

# Autozygosity Mapping, to Chromosome 11q25, of a Rare Autosomal Recessive Syndrome Causing Histiocytosis, Joint Contractures, and Sensorineural Deafness

Leanne M. Moynihan,<sup>1</sup> Sarah E. Bunday,<sup>3</sup> David Heath,<sup>5</sup> E. Lynn Jones,<sup>4</sup> Duncan P. McHale,<sup>1,2</sup> Robert F. Mueller,<sup>2</sup> Alexander F. Markham,<sup>1</sup> and Nicholas J. Lench<sup>1</sup>

<sup>1</sup>Molecular Medicine Unit, University of Leeds, and <sup>2</sup>Department of Clinical Genetics, St. James's University Hospital, Leeds; and Departments of <sup>3</sup>Clinical Genetics, Birmingham Women's Hospital, and <sup>4</sup>Pathology, University of Birmingham, and <sup>5</sup>Department of Medicine, Queen Elizabeth Hospital, Birmingham, United Kingdom

## Summary

We describe a highly consanguineous family, originating from Pakistan, displaying histiocytosis, joint contractures, and sensorineural deafness. The form of histiocytosis exhibited by this family does not fit readily into any of the recognized classes of this disease. It appears to represent a novel form of familial histiocytosis demonstrating autosomal recessive inheritance. Using autozygosity mapping, we have identified a homozygous region of ~1 cM at chromosome 11q25, in affected individuals. A maximum two-point LOD score of 3.42 (recombination fraction  $\theta = .00$ ) was obtained with marker D11S968. This is the first genetic locus to be described that is involved in the molecular pathogenesis of histiocytosis.

## Introduction

The histiocytoses are an unusual group of diseases caused by the abnormal proliferation and/or accumulation of histiocytes (Cline 1994; Pritchard and Broadbent 1994). Histiocytes arise, in the bone marrow, from pluripotent CD34+ hemopoietic stem cells and differentiate into either mononuclear phagocytes or antigen-presenting cells. The prime purpose of the monocyte/macrophage lineage is to phagocytose other cells and process antigens. The first class of histiocyte is found in the sinuses of the lymph nodes and spleen and in the liver and lungs. Abnormal proliferation of these cells

leads to excessive phagocytosis of platelets and red and white blood cells and can progress to a malignant monocytic leukemia. Antigen-presenting histiocytes are non-phagocytic and have a dendritic morphology. The second class of histiocyte can be further subdivided into (1) lymphoid dendritic cells of the blood, (2) Langerhans cells, and (3) dendritic and interdigitating reticulum cells of the lymph node sinusoids. Abnormal proliferation of these histiocytes can cause either Langerhans cell histiocytosis (LCH) or malignant histiocytosis.

A classification of histiocytosis syndromes has been proposed by the Writing Group of the Histiocyte Society (1987) and subsequently has been reviewed and updated by Cline (1994) and Pritchard and Broadbent (1994) (table 1). The class I and class II histiocytoses represent the most prevalent forms of the disease, with malignant histiocytosis accounting for  $\leq 1\%$  of all histiocytosis. In general, histiocytoses are nongenetic. Familial forms are rare and have been described only for hemophagocytic lymphohistiocytosis (MIM 267700) and Letterer-Siwe disease (MIM 246400).

We have used autozygosity mapping to identify a linked genetic locus in a large consanguineous Pakistani family segregating histiocytosis, joint contractures, and sensorineural deafness. The histiocytosis observed in this family does not fit clearly into any of the four classes proposed above, since affected individuals survive into adulthood and do not exhibit abnormal destruction of peripheral blood cells. In addition, there is no obvious involvement of the lungs or bones, and patients do not have enlarged livers or spleens. We hypothesize that this is a novel form of autosomal recessive familial histiocytosis, called "Faisalabad histiocytosis."

## Subjects and Methods

### Study Subjects: Clinical Descriptions

*Proband VII:7.*—The index patient presented at the age of 3 years with rubbery swellings in her eyelids. These were excised and histological analysis of these

Received October 15, 1997; accepted for publication March 17, 1998; electronically published April 10, 1998.

Address for correspondence and reprints: Dr. Nicholas J. Lench, Molecular Medicine Unit, Clinical Sciences Building, St. James's University Hospital, Leeds, United Kingdom LS9 7TF. E-mail: desnl@stjames.leeds.ac.uk

© 1998 by The American Society of Human Genetics. All rights reserved. 0002-9297/98/6205-0019\$02.00

**Table 1****Classification of Histiocytosis Syndromes**

Class	Syndromes
I: LCH <sup>a</sup>	Letterer-Siwe disease (MIM 246400) Hand-Schüller-Christian disease
II: hemophagocytic lymphohistiocytosis	Genetic (MIM 267700) Sporadic
III: malignant disorders of histiocytes	Acute monocytic leukaemia Malignant histiocytosis
IV: other histiocytosis syndromes	Sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman syndrome) Xanthogranuloma Reticulohistiocytoma Faisalabad histiocytosis <sup>b</sup>

<sup>a</sup> Formerly known as histiocytosis X.

<sup>b</sup> Described in this report.

swellings revealed deposits of histiocytes with increased numbers of plasma cells and eosinophils. At the ages of 6 and 7 years, the swellings recurred (requiring hospital admission on both occasions), and the proband displayed systemic symptoms, with failure to thrive and generalized lymphadenopathy but no enlargement of liver or spleen. Additional repeated hospital admissions occurred at the ages of 9, 10, and 13 years because of recurrent swellings of the eyelids, cervical lymph glands, and nasal mucosa. Biopsies showed increased numbers of histiocytes and showed lymphoid tissue infiltrated by plasma cells and histiocytes with few eosinophils (Codling et al. 1972). The appearance was of an inflammatory reaction, with destruction of lymphoid follicles and marked proliferation of histiocytes within the sinuses. The histiocytes contained phagocytosed plasma cells and red cells. At the age of 15 years, the proband required emergency hospital admission, for symptoms of dyspnea and stridor, and was found to have fever, lymphadenopathy, proptosis, raised erythrocyte sedimentation rates (ESRs), and hypergammaglobulinemia. No abnormality of circulating red or white blood cells was detected, although her bone marrow showed diverse cytoplasmic inclusions in phagocytes and reticulum cells. There was diffuse infiltration of the nasal mucosa and respiratory tract with lipogranulomatous material. Following an emergency temporary tracheostomy, she was treated with radiotherapy to the larynx and trachea, together with systemic administration of cyclophosphamide and prednisolone, resulting in a marked improvement of her symptoms. On examination for amenorrhea (cyclophosphamide induced), at the age of 17 years, she was noted to have scant secondary sexual characteristics and ovarian failure. Since then she has been readmitted to the hospital on numerous occasions for breathlessness and swellings of the eyelids. Her current clinical condition is managed by daily administration of prednisolone.

The histological findings, together with the clinical features, prompted comparison with both Hand-Schüller-Christian disease and Rosai-Dorfman disease (Rosai and Dorfman 1969). This diagnosis was again considered likely following immunohistochemical analysis that showed strong staining of histiocytes with CD68 (a macrophage marker) and weak staining of only a few histiocytes with S100 (a marker for Langerhans and interdigitating reticulum cells). However, this case probably represents a distinct clinical entity, since the proband has other clinical problems, as do her affected relatives. She developed increasing sensorineural deafness from the age of 10 years and is now totally deaf. From the age of 13 years, she has developed progressive contractures of the fingers and toes. Examination revealed a short woman (<3d centile) with normal intelligence, sensorineural deafness, and scant secondary sexual characteristics, who had contractures of the elbows, fingers, ankle, and toes. Chromosome studies of the patient and her parents were normal, and radiology has shown no bone or joint lesions. There are three relatives (VI:6, VII:3, and VIII:1) living in Faisalabad who are similarly affected with histiocytosis, joint contractures, and deafness, and, by review of their history, three other deceased relatives were to have been affected (V:8, VI:1, and VI:17).

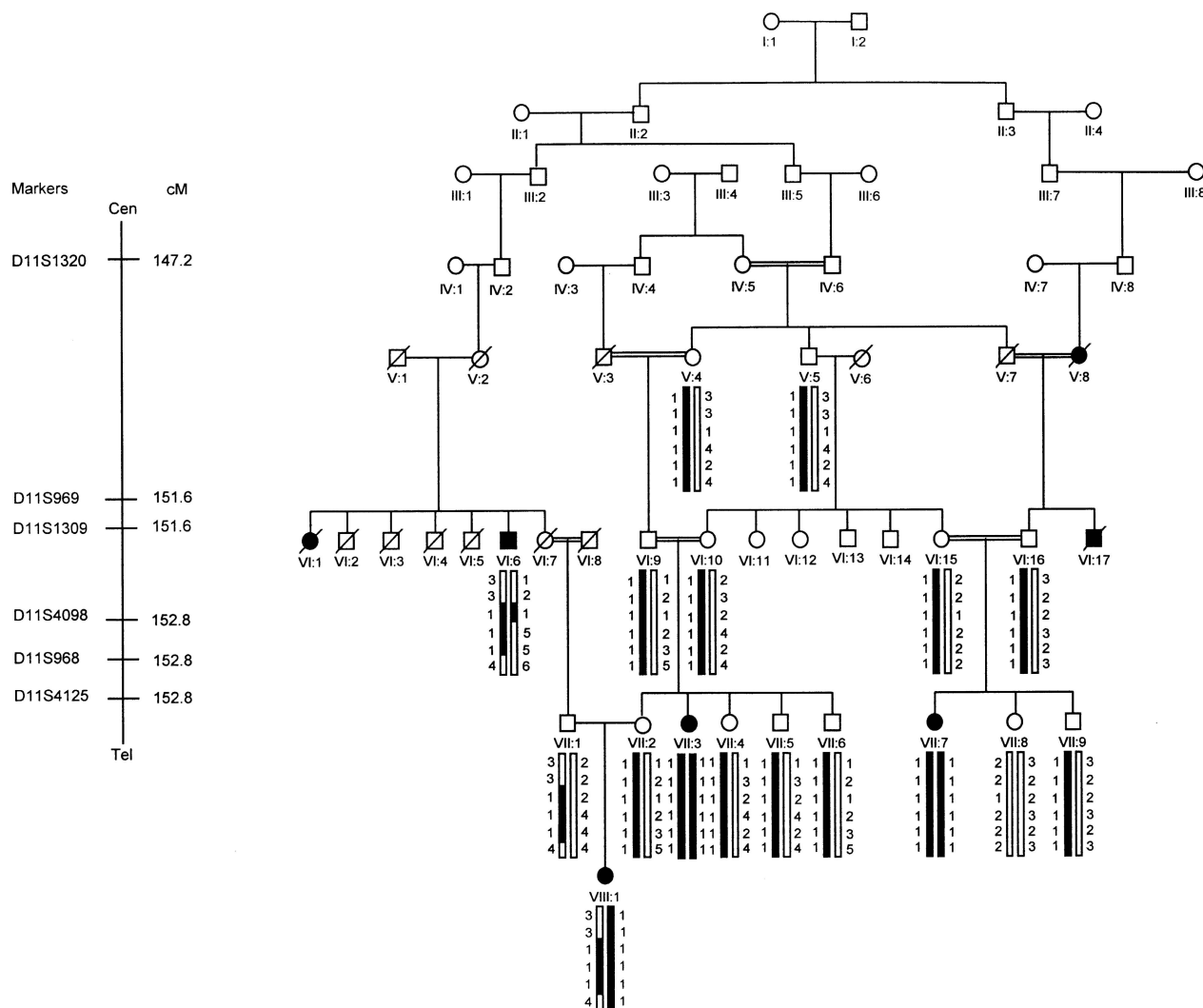
*Subject VI:6.*—Subject VI:6 was born with foot deformities, which have progressed. At the age of 5 years, he was noted to be deaf, and this has progressed so that now he can communicate only by lip reading. At the age of 13 years, he developed swellings above and below both eyes. At the age of 16 years, he developed progressive deformities of his hands. At the age of 50 years, he developed a swelling in his throat, which required surgery. He also had surgery to reduce swelling of his nasal turbinates. On examination at the age of 58 years, he was totally deaf, had a wide and thickened nose, thick ears, a soft swelling under the left eye, flexion deformities of all fingers, and severely deformed feet.

*Subject VII:3.*—Subject VII:3 was noted to have swellings above both eyes, at the age of 6 mo, together with the onset of progressive contractures of her fingers and

**Table 2****Pairwise Linkage Analysis between the Disease Locus and Chromosome 11q25 DNA Markers**

MARKER	LOD SCORE AT $\theta =^a$						
	.00	.001	.050	.1	.2	.3	.4
D11S1320	<u>1.653</u>	1.649	1.425	1.210	.810	.455	.172
D11S969	.061	.343	<u>1.337</u>	1.263	.913	.522	.195
D11S1309	<u>1.376</u>	1.371	<u>1.140</u>	.930	.586	.327	.139
D11S4098	<u>2.017</u>	2.010	1.660	1.299	.632	.152	-.009
D11S968	<u>3.419</u>	3.410	2.955	2.492	1.599	.817	.253
D11S4125	-.057	.224	<u>1.176</u>	1.056	.687	.358	.119

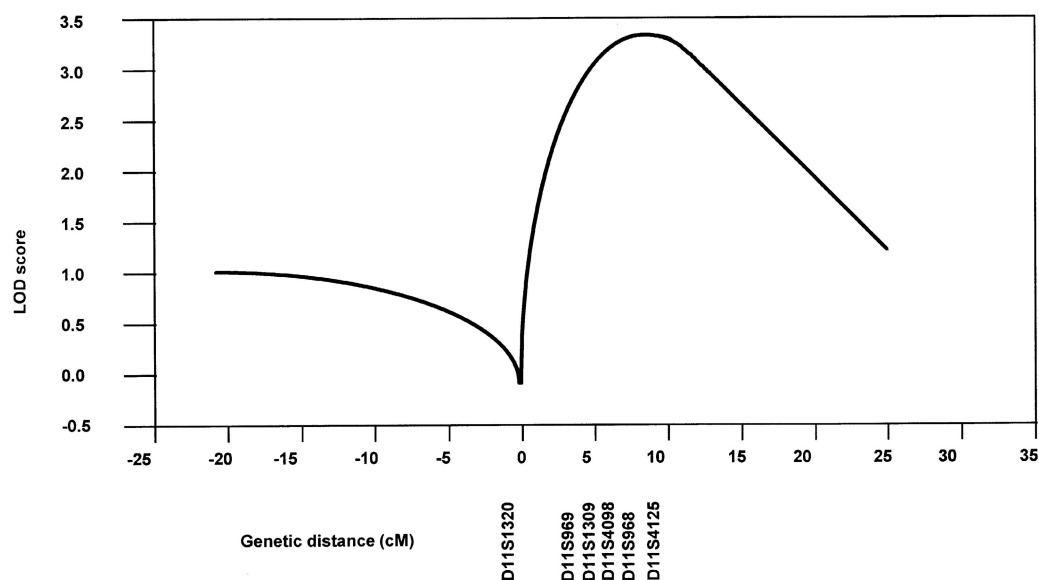
<sup>a</sup> Maximum LOD scores are underlined.



**Figure 1** Pedigree of a highly inbred family displaying features of a syndrome (Faisalabad histiocytosis) causing histiocytosis, joint contractures, and deafness, showing genotypes for six DNA markers from chromosome 11q25. The marker order is cen-D11S1320-D11S4125-tel. Regions of autozygosity are indicated by blackened bars. Blackened symbols denote affected status, and unblackened symbols denote unaffected status. Double lines indicate consanguineous marriages.

toes. At the age of 18 mo, she developed increasing swellings of both cheeks. She was noted to be deaf at the age of 2 years. Examination revealed a short deaf woman without secondary sexual characteristics, with marked swelling of both maxillary salivary glands. She had flexion contractures of elbows, fingers, and toes. There were no bone or joint lesions and no abnormalities of the ears, nose, or mouth, and there was no enlargement of the liver or spleen. Biopsy of a cyst on her right eyelid displayed a thick fibrous wall infiltrated with inflammatory cells, including polymorphs, lymphocytes, eosinophils, and some plasma cells. There was no evidence of malignancy or a viral infection; hematological investigations revealed a low hemoglobin of 8.7 g and a high ESR of 115 mm.

*Subject VIII:1.*—Subject VIII:1 presented, at the age of 6 mo, with progressively enlarging lumps on her eyelids and in the right side of her neck that regressed following administration of steroids. On examination at the age of 3 years, she was not deaf; she had nodules in her eyelids, a swollen right maxillary gland, a few small submandibular nodules, impalpable liver and spleen, and clinodactyly of her little fingers. Her salivary gland was biopsied, and myoepithelial proliferation of the duct epithelium was observed, with massive infiltration by lymphoid tissue, composed of lymphocytes, histiocytes, plasma cells, and reticulum cells. However, there was no evidence of malignancy, and the appearances were those of a benign lymphoepithelial lesion. Twenty milliliters of peripheral blood were collected from 16 consenting



**Figure 2** Multipoint analysis (combined LOD scores from three sibships)

individuals, in accordance with local ethical committee regulations.

#### Genotyping

A low-resolution (15–20 cM) genome screen was performed by use of a fluorescently labeled primer set from the Human Genome Mapping Project of the U.K. Medical Research Council (MRC) (Reed et al. 1994). DNA was prepared from whole blood or from Guthrie card blood spots (individual VIII:1) by use of a standard non-organic method. Polymorphic microsatellite DNA markers were amplified by PCR. PCR was performed in a 10- $\mu$ l reaction volume containing 40 ng genomic DNA, 3 pmoles of each oligonucleotide primer, 1 U *Taq* DNA polymerase (Promega), 10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.1% Triton X-100, 2 mM dNTPs, and MgCl<sub>2</sub> to a final concentration of 1, 1.5, or 2 mM. PCR was performed as follows: an initial denaturation step at 95°C for 5 min, followed by 30–35 cycles at 92°C for 30 s, the annealing temperature for 30 s, and 72°C for 30 s, with a final extension at 72°C for 5 min. PCR products were electrophoresed at 2,400 V, in 1  $\times$  Tris borate-EDTA/6% polyacrylamide gels, for 3.5 h on an ABI 373 DNA sequencer. Gels were processed by use of the Genescan analysis software, version 2.0.2 (Applied Biosystems). Genotypes were generated by use of the Genotyper software, version 1.1 (Applied Biosystems).

#### Statistical Analysis

Linkage analysis was performed by use of the LIPED program for two-point analysis (Ott 1974) and the

HOMOZ program for multipoint analysis (Kruglyak et al. 1995). A fully penetrant autosomal recessive trait was assumed for analysis, and a disease gene frequency of .0001 was used. Equal recombination frequencies between males and females were assumed. Allele frequencies for microsatellite markers were determined by genotyping 80 unrelated, unaffected Pakistani individuals.

#### Results

A low-resolution genome screen was performed by use of 252 fluorescently labeled microsatellite markers. Sixteen individuals from the pedigree were initially genotyped, including three of the four living affected patients. Affected individuals VII:3, VII:7, and VIII:1 were noted to be homozygous for D11S968, the most telomerically mapping panel marker on chromosome 11q. Therefore, genotypes were obtained for an additional five markers (D11S1320, D11S969, D11S1309, D11S4098, and D11S4125) from an ~6-cM interval encompassing D11S968 (fig. 1). Marker order and distances were deduced from published maps (Murray et al. 1994; Dib et al. 1996; Schuler et al. 1996): cen-D11S1320-(D11S969, D11S1309)-D11S4098-(D11S968, D11S4125)-tel. Individuals VII:3 and VII:7 were homozygous for all five additional markers. Individual VIII:1 was homozygous for markers D11S1309, D11S4098, and D11S968 but not for markers D11S1320, D11S969, and D11S4125. Pairwise linkage analysis generated a maximum LOD score of 3.42, at recombination fraction  $\theta = .00$ , with marker D11S968 (table 2). Multipoint linkage analysis was also per-

formed, by use of the HOMOZ program. However, analysis could be performed only when the pedigree was split into three separate sibships and the LOD scores combined (fig. 2). In autozygosity mapping, the minimum critical region is defined as the smallest interval encompassing the region of homozygosity. Analysis of genotypes from individuals VII:3, VII:7, and VIII:1 revealed a common region of homozygosity encompassed by markers D11S969 and D11S4125. Individual VI:6 shares a high-risk haplotype (3-3-1-1-4) with individuals VII:1 and VIII:1 and is homozygous only for marker D11S1309. Therefore, this further reduces the critical interval to an ~1.2-cM region encompassed by D11S969 and D11S4098.

## Discussion

The histiocytoses are an unusual and intriguing group of diseases. The LCHs represent the most widely studied form of the disease, although their etiology and pathogenesis remain unclear (Egeler and D'Angio 1995). The familial histiocytoses are rare, and little is known about the molecular basis of these disorders. Confusion in terminology and classification persists, although guidelines suggested by the Writing Group of the Histiocyte Society (1987) and by Cline (1994) and Pritchard and Broadbent (1994) have helped to clarify this confusion.

Autozygosity mapping of large consanguineous families segregating histiocytosis offers a unique approach to mapping single genes involved in this rare disease process. In this way, we have now identified a novel locus causing histiocytosis, joint contractures, and sensorineural deafness in a consanguineous family originating from Pakistan. The form of histiocytosis found within this family does not fit readily into any of the previously reported classes of the disease. Given the high coefficient of inbreeding within this family and the unique clinical and histological features, we hypothesize that this represents a novel, autosomal recessive form of familial histiocytosis, called "Faisalabad histiocytosis."

We have mapped the smallest region of homozygosity in this family to an ~1.2-cM interval between D11S969 and D11S4098 (Dib et al. 1996). Given the high coefficient of inbreeding within the pedigree, this represents a small homozygous region. However, meiotic recombination events tend to occur more frequently in telomeric regions, thereby reducing the area of observed homozygosity (Watkins et al. 1994). The culture of the population from which this family is derived makes it highly likely that even an individual marrying into the family is distantly related. This increases the likelihood that the mutation could enter the family on more than one occasion. Haplotype data suggest that one of the disease alleles in affected individual VI:6 may have been

inherited from a more distantly related ancestor (possibly entering the family via individual V:1).

It is possible that this form of histiocytosis is caused by a single gene defect, by independent mutations in two adjacent genes at 11q25, or by deletion of a contiguous region of chromosome 11q25 (i.e., a contiguous gene-deletion syndrome). Hemizygous submicroscopic deletions of chromosome 11q23-q25 have been observed in patients with Jacobsen syndrome (Jacobsen et al. 1973; Pivnick et al. 1996) (MIM 147791). However, none of the characteristic clinical features of Jacobsen syndrome are present in the family described in this report, excluding the likelihood that this form of histiocytosis is a contiguous gene-deletion syndrome. The combination of sensorineural deafness and joint contractures with histiocytosis is intriguing and raises the possibility that independent mutations in two adjacent genes at 11q25 may have occurred. We have identified a consanguineous Pakistani pedigree segregating autosomal recessive sensorineural deafness (in the absence of any other clinical features) that is also linked to this region of chromosome 11q25 (L. Moynihan, unpublished data); this suggests the existence of a novel locus, at 11q25, for autosomal recessive sensorineural deafness. Therefore, the symptoms of histiocytosis and joint contractures may be the result of mutations in an adjacent or very closely linked gene. Given the high degree of consanguinity within this family and within the population isolate as a whole, it is not unlikely that parents may be carriers for two independent but closely linked gene mutations. Also, the manifestation of this condition as a result of mutations in a single gene cannot be excluded. Only following exhaustive screening of appropriate candidate genes at 11q25 and mutation identification will the situation be fully resolved.

At present, there are no obvious candidate genes that map to this region of chromosome 11q25. The cloning and identification of gene(s) causing this unusual form of histiocytosis will facilitate elucidation of the molecular mechanisms underlying this and potentially other members of this complex set of diseases.

## Acknowledgments

We are grateful to Art Boylston for his opinions on the biopsies and to Geoff Woods for sample collection. S.E.B. is very grateful to the subjects' family for their hospitality in Pakistan and to N. Ahmad for his opinions on the biopsies, and she thanks the Health Promotion Research Trust for a travel grant. Work in the authors' laboratories is supported by the Birth Defects Foundation, the Wellcome Trust, the MRC, the Yorkshire Cancer Research Campaign, the Northern and Yorkshire Regional Health Authority, and the West Riding Medical Research Trust.

## References

- Cline MJ (1994) Histiocytes and histiocytosis. *Blood* 84: 2840–2853
- Codling BW, Soni KC, Barry DR, Martin-Walker W (1972) Histiocytosis presenting as swelling of orbit and eyelid. *Br J Ophthalmol* 56:517–530
- Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, et al (1996) A comprehensive map of the human genome based on 5,264 microsatellites. *Nature* 380: 152–154
- Egeler RM, D'Angio GJ (1995) Langerhans cell histiocytosis. *J Pediatr* 127:1–11
- Jacobsen P, Hauge M, Henningen K (1973) An (11;21) translocation in four generations with chromosome 11 abnormalities in the offspring: a clinical, cytogenetical, and gene marker study. *Hum Hered* 23:368–385
- Kruglyak L, Daly MJ, Lander ES (1995) Rapid multipoint linkage analysis of recessive traits in nuclear families, including homozygosity mapping. *Am J Hum Genet* 56: 519–527
- Murray JC, Buetow KH, Weber JL, Ludwigsen S, Scherpbier-Heddema T, Manion F, Quillen J, et al (1994) A comprehensive human linkage map with centimorgan density. *Science* 265:2049–2054
- Ott J (1974) Estimation of the recombination fraction in human pedigrees: efficient computation of the likelihood for human linkage studies. *Am J Hum Genet* 26:773–775
- Pivnick EK, Velagaleti GVN, Wilroy RS, Smith ME, Rose SR, Tipton RE, Tharapel AT (1996) Jacobsen syndrome: report of a patient with severe eye anomalies, growth hormone deficiency, and hypothyroidism associated with deletion 11(q23–q25) and review of 52 cases. *J Med Genet* 33: 772–778
- Pritchard J, Broadbent V (1994) Histiocytosis: an introduction. *Br J Cancer Suppl* 70 23:S1–S3
- Reed PW, Davies JL, Copeman JB, Bennett SB, Palmer SM, Pritchard LE, Gough SCL, et al (1994) Chromosome-specific microsatellite sets for fluorescence-based, semiautomated genome mapping. *Nat Genet* 7:390–395
- Rosai J, Dorfman RF (1995) Sinus histiocytosis with massive lymphadenopathy: a newly recognized benign clinicopathological entity. *Arch Pathol* 87:63–70
- Schuler GD, Boguski MS, Hudson TJ, Hui L, Ma J, Castle AB, Wu X, et al (1996) A gene map of the human genome. *Science* 274:540–546
- Watkins WS, Zenger R, O'Brien E, Nyman D, Eriksson AW, Renlund M, Jorde LB (1994) Linkage disequilibrium patterns vary with chromosomal location: a case study from the von Willebrand factor region. *Am J Hum Genet* 55: 348–355
- Writing Group of the Histiocyte Society, The (1987) Histiocytosis syndromes in children. *Lancet* 1:208–209