 region. The top bar shows the order of relevant markers and genes (underlined) on a Mb scale. The horizontal bars indicate the linkage intervals. Arrowheads at the end of the bars indicate that the linkage interval extends beyond the chromosomal region shown. BFLS: Börjeson-Forsman-Lehman syndrome; GS: Gustavson syndrome; CS: Cowchock syndrome; MR-IGHD: mental retardation with isolated growth hormone deficiency; XPH: X linked recessive panhypopituitarism.

from MRX27 and MRX35 in that the variability and severity of the mental handicap is more pronounced and, yet, carrier females never exhibit any mental impairment. Therefore, the whole Xq26 band has to be considered for identification of candidate genes in the family presented here. According to the physical map of Pilia *et al.*,³³ five genes for which the complete coding region is known are located in this region (fig 3). In addition, the human transcript map³⁴ (<http://www.ncbi.nlm.nih.gov/SCIENCE96/>) reports more than 50 expressed sequence tags for this region. Unfortunately, expression data for the corresponding genes are not available and it is therefore not possible at this stage sensibly to select likely candidate genes for MR from this gene pool.

We thank Dr C de Die-Smulders for providing clinical data on the three brothers III.24, III.26, and III.28. We wish to thank the family members for their participation in this study. Professor J P Fryns and Dr P Raeymaekers are greatly acknowledged for providing material from the MRX35 family. We also thank Dr F P M Cremers for critical reading of the manuscript. This research was financially supported by a grant from the Dutch "Praeventiefonds".

- Kerr B, Turner G, Mulley J, Gedeon A, Partington M. Non-specific mental retardation. *J Med Genet* 1991;7:378-82.
- Lubs HA, Chiurazzi P, Arena JF, *et al.* XLMR genes: update 1996. *Am J Med Genet* 1996;64:147-57.
- Lubs H, Chiurazzi P, Arena J, *et al.* XLMR genes: update 1998. *8th International Workshop on Fragile X Syndrome and X-Linked Mental Retardation*, August 1997, Picton, Canada.
- Gedeon AK, Donnelly AJ, Mulley JC, Kerr B, Turner G. How many X-linked genes for non-specific mental retardation (MRX) are there? *Am J Med Genet* 1996;64:158-62.
- Gecz J, Gedeon AK, Sutherland GR, Mulley JC. Cloning of *FMR2*: a gene associated with FRAXE mental retardation. *Nat Genet* 1996;13:105-8.
- D'Adamo P, Gulisano M, Oostra BA, Chelly J, Toniolo D. GDI is responsible for X-linked mental retardation. *Am J Hum Genet* 1997;61:A11.
- Wechsler D. *Wechsler Intelligence Scale for Children-revised*. New York: Psychological Corporation, 1974.
- Van der Steene G, van Haasen PP, de Bruyn EEJ, *et al.* *Intelligence Scale for Children-revised*. Dutch version. Lisse: Swets & Zeitlinger, 1986.
- Raven JC. *Guide to using the Coloured Progressive Matrices*. London: Lewis, 1965.
- Van Bon WHJ. *Raven's Coloured Progressive Matrices; Nederlandse normen en enige andere uitkomsten van onderzoek*. Lisse: Swets & Zeitlinger, 1986.
- Bleichrodt N, Drenth PJD, Zaal JM, Resing WCM. *Revisie Amsterdamse Kinder Intelligentietest; instructie, normen, psychometrische gegevens*. Lisse: Swets & Zeitlinger, 1984.
- Van der Meulen BF, Smrkovsky M. *McCarthy Ontwikkelingsschalen*. Lisse: Swets & Zeitlinger, 1985.
- Bleichrodt N, Resing WCM, Drenth PJD, Zaal JM. *Intelligentiemeting bij kinderen*. Lisse: Swets & Zeitlinger, 1987.
- Grossman HJ, ed. *Manual on terminology and classification in mental retardation*. Washington DC: AAMD, 1977.
- Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215.
- Lathrop GM, Lalouel JM, Julier C, Ott J. Strategies for multilocus analysis in humans. *Proc Natl Acad Sci USA* 1984;81:3443-6.
- Lathrop GM, Lalouel JM. Easy calculations of lod scores and genetic risks on small computers. *Am J Hum Genet* 1984;36:460-5.
- Lathrop GM, Lalouel JM, White RL. Construction of human genetic linkage maps: likelihood calculations for multilocus analysis. *Genet Epidemiol* 1986;3:39-52.
- Nelson DL, Ballabio A, Cremers FPM, Monaco AP, Schlessinger D. Report of the sixth international workshop on X chromosome mapping 1995. *Cytogenet Cell Genet* 1995;71:307-42.
- Gedeon AK, Kozman HM, Robinson H, *et al.* Refinement of the background genetic map of Xq26-q27 and gene localisation for Börjeson-Forssman-Lehmann syndrome. *Am J Med Genet* 1996;64:63-8.
- Huang THM, Hejtmanic JF, Edwards A, *et al.* Linkage of the gene for an X-linked mental retardation disorder to a hypervariable (AGAT)_n repeat motif within the human hypoxanthine phosphoribosyltransferase (HPRT) locus (Xq26). *Am J Hum Genet* 1991;49:1312-19.
- Turner G, Gedeon A, Mulley J, *et al.* Börjeson-Forssman-Lehmann syndrome: clinical manifestations and gene localization to Xq26-27. *Am J Med Genet* 1989;34:463-9.
- Mathews KD, Ardinger HH, Nishimura DY, *et al.* Linkage localization of Börjeson-Forssman-Lehmann syndrome. *Am J Med Genet* 1989;34:470-4.
- Malmgren H, Sundvall M, Dahl N, *et al.* Linkage mapping of a severe X-linked mental retardation syndrome. *Am J Hum Genet* 1993;52:1046-52.
- Gustavson KH, Annerén G, Malmgren H, *et al.* New X-linked syndrome with severe mental retardation, severely impaired vision, severe hearing defect, epileptic seizures, spasticity, restricted joint mobility, and early death. *Am J Med Genet* 1993;45:654-8.
- Cowchock FS, Duckett SW, Streletz LJ, Graziani LJ, Jackson LG. X-linked motor-sensory neuropathy type-II with deafness and mental retardation: a new disorder. *Am J Med Genet* 1985;20:307-15.
- Priest JM, Fischbeck KH, Nouri N, Keats BJB. A locus for axonal motor-sensory neuropathy with deafness and mental retardation maps to Xq24-26. *Genomics* 1995;29:409-12.
- Hamel BCJ, Smits APT, Otten BJ, *et al.* Familial X-linked mental retardation and isolated growth hormone deficiency: clinical and molecular findings. *Am J Med Genet* 1996;64:35-41.
- Lagerström-Fermér M, Sundvall M, Johnsen E, *et al.* X-linked recessive panhypopituitarism with a regional duplication in Xq25-q26. *Am J Hum Genet* 1997;60:910-16.
- Glass LA, White EM, Pope MJ, *et al.* Linkage analysis in a large family with nonspecific X-linked mental retardation. *Am J Med Genet* 1991;38:240-43.
- Gedeon AK, Glass LA, Connor JM, Mulley JC. Genetic localisation of MRX27 to Xq24-26 defines another discrete gene for non-specific X-linked mental retardation. *Am J Med Genet* 1996;64:121-4.
- Gu XX, Decorte R, Marynen P, *et al.* Localisation of a new gene for non-specific mental retardation to Xq22-q26 (MRX35). *J Med Genet* 1996;33:52-5.
- Pilia G, MacMillan S, Nagaraja R, *et al.* YAC/STS map of 9 Mb of Xq26 at 100-kb resolution, localizing 6 ESTs, 6 genes, and 32 genetic markers. *Genomics* 1996;34:55-62.
- Schuler GD, Boguski MS, Stewart EA, *et al.* A gene map of the human genome. *Science* 1996;274:540-6.