

Genetic evaluation of juvenile xanthogranuloma: genomic abnormalities are uncommon in solitary lesions, advanced cases may show more complexity

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Juvenile xanthogranuloma is a rare histiocytic proliferation primarily affecting infants and young children, characterized by aberrant infiltration of histiocyte-derived cells in the skin, soft tissues and more rarely, visceral organs. Juvenile xanthogranuloma is generally considered to be a benign disorder; most lesions are solitary cutaneous nodules that resolve spontaneously without treatment. However, cases with extracutaneous involvement, multiple lesions, and/or systemic disease often require aggressive therapy. Though molecular studies have provided evidence of clonality in juvenile xanthogranuloma, in support of a neoplastic process, little is known about the genetic profile of juvenile xanthogranuloma. We used molecular inversion probe array technology to evaluate the genomic characteristics (copy number alterations or copy neutral-loss of heterozygosity) of 21 archived cases of juvenile xanthogranuloma (19 solitary, 1 diffuse cutaneous, 1 systemic). Four cases (19%) showed acquired, clonal alterations. Two lesions from a case of diffuse cutaneous juvenile xanthogranuloma showed distinct profiles: JXG-1a contained trisomy 5 and 17 and JXG-1b contained loss of heterozygosity in 5q. The systemic juvenile xanthogranuloma (JXG-2) showed multiple genomic alterations. Only two of 19 solitary juvenile xanthogranulomas showed abnormal genomic profiles: JXG-3 showed gains on 1q and 11q and JXG-4 showed a 7.2 Mb loss in 3p. No recurrent abnormalities were observed among these cases. The presence of non-recurrent copy number alterations in a subset of samples implies that copy number changes are unlikely driving pathogenesis in juvenile xanthogranuloma, but may be acquired during disease progression. The presence of genomic abnormalities in more advanced cases (ie, systemic and diffuse cutaneous juvenile xanthogranuloma) supports this notion, particularly as the advanced cases of juvenile xanthogranuloma presented more genomic complexity.

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Juvenile xanthogranuloma is an uncommon proliferation of histiocytic lineage primarily affecting infants and young children, with occasional cases

seen in adults.¹ Characterized by aberrant infiltration of histiocyte-derived cells in the skin, juvenile xanthogranuloma is generally considered to be a benign disorder.^{2–4} Although the incidence of juvenile xanthogranuloma has never been truly evaluated in the general population, a retrospective study of the Kiel Pediatric Tumor Registry reported 129 juvenile xanthogranuloma cases diagnosed out of 24 600 registered patients (incidence rate of 0.52%) over a 36-year span.³

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Juvenile xanthogranuloma typically presents as a solitary cutaneous nodule that develops on the head, neck or trunk. Occasionally extracutaneous presentation of nodules occurs within the eye, soft tissues or visceral organs.^{3–5} Systemic juvenile xanthogranuloma, which involves the visceral organs, is rare, occurring in < 5% of cases.^{2,3} Solitary cutaneous nodules often resolve spontaneously without treatment or are treated by simple tumor excision, however, disseminated cases that present as multiple cutaneous lesions or involve the visceral organs in systemic disease may be serious, requiring aggressive therapy.^{3,4}

Histiocytic lesions are diagnosed and characterized primarily through histopathology and immunohistochemistry. Little is known about the genetics of juvenile xanthogranuloma. In addition to a typically early age of onset, observations including a reported case of juvenile xanthogranuloma in monozygotic twins,⁶ as well as identification of *NF1* and *NF2* germline mutations in a subset of patients,^{7–9} indicate that there may be a constitutional predisposition to develop juvenile xanthogranuloma, at least in some patients. *NF1* and *NF2* encode proteins that function within the mitogen activated protein kinase (MAPK) pathway, a pathway that has a key role in regulating the cell cycle, cell growth and differentiation, and cell senescence.¹⁰ Activating mutations in MAPK genes are found in the germline in several developmental and cancer predisposition syndromes as well as acquired somatically in a wide range of cancers. Juvenile xanthogranuloma patients with *NF1* or *NF2* germline mutations are reported to have an increased risk of juvenile myelomonocytic leukemia, a myeloproliferative neoplasm of childhood associated with both germline and somatic MAPK pathway mutations.^{9,11,12} These observations suggest a potential role for MAPK signaling in juvenile xanthogranuloma.

Studies by whole-exome sequencing have provided additional support for a role for MAPK signaling in juvenile xanthogranuloma. Diamond *et al.*¹³ recently identified mutations involving MAPK pathway genes *NRAS*, *KRAS*, *ARAF*, and *MAP2K1* in 7 out of 12 systemic juvenile xanthogranuloma cases. Another study by Chakraborty and colleagues reported two cases of mixed Langerhans cell histiocytosis/juvenile xanthogranuloma phenotypes that contained the *BRAFV600E* mutation.⁶ Approximately 50% of Langerhans cell histiocytosis and Erdheim-Chester disease—additional histiocytic disorders—are reported to contain the *BRAFV600E* mutation.^{14–16} Although *BRAF* mutations have not been observed in cases with a purely juvenile xanthogranuloma phenotype to date,⁸ Diamond *et al.*¹³ did identify an *RNF11-BRAF* fusion in one of their systemic juvenile xanthogranuloma cases. This finding may indicate that, at least in some incidences of juvenile xanthogranuloma, (1) *BRAF* involvement cannot be ruled out and (2) translocations may also be involved in juvenile xanthogranuloma. Chakraborty *et al.*⁷ also identified

a *PIK3CD* mutation in serial biopsies of a single patient, suggesting that the PI3K pathway may also be involved in juvenile xanthogranuloma progression.⁸

Although emerging sequencing-based data suggest activating mutations may have an important role in juvenile xanthogranuloma pathogenesis, these findings have not been universal. Little is known about the potential contribution of copy number alterations, which are common across many cancers, in juvenile xanthogranuloma. To date, only normal karyotypes have been observed in cases with concurrent cytogenetic studies.^{17–21} Cytogenomic microarray analysis is a widely used genomic profiling technology, providing much higher resolution for detection of copy number alterations compared to traditional cytogenetic studies, as well as for detection of loss-of-heterozygosity, a common genomic alteration in cancer. Here, we evaluated the genomic profile of juvenile xanthogranuloma lesions by molecular inversion probe array, which is optimized for use on archived samples.^{22–25}

Materials and methods

Patient Samples

Lesions from a total of 21 archived juvenile xanthogranuloma cases were obtained from several institutions. Histological review of H&E-stained slides was performed by the contributing pathologists and the diagnosis of juvenile xanthogranuloma was confirmed by experienced pathologists (AB, YK, JH, DO, LW) based on standard WHO 2008 classification criteria.²⁶ Representative H&E images of selected cases from this cohort are provided (Figure 1). Tumor content (40–90%) was estimated during pathologist review. Patient samples were de-identified and used in accordance with University of Utah Institutional Review Board regulations.

DNA Isolation, Processing, and Array Hybridization

Genomic DNA was extracted using the QIAamp DNA FFPE Tissue Kit (QIAGEN, Valencia, CA, USA) and quantitated by Qubit PicoGreen (Life Technologies, Carlsbad, CA, USA). Approximately 80 ng genomic DNA was utilized for molecular inversion probe array analysis. Genomic analysis was performed using the OncoScan FFPE Assay kit (Affymetrix, Santa Clara, CA, USA). All samples were processed using a modified OncoScan protocol.²⁷ In brief, genomic DNA was hybridized to a series of molecular inversion probes, which target ~40 bp regions across the genome and anneal to genomic DNA such that each probe forms an inverted loop flanking a single nucleotide polymorphism. Complementary dinucleotides were then incorporated to form a circularized probe, followed by exonuclease treatment, which was used to remove genomic DNA and unused probes. Probes were then re-linearized for

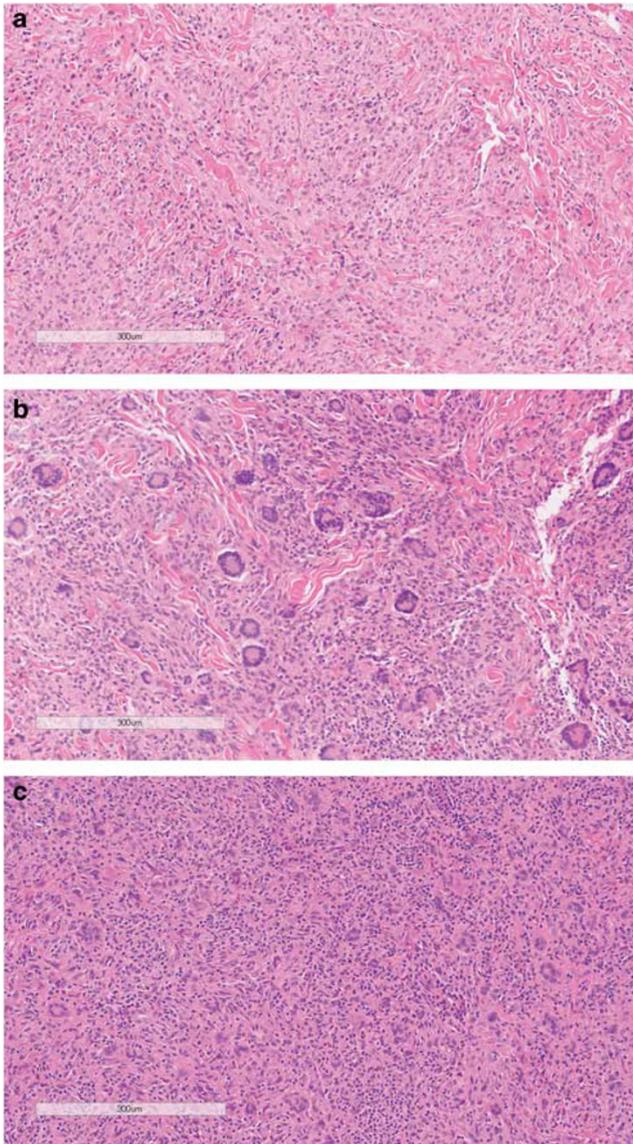


Figure 1 Histopathology of selected juvenile xanthogranuloma lesions with abnormal genomic profiles. (a–c). Hematoxylin and eosin stain ($\times 200$). Juvenile xanthogranuloma lesions display diffuse fibrohistiocytic proliferations with bland round to oval nuclei, admixed lymphocytes and occasional eosinophils. Touton giant cells (shown in b and c) are also common. (a–b). Two distinct lesions (JXG-1a and JXG-1b, respectively) from a single patient with disseminated juvenile xanthogranuloma. (c). A solitary cutaneous lesion (JXG-3).

amplification. Each probe contained a unique sequence identifier tag (barcode), for hybridization to a high-density microarray.

Data Processing and Analysis

Data processing was performed using OncoScan Console (Affymetrix). Analysis was performed using Nexus Express Software for OncoScan 3.1 (Bio-discovery, Hawthorne, CA, USA) with reference to

human genome assembly GRCh37/hg19. All files were processed using Affymetrix TuScan segmentation algorithm with default parameters. All copy number alterations and loss of heterozygosity calls were manually reviewed.

Level of mosaicism (% mosaicism) for each call was determined from genotype data (B-allele frequency and/or allele difference values) using calculations as described.²⁸ Clonal burden was estimated either from the OS-% Aberrant Cells algorithm in Nexus or from average % mosaicism within each case.

Results

Molecular inversion probe array analysis was performed on 21 cases of juvenile xanthogranuloma (Table 1). The cohort included 14 female and 7 male patients. Ages ranged from 11 months to 60 years, (median age: 3 years). The majority of cases were solitary lesions ($n = 19$; 90.5%), including 1 subcutaneous and 18 cutaneous lesions (JXG-3–21). There was a single case of diffuse cutaneous juvenile xanthogranuloma, from which two separate lesions (JXG-1a and 1b) were submitted, and a single case of systemic juvenile xanthogranuloma (JXG-2).

Four cases (19.0%) showed copy number alterations and/or loss of heterozygosity (JXG-1–4; Table 1, Figure 2). Clonal burden ranged from 15 to 60%, consistent with an acquired, somatic origin for these alterations. No recurrent genomic alterations were observed across these cases, and of the genes with reported mutations in juvenile xanthogranuloma, only *PIK3CD* coincided with the altered genomic regions. A general trend of increasing genomic complexity with staging was noted as the abnormalities in the two more advanced stage cases (JXG-1 and 2) involved a larger portion of the genome than the two abnormal solitary lesions (JXG-3–4; Table 1). The remaining 17 cases (81.0%) of solitary, cutaneous or subcutaneous lesions showed normal diploid genomes (JXG-5–21; Figure 2e).

Descriptions of Each Abnormal Case Follow

JXG-1. A 1-year-old male presented with extensive cutaneous juvenile xanthogranuloma with ocular involvement. Biopsies were obtained from two separate lesions (JXG-1a from the abdomen and JXG-1b from the right arm; Table 1). Morphologic differences were observed in the two individual lesions with giant Touton cells identified in JXG-1b, but lacking from JXG-1a (Figures 1a and b). Although genomic alterations were identified in both lesions, distinct and relatively large genomic changes occurred in each lesion (Figure 2a). JXG-1a contained trisomies of chromosomes 5 and 17, whereas JXG-1b had a 110 Mb terminal region of homozygosity, consistent with copy neutral-loss of heterozygosity, along the q-arm of chromosome 5. A skin exam 6 years after diagnosis showed deflated lesions on

Table 1 Characteristics and genomic alterations of juvenile xanthogranuloma cases

<i>JXG ID</i>	<i>Lesion type</i>	<i>Sex</i>	<i>Age</i>	<i>Biopsy site</i>	<i>CNA/LOH</i>	<i>Clonal burden</i>	<i>Genomic coordinates (GRCh37/hg19)</i>	<i>Genome involvement</i>
1	Extensive cutaneous with ocular involvement	Male	1y	A: Abdomen B: Right arm	Lesion A: +5,+17 Lesion B: LOH 5q13.2-q35.3	A: 50% B: 35%	A: Trisomy 5, Trisomy 17 B: Chr5:70,306, 678-180,698,312	8.47%
2	Systemic	Female	49y	Right upper arm/ Right femoral mass (lymph node)	LOH 1p36.33-p36.31 LOH 6p25.3-p21.2 LOH 9p24.3-p13.3 LOH 16p13.3-p12.3 LOH 19p13.3 Gain 7q21.11-q32.3 Loss 10q23.1-q25.1 Loss 19p13.3-p13.2	15%	Chr1:1-19,679,873 Chr6:1-36,766,292 Chr9:1-35,482,075 Chr16:1-17,469,494 Chr19:1-4,669,675 Chr7:89,719, 729-132,706,981 Chr10:84,918, 329-109,814,480 Chr19:4,674, 113-13,752,280	6.49%
3	Solitary cutaneous	Female	12y	Right elbow	Gain 1q21.1-q32.1 Gains 11q11-q13.4 11q23.1-q23.3 11q24.1-q25	40%	Chr1:144,009, 053-249,212,878 Chr11:54,795, 357-73,167,739 Chr11:110,777, 257-120,067,082 Chr11:123,471, 031-134,938,847 Chr3:45,170, 095-52,338,852	3.26%
4	Solitary cutaneous	Female	9y	Columella	Loss 3p21.31-p21.1	25%	Chr3:45,170, 095-52,338,852	0.23%
5	Solitary cutaneous	Female	3y	Right Lower Eyelid	–	–	N/A	N/A
6	Solitary cutaneous	Female	3y	Left thigh Mass	–	–	N/A	N/A
7	Solitary cutaneous	Male	4y	Right scalp	–	–	N/A	N/A
8	Solitary cutaneous	Male	1y	Right Abdomen	–	–	N/A	N/A
9	Solitary cutaneous	Female	1y	Right scalp	–	–	N/A	N/A
10	Solitary cutaneous	Female	20y	Left inner buttock	–	–	N/A	N/A
11	Solitary cutaneous	Male	2y	Right forehead	–	–	N/A	N/A
12	Solitary cutaneous	Female	1y	Posterior scalp	–	–	N/A	N/A
13	Solitary cutaneous	Female	1y	Left mid back	–	–	N/A	N/A
14	Solitary cutaneous	Male	19y	Right chin	–	–	N/A	N/A
15	Solitary cutaneous	Female	11 m	Right lower posterior leg	–	–	N/A	N/A
16	Solitary cutaneous	Male	1y	Left inner thigh	–	–	N/A	N/A
17	Solitary cutaneous	Male	31y	Left thigh	–	–	N/A	N/A
18	Solitary cutaneous	Female	2y	Left cheek	–	–	N/A	N/A
19	Solitary cutaneous	Female	15y	Thigh	–	–	N/A	N/A
20	Solitary cutaneous	Female	60y	Right nasal vestibule	–	–	N/A	N/A
21	Solitary cutaneous	Female	16y	Abdominal wall	–	–	N/A	N/A

Abbreviations: CNA: copy number alterations; LOH: loss of heterozygosity.

the nose and depressed scars on the trunk, although a new lesion was present on the left ear. The patient was on a weekly methotrexate treatment with a daily supplement of folate.

JXG-2. A 49-year-old female with systemic juvenile xanthogranuloma. Genomic analysis was performed on a combined tissue sample from this patient that included a biopsied lymph node from upper, right leg and a skin lesion from the right arm (Table 1). Full clinical information of this patient indicating involvement of additional organs or viscera was not available. Eight alterations were identified on seven chromosomes: three involved copy number changes including a 46.8 Mb interstitial gain in 7q, a 24.9 Mb interstitial loss in 10q and a 9.1 Mb interstitial loss in 19q, and 5 were terminal regions of homozygosity including 19.7 Mb overlapping

PIK3CD on 1p, 36.8 Mb on 6p, 35.5 Mb on 9p, 17.5 Mb on 16p and 4.7 Mb on 19p (Figure 2b). Follow-up information was unavailable.

JXG-3. A 12-year-old female presented with a solitary cutaneous lesion (Figure 1c). Array analysis showed four gains involving chromosomes 1q and 11q: a 61.9 Mb gain in proximal 1q and three non-contiguous gains in 11q: 18.4 Mb from 11q11 to 11q13.4; 9.3 Mb from 11q23.1 to 11q23.3; and 11.5 Mb from 11q24.2 to 11q25 (Figure 2c). Follow-up information was not available for this patient.

JXG-4. A 9-year-old female presented with a solitary cutaneous lesion on the nose. Array analysis identified a single 7.2 Mb interstitial deletion from 3p21.31 to 3p21.1 (Figure 2d). The lesion was removed 3 months after diagnosis, and follow-up

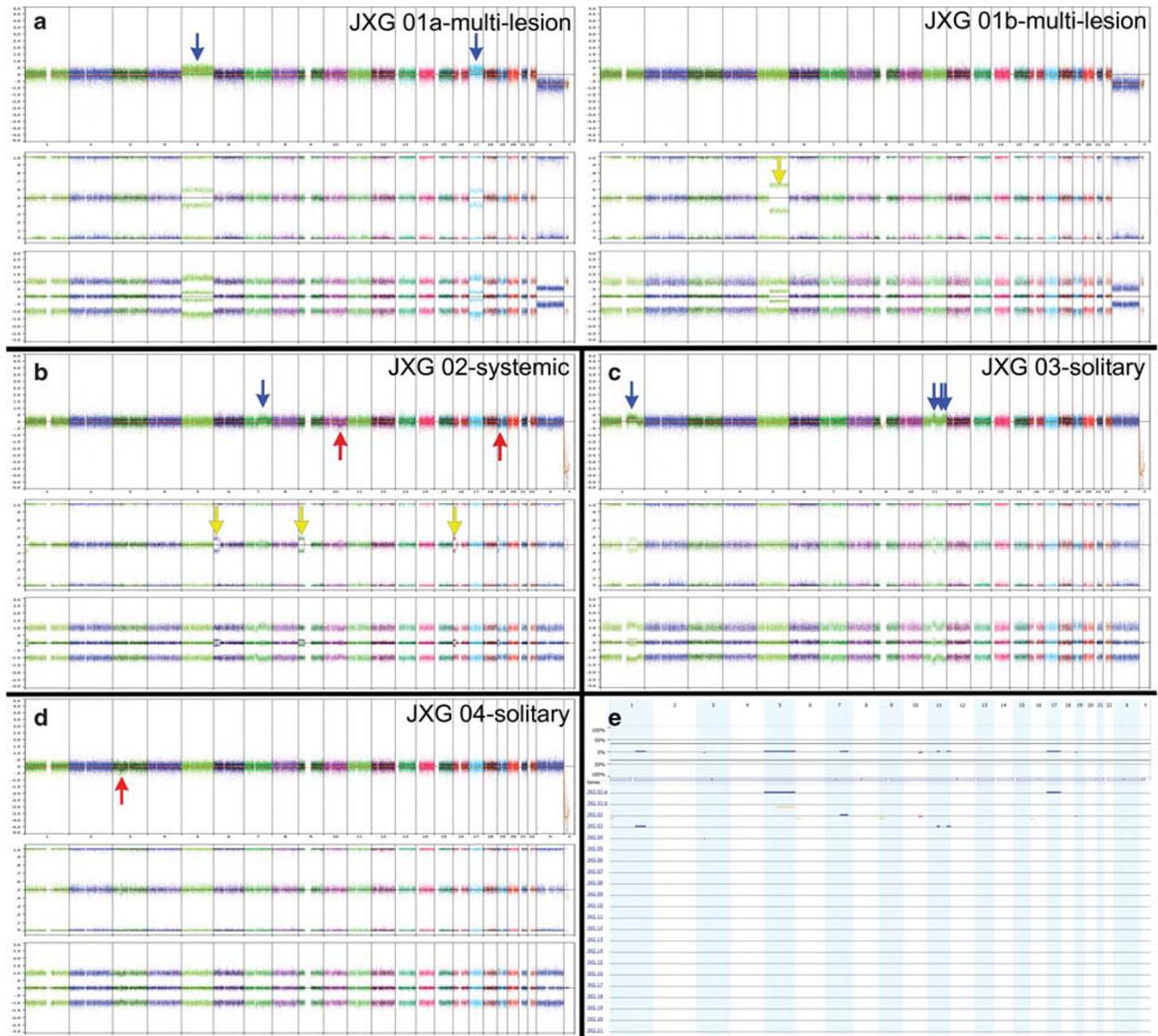


Figure 2 Genomic profiles of 21 juvenile xanthogranuloma cases. Copy number alterations were identified in five specimens from four patients (JXG-1–4). (a) Non-recurrent copy number alterations in two lesions from disseminated juvenile xanthogranuloma. JXG-1a (left) showed trisomy 5 and trisomy 17 (blue arrows). JXG-1b (right) showed copy neutral-loss of heterozygosity in chromosome 5q (yellow arrow). (b) Systemic juvenile xanthogranuloma (JXG-2) with complex genomic profile including copy neutral-loss of heterozygosity in 1p, 6p, 9p, 16p and 19p (yellow arrows), a gain in 7q (blue arrow), and losses in 10q and 19p (red arrows). (c) Solitary cutaneous lesion (JXG-3) with gains in 1q and 11q. (d) Solitary cutaneous lesion (JXG-4) with a loss in 3p. (e) Whole genome view of this cohort. Five lesions (cases 1–4) contained genomic alterations (red = single copy loss, blue = single copy gain, yellow = LOH). The remaining 17 cases (all solitary cutaneous juvenile xanthogranuloma) had normal diploid genomes. Chromosomes represented in order (1-22,X,Y) by individual columns.

showed no further evidence of disease over a 6-year period.

Discussion

The purpose of this study was to further define the genomic landscape of juvenile xanthogranuloma by identifying copy number alterations and loss of heterozygosity in juvenile xanthogranuloma lesions. Genomic analysis of 21 cases of juvenile

xanthogranuloma by molecular inversion probe array revealed that the majority of solitary cutaneous juvenile xanthogranuloma lesions are genomically quiet, with no identifiable genomic abnormalities. These findings suggest that copy number alterations and loss of heterozygosity do not primarily contribute to development of these lesions. Though recent sequence-based analyses of juvenile xanthogranuloma have mostly focused on advanced stage juvenile xanthogranuloma, our findings support the generally benign nature of solitary cutaneous

juvenile xanthogranuloma. The presence of copy number changes in only two solitary cutaneous lesions of this cohort indicates that these types of genomic alterations are uncommon in solitary juvenile xanthogranuloma. Clinical follow-up was available for one of the two solitary lesions with copy number alterations. Tumor excision was performed, and over a 6-year period the patient showed no recurrence or progression of disease, further suggesting that copy number changes do not drive disease development or progression.

Whole-exome sequencing studies have suggested a role for the MAPK and PI3K signaling pathways in juvenile xanthogranuloma development. Specifically, somatic mutations have been identified in *KRAS*, *NRAS*, *ARAF*, and *MAP2K1*¹³ and *PIK3CD*.⁷ As copy number alterations and loss of heterozygosity provide mechanisms for unmasking haploinsufficient and recessive-acting loss-of-function alleles, or for duplication of gain-of-function mutations, we investigated whether any of these genes reside within the genomic alterations identified by our analysis. None of the MAPK genes with reported mutations in juvenile xanthogranuloma coincided with our observed genomic alterations, but *PIK3CD* mapped to the region of chromosome 1 with copy neutral-loss of heterozygosity in JXG-2. Additional gene mutations reported in juvenile xanthogranuloma that are not involved in the MAPK or PI3K pathways⁷ also map outside of these regions. In addition to sequence-level alterations, balanced rearrangements, including translocations—such as that reported by Diamond *et al.*¹³—and inversions cannot be detected by molecular inversion probe array. Therefore, additional studies are needed to incorporate genomic alterations with mutation and translocation detection.

Four cases in this cohort showed abnormal genomic profiles, with no recurrent abnormalities. A review of these four cases with genomic changes showed that no histologic differences were observed, but that each of these lesions had typical histology for juvenile xanthogranuloma. In addition, the profiles of two separate lesions from the same patient (JXG-1) were distinct. These findings suggest that these clonal genomic alterations were acquired secondarily, rather than occurring in the initial development of these lesions. Though limited in number, the systemic and disseminated cases showed greater genomic complexity and provide the basis to further study how copy number alterations/loss of heterozygosity correlates with staging in juvenile xanthogranuloma.

It is also important to consider cases of juvenile xanthogranuloma which occur in association with other related disorders, including juvenile myelomonocytic leukemia, Langerhans cell histiocytosis and other *BRAF*-mutated dendritic and histiocytic neoplasms. These cases may share immunophenotypic features with juvenile xanthogranuloma, but may have an underlying clonal relationship to neoplastic cells. For example, adults with solitary and systemic xanthogranuloma lesions may be a

manifestation of Erdheim-Chester disease, and may warrant further evaluation, including testing for gain-of function mutations in *BRAF*, *NRAS*, *KRAS*, or *MAP2K1*, which are associated with Erdheim-Chester disease. Radiologic examination for typical bone features of Erdheim-Chester disease may also be of benefit. Our adult cases (JXG-2 and JXG-20) did not have available clinical or radiographic follow-up for further evaluation. Further studies of juvenile xanthogranuloma, including mutation profiles, relationship of systemic to solitary cutaneous disease and coincidence with other histiocytic/dendritic cell neoplasms is warranted.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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