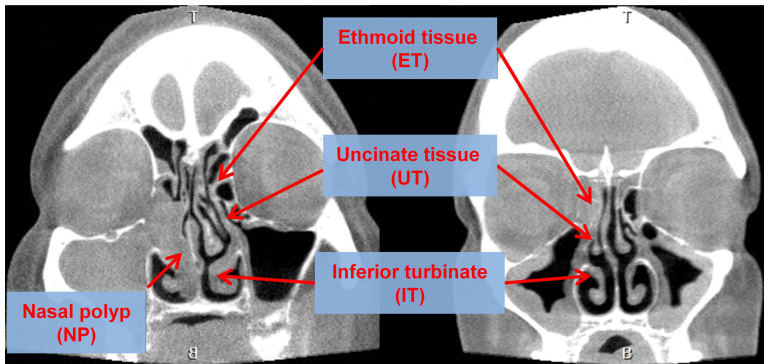
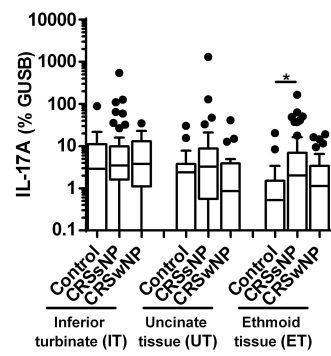
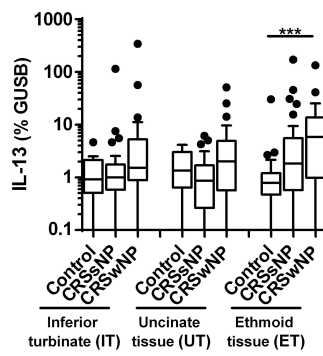
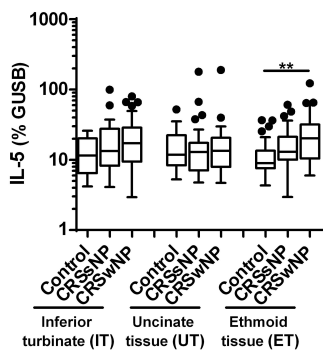
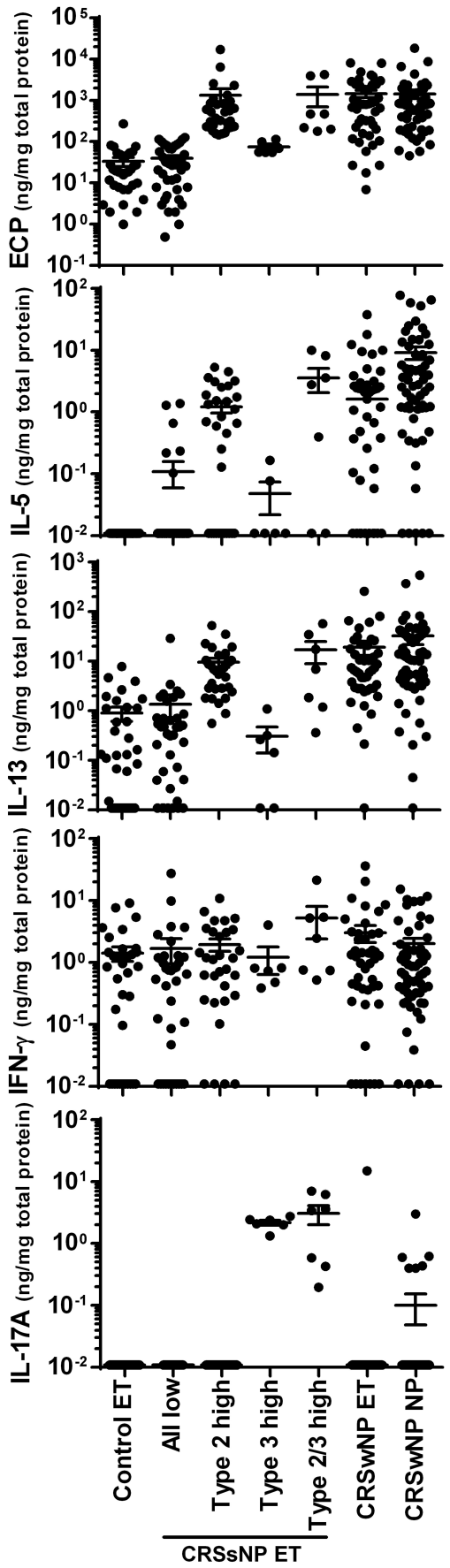


A. Unilateral CRSwNP

B. CRSsNP







1 **ONLINE REPOSITORY**

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3 **Heterogenous inflammatory patterns in chronic rhinosinusitis without nasal polyps in**

4 **Chicago, Illinois**

5

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18 METHODS:

19

20 *Patients and biopsies*

21 Patients with chronic rhinosinusitis (CRS) were recruited from the Otolaryngology clinic
22 and the Northwestern Sinus Center of Northwestern Medicine. Inferior turbinate (IT) tissue,
23 uncinata process tissue (UT), ethmoid tissue (ET) and nasal polyp (NP) tissue were obtained
24 during routine endoscopic sinus surgery performed on patients with CRS. All patients met the
25 criteria for CRS as defined by the American Academy of Otolaryngology-Head and Neck
26 Surgery Chronic Rhinosinusitis Task Force.^{E1, E2} Patients with an established immunodeficiency,
27 pregnancy, coagulation disorder or diagnosis of aspirin hypersensitivity, classic allergic fungal
28 sinusitis, Churg-Strauss syndrome or cystic fibrosis were excluded from the study. Disease-free
29 sinus tissues from normal control patients without a history of CRS were obtained during
30 procedures for conditions other than CRS (septoplasty for nasal obstruction, transnasal
31 endoscopy skull base procedures, repairs of facial fractures, treatment of nasal disorders in
32 obstructive sleep apnea, etc.). Patients were skin-tested for pollens, dust mites, pets, molds, and
33 cockroach using Hollister-Stier (Spokane, WA) extracts. Several patients were taking a variety of
34 medications, including corticosteroids (Table E1 and E2). Details of patients' characteristics are
35 included in Table E1 and E2. All patients signed informed consent forms and the protocol
36 governing procedures for this study was approved by the Institutional Review Board of
37 Northwestern University Feinberg School of Medicine (IRB Project Number: STU00016917).

38

39 *Real-time RT-PCR*

40 A portion of nasal tissues for isolation of RNA was transferred in RNeasy (Ambion,
41 Austin, TX) and stored at -20°C. Total RNA from sinus tissue was extracted using QIAzol

42 (Qiagen, Valencia, CA) and was cleaned and treated with DNase I using NucleoSpin RNA
43 (Clontech Laboratories, Mountain View, CA) according to the manufacturer's instructions. The
44 quality of total RNA from sinus tissue was assessed with a 2100 Bioanalyzer (Agilent
45 Technologies, Santa Clara, CA) using a RNA 6000 Nano LabChip (Agilent Technologies). RNA
46 in which RIN was greater than 7.0 was used for cDNA synthesis. Single-strand cDNA was
47 synthesized with SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA) and random
48 primers. Real-time RT-PCR was performed with a TaqMan method using a StepOnePlus Real-
49 Time PCR System (Applied Biosystems, Foster City, CA) in 20 μ l reactions (10 μ l 2x TaqMan
50 Fast Advanced Master Mix (Applied Biosystems), 1 μ l 20x primer and probe mixture for target
51 gene, 1 μ l 20x primer and probe mixture for β -glucuronidase (GUSB) plus cDNA equivalent to
52 10 ng of total RNA). Primer and probe sets for IFN- γ (Hs00989291_m1), CLC
53 (Hs00171342_m1), IL-17A (Hs00936345_m1), IL-5 (sense, 5'-
54 AGCTGCCTACGTGTATGCCA-3'; antisense, 5'-GTGCCAAGGTCTCTTTCACCA-3'; FAM/
55 BHQ1 probe, 5'-CCCCACAGAAATTCCCACAAGTGCA-3'), IL-13 (sense, 5'-
56 AAGGTCTCAGCTGGGCAGTTTA-3'; antisense, 5'-AAACTGGGCCACCTCGATT-3'; FAM/
57 BHQ1 probe, 5'-CCAGCTTGCATGTCCGAGACACCA-3') and GUSB (Human β -
58 glucuronidase endogenous control, PN; 4326320E) were purchased from Applied Biosystems or
59 Integrated DNA Technologies (Coralville, IA). The mRNA expression levels were normalized
60 to the expression of a housekeeping gene, GUSB. Expression of GUSB was not significantly
61 different between control ethmoid tissues and NPs (data not shown).

62

63 *Tissue homogenates and protein assays*

64 Freshly obtained tissue specimens were weighed, and 1 ml of PBS supplemented with

65 0.05 % Tween 20 (Sigma-Aldrich, St. Louis, MO) and 1% protease inhibitor cocktail (PIC, PN;
66 P8340, Sigma-Aldrich) was added for every 100 mg of tissue. The tissue was then homogenized
67 with a Bullet Blender Blue (Next Advance, Averill Park, NY) at setting 7 for 8 min at 4°C. After
68 homogenization, the suspension was centrifuged at 4000 rpm for 20 min at 4°C and the
69 supernatants were stored at -80°C. Before analysis, tissue homogenates were centrifuged at
70 16,000g for 15 min at 4°C and we used those supernatants for each assay.

71 The concentration of ECP in cell free supernatants was determined by a commercial ELISA
72 kit (MBL, Woburn, MA). The minimal detection limit for this kit is 0.125 ng/ml. The
73 concentrations of IFN- γ , IL-5, IL-13 and IL-17A in cell-free supernatants were measured using a
74 MILLIPLEX MAP Human High Sensitivity T Cell Panel from EMD Millipore (Billerica, MA).
75 The minimal detection limits for IFN- γ , IL-5, IL-13 and IL-17A are 0.61, 0.49, 0.24 and 0.73
76 pg/ml, respectively. The concentration of ECP and cytokines in tissue homogenates was
77 normalized to the concentration of total protein as detected by BCA protein assay kit
78 (ThermoScientific, Rockford, IL).

79

80

81 *Statistics*

82 All data are reported as the median (25-75% interquartiles) or as the mean \pm SEM.
83 Differences between groups were analyzed using the 1-way ANOVA Kruskal-Wallis Dunn's
84 multiple comparison test. Correlations were assessed using the Spearman's rank correlation. All
85 statistical analyses were performed using GraphPad prism 6.07 software (La Jolla, CA). A p
86 value of less than 0.05 was considered significant. In the log scaled figures, we plotted 0 as 0.01
87 although we did statistical analysis using all the raw data including 0 (undetectable).

89 **Table E1 Patient characteristics in RNA study**

	Control IT (n=19) n (%)	Control UT (n=22) n (%)	Control ET (n=33) n (%)	CRSsNP IT (n=53) n (%)	CRSsNP UT (n=44) n (%)	CRSsNP ET (n=61) n (%)	CRSwNP IT (n=28) n (%)	CRSwNP UT (n=29) n (%)	CRSwNP ET (n=40) n (%)	CRSwNP NP (n=48) n (%)
Female	8 (42)	15 (68)	22 (67)	35 (66)	26 (59)	34 (56)	8 (29)	9 (31)	11 (28)	13 (27)
Atopy	2 (11)	3 (14)	7 (21)	21 (40)	18 (41)	29 (48)	13 (46)	14 (48)	22 (55)	29 (60)
Asthma	0 (0)	0 (0)	3 (9)	7 (13)	9 (20)	17 (28)	16 (57)	13 (45)	20 (50)	26 (54)
Nasal steroid	0 (0)	0 (0)	5 (15)	5 (9)	5 (11)	18 (30)	4 (14)	5 (17)	8 (20)	8 (17)
Inhaled steroid	0 (0)	1 (5)	0 (0)	2 (4)	3 (7)	10 (16)	2 (7)	2 (7)	8 (20)	9 (19)
Oral steroid	0 (0)	0 (0)	1 (3)	1 (2)	4 (9)	3 (5)	5 (18)	7 (24)	6 (15)	8 (17)
Age (y), median (range)	36.5* (17-72)#	46.5 (16- 75)	58.0 (22- 75)	37.0 (21- 71)	38.5 (20- 73)	43.0 (20- 70)	45.0 (27- 70)	47.0 (23- 67)	47.0 (23- 72)	45.0 (20- 72)

90 *median, #(range).

91 **Table E2 Patient characteristics in protein study**

92

	Control ET (n=34) n (%)	CRSsNP ET (n=83) n (%)	CRSwNP ET (n=45) n (%)	CRSwNP NP (n=60) n (%)
Female	17 (50)	51 (61)	14 (31)	25 (42)
Atopy	6 (18)	42 (51)	24 (53)	39 (65)
Asthma	4 (12)	32 (39)	19 (42)	28 (47)
Nasal steroid	0 (0)	27 (33)	12 (27)	14 (23)
Inhaled steroid	1 (3)	22 (22)	9 (20)	13 (22)
Oral steroid	1 (3)	12 (14)	9 (20)	6 (10)
Age (y), median (range)	56.5* (22-75)#	38 (19-74)	47.0 (24-72)	45.0 (24-76)

93 *median, #(range).

94

95 **References**

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101

102 **FIGURE LEGENDS**

103

104 **Figure E1. Diagram of nasal and paranasal sinuses.**

105 Illustrative computed tomography (CT) images demonstrating the anatomic location of
106 studied tissue from A) a patient with typical changes of CRSwNP on one side but more normal
107 anatomy on the contralateral side; and B) a patient with radiographic changes typical for
108 CRSsNP.

109

110 **Figure E2. Expression of IL-5, IL-13 and IL-17A in IT, UT and ET.**

111 Total RNA was extracted from whole tissue of control IT (n=19), control UT (n=21), control
112 ET (n=33), CRSsNP IT (n=53), CRSsNP UT (n=44), CRSsNP ET (n=61), CRSwNP IT (n=28),
113 CRSwNP UT (n=29) and CRSwNP ET (n=40). Expression of mRNAs for IL-5, IL-13 and IL-
114 17A was analyzed using real-time RT-PCR. Gene expression was normalized by a housekeeping
115 gene, GUSB and expression levels were shown as % expression of GUSB. Results are shown as
116 medians (25% to 75% interquartile ranges). * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001,
117 by one-way ANOVA.

118

119 **Figure E3. CRSsNP can be divided into several groups.**

120 Total RNA was extracted from whole tissue of control ET (n=33), CRSsNP ET (n=61),
121 CRSwNP ET (n=40) and CRSwNP NP (n=48). CRSsNP was further divided into type 1 cytokine
122 high group (n=5), type 2 cytokine high group (n=17), type 3 cytokine high group (n=4), type 1

123 and 2 cytokines high group (type 1/2, n=4), type 1 and 3 cytokines high group (type 1/3, n=4),
124 type 1, 2 and 3 cytokines high group (type 1/2/3, n=1) and type 1, 2 and 3 cytokine all low
125 group (all low, n=26). Type 1, 2 and 3 high groups were classified based on the 95th percentile
126 expression of IFN- γ (13.2), CLC (96.4) and IL-17A (12.1), respectively, in control ET.
127 Expression of mRNAs for CLC, IL-5, IL-13 and IFN- γ was analyzed using real-time RT-PCR.
128 Gene expression was normalized to a housekeeping gene, GUSB and expression levels were
129 shown as % expression of GUSB. Results are shown as mean \pm SEM. In order to display
130 undetectable data, we plotted 0 as 0.01 in the log scaled figures.

131

132 **Figure E4. Sub-classification of CRSsNP by protein levels of type 2 and 3 cytokines.**

133 Protein extracts were generated from whole ET tissues of control (n=34), CRSsNP (n=83),
134 CRSwNP (n=45) and NP tissues (n=60). CRSsNP was further divided into type 2 cytokine high
135 group (n=31), type 3 cytokine high group (n=6), type 2 and 3 cytokines high group (type 2/3,
136 n=7) and type 2 and 3 cytokine all low group (all low, n=39). Expression of ECP, IL-5, IL-13,
137 IFN- γ and IL-17A proteins in tissue homogenates was measured using ELISA and Luminex and
138 normalized to the concentration of total protein. Type 2 and 3 high groups were classified based
139 on the 95th percentile expression of ECP (131.5 ng/mg) and IL-17A (0.02 pg/mg (detectable)),
140 respectively, in control ET. In order to display undetectable data, we plotted 0 as 0.01 in the log
141 scaled figures.

142

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