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### ► **To cite this version:**

Nina Anesi, Mathilde Calmels, Laurent Guilleminault, Flora Abbas, Claire Cenac, et al.. In Asthmatic Patients, Sexual Dimorphism correlates With Androgen Receptor Expression in ILC2s at Single Cell-Resolution. *Clinical and Experimental Allergy*, 2025, 55 (9), pp.851-853. <10.1111/cea.70047>. <hal-05187200>

**HAL Id: hal-05187200**

**<https://hal.science/hal-05187200v1>**

Submitted on 7 Nov 2025

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## RESEARCH LETTER OPEN ACCESS

# In Asthmatic Patients, Sexual Dimorphism correlates With Androgen Receptor Expression in ILC2s at Single Cell-Resolution

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**Received:** 22 April 2024 | **Revised:** 10 February 2025 | **Accepted:** 24 March 2025

**Funding:** N.A. and M.C. were supported by fellowships from the Ministère de l'Éducation Nationale, de la Recherche et de la Technologie, the Agence Nationale de la Recherche (ANR-20-CE15-0027), and the Fondation pour la Recherche Médicale, respectively. This work was supported by grants from the French Society of Allergology, the Fondation du Souffle, the Fondation pour la recherche Médicale (FRM DEQ20180339187 to J.-C.G.) and the Agence Nationale de la Recherche (ANR-20-CE15-0027 to S.L.).

**Keywords:** androgen receptor | selective androgen receptor modulator | sex bias in asthma | type 2 innate lymphoid cells (ILC2)

To the Editor,

Asthma, a chronic inflammatory airway condition, is the most common respiratory disease. There is a sex bias in asthma with a predominance of females affected by this disease in adulthood [1]. Type 2 innate lymphoid cells (ILC2s) have been identified as key players during allergic and/or asthmatic responses [2] through their capacity to licence dendritic cells (DC) for optimal Th2 cell priming [3]. The ILC2/IL33 axis was also reported to maintain airway hyperreactivity and remodelling in a chronic asthma model independently of T cells [4]. Indeed, the non-redundant role of ILC2 in recruiting eosinophils in the airway in allergic asthma models has been established in mice selectively lacking ILC2 [5]. ILC2 are found at higher numbers in female tissues, including the lung, compared to males, and express high levels of the *Nr3c4* androgen receptor (AR) [6, 7]. Androgen, via the AR receptor, acts directly within ILC2s to limit their expansion and cytokine production, thus protecting males and females from ILC2-dependent lung inflammation [6, 7]. Interestingly, children and adults with complete androgen insensitivity syndrome due to inherited loss of AR activity exhibit an increased asthma risk [8].

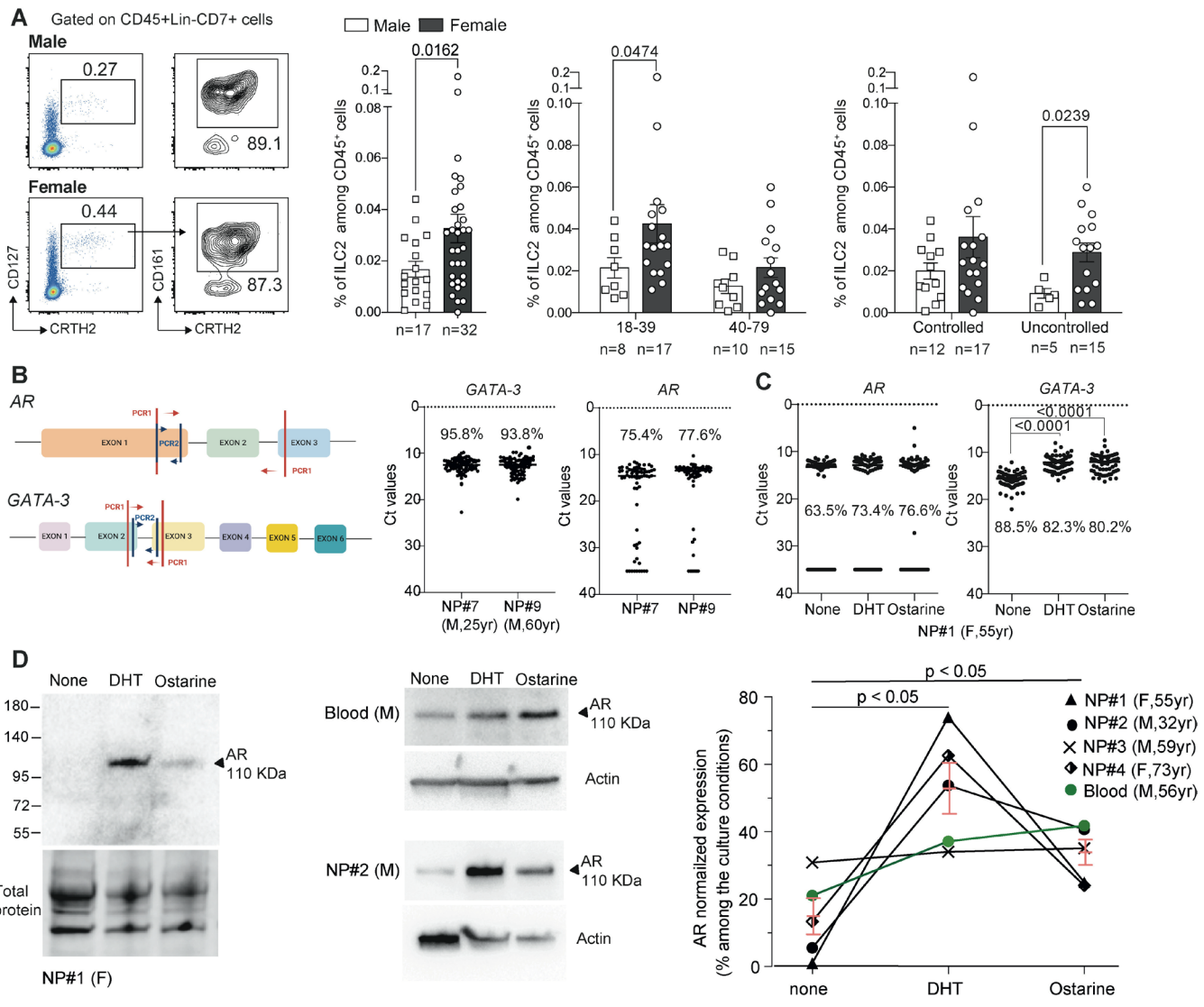
Studies investigating sex-bias in ILC2 in asthmatic subjects, as well as AR gene expression in human ILC2, are scarce. Here, we analysed the frequency of circulating ILC2s in adult male and female asthmatic patients without pre-selection criteria based on sex (Table S1). However, since the prevalence of asthma is higher in women compared to males, as reported by others [1], fewer males were recruited during the course of the study. ILC2s were identified as CD45<sup>+</sup>Lin<sup>-</sup>CD7<sup>+</sup>CD127<sup>hi</sup>CD161<sup>+</sup>CRTH2<sup>+</sup> (Figure 1A; Figure S1) and their frequency was significantly higher in asthmatic females compared to males. The sex-bias was more pronounced in younger individuals than in patients above 40, suggesting a role for sexual hormones. Interestingly, the frequency of ILC2 was markedly higher in females with uncontrolled asthma when compared to males (Figure 1A). Since androgen-signalling has been reported to negatively control ILC2 numbers in mice, we next investigated whether human inflammatory ILC2 express AR gene at single-cell resolution (Figure 1B). Because tissue ILC2s could be easily obtained from nasal polyp (NP), we isolated NP-ILC2 and expanded them in vitro (Figure S2A). Moreover, the presence of inflammatory CD45RO<sup>+</sup> ILC2s in NP has been linked to severe asthma and

**Abbreviations:** AR, androgen receptor; DHT, dihydrotestosterone; NP, nasal polyp; SARM, selective androgen receptor modulator.

Nina Anesi and Mathilde Calmels contributed equally to the work. Sophie Laffont and JC Guéry jointly supervised this study.

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**FIGURE 1** | ILC2 Frequencies are decreased in male asthmatic patients and ILC2s from both sexes express *AR* at single-cell resolution. (A) Gating strategy analysis of ILC2 (CD45+Lin-CD7+CD127<sup>hi</sup>CD161+CRTH2<sup>+</sup>) in PBMCs. Frequency of ILC2 among CD45+ cells from PBMCs of adult male ( $n = 17$ ) and female ( $n = 32$ ) asthmatic patients. Patients were further stratified according to their age or their capacity to control asthma. Difference between groups was calculated with the Student's t-test with Welsh's correction. (B) Localization of primer pairs used for the pre-amplification PCR (PCR1) and the qPCR (PCR2) of *AR* and *GATA-3* gene (Table S2). ILC2 were magnetically enriched from nasal polyp of asthmatic patients and further amplified for several days in presence of IL-2, -7, -25 and -33. ILC2 were then single-cell sorted to evaluate *AR* and *GATA-3* expression per cell (raw Ct value in real-time PCR). Numbers represent the frequency of ILC2 expressing *GATA-3* and *AR* mRNA among *GATA-3*+ ILC2s from 2 male donors and (C) from a female donor (NP#1) cultured in presence or not of DHT or ostarine. Two-way ANOVA was used to assess statistical differences between groups. (D) *AR* expression quantification by western blot from ILC2s cultured in presence or absence of the indicated *AR*-agonist ligands. *AR* protein expression was normalised to total protein or  $\beta$ -Actin and expressed as percentage from the 3 culture conditions tested ( $n = 5$ ). ILC2 cultures from NP donors (3 males and 2 females) and one male blood donor. Two-way ANOVA was used to assess statistical differences between culture conditions.

steroid resistance [9]. After 7 to 10 days of culture, we obtained homogeneous populations of lymphoid cells expressing the expected ILC2 markers, CD7, CD161 and *GATA-3*, and lacking T cell markers such as CD3 (Figure S2B) and CD4 (data not shown). After PMA/ionomycin activation, these ILC2s produced IL-13 (Figure S2C). We designed a single-cell RT-PCR method to detect both *AR* and *GATA-3* genes simultaneously in cDNAs obtained from a single ILC2 (Figure 1B). We tested ILC2s from the NP of 2 males. *GATA-3* transcripts were detected in more than 93% of the wells, confirming ILC2 lineage identity. Within *GATA-3*+ ILC2s, we then measured *AR* gene expression,

which we could detect in more than 75% of cells (Figure 1B). We then tested NP-ILC2s from a female donor cultured in vitro in the presence or absence of 5 $\alpha$ -dihydrotestosterone (DHT) or the non-steroidal selective androgen receptor modulator (SARM) ostarine. Ostarine is one of the most potent and tissue-selective nonsteroidal SARMs exhibiting strong in vivo androgenic and anabolic activity. More than 88% of the wells scored positive for *GATA-3* transcripts, and within these cells, 63% to 76% expressed *AR* (Figure 1C). We observed enhanced *GATA-3* gene expression in ILC2 cultured in the presence of *AR*-agonist ligands, whereas *AR* expression was unchanged (Figure 1C). Because

## Summary

- Sex differences in asthma are associated with higher circulating ILC2 numbers in females.
- Male and female ILC2s express AR gene at single-cell resolution as well as AR protein.

the majority of ILC2 in both sexes express AR mRNA, we then investigated whether AR protein in ILC2 could be targeted by agonist ligands. Western blot analysis identified a clear signal at 110 KDa corresponding to the molecular weight of AR protein in the five ILC2 preparations expanded from NP (2F, 2M) or the blood of a male asthmatic patient (Figure 1D). AR expression was significantly enhanced when ILC2s were cultured in the presence of DHT or ostarine (Figure 1D). It is well established that AR agonist ligands, by binding to AR, stabilise the protein both in tumour cells and murine ILC2 [7].

In summary, our data show that the frequency of circulating ILC2 is higher in asthmatic females compared to males, particularly in young individuals below 40 years, and that this sex bias was even more pronounced in uncontrolled asthma. The enhanced proportion of blood ILC2 in young asthmatic females is unlikely to reflect a positive action of ovarian hormones, including oestrogens, directly on ILC2 or their progenitors as ovariectomy, oestrogen supplementation and oestrogen receptor deficiency had no impact on ILC2 frequency in mice [6]. By contrast, previous studies have clearly established that murine ILC2 are amenable to in vivo and in vitro pharmacological manipulations with AR agonist ligands [7]. Like in mice, human ILC2s express AR, suggesting that they are programmed to respond to androgens in a cell-intrinsic manner in both sexes. Of note, the non-steroidal SARM ostarine also binds to human AR protein thereby stabilising its expression in ILC2s. SARMs represent interesting alternatives to Testosterone for in vivo therapy as they can maintain tissue-selective agonist properties with greatly reduced virilising and proliferative side effects on reproductive organs. Given the emerging beneficial role of androgens in human asthma [8], our data, by establishing that human ILC2 express functional AR protein, reinforce the notion that AR-agonist ligands could be exploited to therapeutic benefit in asthma.

## Author Contributions

L.G., S.L., J.-C.G. contributed to the conceptualisation of the study, data analysis and interpretation, drafting the manuscript and critical revision of the manuscript. N.A., M.C., F.A. and C.C. contributed to the acquisition, analysis and interpretation of data. L.G., M.C., T.V., G.d.B. contributed to the recruitment of blood samples and tissues from asthmatic patients. J.-C.G., N.A., S.L. wrote the manuscript with input from the co-authors. All authors read and approved the final version of the manuscript.

## Acknowledgements

Flow cytometry experiments were done at the INFINITY-INSERM UMR1291 core facility connected to the Toulouse Réseau Imagerie network, a member of the France-BioImaging national infrastructure supported by the French National Research Agency (ANR-10-INBS-04). We thank Valérie Duplan, Hugo Garnier, A.L. Iscache, and F.L'Faqihi for the help with cell sorting and data acquisition.

## Conflicts of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

All of the data generated or analysed during this study are included in this article and the Supporting Information. All data supporting the findings of this study are also available upon reasonable request from the corresponding authors (S.L. or J.-C.G.). Additional information about study methods and findings is available in the following repository at Zenodo <https://doi.org/10.5281/zenodo.14361771>.

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