



A Chromosomal Duplication Encompassing Interleukin-33 Causes a Novel Hyper IgE Phenotype Characterized by Eosinophilic Esophagitis and Generalized Autoimmunity

Interleukin (IL)-33 is a member of the IL-1 cytokine family and is secreted by fibroblasts, endothelial cells, and epithelial cells in tissues (lung, skin, and gastrointestinal) that are exposed to the environment.¹ IL-33 is stored in the nucleus and environmental allergens trigger RIPK1-caspase 8 ripoptosome activation in epithelial cells to enable IL-33 maturation and release.² IL-33 signals via its receptor ST2, which is found on multiple immune cells, such as eosinophils, mast cells, T cells, macrophages, basophils, and type 2 innate lymphoid cells, which all promote type-2 innate immune reactions.³ Therefore, IL-33 plays a central role in promoting allergic inflammatory responses in diseases such as eosinophilic esophagitis (EoE), allergy to food/inhalants, asthma, and atopic dermatitis. Genome-wide association data of these conditions consistently report polymorphisms in the *IL-33* gene as a risk factor.⁴ Thus, IL-33 has been proposed as a therapeutic target for these conditions. In animal models, IL-33 has been mechanistically linked with allergic, autoimmune, rheumatologic, and inflammatory bowel diseases.⁵ However, monogenic human diseases caused by either gain-of-function or loss-of-function variants in the *IL-33* gene have not been reported previously.

Here, we describe a patient with a rare and novel chromosomal duplication that encompasses the entire *IL-33* gene. Among other clinical features, this patient presented with EoE, and other typical atopic disease manifestations, such as hypereosinophilia, elevated IgE, recurrent eosinophilic skin infiltrations, food allergy, and mild asthma. To our knowledge, this is the first report of a potential gain-of-function phenotype for IL-33 in a human and gives us some important insights into the effects and tropism of constitutive overexpression of this cytokine in multiple tissues in vivo.

Informed consent was obtained from the participant's parents and this study was approved by the research ethics board at The Hospital for Sick Children, Toronto, Canada (Research Ethics Board: REB1000024905). The patient is a 12-year-old boy of white European ancestry, born to non-consanguineous parents, who presented with painful skin nodules starting in infancy (Figure 1A), mild asthma, encopresis, milk allergy, and eosinophilic gastrointestinal disease. His gastrointestinal disease began with food intolerance in infancy, occasional difficulty swallowing, and failure to thrive. He was eventually diagnosed with EoE after endoscopy at age 9 years, which showed edema and loss of esophageal vascularity. There were no signs of fibrostenosis. Pathology review of the esophageal biopsies showed that intraepithelial eosinophilic infiltration was present at multiple levels (>15/high-power field, highest up to 55/high-power field) with basal hyperplasia and spongiosis,

consistent with a diagnosis of EoE; repeat biopsies while on a dairy- and soy-free diet after 1 year of treatment showed histologic remission. Despite ongoing encopresis, his upper and lower gastrointestinal tract biopsies showed only EoE. Skin biopsies revealed eosinophilic dermal infiltrates with necrobiosis of collagen. There were no signs of histiocytic disease pathology. A bone marrow biopsy showed increased eosinophils at all stages of maturation (18%). He was found to have constitutive peripheral eosinophilia ($1.68\text{--}5.12 \times 10^9$), elevated total IgE (896–2287 IU/mL), and increased tryptase levels (14.8–19.9 $\mu\text{g/L}$). He also displayed some dysmorphic features, including deeply set eyes, a low anterior hairline, a large nose with prominent nasal bridge, micrognathia, and jagged uneven teeth. He had poor growth (remaining below the third percentile for height and weight), joint hypermobility, myopia, and moderate intellectual disability. A skeletal survey revealed skull lytic lesions (Figure 1B). As part of the extended clinical workup, other underlying causes of immunodeficiency and hyper-eosinophilia were ruled out, including PDGFRA-associated leukemia (Supplementary Table 1). Neither cyclosporine nor mycophenolate mofetil reduced the clinical symptoms, but his skin nodules responded to intermittent courses of high-dose oral prednisone.

As part of a systematic genetic workup, a clinical single nucleotide polymorphism microarray initially showed a de novo 0.58-Mb duplication of unknown significance at chromosome 9p24.1, encompassing the entire *IL33* gene (arr[hg19]9p24.1(6,194,475-6,774,846)). Whole-genome sequencing confirmed that the duplication was in tandem and that there were no other pathogenic or likely pathogenic variants in other genes that could explain his phenotype. We did not find any similar duplications in the DECIPHER database, indicating that the duplication was novel or very rare.

Mass cytometry did not demonstrate peripheral T cell polarization (Supplementary Figure 1) and serum analysis of circulating peripheral IL-33 or soluble ST2 levels were not elevated (data not shown). However, messenger RNA (mRNA) sequencing showed significant elevation in *IL-33* mRNA expression in whole blood compared with tissue-matched samples from GTEx (gtexportal.org) and the patient's parents (Figure 1C and D). In line with predominantly mucosal-, skin-, and tissue-based clinical manifestations, the

Abbreviations used in this paper: EoE, eosinophilic esophagitis; IL, interleukin; mRNA, messenger RNA.

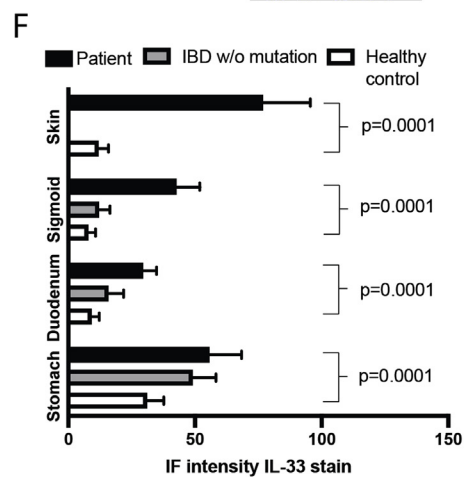
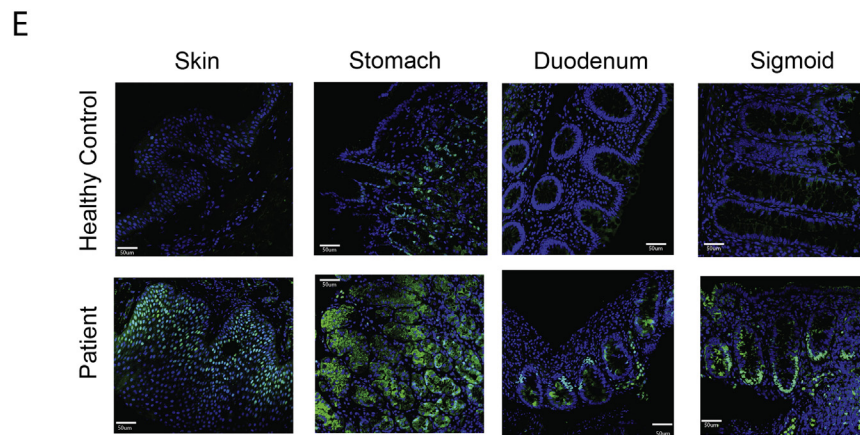
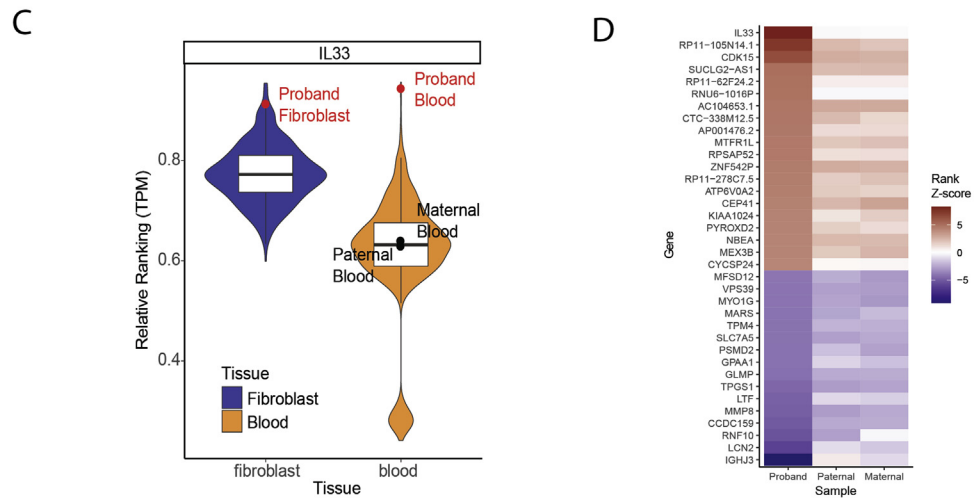
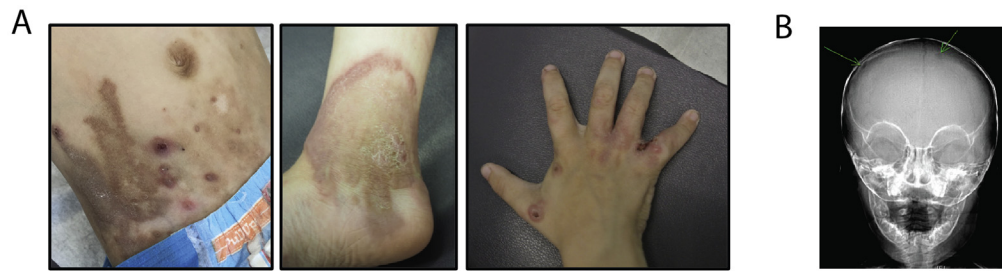


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elevation in IL-33 mRNA expression was also found in the patient's fibroblast samples. Tissue protein expression of IL-33 was conducted via immunofluorescence in skin and gut biopsy samples (ie, stomach, sigmoid, and duodenum). We found a significant elevation of IL-33 protein in the patient's gastrointestinal biopsy samples compared with 9 healthy control subjects ($P = .0001$) and 9 children with inflammatory bowel disease (Figure 1E and F). Baseline elevation of IL-33 on immunofluorescence was noted not only in skin biopsies with active eosinophilic-mediated inflammation (nuclear localization), but also in noninflamed gut biopsy samples from the patient (cytoplasmic localization). An increase in IL-33 expression has been reported previously in EoE.⁶ In vivo evidence choosing a transgenic overexpression approach of secreted/active IL-33 in the esophagus resulted in EoE.⁷ The difference in subcellular localization of IL-33 in different tissues could explain tissue-specific pathology and have implications for the application and efficacy of IL-33 targeting therapies in different anatomic locations.

Whole-genome sequencing has the benefit of detecting copy number variants that are beyond the limit of detection for whole-exome sequencing. The diagnostic workup in this case highlights the utility of multi-omic analyses, such as mRNA sequencing to help interpret whole-genome sequencing data. This case also cements the central role for IL-33 in controlling the type 2-mediated response in tissues facing the external environment. Although the patient had several features of the atopic type 2-mediated response, such as mild asthma, a milk allergy, and meets criteria for a hypereosinophilia-like syndrome, interestingly, he did not present with atopic dermatitis but instead with an eosinophil-dominated skin inflammation condition. He was originally given a diagnosis of Well's syndrome (eosinophilic cellulitis) and it is possible that people with this phenotype could have an IL-33-driven disease. The description of pathology in multiple other organ systems in this patient also indicates a possible role for IL-33 in bone structure, dental tissue, connective tissue, and neurodevelopment.

There has been interest in pursuing monoclonal antibodies that target IL-33 as treatments for atopy.⁸ Although there was limited success in atopic dermatitis, a phenotype not described in our patient, anti-IL-33 therapies have been found to be potentially efficacious in moderating peanut

allergy.⁹ It will be of interest to see whether anti-IL-33 monoclonal antibodies could be a treatment option for patients with rare disease with hypereosinophilia and autoimmunity who have treatment-refractory, debilitating end organ disease.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at <https://doi.org/10.1053/j.gastro.2022.04.026>.

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Figure 1. Patient phenotype and functional data. (A) Patient pictures of skin rash caused by eosinophilic infiltration. (B) Skull x-ray with lytic lesions shown by green arrows (C, D) mRNA-sequencing data showing relative ranking of IL-33 expression in proband vs control tissues. Y-axis shows percentile-ranked IL-33 expression calculated by ranking IL-33 tags per million (TPM) values and dividing by the total number of genes for each dataset. Data from the proband (red dots) and mother and father (labeled black dots) are shown. Box and whisker plots show IL-33 percentile-ranking from $n = 504$ fibroblast and $n = 755$ blood RNA-sequencing (RNA-seq) datasets from GTEx. Boxes show the 25th and 75th percentiles; whiskers extend to $1.5 \times$ interquartile range. IL-33 expression ranking in the proband (red dot) ranked seventh ($>98.6\%$) among fibroblast and second ($>99.7\%$) among whole-blood samples from GTEx. A heatmap (D) of the top genes identified as outliers in patient but not parental whole-blood RNA-seq. Color scale shows Z score of gene rankings calculated across all GTEx whole-blood RNA-seq samples. Only genes with a Z score <3 in both parents and >4 in the patient are shown. (E, F) Data showing an increase in IL-33 tissue expression (green) in the patient (9 sections used for analysis) via immunofluorescence in skin, stomach, and colon biopsy tissues compared with 9 samples from healthy control subjects and 9 samples from patients with IBD. P values were calculated using the *t* test comparison between patient and healthy control subjects. Aggregate morphometric data from multiple samples ($n = 9$ in each group) are shown (F), as well as representative staining for each site in healthy control subject and patient (E).

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Data Availability

Data, analytic methods, and study materials will be made available to other researchers upon request to senior author, Thomas Eiwegger.

Conflicts of interests

The authors disclose no conflicts.

Supplementary Methods

Genetic Testing

Clinical microarray testing was performed with the CytoScan HD SNP Array (Affymetrix) and analyzed using ChAS (Affymetrix) software according to clinical reporting protocols. Genome sequencing was performed at the Centre for Applied Genomics (Toronto, Ontario, Canada) with high-quality DNA extracted from whole blood using established methods.¹

Library preparation was performed from 500 ng of DNA using the TruSeq Nano DNA Library Preparation Kit (Illumina), omitting the polymerase chain reaction amplification step, followed by sequencing on a HiSeq X platform (Illumina) per recommended protocols. Base calling and data analysis were performed using Bcl2FASTQ or HiSeq Analysis Software version 2-2.5.55.1311 (Illumina) and reads were mapped to the hg19 reference sequence using Burrows-Wheeler Aligner, version 0.7.12 (Illumina). Single-nucleotide variations and insertions and deletions were detected using Genome Analysis Toolkit, version 3.4-46 or version 3.7 (Broad Institute). Detected variants were annotated using a custom pipeline based on ANNOVAR (ANNOtate VARIation; Center for Applied Genomics, University of Pennsylvania) and with the addition of SpliceAI (Illumina). Copy number variations (CNVs) were detected using the read depth methods ERDS (estimation by read depth with single-nucleotide variants; Duke University) and CNVnator (Yale University) with a window size of 500 bp. High-quality CNVs were defined as those detected by ERDS that were also detected by CNVnator with >50% reciprocal overlap. Structural variants were detected using the algorithms Manta (Illumina), LUMPY (University of Virginia), and DELLY (European Molecular Biology Laboratory). Structural variants that were detected by at least 2 callers were prioritized, with variants supported by at least 5 paired or split reads considered as higher stringency. Short tandem repeats were genotyped at 54 targeted loci of known or potential clinical relevance using ExpansionHunter, version 3.1.2 (Illumina). Mitochondrial variants were converted to NC_012920 coordinates with a custom script and then annotated using MitImpact19, version 2.444 (Laboratory of Bioinformatics, IRCCS Casa Sollievo della Sofferenza). Where necessary, read alignments were manually inspected using Integrative Genomics Viewer (Broad Institute). Rare single-nucleotide variations and insertions and deletions were defined as those present at <1% allele frequency in large population control data sets, and rare structural variants and CNVs were those present in <1% of unaffected parents in the Autism Speaks MSSNG data set. CNVs were also annotated with respect to the degree of overlap with those in the Database of Genomic Variants.

RNA Sequencing

RNA sequencing (RNA-seq) libraries were made by means of polyA enrichment of total RNA; alignment and

quantification of RNA-seq datasets were performed in accordance with common practices in the field, as described in detail in the [Supplementary Methods](#). Transcripts per million values were calculated by dividing read counts per gene by the total number of reads successfully assigned to genes per library. Precalculated transcripts per million values for GTEx v8 datasets were obtained from the GTEx portal on March 15, 2021. Relative expression ranking was calculated in R using the rank() function and dividing by the total number of genes.

RNA quality was checked with Bioanalyzer (Agilent) at The Centre for Applied Genomics and quantified with Qubit RNA HS assay (Thermo Fisher). RNA input of 250 ng of total RNA was used per sample and spiked with SIRV Set 3 (Lexogen). Whole-blood libraries were sequenced at The Centre for Applied Genomics on a HiSeq2500 with paired-end 50 bp using CATSseq custom read 2 primer. The fibroblast library was sequenced at The Centre for Applied Genomics on a NovaSeq6000 with paired-end 100 bp.

RNA-seq libraries were prepared with CATS mRNA-seq kit with polyA selection (Diagenode, Belgium) for whole-blood samples and NEBNext Ultra II Directional RNA library prep kit for Illumina with NEBNext Poly(A) mRNA Magnetic Isolation Module (New England Biolabs) for fibroblast samples according to manufacturer's protocols. RNA-seq data were obtained in paired-end FASTQ format and processed as follows. CAT-seq libraries (proband, maternal, and paternal whole blood) were processed for read trimming, as recommended by the supplier (Diagenode trimming tools for CATS library preparation [read1]). Read trimming was not performed for the proband fibroblast library. FASTQ sequences were aligned to the human hg19 genome assembly using STAR v2.5.1b using default settings. Read counting to GENCODE v19 gene models was performed using featureCounts v1.5.0 in stranded mode with the settings -p -C.

Immunohistochemistry Staining

Colon-sigmoid mucosa and skin tissue samples were retrieved from the Division of Pathology, The Hospital for Sick Children. These samples included the patient, individuals with inflammatory bowel disease, and healthy controls. The details for immunofluorescence staining on formalin-fixed, paraffin-embedded sections have been published previously.^{e1}

Briefly, as a first step, paraffin was removed using Xylene, and afterwards rehydrated with different percentages of ethanol. Antigen retrieval was performed with high-pressure cooking in EDTA-borax buffer (1 mM EDTA, 10 mM borax [sodium tetraborate]; Sigma, St Louis, MO), 10 mM boric acid (Sigma) with 0.001% Proclin 300 (Supleco, Bellefonte, PA) at pH 8.5. To block nonspecific staining, the slides were incubated for 1 hour at room temperature in 4% bovine serum albumin in 1X phosphate-buffered saline (Multi Cell) 20% normal donkey serum. A properly diluted primary antibody stain with human anti-IL-33 antibody AF3625 (R&D Systems) was performed

overnight at 4°C. On the following day, stained slides were washed 3 times for 5 minutes with 1X phosphate-buffered saline. Secondary antibody, donkey anti-rabbit IgG Fab2 fragment-rhodamine conjugate mixed with donkey anti-mouse IgG Fab2 fragment-fluorescein isothiocyanate conjugate (Jackson ImmunoResearch Laboratory, West Grove, PA) incubation was performed at room temperature in darkness for 2 hours, and slides were washed afterwards 3 times for 10 minutes in darkness. As a nuclear counterstain reagent, RedDot2 far red fluorescence (Biotium, Fremont, CA) was used at a dilution of 1:200. Finally, sections were mounted overnight with VECTASHIELD fluorescence mounting medium (Vector Laboratories, Burlington, Ontario, Canada). Double/triple-immunostained sections

were imaged using a Leica confocal laser scanning microscope (model TCS-SP8) and LAS-AF software (Leica Microsystems, Wetzlar, Germany). The variable excitation wavelengths of the krypton/argon laser were 488 nm for fluorescein isothiocyanate conjugate, 568 nm for Texas Red complex, and 695 nm for Alexa Fluor 680 conjugate/RedDot 2 (nuclear counterstaining). Image processing, including color resolution, color separation, and merging of fields were carried out using Adobe PhotoShop CS5 software (Adobe Systems Incorporated, San Jose, CA).

Supplementary Reference

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Supplementary Table 1. Summary Clinical Investigations for Patient

Test	Value	Normal range	Unit
TCR V beta analysis	No significant expansion of any TCRV beta clones	NA	NA
CD3 ⁺ %	72.5	63.2–77.8	%
CD3 ⁺ absolute	3185	1239–2611	cells/ μ
CD3 ⁺ CD8 ⁺ %	36.2	17.1–30.0	%
CD3 ⁺ CD8 ⁺ absolute	1593	365–945	cells/ μ
CD3 ⁺ CD4 ⁺ %	28.6	31.7–47.0	%
CD3 ⁺ CD4 ⁺ absolute	1259	646–1515	cells/ μ
CD16 ⁺ CD56 ⁺ %	11.2	5.4–18.6	%
CD16 ⁺ CD56 ⁺ absolute	494	120–483	cells/ μ
CD19 ⁺ %	15.3	12.0	%
CD19 ⁺ absolute	672	276–640	cells/ μ L
CD4/CD8 ratio	0.79	1.18–2.65	NA
CD45RA:CD45RO T cells %	normal	NA	NA
Treg %	5	4–20	%
Treg absolute	36.9	33–190	$\times 10^6$ /L
Lymphocyte PHA proliferation	Normal	NA	NA
IgA	0.6	0.5–2.2	g/L
IgG	10.4	6.6–15.3	g/L
IgM	0.6	0.5–1.5	g/L
IgE	Minimum 896 and maximum 2287 (4 y of sampling)	<450	IU/mL
Measles, mumps, rubella IgG	Reactive	NA	NA
VZV antibody	Positive	NA	NA
Isohemagglutinin titer anti-B	Normal (0.32)	NA	NA
Tetanus toxoid IgG	Normal (0.21)	NA	NA
Blood FISH for PDGFA/B rearrangement	Negative	NA	NA
Bone marrow FISH for PDGFA/B rearrangement	Negative	NA	NA
Chest x-ray	Mild parabrachial perihilar thickening bilaterally, small airway disease	NA	NA
Echocardiogram and ECG	Normal	NA	NA
MRI brain	Normal	NA	NA
Bone marrow biopsy	Eosinophilia with no morphologic evidence of malignant infiltration (18% eosinophils)	NA	NA
CMV/HHV/EBV PCR	Negative	NA	NA
HIV	Negative	NA	NA
Blood eosinophils	Minimum 1.68 and maximum 5.12 (11 y of sampling)	0.03–0.52	$\times 10^9$
Esophageal biopsy eosinophils	Proximal esophagus 15/HPF Mid esophagus 25/HPF Distal esophagus 55/HPF	<15	per HPF

CMV, cytomegalovirus; EBV, Epstein-Barr virus; ECG, electrocardiogram; FISH, fluorescence in situ hybridization; HHV, human herpesvirus; HIV, human immunodeficiency virus; HPF, high-power field; NA, not applicable; MRI, magnetic resonance imaging; PCR, polymerase chain reaction; PHA, phytohemagglutinin; TCR, T cell receptor; Treg, regulatory T cell; VZV, varicella-zoster virus.