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Langerhans' cell histiocytosis: is there a role for genetics?

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Langerhans' cell histiocytosis is an intriguing disorder of undefined pathogenesis, affecting mainly children but with a possible onset at any age. Occasional reports of affected twins are apparently in contrast with the general belief that this is a sporadic disorder. To address the issue of whether a genetic component is in play in Langerhans' cell histiocytosis (LCH), over the last few years we have performed different studies, including a search for familial cases among large patient series, and evaluation of chromosomal aberrations during different stages of the disease. We have documented a high (86%) concordance rate for the disease among identical, but not dizygotic, twins. Furthermore, at least 1% of patients with LCH have another affected member in the same or another generation of the same family.

Altogether our findings of chromosomal instability and familial recurrence, together with the reported increased tumor risk and association with specific alleles in potentially predisposing genes, support the hypothesis that Langerhans' cell histiocytosis should be potentially regarded as a genetic disorder.

The clinical manifestations of Langerhans' cell histiocytosis are widely heterogeneous[1] and efforts to define stringent clinical and pathologic diagnostic criteria[2-4] resulted in better comparison of pathologic and clinical data. Retrospective evaluation of large series of patients has improved our clinical knowledge of LCH, including the isolated or uncommon manifestations,[5,6] and international prospective trials allowed definition of standard treatment.[7] The morbidity of LCH is related to the level of dissemination of the disease, in particular to the number and type of involved tissues. This results in a wide range of clinically relevant manifestations, as well as in sub-clinical alterations. Recognition of the different patterns of clinical involvement have characterized the former history of LCH. Lichtenstein had the great intuition that eosinophilic granuloma,[8,9] Hand-Schuller-Christian[10-12] and (Abt)-Letterer-Siwe[13-15] diseases were not independent diseases. On the contrary, he felt that they shared pathogenesis and enough clinical aspects to be encompassed under the term *histiocytosis X*.[16] This was a breakthrough and the first step towards understanding that LCH is a unique nosologic entity with heterogeneous clinical manifestations.

Pathogenesis of LCH

Over a century after the first reports, the etiology and pathogenesis of LCH remain largely to be defined. Even its incidence remains poorly defined. The disease is readily identified and diagnosed in its disseminated aggressive forms, while in contrast it may be under diagnosed when localized: bone lesions may be painless lumps ascribed to trauma, and mild skin disease may be mistaken for seborrhoic eczema.

LCH has always been considered a sporadic disorder affecting both sexes at any age. Epidemiological studies failed to identify predisposing factors,[17] but an increased risk of hematologic and solid tumors has been clearly identified.[18]

Although localized skin or bone disease is usually regarded as a benign, self-healing disease, a struggling debate has been raised on the possible neoplastic origin of the disseminated form of LCH, associated with a severe course and a high mortality rate.[7] Evidence of clonality obtained by HUMARA assay in the lesions of nine patients led Willman *et al.*[19] to suggest that LCH is a clonal neoplastic disorder with highly variable biological behavior and that gene mutations might be identified. Alternative pathogenic hypotheses include viral infections, whose role in the development of LCH remains controversial despite recent work on screening viral genomes.[20-22]

On the other hand genetic factors may be involved in the pathogenesis of LCH in different ways. Changes in regulatory gene(s) could predis-

Table 1. LCH in monozygotic twins: summary of published cases.

Source (Ref)	*Sex*	*Age at onset (months)*	*Zygosity indicators*	*Disease extent (sites)*
Lightwood 1954	M	6	Blood groups, fingerprint	Disseminated (skin, bone, lymph nodes)
	M	-		None; asymptomatic at 7+ years
Bierman 1966	M	8	Not defined	Disseminated (including bone)
	M	8		Disseminated (including bone)
Caldarini 1966	M	6	Blood groups, fingerprint	Disseminated (skin, hepatosplenomegaly)
	M	6		Disseminated (skin, hepatosplenomegaly)
Juberg 1970	M	12	Blood group	Disseminated (skin, ear)
	M	12		Disseminated (including bone, diabetes insipidus)
Jacobson 1987	M	18	Not defined	Disseminated (bone, proptosis)
	M	11		Disseminated (skin, bone)
Kuwabara 1990	NS	NS	Not defined	Localized (bone)
				Localized (bone)
Katz 1991	M	11	Not defined	Disseminated (with skin + ear)
	M	11		Disseminated (with skin + ear)
Enjolras 1992	M	Birth	Not defined	Localized (skin)
	M	Birth		Localized (skin)
Kanold 1994	F	4	HLA type, DNA analysis	Disseminated (with bone)
	F	4		Disseminated (with bone)

pose to a deranged control of immune response which in turn leads to exuberant cell proliferation or to defective apoptotic mechanisms. Yet, the large number of genes potentially involved in such mechanisms prevents their routine screening in patients with LCH.

Twin studies

Familial clustering has been described in the past, mostly in twins reported or known to be monozygotic (Table 1).[23] We have identified 19 patients with a twin sibling and evaluated the rate of concordance for LCH. Seven of them were assumed to be monozygotic (concordance for sex, blood groups or HLA). Six out of seven pairs were concordant for LCH with similar clinical features, pattern of dissemination, age of onset, which was simultaneous in five (Table 2). Of eight patients with a dizygotic twin, one twin was affected by LCH (Table 3). Of the five twin pairs with unknown zygosity, none was concordant for the disease (Table 4).

These data show a higher rate of concordance for LCH (86%) in presumed monozygotic twins, versus dizygotic ones (12%) and strongly suggests that a genetic component is relevant in the pathogenesis of LCH.

Familial clustering of LCH

If a genetic component is in fact present in LCH, we should expect to observe families in which more than one subject develops the disease. Indeed we have recently reported three families with more than one affected sibling or cousin;[24] the fourth case (Family M) in the same report is now excluded because of unconfirmed paternity. Overall, at present we have evidence of five families in which two siblings (n=3) or first cousins (n=2) developed LCH (Table 5).

Familial recurrence of LCH is not restricted to the same generation: two such families have been previously reported; we have described one father-and-son pair[24] and another mother-and-son pair has been recently identified. One additional two-generation family is currently under evaluation.

Overall, on the basis of available data, one percent of children with LCH have an affected relative;[23] a similar finding has been found in the preliminary data-set obtained by the Histiocyte Society Adult Study Group on adult patients (*Aricò et al., personal communication*).

Genetic models to explain "familial LCH"

The recurrence of LCH in a minority of families may be explained by several models. LCH might occur as a consequence of the expression of a combination of specific alleles of a group of genes con-

Table 2. Main features of seven presumed monozygotic twins with LCH.

Family	*Sex*	*Age at Dx (months)*	*Zygosity indicators*	*Clinical manifestations*	*Course and outcome*
1	M	8	Sex, blood groups	Intrauterine growth retardation; skin (since birth); subsequently bone, spleen, LN, cytopenia, CNS, mouth	Reactivation; CR at 7+ years
	M	8		Intrauterine growth retardation; skin (since birth); subsequently bone, ear, liver, spleen, LN	Reactivation; CR at 7+ years
2	M	4	Sex, blood groups, HLA	Bone, ear, thymus; subsequently LN	Reactivation; CR at 7+ years
	M	4		Bone, ear, LN, thymus	Reactivation; AWD (otitis) at age 7 years
3	M	11	Sex, blood groups, HLA	Skin, ear, lung, bone, BM, HSM	No follow-up
	M	11		Skin, ear, lung, HSM	No follow-up
4	F	1	Sex, HLA	Skin, spleen, thrombocytopenia, lung	CR at age 6 years
	F	NA		None	Asymptomatic at age 6 years
5	M	3	Sex, blood groups, placental examination	Skin, soft tissues, bone, HM	Reactivation; APL at age 8.9; died at age 11 years
	M	3		Skin, soft tissues, bone, HM	Alive and well at age 13.5 years
6	F	4	Sex, HLA	Bone, skin, LN, liver, BM, lung,	Reactivation; CR at an age of 2.9 years
	F	4		Lymph nodes, lung, bone	Reactivation; CR at age of 2.9 years
7	M	2	Phenotype	Ear, maybe bone	CR
	M	42		lymph nodes	CR

Abbreviations. Dx: diagnosis; H(S)M: hepato(spleno)megaly; BM: bone marrow; LN: lymph node(s); CNS: central nervous system; APL: acute promyelocytic leukemia; CCR: complete remission; AWD: alive with disease; NA: not applicable.

Table 3. Main features of eight dizygotic twins with LC.

Family	*Sex*	*Age at Dx (months)*	*Zygosity indicators*	*Clinical manifestations*	*Course and outcome*
8	F	21	Discordant for HLA	Skin, bone, HSM, BM, LN, ear, proptosis	Reactivation; APL at age 5 years
	F	21		Skin, bone, HSM, LN, ear,	CR at age 6 years
9	M	32	Sex	Bone, skin	Reactivation; CR age 9.5 years
	F	NA		None	Asymptomatic at age 9.5 years
10	F	14	Discordant for HLA	Skin, LN, liver	DOD at age 22 months
	F	NA			Asymptomatic at age 3.5 years
11	M	12 y	Bi-amniotic	Bone, skin, LN, ear	Asymptomatic at age 18 years
	M	NA		None	Asymptomatic at age 18 years
12	M	38	Sex	Bone	CR at age 4 years
	F	NA		None	Asymptomatic at age 4 years
13	M	17	Sex	Skin, liver, BM	DOD (early progression?)
	F	NA		None	No FUP
14	F	4	Sex	Bone	Reactivations; CR at age 2.2 years
	M	NA		None	No FUP
15	M	11.5 y	Sex	Bone multifocal	CR at age 26 years
	F	NA		None	Asymptomatic at age 26 years

Abbreviations. Dx: diagnosis; HSM: hepato(spleno)megaly; BM: bone marrow; LN: lymph node(s); DOD: died of disease; CNS: central nervous system; APL: acute promyelocytic leukemia; CCR: complete remission; AWD: alive with disease; NA: not applicable.

Table 4. LCH in twins with unknown zygosity.

Family	Sex	Age at diagnosis	Clinical manifestations	Course and outcome
16	F	1 month	Skin	CR at age 3 years
	F	NA	None	Asymptomatic at age 3 years
17	F	24 days	Skin, bone, lung	CR at age 5.5 years
	F	NA	None	No follow-up
18	M	13 years	Bone	CR at age 19 years
	M	NA	None	No follow-up
19	F	4 months	LN	No follow-up
	F	NA	None	No follow-up
20	M	25 years	Bone multifocal	CR at age 27 years
	M	NA	None	Asymptomatic at age 27 years

ferring susceptibility to the disease. All members of the family who share such alleles also share a propensity to develop the disease. The non-random association between LCH and a specific mannose-binding lectin (MBL) allele constitutes preliminary evidence for such a model.[25] Additional genetic or acquired conditions which may substantially affect the immune response could play a role in a propensity to develop LCH. The observation of histiocytosis in children with associated conditions should be considered carefully. The identification of mutations in the perforin gene as the cause of the cellular cytotoxicity defect in some patients with hemophagocytic lymphohistiocytosis (HLH)[26,27] suggests that histiocytoses may occur as a result of uncontrolled proliferation of lymphocytes and histiocytes in subjects unable to mount an effective immune response against viruses or other infectious agents. Therefore the association of HLH or LCH with Di George/Catch 22 syndromes[28-30] (*Aricò et al, unpublished data*) or with severe combined immune deficiency[31] in five patients is not casual.

An alternative model is the presence of mutations in an *LCH-gene*: in most cases mutation of this putative gene are somatic mutations; in a minority of cases germinal mutations occur and explain familial cases. Genetic heterogeneity and the relationship between simple and complex inheritance are further issues that need to be investigated.

Chromosome studies in LCH

As a genetic component has long been suspected to play a role in the pathogenesis of the disease, extensive chromosomal studies in LCH patients may provide additional insights. Normal karyotypes are usually observed in LCH patients. In 1998 Betts

Table 5. Clinical features and outcome in non-twin cases of familial LCH.

Family	Sex	Age at Dx (months)	Clinical manifestations	Course and outcome
Siblings				
21	M	29	Liver; subsequently spleen, ear, bone, lung, cytopenia	Reactivation; DOD at age 4.5 years
	F	27	Asthma; subsequently skin, bone, lung, LN, anemia	Reactivation; DOD at age 3.5 years
22	F	<12	Bone, skin, liver	Reactivation; DOD at age 15 mo
	M	36	Cheek, soft tissue, skin	No follow-up
23	M	5	Bone	Asymptomatic at 24 years
	M	19	Lung	Asymptomatic at 21 years
First cousins				
24	F	20	Bone, LN, anemia, diabetes insipidus	Reactivation; asymptomatic at 12 years
	M	24	Bone	Reactivation; asymptomatic at 12 years
25	M	9.3 years	Bones	CR at 18 years
	M	<1	Skin, gum	CR, 5 months off therapy (11 months post-diagnosis)
Two generations				
26	F	7 years	Bone (skull)	AWD at age 35 years, one year after osteolytic reactivation
	M	2.5 years	Bone + ST (orbit)	AWD at 4 years

AWD: alive with disease; DOD: died of disease; CR: alive in complete remission.

et al. documented a t(7;12)(q11.2;p13) translocation in unsorted cells cultured from an eosinophilic granuloma.[32] This report prompted many investigators to verify whether the reported breakpoints contained a potential *LCH gene*, which has so far not yet been identified.

We have analyzed the karyotypes of phytohemagglutinin-stimulated peripheral blood lymphocytes from 16 patients with LCH at diagnosis or during the course of the disease. An increased number of chromatid and chromosomal breaks was observed in 13 patients, more often in cases with disseminated disease. Polyploid cells or cells with chromosomal pulverization were also observed in some cases.[33] Our data demonstrate that chromosomal instability is frequent in LCH, in keeping with data reported by Betts, although at that time instability was considered less relevant than the translocation.[32]

Constitutional chromosomal instability is a well known feature of some human conditions[34] which are also associated with an increased risk of neoplasia. Yet these diseases are characterized by a specific phenotype not present in our patients nor ever reported in LCH. Interestingly, instability can be observed in any stage of the disease, including during asymptomatic long-term follow-up, suggesting that it is not dependent on the acute phase of the disease nor on treatment. Nevertheless no evidence is currently available to suggest that instability results from a genetic defect of the DNA-handling mechanisms, and that LCH may represent a new chromosomal instability syndrome.

The association between chromosomal instability and exposure to viral agents has been known for over 25 years[35,36] and was recently confirmed.[37] Evidence of chromosomal pulverization in association with herpes virus[35] and respiratory syncytial virus (*Scappaticci MR, unpublished data*) in children provides further support to the hypothesis that viruses represent a possible trigger of LCH.[20-22] The presence of different types of chromosomal alterations, together with the variability in the number of chromosomal breaks, are in keeping with the hypothesis that the abnormalities are virus-driven. Even in patients in whom clinical viral infections are not reported, a preclinical infection may be difficult to rule out. If chromosomal aberrations are virus-related, this would fit with the hypothesis of a defect in some step of anti-infective mechanisms.

Conclusions

Although the pathogenesis of LCH remains far from being clarified, recently available data strongly suggest a relevant genetic component in the development of the disease. Since familial cases are likely to be underreported, we suggest that a careful family history is taken for every patient with LCH, with special attention to possible localized or oligo-symptomatic diseases in relatives. A joint effort between pediatricians and adult specialists could be the key to revealing the real incidence of the disease and identifying the true genetic component of LCH. Future studies should address the issue of whether a single rare mutation of an *LCH-gene* may account for the disease or otherwise several predisposing factors may concur in the development and expression of LCH. Whenever possible, constitutional and lesional DNA samples from the patient and affected relatives in familial cases should be collected and stored for further investigations. The results of such studies will allow more specific counseling for families and provide tools to refine treatment programs.

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