Novel somatic alterations in unicentric and idiopathic multicentric Castleman disease

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Objectives: Castleman disease (CD) is a heterogeneous group of disorders involving systemic inflammation and lymphoproliferation. Recently, clonal mutations have been identified in unicentric CD (UCD) and idiopathic multicentric CD (iMCD), suggesting a potential underlying neoplastic process.

Methods: Patients with UCD or iMCD with next generation sequencing (NGS) data on tissue DNA and/or circulating tumor DNA (ctDNA) were included.

Results: Five patients were included, 4 with iMCD and 1 with UCD. Four patients (80%) were women; median age was 40 years. Three of five patients (60%) had ≥1 clonal mutation detected on biopsy among the genes included in the panel. One patient with iMCD had a 14q32-1p35 rearrangement and a der(1)dup(1)(q42q21)del(1)(q42) (1q21 being IL-6R locus) on karyotype. This patient also had a *NF1* K2459fs alteration on ctDNA (0.3%). Another patient with iMCD had a *KDM5C* Q836* mutation, and one patient with UCD had a *TNS3-ALK* fusion but no ALK expression by immunohistochemistry.

Conclusions: We report 4 novel somatic alterations found in patients with UCD or iMCD. The 1q21 locus contains IL-6R, and duplication of this locus may increase IL-6 expression. These findings suggest that a clonal process may be responsible for the inflammatory phenotype in some patients with UCD and iMCD.

Supplemental Table 1. Patient information and fulfillment of the diagnostic criteria[1]

Patient	1	2	3	4	5	Reference range
Age[†]/Sex	28.7/F	30.4/F	58.8/F	39.7/M	39.8/F	
Diagnosis	iMCD	iMCD	iMCD	iMCD	UCD	
Pathology	Hyaline vascular	Plasmacytic	Plasmacytic	Plasmacytic	Hyaline vascular	
Slides Reviewed at UCSD[‡]	No (Stanford)	Yes	Yes	No	No	
Biopsy site	Right axillary LN	Left inguinal LN	Submental LN	Inguinal LN	Supraclavicular LN	
HHV-8 IHC/PCR	Neg	Neg	Neg	Neg	Neg	Neg
Imaging	PET/CT with cervical, axillary, pericardiac, retroperitoneal, pelvic, and mesentery LAD	CT with mediastinal, abdominal, and pelvic LAD	PET/CT with cervical, mediastinal, and abdominal LAD	CT with bilateral axillary and inguinal LAD	PET/CT with no LAD	
CRP (mg/L)[†]	ND	88	ND	ND	0.1	<0.5
ESR (mm/hr)	100	N/A	84	106	6	0-20
Hgb (g/dL)	9.5	10.2	12.9	11.9	13	13.6-17.0
Platelet (1000/mm^3)	387	468	487	273	295	140-400
Albumin (g/dL)	2.2	4.3	3.9	2.8	4.6	4.0-
eGFR (mL/min)	ESRD	ESRD	>60	>60	>60	
IgG (mg/dL)	1542	1140	2969	4880	1078	700-1600
B symptoms	N/A	N/A	No	No	No	
Hepatomegaly splenomegaly	Yes	No	No	No	No	
Edema	Yes	Yes	No	No	No	
Cherry hemangioma /violaceous rash	No	No	Yes	No	No	
Lymphocytic interstitial pneumonitis	No	No	No	Possibly[§]	No	
IL-6 (pg/mL)	41	ND	20	13	ND	≤5

Abbreviations: CRP: C-reactive protein; CT: computed tomography; eGFR; estimated glomerular filtration rate; ESR: erythrocyte sedimentation rate; ESRD: end stage renal disease; F: female; Hgb: hemoglobin; HHV-8: Human herpesvirus-8; IHC: immunohistochemistry; IL-6: interleukin-6; iMCD: idiopathic multicentric Castleman disease; LAD: lymphadenopathy; LN: lymph node; N/A: not available; Neg: negative; ND: not done; PCR: polymerase chain reaction; PET: positron emission tomography; UCD: unicentric Castleman disease; UCSD: University of California San Diego

[†]At diagnosis

[‡]Pathology reports were reviewed by a hematopathologist (HYW) when slides were unavailable for review

[§]Not biopsy confirmed

Supplemental Table 2. Summary of reported genetic alterations in iMCD and UCD

Study	N†	Method	Genetic alteration‡	Somatic vs germline	VAF	Proposed mechanism
iMCD						
Nakamura et al.(24) 1993	1	Karyotype	t(7;14)(p22;q22)	Somatic	NR	*IL6* gene is located on 7p21-22 and translocation potentially resulted in high level of IL-6
Kone-Paut et al.(21) 2009	1	Single gene targeted sequencing	*MEFV*	Germline	NR	Reduced activity of pyrin in white blood cells by MEFV mutation causing uncontrolled inflammation
Patel et al.(25) 2017¶	1	Targeted NGS	*JAK1* V310I	Somatic	NR	Predicted that JAK1 is more readily activated through IL-6R due to loss of inhibitory interactions.
You et al.(30) 2019	22	WES	*NCOA4* L261F§ (4)	Somatic	NR	NCOA4 may influence the MAPK/AR-ARA signaling pathway
			DARS2§			
			MTCL1§			
			RABEP1§			
			DNAH11§			
Yoshimi et al.(29) 2020	3	Targeted NGS	*MEK2* P128L	Somatic	10%	Activate MAPK signaling. In vitro, cells demonstrated hypersensitive growth with low IL-3. These cells were also sensitive to MEK inhibitors.
			RUNX1 G60C	Germline	49%	Predicted to affect heterodimerization of RUNX1 with CBFβ, which is essential for its function. In vitro, bone marrow progenitors had enhanced self-renewal capacity.
UCD						
Pauwels et al.(26) 2000	1	Karyotype	t(1;16)(p11;p11), del(7)(q21q22), del(8)(q12q22) [15/20]	Somatic	NR	Clonal proliferation of stromal elements contribute to pathophysiology.
Cokalaere et al.(20) 2002	1	Karyotype	add(1)(q21), der(6)t(6;12)(q23;q15), add(7)(p22), -9,inv(9)(p11q13), del(12)q(15), +mar	Somatic	NR	Results in rearrangement of *HMGIC* gene which was found in anti-CD21 reactive follicular dendritic cells.
Chen et al.(19) 2006	2	Karyotype	t(1;22)(p22;q13) [18/20]	Somatic	NR	Genetic changes attributed to stromal cells.
			t(7;8)(q37.3;q12) [1/20]			
Reichard et al.(27) 2011	1	Karyotype	add(6)(p23),add(7)(p15), del(7)(p15),add9(q22) [4] inv(9)(p13q22) [2] -3,+r [2]	Somatic	NR	Genetic changes were attributed to non-lymphoid cells, most likely stromal, dendritic, or endothelial origin.
Chang et al.(18) 2014	29	Single gene PCR	*HUMARA* (22)	Somatic	NR	Stromal cell clonal proliferation
Legras et al.(22) 2017	9	Targeted NGS	*VHL* F119L	Somatic	35%	Loss of tumor suppressor activity
			JAK3 V718L	Germline	53%	Activation of STAT, AKT, ERK pathways which lead to proliferation

iMCD and UCD						
Stone et al.(28) 2012	58	PCR of selected SNPs of *IL6* promoter, *IL6R*, and *gp130*	*IL6R* rs4537545˚ *IL6R* rs2228145˚	Germline	NR	Increased level of soluble IL6R in the serum leads to increased level of IL-6 signaling
Baker et al.(17) 2018	2	WGS	*FAS* R68G (2)	Germline	NR	Defective Fas-induced lymphocyte apoptosis
Nagy et al.(23) 2018	18	Targeted NGS and WES for FDCS patients	*DNMT3A* L295Q※	Somatic	9%	Disrupts PWWP domain, the DNA binding site, of DNMT3A, resulting in increased proliferation of these cells that produce IL-6.
Li et al.(6) 2018	75	WES Targeted sequencing of *PDGFRB* p.N666S	*PDGFRB*ˆ (7)	Somatic	NR	Activate KIT kinase through ligand-independent autophosphorylation

Abbreviations: AKT: AKT serine/threonine kinase; AR: androgen receptor; ARA: androgen-associated receptor; DNMT3A: DNA methyltransferase 3 alpha; ERK: extracellular signal-regulated kinases; FAS: Fas cell surface death receptor; FDCS: follicular dendritic cell sarcoma; HMGIC: high-mobility group protein isoform C; HUMARA: human androgen receptor α; IL-3: interleukin-3; IL-6: interleukin-6; IL-6R: interleukin-6 receptor; iMCD: idiopathic multicentric Castleman disease; JAK: Janus kinase; KIT: KIT proto-oncogene receptor tyrosine kinase; MAPK: mitogen activated protein kinase; MEK: mitogen activated protein kinase kinase; NCOA4: nuclear receptor coactivator 4; NGS: next generation sequencing; NR: not reported; PCR: polymerase chain reaction; PDGFRB: platelet-derived growth factor receptor b; RUNX1: runt related transcription factor 1; SNP: single nucleotide polymorphism; STAT: signal transducer and activator of transcription; UCD: unicentric Castleman disease; VAF: variant allele frequency; VHL: von Hippel-Lindau; WES: whole exome sequencing; WGS: whole genome sequencing; FDCS:

†Number of patients with CD who were sequenced

‡Reported genetic alterations are single patients among those who are tested except for indicated in parentheses

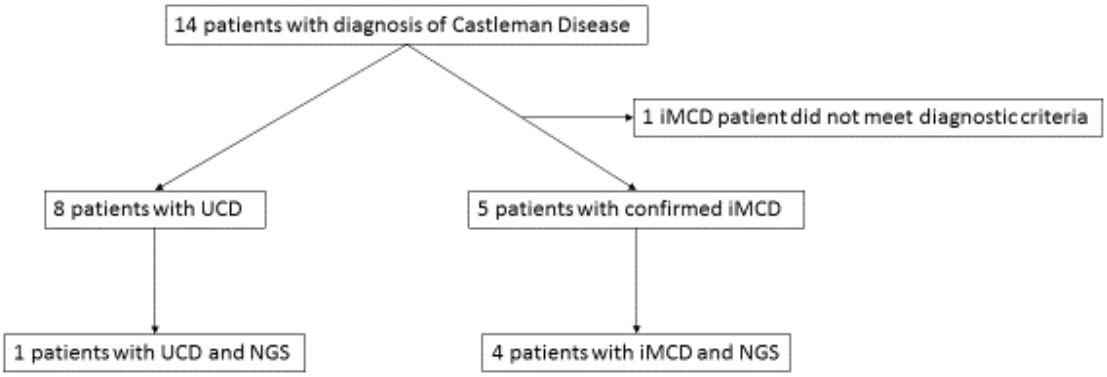
§Representative list mutations that are claimed to be significantly associated with adverse prognosis

¶Patient did not meet strict diagnostic criteria for iMCD upon further review

˚Allele frequency for IL6R rs4537545 was 0.49 and IL6R rs2228145 was 0.42, statistically significantly higher compared to healthy controls

※Several copy number variants were also found. *DNMT3A* mutation was found in patient with iMCD

ˆOther mutations were also identified as part of NGS. *PDGFRB* mutation was seen in 7 of 41 UCD patients and none of the iMCD patients



Supplemental Figure 1. CONSORT diagram
Abbreviations: iMCD: idiopathic multicentric Castleman disease; NGS: next generation sequencing; UCD: unicentric Castleman disease.