Am. J. Hum. Genet. 46:486–491, 1990

Linkage of an Autosomal Dominant Clefting Syndrome (Van der Woude) to Loci on Chromosome 1q

J. C. Murray,* D. Y. Nishimura,* K. H. Buetow,† H. H. Ardinger,* M. A. Spence,† R. S. Sparkes,‡ R. E. Falk,§ P. M. Falk,§ R. J. M. Gardner,‖ E. M. Harkness,# L. P. Glinski,** R. M. Pauli,** Y. Nakamura,†† P. P. Green,‡‡ and A. Schinzel§§

*Department of Pediatrics, University of Iowa, Iowa City; †Fox Chase Cancer Center, Philadelphia; †Department of Psychiatry, University of California, and §Children's Hospital of Los Angeles, University of Southern California, Los Angeles; Departments of ‖Paediatrics and ‖Orthodontics, University of Otago, Dunedin, New Zealand; **Departments of Pediatrics and Medical Genetics, University of Wisconsin, Madison; ††Howard Hughes Medical Institute, Salt Lake City; ‡‡Collaborative Research, Incorporated, Waltham, MA; and §§Institut für Medizinische Genetik der Universität Zurich, Zurich

Summary

Van der Woude syndrome (VWS) is an autosomal dominant disorder in which affected individuals have one or more of the following manifestations: cleft lip, cleft palate, hypodontia, or paramedian lower-lip pits. VWS is a well-characterized example of a single-gene abnormality that disturbs normal craniofacial morphogenesis. As a first step in identifying genes involved in human development, we used a candidate-gene-and-region approach to look for a linkage to VWS. Six families with 3 or more generations of affected individuals were studied. Evidence for linkage (θ = 0.02, lod score = 9.09) was found between the renin (REN) gene on 1q and VWS. Other linked loci included CR1, D1S58, and D1S53. The genes for laminin B2 (LAMB2), a basement-membrane protein, and for decay-accelerating factor (DAF) were studied as possible candidate genes on 1q. Recombinants between VWS and both LAMB2 and DAF excluded these genes from a causal role in the etiology of VWS for the families studied in this report. Multipoint linkage analysis indicated that the VWS locus was flanked by REN and D1S65 at a lod score of 10.83. This tight linkage with renin and other nearby loci provides a first step in identifying the molecular abnormality underlying this disturbance of human development.

Introduction

Identification of the molecular abnormalities underlying human developmental disturbances will provide insights into the mechanisms of normal morphogenesis and may suggest strategies for the amelioration or prevention of such defects. One opportunity for elucidating such pathways is to use genetic techniques, particularly linkage analysis, to identify the genes involved in such disorders. Such strategies have successfully identified genes for Duchenne muscular dystrophy (Koenig et al. 1987), retinoblastoma (Cavenee et al. 1983), and chronic granulomatous disease (Royer-Pokora et al. 1986). Linkage analysis has also proved successful in identifying the genetic location, if not the specific gene, for a number of other human inherited disorders, including at least one with craniofacial anomalies—an X-linked form of cleft palate (Moore et al. 1987). Such linkages provide the first step in localizing particular genes, although the subsequent move from linkage to gene may prove difficult without the benefit of focusing cytogenetic abnormalities.

The Van der Woude syndrome (VWS) is a human autosomal dominant disorder associated with cleft lip and/or cleft palate and/or hypodontia with paramedian lower-lip pits (Schinzel and Klausler 1986). It has been estimated to account for 1%–3% of all cases of cleft lip and palate and has been described in over 160 published pedigrees (Burdick et al. 1985). We have used both candidate genes and a candidate cytogenetic region to search for a linkage to the gene for VWS, as

Received August 1, 1989; revision received October 17, 1989.

Address for correspondence and reprints: J. C. Murray, M.D., Department of Pediatrics, Division of Medical Genetics, University of Iowa Hospitals, Iowa City, IA 52242.

0002-9297/90/4603-0011$02.00

the preliminary step in establishing the underlying molecular abnormality of this disturbance of human morphogenesis. Six extended families with VWS were identified, and linkage analysis was used to study a battery of RFLPs for candidate genes and loci in a candidate region. Several growth factors and/or their receptors were studied after being identified, on the basis of their timing of expression or presence in fetal palatal tissue, as candidates for involvement in human cleft lip and palate disorders (Ferguson 1987). These growth factors and receptors included the epidermal growth factor (EGF), the transforming growth-factor alpha (TGFA), the epidermal growth-factor receptor (EGFR), and the glucocorticoid receptor (GRL). We also focused attention on the long arm of chromosome 1 as a candidate cytogenetic region, on the basis of two classes of reports. In one, Bocian and Walker (1987) described an individual with a VWS-like phenotype who had an interstitial deletion for 1q32–1q41. Second, data from previous linkage studies of VWS (Eastman et al. 1978; Spence et al. 1983; Wienker et al. 1987) gave a lod score (Z) of 1.13 with a recombination fraction (θ) of .10 for the Duffy gene, which maps to 1q (Sherman and Bruns 1988). These results suggested that 1q would make a logical first chromosomal location to screen for linkage if candidate genes not on 1q were excluded.

Material and Methods

Clinical Material

Six families (fig. 1) with VWS who had not previously been studied for linkage were ascertained through contact with geneticists, orthodontists, or cleft-palate clinics. Patients were contacted regarding their involvement in this study and were asked to participate, and signed, informed consent was obtained. All patients were examined by one or more clinical geneticists (J.C.M., H.H.A., R.E.F., R.J.M.G., E.M.H., R.M.P., A.S., and Victor McKusick). Individuals who had one or more of cleft lip, cleft palate, hypodontia, or lower-lip pits were considered to be affected. All families contained at least two individuals with lip pits and one of the other manifestations of VWS. Only a single person (IV-1 in VDWS5) had isolated hypodontia and was considered affected. Individuals who were examined and found to have none of the above-listed anomalies were considered unaffected for the purposes of the present study. Medical records of deceased or unavailable individuals were also used to ascertain the presence of one or more of the above-listed physical findings. Thirty-milliliter samples of whole blood were obtained from

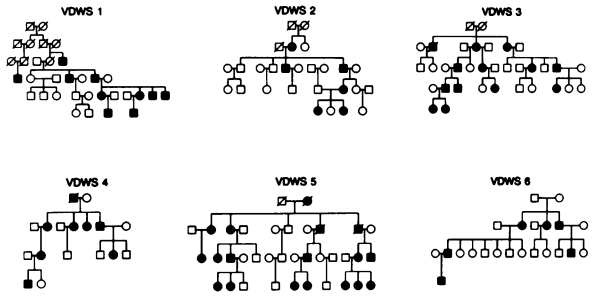

Figure 1 Pedigrees of the six unrelated families used in the linkage study. Darkened symbols indicate affected individuals. Open symbols indicate unaffected individuals, except for the first-generation couple in VDWS 6, who were not examined.

the adults, and 1 ml whole blood/kg body weight was obtained from each of the children in the families studied. Lymphoblastoid cell lines were established for most individuals.

DNA Methodology

Twenty-milliliter samples of blood were used to extract DNA according to a modification of the procedure of Poncz et al. (1982). Following DNA extraction, samples from all family members were digested with restriction enzymes demonstrating the polymorphisms shown in table 1, and the digests were subjected to electrophoresis on 0.8% or 1.2% agarose gels. The DNA was then transferred to Zetabind filters (AMF Cuno) by using 0.4 M NaOH. The filters were prehybridized, hybridized, and washed according to the manufacturer's instructions. DNA probes for the loci reported in table 1 were labeled using the random primer method of Feinberg and Vogelstein (1983, 1984), and the blots were autoradiographed using intensifying screens. RFLPs were scored, and the data was coded in linkage format.

Linkage Studies

Linkage analysis between the VWS locus and marker loci was performed in the six families by using the lod-score method (Morton 1955). The VWS locus was modeled as an autosomal dominant, two-allele system. The disease allele was assumed to have a frequency of .001 and was taken to be 90% penetrant in heterozygotes (Schinzel and Klausler 1986). Lod tables for the pairwise recombination estimates were calculated assuming $\theta_m = \theta_f$ and using the LINKAGE (version 4.7) program MLINK (Lathrop et al. 1984, 1985). Maximum-likelihood estimates of sex-averaged and sex-specific recombination were obtained by using the LINKAGE program ILINK. Significance was evaluated

Table 1

Description of Linkage Markers

Gene/Locus	Probe Name	Polymorphic Enzyme(s)	Map Location	Reference
REN	pHRnES1.9	*Hin*dIII, *Bgl*I, and *Mbo*I	1q32-q42	McGill et al. 1987; Nakai et al. 1988
D1S52.....	CRI-L112	*Bam*HI	1	Donis Keller et al. 1987
D1S53.....	CRI-L673	*Msp*I	1	Donis-Keller et al. 1987
LAMB2.....	lamb2	*Msp*I and *Alu*I	1q25-q31	Nishimura et al. 1988
D1S58.....	pYNZ23	*Msp*I	1q	Nakamura et al. 1987
D1S59.....	pHHH119	*Msp*I	1q	Hoff et al. 1988
D1S65.....	pEKH7.4	*Taq*I	1	Kumlin-Wolff et al. 1987
AT3	pA62	*Pst*I	1q23	Bock et al. 1982
CR1	pCR1	*Hin*dIII	1q32	Carroll et al. 1988
CR2	pCR2-1.6	*Taq*I	1q32	Carroll et al. 1988
DAF	pRSV.DAF	*Hin*dIII	1q32	Caras et al. 1987
EGFR	A64	*Hin*dIII, *Stu*I, and *Pst*I	7p13-p12	Smith et al. 1987
TGFA	phTGF1-10-925	*Bam*HI and *Rsa*I	2p13	Murray et al. 1986*a*
EGF	phEGF121	*Hin*cII and *Sac*I	4q25	Murray et al. 1986*b*
GRL	OB7	*Bcl*I	5q11-q13	Murray et al. 1987

NOTE.—Additional information on map locations and RFLPs can be obtained through the Human Gene Mapping Library at Yale University.

using the standard criterion ($Z \geq 3.0$). Multipoint linkage analysis was performed with VWS and with markers in the chromosomal region showing significant linkage with the disease locus. The localization within a multipoint map was performed utilizing the location-score method (Cook et al. 1974; Lathrop et al. 1985) as implemented in the LINKAGE program LINKMAP. Specifically, the likelihood of the VWS locus was evaluated in all possible locations of a fixed multilocus map. A multilocus Z was calculated for each possible location by contrasting the likelihood of this position with the likelihood that the VWS locus was unlinked to the multilocus map. An interval was excluded as containing the VWS locus if its relative likelihood was 1,000 times less likely than the best (maximum-likelihood) location. A 1-lod difference from the maximum-likelihood estimate was used to construct a support (confidence) interval (Conneally et al. 1985).

Results

No significant linkage was detected between VWS and EGF, EGFR, TGFA, or GRL (data available from J.C.M. on request). However, four chromosome 1q markers showed significant linkage with VWS: REN, D1S53, CR1, and D1S58. Two others, D1S52 and CR2, approached significance. A summary of the pairwise Z analysis for chromosome 1 markers is presented in table 2. Z values for linkage of REN and VWS, for each family studied, are given in table 3. There was

Table 2

VWS Linkage with 1q Markers

	$\theta_m = \theta_f$											
VWS vs.	0	.001	.05	.10	.20	.30	.40	Z_{max}	$\hat{\theta}$	$Z(m,f)$	$\hat{\theta}_m$	$\hat{\theta}_f$
REN.....	7.15	8.22	8.85	8.05	6.03	3.79	1.61	9.09	.02	9.28	0	.03
D1S53	−.94	1.12	3.83	3.79	3.11	2.18	1.14	3.87	.07	4.73	0	.18
CR1	−3.95	−1.47	3.14	3.43	2.87	1.83	.74	3.43	.10	3.43	.09	.10
D1S58	−.08	1.13	2.83	2.82	2.21	1.34	.49	2.88	.07	3.16	.12	0
D1S52	0.57	1.31	2.71	2.69	2.19	1.47	.66	2.74	.07	2.99	0	.11
CR2	−.22	.76	2.40	2.44	1.93	1.21	.53	2.46	.08	2.74	0	.12
DAF	−2.14	−1.86	1.35	1.92	1.81	1.18	.50	1.99	.13	2.02	.09	.15
D1S65	−.90	−.43	1.82	1.95	1.58	.10	.42	1.95	.09	1.97	.13	.06
LAMB2	−6.27	−4.10	−.36	.54	1.13	1.01	.56	1.14	.22	1.17	.26	.19

Table 3

REN-VWS Linkage by Family

Family	$\theta_m = \theta_f$ 0	.001	.05	.10	.20	.30	.40	Z_{max}	$\hat{\theta}$	$Z(m,f)$	$\hat{\theta}_m$	$\hat{\theta}_f$
VWS 1	2.77	2.77	2.47	2.17	1.54	.91	.34	2.77	0	2.77	0	0
VWS 2	1.68	1.68	1.55	1.41	1.06	.68	.31	1.68	0	1.69	.10	0
VWS 3	1.39	1.39	1.25	1.10	.79	.48	.21	1.39	0	1.39	0	0
VWS 4	−2.04	−.96	.56	.70	.67	.56	.27	.72	.14	1.20	0	.35
VWS 5	.81	.81	.73	.65	.48	.31	.15	.81	0	.81	0	0
VWS 6	2.54	2.54	2.29	2.02	1.48	.91	.34	2.54	0	2.54	0	0
Overall	7.15	8.23	8.85	8.05	6.02	3.85	1.62	9.91	.02	10.40	0	.03

no evidence of heterogeneity of linkage (data not shown) when Smith's test of homogeneity of θ values was utilized (Smith 1963).

Multipoint linkage analysis was used to determine the best location for VWS in this region of chromosome 1. Location-score analysis was performed utilizing a five-locus map of anchor loci surrounding the REN locus. The anchor map was constructed utilizing chromosome 1 RFLP typing from the Centre d'Etude du Polymorphism Humain reference pedigree panel (Buetow et al. 1989). The location-score analysis and anchor map are presented in figure 2. The best location for VWS was observed to be flanked by REN and D1S65, with a θ of .04 with REN and .10 with D1S65. The 1-lod support interval placed VWS in the region .01–.11 proximal to REN (.12–.03 distal to D1S65). VWS could be excluded, with odds of 175:1, from the more distal REN-D1S58 interval. VWS could be excluded, with odds of greater than 1,000:1, from lying in the region proximal to D1S65. VWS could not be excluded, by this criterion, from other intervals within the anchor map.

Pairwise and multipoint linkage analysis were utilized to eliminate two genes localized to this region of chromosome 1 as the candidate genetic lesion in VWS. The first, laminin B2 (LAMB2), is a component of the laminin molecule found in basement membranes. LAMB2 has been shown to map proximal to REN and D1S65 (Nishimura et al. 1988; Buetow et al. 1989). Recombinants in affected individuals (table 2) and multipoint analysis (fig. 2) exclude with high likelihood both LAMB2 and genes immediately adjacent to it as being causative in VWS. The second candidate, decay-accelerating factor (DAP), is a part of a gene family whose members participate in programmed cell death. Recombinants in affected pedigree members exclude, with high likelihood, DAF as the VWS gene. However, multipoint analysis does not exclude other gene family members in the same region as possible candidates.

We did not observe any aberrant bands on Southern blots of VWS families when the REN, DAF, CR1, or D1S53 probes were used. This would exclude gross structural rearrangements of the DNA in the immediate vicinity of these loci as being the cause of VWS. Studies using transverse alternating-field electrophoresis are now underway and should be able to screen a larger region surrounding these loci for structural rearrangements leading to VWS.

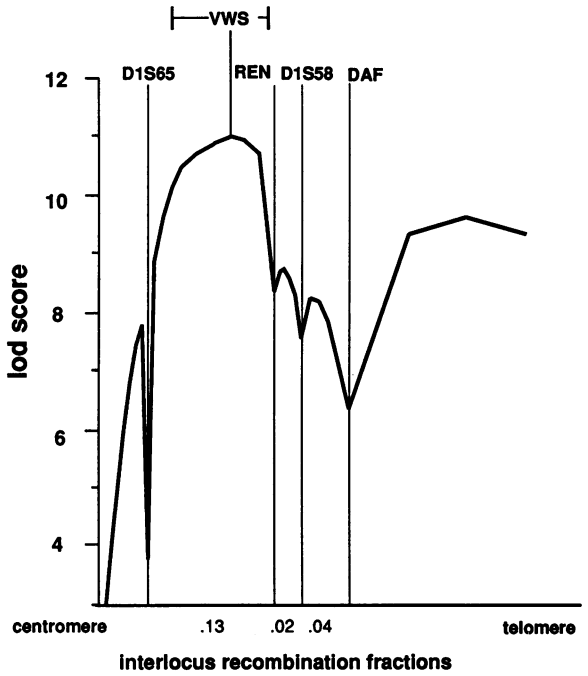

Figure 2 Results of location-score analysis of VWS for region surrounding REN. Brackets flanking VWS indicate 1-lod support interval. Anchor-map distances are presented as frequency of recombination for each interval.

Discussion

The present study reports evidence for linkage between an autosomal dominant form of cleft lip and cleft palate (i.e., VWS) and four loci whose shortest region of overlap is 1q32–1q41. A maximum multipoint Z of 10.83 (fig. 2) with this cluster of markers provides strong evidence that, at least in the families studied, the gene for VWS maps to this region. The 95% confidence interval (1-lod interval) would place VWS within 9 map units—or roughly 9×10^6 bp—of DNA.

Development of the lip and palate is a complex process that is affected by genetic and environmental components. The opportunity to identify specific mechanisms underlying such disturbed development may provide insights into other normal and abnormal developmental processes in humans. The 1q32-q41 regional assignment for the renin gene provided two other nearby genes which were potential candidates for VWS. These were the LAMB2 gene and DAF genes. Recombinants between LAMB2 RFLPs and DAF RFLPs with affected individuals with VWS excluded these genes. Several other candidate genes had been excluded in the initial linkage search.

The genetic analysis of cleft lip and cleft palate done by Fogh-Anderson (1942) in the 1940s and continued by Fraser (see Curtis et al. 1961), Carter et al. (1982), and others has suggested that, in the nonsyndromic forms of cleft lip and cleft palate, cleft lip with or without cleft palate is genetically distinct from cleft palate alone. VWS is one of only a small number of disorders in which the two anomalies may be seen to segregate as components associated with the same gene (Burdick et al. 1985). Thus, it will be of considerable utility for our understanding of normal palatal development to identify an abnormality that can allow either or both of these conditions to result from the same genetic disturbance.

The present study provides an initial step toward the identification of the VWS gene. Opportunities for prenatal diagnosis and detection of asymptomatic carriers of VWS can be undertaken either in families showing significant linkage or when heterogeneity has been minimized by studies of additional families. Identification of the molecular abnormality underlying one form of cleft lip and cleft palate and its variants will provide the groundwork on which additional studies of biochemistry, cell-cell interaction, and tissue localization and expression can be built. This gene may have homology with other genes involved in common human developmental disturbances, such as neural tube defects or congenital heart disease. Future studies may thus provide insights into the detection, amelioration, or prevention of a number of different birth defects.

Acknowledgments

We thank Nancy Newkirk for outstanding administrative and secretarial assistance and Dr. Janusz Bardach, Dr. Duane VanDemark, Barbara Bernhardt, and Dr. Victor McKusick for providing us with the names of VWS families. Drs. R. Lawn (Genentech; AT3), I. Caras (Genentech; DAF), A. Ullrich (Genentech; EGFR), R. Evans (Salk Institute; GRL), G. Bell (Howard Hughes Medical Institute; EGF and TGFA), and Y. Yamada (NIH/NIDR; LAMB2) supplied probes, as did the American Type Culture Collection (REN and CR2). CEPH supplied DNA for family studies and software for analysis. Kaitlin Murray, Andrew Ardinger, Patrick Ardinger, and Claire Buetow provided technical support. We are particularly appreciative of the help and cooperation of the families themselves, without whom this study would not have been possible. The coordinating efforts of Bonnie Farland and Cheryl Tumminia are specially noted. This work was supported by NIH grants DE08559 (J.C.M.) and GM07091 (D.Y.N.).

References

Bocian M, Walker AP (1987) Lip pits and deletion 1q32-41. Am J Med Genet 26:437–443

Bock SB, Wion KL, Vehar GA, Lawn RM (1982) Cloning and expression of the cDNA for human antithrombin III. Nucleic Acids Res 10:8113–8125

Buetow KH, Nishimura D, Green P, Nakamura Y, Schull M, Weber J, Murray JC (1989) A multipoint genetic map and new RFLPs for human chromosome 1. Cytogenet Cell Genet 50:972

Burdick AB, Bixler D, Puckett CL (1985) Genetic analysis in families with Van der Woude syndrome. J Craniofac Genet Dev Biol 5:181–208

Caras IW, Davitz MA, Rhee L, Weddell G, Martin DW, Nessenzweig V (1987) Cloning of decay-accelerating factor suggests novel use of splicing to generate two proteins. Nature 325:545–549

Carroll MC, Alicot EM, Katzman PJ, Klickstein LB, Smith JA, Fearon DT (1988) Organization of the genes encoding complement receptors type 1 and 2, decay-accelerating factor, and C4-binding protein in the RCA locus on human chromosome 1. J Exp Med 167:1271–1280

Carter CO, Evans K, Coffey R, Fraser Roberts JA, Fraser Roberts M (1982) A three-generation family study of cleft lip with or without cleft palate. J Med Genet 19:246–261

Cavenee WK, Dryja TP, Phillips RA, Benedict WF, Godbout R, Gallie BL, Murphree AL, et al (1983) Expression of recessive alleles by chromosomal mechanisms in retinoblastoma. Nature 305:779–784

Conneally PM, Edwards JH, Kidd KK, Lalouel J-M, Morton

NE, Ott J, White R (1985) Report of the Committee on Methods of Linkage Analysis and Reporting. Cytogenet Cell Genet 40:356–359

Cook PJL, Robson EB, Buckton KE, Jacobs PA, Polani PE (1974) Segregation of genetic markers in families with chromosome polymorphisms and structural rearrangements involving chromosome 1. Ann Hum Genet 37:261–274

Curtis EJ, Fraser FC, Warburton D (1961) Congenital cleft lip and palate: risk figures for counseling. Am J Dis Child 102:853–857

Donis-Keller H, Green P, Helms C, Cartinhour S, Weiffenbach B, Stephens K, Keith TP (1987) A genetic linkage map of the human genome. Cell 51:319–337

Eastman JR, Bixler D, Escobar V (1978) Linkage studies in Van der Woude syndrome. J Med Genet 15:217–218

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

——— (1984) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity: addendum. Anal Biochem 137:266–267

Ferguson MWJ (1987) Palate development: mechanisms and malformations. Ir J Med Sci 156:309–315

Fogh-Anderson P (1942) Inheritance of harelip and cleft palate. Busck, Copenhagen

Hoff M, Nakamura Y, Holm T, Ballard L, O'Connell P, Leppert M, Lathrop GM, et al (1988) Isolation and mapping of a polymorphic DNA sequence (pHHH119) on chromosome 1 (D1S59). Nucleic Acids Res 16:4741

Koenig M, Hoffman EP, Bertelson CJ, Monaco AP, Feener C, Kunkel LM (1987) Complete cloning of the Duchenne muscular dystrophy (DMD) cDNA and preliminary genomic organization of the DMD gene in normal and affected individuals. Cell 50:509–517

Kumlin-Wolff E, Nakamura Y, Hoff M, O'Connell P, Leppert M, Lathrop GM, Lalouel JM, et al (1987) Isolation and mapping of a polymorphic DNA sequence pEKH7.4 to chromosome 1 (D1S65). Nucleic Acids Res 15:9621

Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 81:3443–3446

——— (1985) Multilocus linkage analysis in humans: detection of linkage and estimation of recombination. Am J Hum Genet 37:482–498

McGill JR, Chirgwin JM, Moore CM, McCombs JL (1987) Chromosome localization of the human renin gene (REN) by in situ hybridization. Cytogenet Cell Genet 45:55–57

Moore GE, Ivens A, Chambers J, Farrall M, Williamson R, Page DC, Bjornsson A, et al (1987) Linkage of an X-chromosome cleft palate gene. Nature 326:91–92

Morton NE (1955) Sequential tests for the detection of linkage. Am J Hum Genet 7:277–318

Murray JC, Buetow KH, Bell GI (1986*a*) RFLPs for transforming growth factor alpha (TGFA) gene at 2p13. Nucleic Acids Res 14:7136

Murray JC, DeHaven CR, Bell GI (1986*b*) RFLPs for epidermal growth factor (EGF), a single copy sequence 4q25-4q27. Nucleic Acids Res 14:5117

Murray JC, Smith RF, Ardinger HA, Weinberger C (1987) RFLP for the glucocorticoid receptor (GRL) located at 5q11-5q13. Nucleic Acids Res 15:6765

Nakai H, Inoue S, Miyazaki H, Murakami K, Tada K (1988) Human renin gene assigned to chromosome band 1q42 by in situ hybridization. Cytogenet Cell Genet 47:90–91

Nakamura Y, Culture M, Gillilan S, O'Connell P, Leppert M, Lathrop GM, Lalouel JM, et al (1987) Isolation and mapping of a polymorphic DNA sequence pYNZ23 to chromosome 1 (D1S58). Nucleic Acids Res 15:9620

Nishimura D, Buetow KH, Yamada Y, Murray JC (1988) RFLPs and linkage relationships of the human laminin B2 gene. Genomics 3:393–395

Poncz M, Solowiejczyk D, Harpel B, Mory Y, Schwartz E, Surrey S (1982) Construction of human gene libraries from small amounts of peripheral blood: analysis of β-like globin genes. Hemoglobin 6:27–36

Royer-Pokora B, Kunkel LM, Monaco AP, Goff SC, Newburger PE, Baehner RL, Cole FS, et al (1986) Cloning the gene for an inherited human disorder—chronic granulomatous disease—on the basis of its chromosomal location. Nature 322:32–38

Schinzel A, Klausler M (1986) The Van der Woude syndrome (dominantly inherited lip pits and clefts). J Med Genet 23:291–294

Sherman SL, Bruns GA (1988) Report of the Committee on the Genetic Constitution of Chromosome 1. Cytogenet Cell Genet 49:39–45

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

Smith RF, Ardinger HH, Murray JC (1987) Multiple RFLPs demonstrated for epidermal growth factor receptor (EGFR) on chromosome 7. Nucleic Acids Res 15:6764

Spence MA, Glass L, Crandall BF, Stewart RE, Miles J, Falk RE, Field LL, et al (1983) Genetic linkage studies with cleft lip and palate: report of two family studies. J Craniofac Genet Dev Biol 3:207–212

Wienker TF, Hudek G, Bissbort S, Mayerova A, Mauff G, Bender K (1987) Linkage studies in a pedigree with Van der Woude syndrome. J Med Genet 24:160–162