Immune function during early adolescence positively predicts adult facial sexual dimorphism in both men and women

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Abstract

Evolutionary theories suggest that humans prefer sexual dimorphism in faces because masculinity in men and femininity in women may be an indicator of immune function during development. In particular, the immunocompetence handicap hypothesis proposes that sexual dimorphism indicates good immune function during development because the sex hormones, particularly testosterone in men, required for the development of sexually dimorphic facial features also taxes the immune system. Therefore, only healthy males can afford the high level of testosterone for the development of sexually dimorphic traits without compromising their survival. Researchers have suggested that a similar mechanism via the effects of estrogen might also explain male preferences for female femininity. Despite the prominence of the immunocompetence handicap hypothesis, no studies have tested whether immune function during development predicts adult facial sexual dimorphism. Here, using data from a longitudinal public health dataset, the Western Australian Pregnancy Cohort (Raine) Study (Generation 2), we show that some aspects of immune function during early adolescence (14 years) positively predict sexually dimorphic 3D face shape in both men and women. Our results support a fundamental assumption that facial sexual dimorphism is an indicator of immune function during the development of facial sexual dimorphism.

Key words: sexual dimorphism, immunocompetence handicap hypothesis, facial attractiveness

1. Introduction

Men and women differ in their facial appearance. Men have more prominent brow ridges, stronger jawlines, more prominent cheekbones, and thinner lips compared to women (D. M. Enlow, 1982). Noting the highly elaborate sexually dimorphic traits found in many species, Darwin proposed that secondary sexual traits could have evolved via sexual selection for two main purposes: attracting the opposite sex (mate choice) and/or competing with same-sex conspecifics for access to opposite-sex partners (mating competition) (Darwin, 1871). Since the inception of the theory of sexual selection, mate choice for sexually dimorphic traits has been demonstrated in a wide range of taxa, including insects, fishes, birds, reptiles, and mammals (Andersson, 1994). In humans, there is considerable evidence that facial sexual dimorphism (i.e. masculinity in men and femininity in women) is linked to attractiveness in both sexes (Foo, Simmons, & Rhodes, 2017; Holzleitner & Perrett, 2017; Little, Jones, & DeBruine, 2011; Rhodes, 2006; Thornhill & Gangestad, 1999). Across studies, masculinity in men has been found to elicit a range of preferences that depend on factors such as mating context (short-term vs long-term mating), the women's own attractiveness and partner violence (Borras-Guevara, Batres, & Perrett, 2017; Li et al., 2014; Little et al., 2011; Rhodes, 2006; Scott, Clark, Boothroyd, & Penton-Voak, 2013). Variation in preferences for masculinity have often been interpreted in terms of balancing the pros of having a high-quality man against the increased likelihood of partner violence and unfaithfulness (Little et al., 2011). In contrast, femininity in women has consistently been found to be preferred by men (Little et al., 2011; Rhodes, 2006). Overall, a meta-analysis showed that both men and women with more sexually dimorphic faces are rated by the opposite sex as more attractive than their less sexually dimorphic counterparts (Rhodes, 2006). Facial sexual dimorphism is also positively related to self-reported mating success in both sexes (Rhodes, Simmons, & Peters, 2005). Together, the evidence indicates that sexual

selection via mate choice may have played a role in the evolution of facial sexual dimorphism in humans. But why have we evolved to prefer facial sexual dimorphism in opposite-sex partners?

One prominent theory is that sexually dimorphic traits provide honest indicators of quality, particularly immune function (Folstad & Karter, 1992; Hamilton & Zuk, 1982). Individuals are exposed to pathogens on a daily basis. Some pathogens can cause serious infections that threaten our health or even survival. Indeed, data from the World Health Organization (WHO) show that disease-causing infections such as lower respiratory infections, diarrhoeal diseases, and tuberculosis are among the top ten causes of mortality in humans (World Health Organization, 2018). In response to the debilitating effects of infectious diseases, our immune system has evolved a complex range of defences to neutralize pathogens (Parkin & Cohen, 2001). From a sexual selection perspective, therefore, choosing a partner based on indicators of superior immunity can provide us with a number of direct benefits, including infectious disease avoidance, better parenting, and more resources, and indirect benefits such as genes that code for good immune functioning for our offspring (Andersson, 1994; Andersson & Simmons, 2006).

In terms of the mechanistic links, life history theory has proposed that sexually dimorphic traits honestly indicate superior immune function because individuals are subject to trade-offs in the allocation of limited resources to developing attractive traits vs maintaining immune functioning (Rolff, 2002; Stearns, 1977, 1992; Wedekind & Folstad, 1994). Therefore, healthy individuals with superior immunity can afford to allocate more resources to develop highly sexually dimorphic traits. According to the immunocompetence handicap hypothesis, in vertebrates, the trade-off in developing sexually dimorphic traits vs maintaining immune functioning in males is mediated partly by the immunosuppressive effect of the sex hormone testosterone (Folstad & Karter, 1992). Indeed, results from a recent

meta-analysis using data from 38 species supported the assumption of the immunocompetence handicap hypothesis that testosterone suppresses the immune system in males (Foo, Nakagawa, Rhodes, & Simmons, 2017).

Despite the prediction of the immunocompetence handicap hypothesis, researchers have found mixed support for a relationship between facial masculinity and immune function in men. Early studies relied primarily on indirect and non-physiological measures of potential immune function. For instance, men with masculine faces report experiencing fewer infection-related conditions (e.g. flu incidence and antibiotic intake, Thornhill & Gangestad, 2006; but see Boothroyd, Scott, Gray, Coombes, & Pound, 2013) and are rated by doctors as healthier during adolescence based on their past medical records (Rhodes, Chan, Zebrowitz, & Simmons, 2003). More recent studies have utilised putative genetic correlates of immune function. One example is the degree of heterozygosity in the major histocompatibility complex (MHC) genes, which predicts the range of antigens that the immune system can recognize (Apanius et al., 2017). MHC heterozygosity was shown not to be linked to facial masculinity in men (Lie, Rhodes, & Simmons, 2008; Zaidi et al., 2019). However, MHC heterozygosity only measures the range of pathogens to which one can raise a response and not the magnitude of the actual response (e.g. the amount of circulating immune cells/antibodies or those produced in response to a threat), which is a key aspect of eliminating foreign antigens. Few studies have used physiological markers of immune function, such as adaptive immunity (Rantala et al., 2013), innate immunity (Foo, Simmons, et al., 2017), or cytokine responses to antigen challenges (Phalane, Tribe, Steel, Cholo, & Coetzee, 2017). Among these studies, Rantala et al. (2013) and Phalane et al. (2017) found support for an association between masculinity and immune function but Foo et al. (2017) did not.

Although the immunocompetence handicap hypothesis was initially proposed to explain the attractiveness of masculine traits in males, researchers have raised the possibility that feminine traits may also serve as honest indicators of immune function in females, particularly in humans, where both female and male mate choice occur (Gray & Boothroyd, 2012; Jones, 2018; Rhodes et al., 2003). It was further speculated that a similar trade-off mechanism via the effects of the female hormone oestrogen might underpin the relationship between facial femininity and immune function in women (Rhodes et al., 2003). However, extending the analogy from men's to women's faces is questionable for two reasons. First, much of the pubertal facial changes that lead to sexual dimorphism (e.g. brow, jaw, cheekbones, except the lips), occur in men while women's faces remain relatively immature (D. H. Enlow & Hans, 1996; D. M. Enlow, 1982). Second, a meta-analysis by Foo et al (2017) found that oestrogen could have either a positive or negative effect on female immune function, depending on the aspect of the immune system measured. Such mixed findings call into question the hypothesis that attractive feminine traits honestly indicate female immune function because oestrogen suppresses female immune function. Indeed, the relationship between facial femininity and immune function in women is mixed. While women with feminine faces have lower self-reported prevalence of infection-related conditions (Gray & Boothroyd, 2012), studies have failed to find significant relationships between facial femininity in women and their adolescent health as rated by doctors (Rhodes et al., 2003), MHC genetic heterozygosity (Lie et al., 2008), innate immunity (Foo, Simmons, et al., 2017), or salivary IgA levels (Cai et al., 2019).

The majority of the abovementioned studies have focused on the relationship between facial sexual dimorphism and current adult immune function. In general, theories of sexual signalling predict a positive relationship between sexual dimorphism and current adult immune function. Particularly, under direct-benefit models of mate choice, we expect a

positive relationship because we obtain direct benefits (e.g. infectious disease avoidance, better parenting, and more resources) only when an attractive partner also has a better immunity compared to a less attractive individual (Andersson & Simmons, 2006). However, under indirect-good-genes models, trade-offs can lead to null or even negative relationships between facial sexual dimorphism and current adult immune function, which could explain the mixed findings in the current literature (Getty, 2006).

Rather than indicating current adult immune function, life-history theory predicts that sexually dimorphic traits should indicate immune function during the period in which they develop. In many species, secondary sexual traits do not develop until the individuals approach sexual maturity (Birkhead, Fletcher, & Pellatt, 1999; D. M. Enlow, 1982; Fry, 2006; Gilbert, Karp, & Uetz, 2016). In humans, for instance, individuals experience substantial development during puberty in facial sexually dimorphic traits, especially shape features like the brow ridges, jawlines, cheekbones, and lips (D. M. Enlow, 1982). In such species, we expect adult secondary sexual traits to indicate the ability to divert resources to the formation of attractive traits while remaining able to cope with parasitic challenges during development. This prediction is well-supported in non-human animal studies, with findings linking adult male secondary traits to various aspects of juvenile health such as diet and immune function in taxa such as insects, fishes, and birds (Geary, 2015; Irschick, Briffa, & Podos, 2015).

Critically, this developmental prediction has received very little empirical attention in the human literature, primarily due to the difficulty of conducting protracted longitudinal studies to test the relationship between immune function during the period in which secondary sexual traits develop (i.e. adolescence) and facial sexual dimorphism in adulthood. So far, only one study has done so (Rhodes et al., 2003). In line with the developmental prediction, men who were rated by doctors as healthier during adolescence based on their

past medical records had more masculine faces in adulthood. The study also tested women but failed to find any relationship between adolescent health and adult facial femininity. The health data in that study was based on data from the 1920s, before population health was impacted by the adoption of modern medical advances such as vaccinations and antibiotics. Therefore, the measure is likely to reflect in part the ability to resist pathogens. However, it remains plausible that the measure also captured variation in other facets of health, such as reproductive or even mental health.

Here, using data from a large-scale (N > 400) longitudinal public health dataset, the Western Australian Pregnancy Cohort (Raine) Study (Straker et al., 2017), we examine the relationship between early adolescent immunity and adult facial sexual dimorphism in both men and women. The Raine Study was established between 1989-1991 initially with a sample of 2900 pregnant women that was representative of the general (Perth) Australian community for the purpose of testing the effect of repeated ultrasound scans on pregnancy outcomes. Multiple follow-ups with the offspring of participants (i.e. Generation 2) involving detailed assessments of health, lifestyle, and family background have since been conducted at 1, 2, 3, 5, 14, 17, 18, 20 and 22 years of age, providing an invaluable opportunity for testing the developmental prediction. Based on life history theory, we hypothesize that immune function during development (i.e. adolescence) positively predicts sexual dimorphism in 3D facial shape in both men and women.

Besides providing valuable developmental data for us to test our hypothesis, this dataset has two other advantages. First, it contains more than 40 immune physiological markers. Generally, evolutionary studies on the links between sexually-selected traits and immune function have relied on small number of immune measures. However, the immune system is a highly complex collection of mechanisms that work in tandem to protect against pathogens (Parkin & Cohen, 2001). Therefore, when studying the immune system, it is

crucial to employ multiple measures that index different aspects of immunity (Blount, Houston, Møller, & Wright, 2003; Demas & Nelson, 2012; Møller & Petrie, 2002; Norris & Evans, 2000; Nowak, Pawłowski, Borkowska, Augustyniak, & Drulis-Kawa, 2018). Here, our immune measures include white blood cell counts, generic and antigen-specific antibodies, inflammatory markers, and cytokine responses. Together, these measures cover a wide range of functional aspects of both innate and adaptive immunity, including anti-bacterial immunity, anti-viral immunity, cellular immunity, inflammation, and allergies. The richness of the immune dataset allows us to provide one of the most comprehensive tests of the relationship between immune function and facial sexual dimorphism to date. Second, the participants from the Raine dataset are representative of the wider (Perth) Australian community (Straker et al., 2017), which increases the generalizability of the results compared to previous studies in the field, which have typically been conducted with university student samples.

2. Methods

2.1 Participants

We used the 14-year immune function and 22-year 3D face scans data from 454 Caucasian Generation 2 participants (218 men and 236 women) from the Raine Study. The majority of participants in the dataset had parents who were both self-reported Caucasians (82.6%). We decided a-priori to exclude the data of participants who had at least one self-reported non-Caucasian parent to control for potential racial differences in immune function and face shape.

2.2 Ethics statement

The current research was approved by the Human Ethics Committee at the [omitted for blind review]. The 14-year follow-up was approved by the Human Ethics Committee of [omitted for blind review], and parents provided informed consent, with assent provided by

the teenage participants. The 22-year follow-up was approved by the Human Research Ethics Committees at the [omitted for blind review] and [omitted for blind review], and informed consent was provided by the adult participants.

2.3 Immune function at 14 years old

The teenage participants were visited in their homes by a phlebotomist/enrolled nurse to collect early-morning fasting blood and urine samples from the participants. The following immune measures were assessed from the blood samples:

2.3.1 Hematology. A full white blood cell count, including circulating eosinophil, lymphocyte, neutrophil, monocyte, and basophil, was conducted.

2.3.2 Antibodies. Total IgE, IgE to a panel of common inhalant allergens (i.e. phadiatop IgE), and IgE to a mixture of SA enterotoxins (SAE) (Staphylococcal enterotoxin A, Staphylococcal enterotoxin B and toxic shock syndrome toxin 1) were measured in serum using the ImmunoCAP assay (Phadia AB, Uppsala, Sweden).

IgE, IgG1, and IgG4 antibodies to outer membrane protein P4 and P6 from *H influenza*, and PspA1, PspA2 and PspC from *S pneumoniae* were assayed by a microtitre plate dissociation-enhanced immunofluorescence assay (DELFIA) using humanised chimeric antibodies for absolute quantitation.

2.3.3 Cytokine responses. Peripheral blood mononuclear cells were cultured and stimulated with house dust mites, rye, phytohemagglutinnin, poly(I:C), and LPS both with and without IFN-γ. IL-5, IL-10, IL-12, IL-13, IFN-γ, and tumor necrosis factor-α responses were measured by time-resolved fluorometry as a difference from unstimulated control. IL-4 and IL-9 responses were measured by quantitative mRNA reverse-transcriptase polymerase chain reaction as a difference from unstimulated control.

2.3.4 Inflammatory marker. The inflammatory marker soluble CD14 was measured in plasma by ELISA (BioScientific, Gymea, Australia).

The inflammatory eosinophil product eosinophil protein X was measured from urine and normalized against creatinine using ELISA (Cayman Chemical, Ann Arbor, MI; Phadia AB). Full details of the immune assays were presented in Hollams et al (Hollams et al., 2009)

2.4 3D shape sexual dimorphism at 22 years old

3D facial photographs of the participants were taken at the 22-year follow-up using the 3dMDFace system (www.3dMD.com). Participants were seated at a fixed distance from the camera system. The height of the participants' heads was standardized by adjusting the height of their chair such that the participants were at eye-level to a fixed point on the wall in front of them. Participants were asked to look straight and keep a neutral expression with their mouth closed. The 454 faces in this study represent a subset of an initial pool of 930 faces. We selected faces based on the following a-priori inclusion/exclusion criteria: (1) No excess facial hair that might obstruct the quantification of face shape (e.g. full beard); (2) No excessive missing regions that would prevent quantification of face shape (e.g. missing the entire jaw); (3) Neutral expression with mouth closed, eyes open and looking straight at the camera; (4) Caucasian; (5) Had immune data from the 14-year follow-up.

We quantified 3D shape sexual dimorphism using a well-established geometric morphometric method in the program Morphanalyser 2.4 (Cai et al., 2019; Holzleitner & Perrett, 2017). Twenty-four of the faces (17 men and 7 women) were missing regions of their foreheads due to technical reasons (e.g. occlusion by hair or faulty scans) and a minority of men were exhibiting signs of receding hairline due to male pattern baldness. Therefore, in order to maximise statistical power and avoid confounds in forehead shape due to male pattern baldness, we trimmed the forehead from all faces and analysed face shape using only the region starting from the brow ridge and downwards. We also trimmed away the neck from the face scans to prevent variations in the neck from confounding our face shape

measurements. We delineated the *xyz* positions of 48 landmarks on the faces (see Figure 1 for an example. The example depicts the average of all the faces in our dataset and does not correspond to any individual identity. Other studies using this method have used similar numbers of landmarks (Cai et al., 2019; Holzleitner & Perrett, 2017). The original faces each contain different numbers of tessellations. In order for us to make systematic comparisons across the faces, we resampled the surface maps in reference to that of one of the faces such that all faces contained the same number of tessellations between landmarks. To ensure that our sexual dimorphism measure was capturing variations in shape only, Procrustes alignment was applied to the resampled surface maps to standardize their size, position in space, tilt, and orientation. Size of the head is sexually dimorphic, with men having bigger heads than women. However, the size of the head is highly correlated with body size. Therefore, we removed variation in face size using the Procrustes alignment to control for size variations due simply to magnification. This method maintains allometric shape differences between male and female faces (i.e. shape changes arising due to size changes) despite removing geometric size. Such procedures are commonly adopted in studies of face shape (Cai et al., 2019; Holzleitner et al., 2014; Holzleitner & Perrett, 2017).

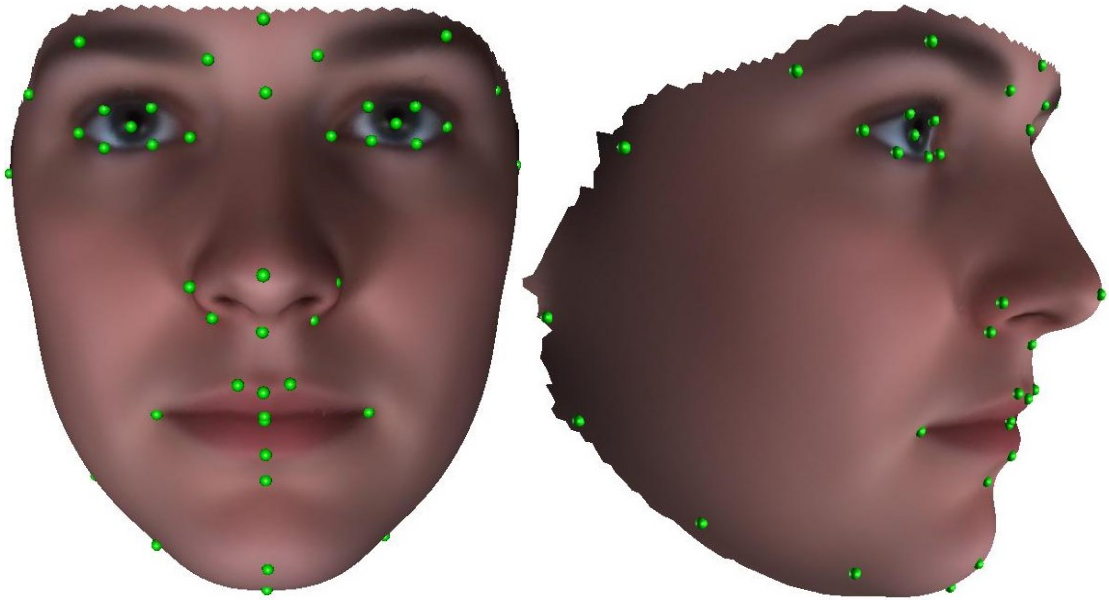

Figure 1. Example face landmark placements. Example here depicts the average of all the faces in our dataset, including both men and women, and does not correspond to any individual identity.

Principal components analysis (PCA) was then conducted on the surface maps (i.e. the entire network of tessellations, not just the landmarks). Forty PCs were retained (eigenvalue > 1). To determine which of these 40 PCs were sexually dimorphic, we conducted independent samples t-tests with sex as the independent variable on the 40 PCs. 12 were significantly different based on the criterion of $p < 0.05$. Therefore, we computed a composite 3D shape sexual dimorphism score (hereafter termed sexual dimorphism) for each face by summing the standardized Z scores on the 12 sexually dimorphic PCs weighted by the relative contribution of each PC to the overall variance in face shape. The higher the score, the more masculine is the shape of the face. To facilitate ease of interpretation, women's sexual dimorphism score was reverse-scored such that a higher score indicated more feminine face shape in women. Given the lack of a large and separate training set for deriving face shape components that are sexually dimorphic, there is a possibility that some of the sex differences are due to idiosyncratic differences arising from our sample. However, given the

relatively large number of individuals tested in both sexes (218 men and 236 women) and findings that our sample is representative of the larger Perth community on other health and life-history variables (Straker et al., 2017), we believe that the contributions of such idiosyncrasies to the sexual dimorphism score are likely to be small.

To confirm that our composite score was indeed capturing variations in sexual dimorphism, we entered the score into a discriminant analysis with sex as the classifying criterion. Our composite score correctly classified 80.8% of individuals, Wilks' $\lambda = 0.57$, $\chi^2(1) = 255.71$, $p < 0.0001$ (see Figure 2 for density plot of sexual dimorphism score by sex and the average male and female faces of our sample).

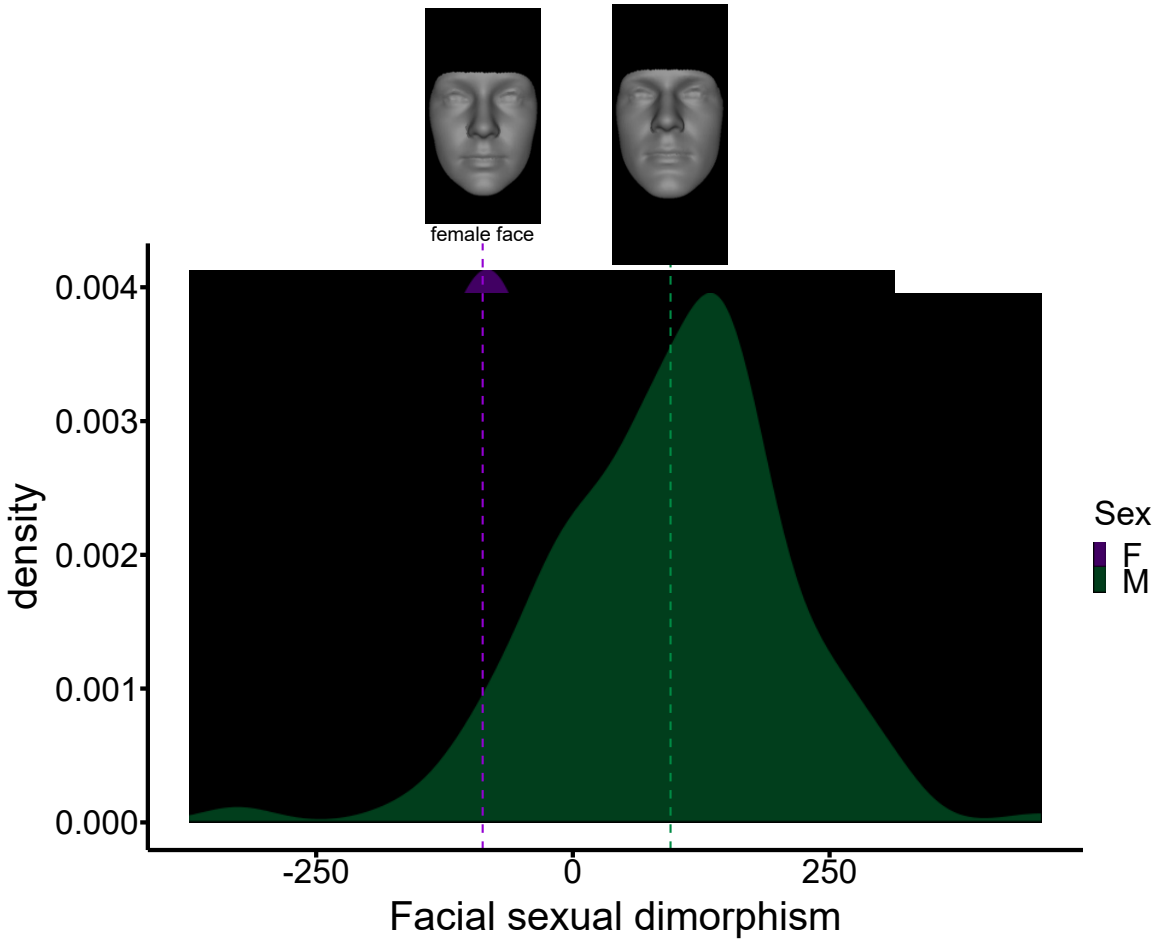

Figure 2. Density plot of 3D shape facial sexual dimorphism scores by sex of participants. Vertical lines denote the average sexual dimorphism score for each sex with the average face of the sexes presented above the lines (Mean sexual dimorphism score = -87.85 for women and 95.11 for men).

3. Results

Table 1 presents the descriptive statistics.

Table 1. Descriptive statistics of facial sexual dimorphism and immune function by sex of participants (note that the facial femininity scores were reversed-scored from the sexual dimorphism scores to facilitate interpretation, i.e. higher score, more feminine).

	Men					Women				
	N	Mean	SD	Skew	Kurtosis	N	Mean	SD	Skew	Kurtosis
Masculinity/Femininity at 22-year follow-up	218	95.11	110.93	-0.42	1.30	236	87.85	99.12	0.02	0.15
Immune function at 14-year follow-up										
Antibodies										
Total IgE (kU/L)	218	247.14	531.16	5.77	44.23	236	214.01	452.34	6.67	64.39
Phadiatop (common inhalant allergens) IgE (PAU/L)	218	18.39	41.42	4.28	23.30	236	16.01	45.68	5.60	39.92
Strep. enterotoxin A IgE (kUA/L)	218	0.60	2.28	9.19	102.05	236	0.33	0.98	6.65	47.07
Outer membrane protein P4 from H. influenzae IgE (ng/ml)	218	0.27	0.66	4.86	31.12	236	0.44	1.39	9.67	118.44
Outer membrane protein P6 from H. influenzae IgE (ng/ml)	218	0.47	0.88	3.81	19.75	236	0.66	1.41	6.11	54.40
Pneumococcal surface protein C (PspC) IgE (ng/ml)	218	0.88	1.66	4.17	23.00	236	0.93	1.44	3.37	18.22
P4 IgG1 (ng/ml)	218	102742.05	664524.92	10.09	107.16	236	239916.12	1653246.22	9.12	89.44
P6 IgG1 (ng/ml)	218	5749.28	24699.69	7.99	70.42	236	6519.46	23098.80	6.84	57.96
Pneumococcal surface protein A family 1 of S. pneumoniae (PaspA1) IgG1 (ng/ml)	218	147732.07	258428.08	2.44	6.36	236	96394.77	279224.77	5.89	45.51
Pneumococcal surface protein A family 2 of S. pneumoniae (PaspA2) IgG1 (ng/ml)	218	353097.71	580535.54	4.92	41.54	236	313006.56	577234.78	3.39	14.99
PaspC IgG1 (ng/ml)	218	424497.94	584243.50	3.47	20.59	236	476478.16	956491.11	4.99	30.87

P4 IgG4 (ng/ml)	218	69.71	224.62	6.74	56.65	236	88.58	377.16	6.55	44.30
P6 IgG4 (ng/ml)	218	106.37	525.14	7.45	61.87	236	180.76	1119.19	9.82	110.24
PaspA1 IgG4 (ng/ml)	218	644.56	7301.20	14.50	212.53	236	87.39	485.70	7.92	66.35
PaspA2 IgG4 (ng/ml)	218	190.49	1121.96	10.41	123.37	236	108.90	620.93	12.22	165.84
PaspC IgG4 (ng/ml)	218	139.08	306.30	2.81	7.63	236	177.76	391.43	2.55	5.56
Inflammatory markers										
Soluble CD14 (SCD14; ng/ml)	218	1858.48	373.98	1.12	2.15	236	1873.27	366.30	0.63	0.94
Urinary Eosinphil Protein X (EPX; µg/mmol Creatinine)	211	90.92	56.08	1.66	3.12	220	58.36	36.84	2.77	14.68
White blood cell counts										
Eosinophil (x109/L)	218	0.34	0.28	2.98	13.22	234	0.32	0.26	1.99	4.35
Lymphocyte (x109/L)	218	2.47	0.60	0.59	0.73	234	2.68	0.67	0.71	1.04
Neutrophil (x109/L)	218	2.74	1.14	5.00	46.09	234	3.24	1.16	0.78	0.96
Monocyte (x109/L)	218	0.56	0.16	0.92	1.75	234	0.55	0.18	0.80	1.15
Basophil (x109/L)	218	0.07	0.04	-0.85	-0.72	234	0.08	0.05	-0.25	1.66
Cytokine responses to house dust mite										
IL-5 (pg/ml)	218	13.76	39.70	4.15	20.09	236	11.71	36.18	4.15	20.32
IL-10 (pg/ml)	218	4.43	14.23	4.67	30.77	236	4.09	11.74	3.11	10.29
IL-13 (pg/ml)	218	41.48	90.65	5.23	41.24	236	39.23	90.53	5.51	44.74
Interferon γ (IFNγ; pg/ml)	218	8.50	48.06	10.22	120.61	236	8.43	36.78	8.10	82.66
Cytokine responses to rye										
IL-5 (pg/ml)	218	2.32	9.73	4.48	20.73	236	1.47	8.48	6.75	51.17
IL-10 (pg/ml)	218	28.03	54.75	4.38	29.29	236	26.64	51.38	4.01	25.57
IL-13 (pg/ml)	218	17.21	49.43	4.77	27.18	236	14.92	36.84	3.83	19.03
IFNγ (pg/ml)	218	16.08	79.84	7.97	72.51	236	39.46	342.79	10.67	114.05

Cytokine responses to phytohaemagglutinin (PHA)										
IL-5 (pg/ml)	218	224.24	196.76	2.61	10.13	236	208.58	178.24	2.54	9.53
IL-10 (pg/ml)	218	519.11	371.70	1.69	4.46	236	591.77	394.56	1.03	1.09
IL-13 (pg/ml)	218	1414.05	782.59	1.47	3.43	236	1320.33	834.87	2.42	11.80
IFNγ (pg/ml)	218	12187.73	10335.42	2.40	9.62	236	12558.43	9199.67	1.48	3.11
Cytokine responses to staph. enterotoxin B (SEB)										
IL-5 (pg/ml)	218	182.68	153.54	2.44	9.17	233	152.65	121.27	2.66	11.09
IL-10 (pg/ml)	218	610.74	439.12	2.15	7.80	233	636.59	381.12	1.24	2.45
IL-13 (pg/ml)	218	820.32	489.66	1.71	4.64	233	756.14	411.94	1.09	1.90
IFNγ (pg/ml)	218	23830.78	12945.11	0.91	1.03	233	22156.68	12262.71	0.99	1.47
Innate cytokine responses										
IL-10 to lipopolysaccharide (LPS; pg/ml)	217	1039.35	663.37	2.38	12.30	234	1042.09	641.46	1.04	1.23
IFNγ to LPS (pg/ml)	217	221.37	431.17	6.47	59.13	234	148.60	261.82	4.85	29.43
Tumour necrosis factor α (TNFα) to LPS (pg/ml)	217	1117.76	676.64	1.33	2.20	234	905.28	684.43	1.64	2.87
IL-10 to LPS+IFNg (pg/ml)	217	393.00	240.85	1.65	4.12	233	420.54	289.14	1.94	5.93
IL-12 to LPS+IFNg (pg/ml)	218	639.20	692.83	3.73	21.45	231	545.00	541.62	2.25	6.08
TNFα to LPS+IFNg (pg/ml)	217	3764.42	1932.38	1.25	2.50	233	3050.14	1730.41	1.18	1.50
IL-10 to Poly I:C (pg/ml)	217	1736.37	1266.49	1.35	1.90	233	1784.20	1210.01	1.45	2.97
IFNγ to Poly I:C (pg/ml)	217	1955.91	1952.31	2.09	5.97	233	1542.87	1393.17	1.71	3.20
TNFα to Poly I:C (pg/ml)	217	842.03	571.81	1.66	5.25	233	687.92	547.71	1.63	2.85

3.1 The relationship between immune function and facial masculinity in men

3.1.1 Male immune data reduction. PCA with varimax rotation was used to summarize the interrelated immune function measures in the male data. Sixteen PCs were retained (eigenvalue > 1), accounting for 73.49% of the total variance. The factor loadings of the PCs are presented in Supplementary Table 1. The PCs were loaded by immune measures that measured similar aspects of the immune system or reactions to the same antigen, suggesting that our PCA succeeded at identifying clusters of related measures.

3.1.2 Immune predictors of facial masculinity in men. To identify the immune PCs that predicted male facial masculinity, we conducted an Akaike Information Criterion (with sample size correction; AICc) based model selection and averaging procedure (Burnham & Anderson, 2002; Grueber, Nakagawa, Laws, & Jamieson, 2011; Symonds & Moussalli, 2011) using the R package MuMIn (Barton, 2014). This analysis was conducted using only participants with complete data (i.e. no missing data) to ensure that the AICc values of the candidate models generated for selection were comparable. We first generated a list of candidate linear regression models that covered all possible combinations of the 16 immune function PCs predicting facial masculinity in men. Next, we identified the "best" model based on the lowest AICc value. We then averaged the model coefficients (without shrinkage) of all models that were within two AICc values from the "best" model and were, therefore, considered comparable in terms of model fit. The Information Theoretic Akaike Information Criterion (IT-AIC) model selection and averaging approach allows us to compare the information value of multiple models and make conclusions based on multiple models that are similar in information value simultaneously (Burnham & Anderson, 2002; Grueber et al., 2011; Symonds & Moussalli, 2011). The model-averaged results also have the advantage of being more stable than those derived from just the best model or full model (Richards, 2005; Richards, Whittingham, & Stephens, 2011).

Visual examination of the scatterplots of facial masculinity in men and each of the 16 immune PCs revealed that the linear regression models might be influenced by potential outliers on immune PCs 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16 (Supplementary figure 1). Therefore, we winsorized all immune PCs to ±4SDs before conducting the model selection and averaging. The zero-order correlations between facial masculinity and each of the individual immune PCs after winsorization are presented in Supplementary Table 2.

Thirty models, including the "best" model and 29 others that were within two AICc values from the "best" model, were selected from a total of 65535 models. The "best" model was 7.98 AICc points lower than the null model, AICcs = $2575.26_{\text{"best" model}}$ vs $2583.24_{\text{null model}}$, indicating that even the least informative model that was selected was a better fit compared to the null model based on the criterion of two of more AICc points lower than the null (Burnham & Anderson, 2002). This result indicated that the averaged model was indeed more informative than the null model. Evidence ratios of the selected models relative to the null (i.e. how many times more likely the model results are relative to the null) ranged from 19.9 to 54.1.

We tested our hypothesis based on the parameter estimates of the averaged model. The model-average results are presented in Table 2. The skew and kurtosis values of the residuals indicate that our average model met the linear regression model assumption of having normally-distributed residuals, skew = -0.48, kurtosis = 1.44. Breusch-Pagan test indicated no heterocedasticity in our average model, studentized Breusch-Pagan = 9.37, $p = 0.59$. Visual examination of the residuals vs leverage plot did not reveal any data points with unusually high leverage or influence. Overall, the predicted values from the average model shared 9.17% variance with actual sexual dimorphism in men, $r_{208} = 0.30$, $p < 0.001$ indicating that immune function of men when they were 14 years old significantly predicted their facial masculinity at 22 years old. The relative importance values of the predictors, which were

calculated based on the sum of the 'Akaike weights' over all models that included that particular predictor, indicated that there were four important predictors, namely PC15, PC4, PC6, and PC9 (Table 2). Out of these four predictors, facial masculinity in men was positively and significantly predicted by immune PC15 and PC9, but only marginally by immune PC4 and PC6 (Table 2, Figure 3). These four immune PCs were loaded substantially by measures related to allergies (PC15, PC9, and PC4), antibacterial immunity (PC15 and PC6), and cellular immunity (PC15). To help visualise the facial differences between those high and low on the two immune PCs that significantly predicted facial masculinity, we presented the high-immune and low-immune average faces in Supplementary Figure 2. The high-immune average was created from a total of six faces, the top three faces on immune PC15 and the top three faces on immune PC9. The same was done for the low-immune average using the bottom three faces from each of the two PCs.

Table 2. Model-averaged results of immune function PCs predicting men's facial masculinity (N = 210)

	B	SE	z	p	95% CI lower bound	95% CI upper bound	Effect size r	Relative variable importance
(Intercept)	97.35	7.59	12.75	< 0.001	82.38	112.31		
Immune PC 15	15.90	7.63	2.07	0.0383	0.85	30.95	0.15	1.00
Immune PC 4	16.97	9.10	1.86	0.0636	-0.96	34.91	0.13	1.00
Immune PC 6	23.57	12.44	1.88	0.0595	-0.95	48.09	0.13	1.00
Immune PC 9	19.47	9.39	2.06	0.0393	0.95	37.98	0.15	0.88
Immune PC 14	-11.98	8.86	1.34	0.1790	-29.46	5.49	0.10	0.42
Immune PC 5	18.44	13.52	1.36	0.1751	-8.21	45.08	0.10	0.45
Immune PC 10	-9.88	8.15	1.21	0.2283	-25.95	6.19	0.09	0.21
Immune PC 8	-7.02	7.84	0.89	0.3733	-22.48	8.44	0.06	0.14
Immune PC 13	-8.50	9.53	0.89	0.3756	-27.29	10.30	0.06	0.14
Immune PC 11	5.79	8.03	0.72	0.4733	-10.04	21.62	0.05	0.08
Immune PC 3	4.41	8.14	0.54	0.5900	-11.64	20.46	0.04	0.03

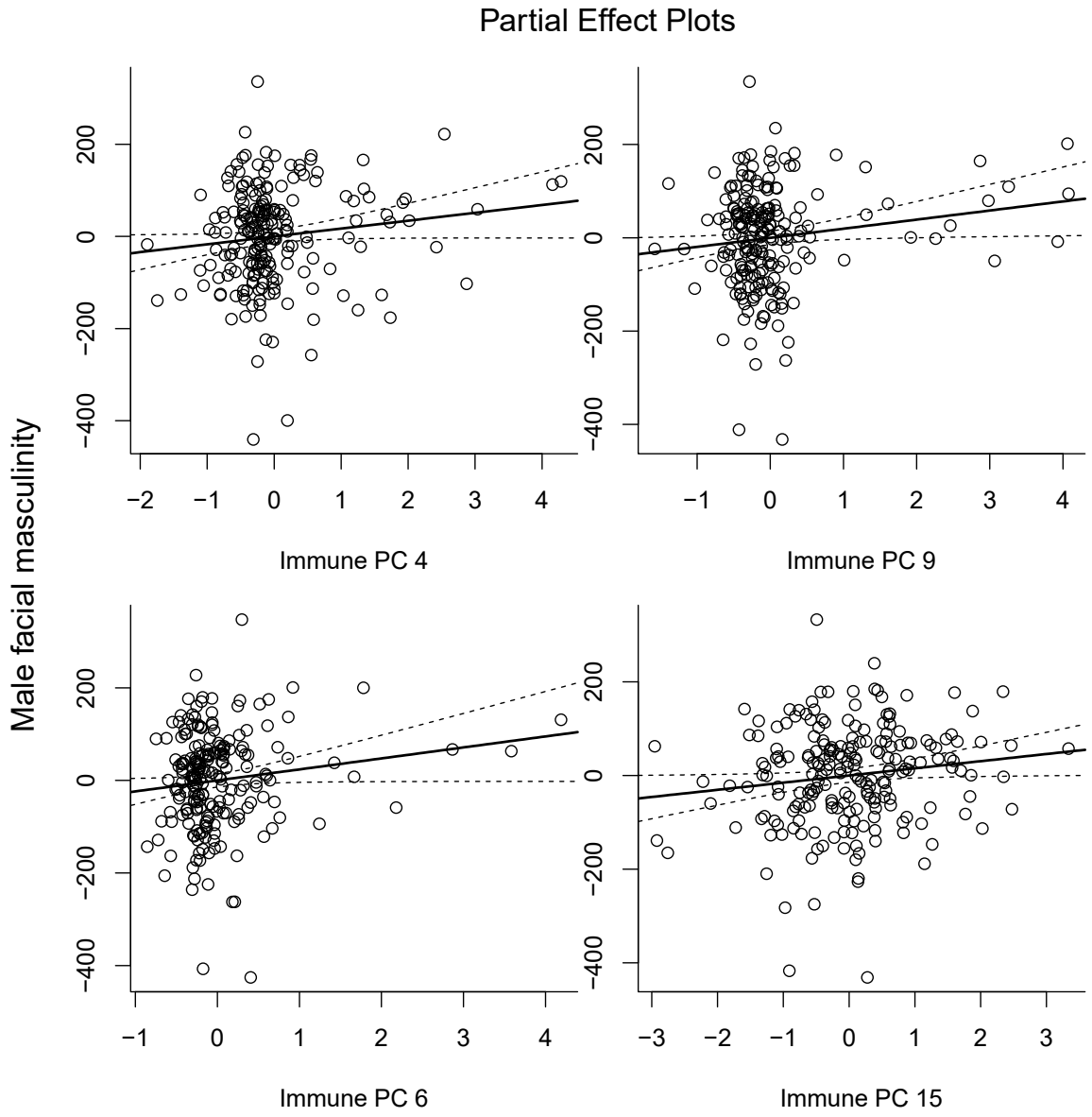

Figure 3. Partial effect plots of the immune PCs that predicted male facial masculinity. PC4 and PC6 were only marginally significant.

We checked the robustness of the male model results by testing whether any of the immune PCs were significantly correlated with pubertal developmental stage (measured based on the Tanner's pubertal developmental stage classification scale). Immune function may be confounded by the immunosuppressive effects of testosterone as testosterone levels surge during puberty. None of the immune PCs were correlated with pubertal developmental

stage, indicating that our results were not subject to confounds due to pubertal developmental stage. The statistics are presented in Supplementary Table 3.

A previous study suggested that facial adiposity might account for the relationship between immune function and facial masculinity (Rantala et al., 2013). Therefore, we checked whether the immune PCs were correlated with body mass index of the male participants at 22 years (BMI), a correlate and proxy of facial adiposity (de Jager, Coetzee, & Coetzee, 2018), as well as the body measurements that constitute the BMI measure, namely weight and height at 22 years. None of the immune PCs were significantly correlated with BMI at 22 years (Supplementary Table 4). Immune PC 14 was positively correlated with height at 22 years (Supplementary Table 4). But the relationship did not survive Bonferroni corrections (Supplementary Table 4). We also note that immune PC 14 was not one of the predictors of facial masculinity (Table 2). We note that BMI does not distinguish between different sources of mass (fat, muscles, water, bones etc.). However, given that it is substantially correlated with facial adiposity (de Jager et al., 2018), the lack of a relationship between BMI and facial masculinity suggests that the relationship between immunity at 14 years and facial masculinity at 22 years is unlikely to be associated with facial adiposity at 22 years. The lack of a robust relationship with height and weight also indicates that immune function is unlikely to be related with sexual dimorphism in overall body (and face) size.

3.2 The relationship between immune function and facial femininity in women

3.2.1 Female immune data reduction. PCA with varimax rotation was used to summarize the female immune data. The immune measure EPX was excluded from the PCAs and all subsequent analyses due to missing EPX data from 16 participants (6.78%). Sixteen PCs were retained (eigenvalue > 1), accounting for 73.90% of the total variance (Supplementary Table 5). The PCs were loaded by immune measures that measured similar

aspects of the immune system or reactions to the same antigen, suggesting that our PCA succeeded at identifying clusters of related measures.

3.2.2 Immune predictors of facial femininity in women. Visual examination of the scatterplots of facial femininity and each of the 16 immune PCs revealed similar outlier issues as in the men's data on immune PCs 1, 2, 3, 5, 6, 7, 9, 10, 12, 13, 14, 15 (Supplementary figure 3). Therefore, we winsorized all immune PCs to ±4SDs before conducting the model selection and averaging. The zero-order correlations between facial masculinity and each of the individual immune PCs after winsorization are presented in Supplementary Table 6.

For the female data, 31 models, including the "best" model and 30 others that were within two AICc values from the "best" model, were selected from a total of 65535 models. The "best" model was 12.38 AICc points lower than the null model, AICcs = $2695.19_{\text{"best" model}}$ vs $2707.57_{\text{null model}}$, indicating that even the least informative model that was selected was a better fit compared to the null model based on the criterion of two of more AICc points lower than the null (Burnham & Anderson, 2002). This result indicated that the averaged model was indeed more informative than the null model. Evidence ratios of the selected models relative to the null (i.e. how many times more likely the model results are relative to the null) ranged from 179.5 to 487.8.

The model-average result is presented in Table 3. The skew and kurtosis values of the residuals indicate that the average model met the linear regression model assumption of having normally-distributed residuals, skew = 0.01, kurtosis = -0.08. Breusch-Pagan test indicated no heterocedasticity in our average model, studentized Breusch-Pagan = 19.25, $p = 0.08$. Visual examination of the residuals vs leverage plot did not reveal any data points with unusually high leverage or influence. Overall, the predicted values from the average model shared 10.63% variance with actual facial femininity in women, $r_{223} = 0.33$, $p < 0.001$,

indicating that immune function of women when they were 13 years old significantly predicted their facial femininity at 22 years old. The relative importance values of the predictors, which were calculated based on the sum of the 'Akaike weights' over all models that included that particular predictor, indicated that there were five important predictors, namely PC13, PC15, PC3, PC4, and PC8 (Table 3). Out of these 5 predictors, female femininity was positively and significantly predicted by PC13 and negatively by PC15 (Table 3, Figure 4). Although the regression coefficient for PC15 was negative, the immune measures that loaded substantially onto this PC were in the negative direction (Supplementary Table 4). Therefore, for both PC13 and PC15, the more feminine the face shape, the better the woman's immunity. PC13 was loaded substantially by measures linked to allergic responses and PC15 by those linked to antibacterial immunity. The high-immune and low-immune average faces are presented in Supplementary Figure 4 using the top vs bottom three faces from each of the two PCs.

Table 3. Model-averaged results of immune function PCs predicting women's facial femininity (N = 225)

	B	SE	z	p	95% CI lower bound	95% CI upper bound	Effect size r	Relative variable importance
(Intercept)	90.23	6.36	14.11	0.0000	77.70	102.76		
Immune PC 13	23.22	8.41	2.75	0.0061	6.64	39.80	0.19	1.00
Immune PC 15	-18.55	7.29	2.53	0.0114	-32.90	-4.19	0.17	1.00
Immune PC 3	-13.59	8.15	1.66	0.0972	-29.65	2.47	0.11	0.73
Immune PC 4	10.92	6.36	1.71	0.0875	-1.60	23.44	0.12	0.77
Immune PC 7	-14.58	9.54	1.52	0.1286	-33.39	4.22	0.10	0.56
Immune PC 8	10.57	6.35	1.66	0.0979	-1.95	23.08	0.11	0.75
Immune PC 9	-7.64	6.37	1.19	0.2331	-20.19	4.92	0.08	0.25
Immune PC 14	-6.13	7.03	0.87	0.3862	-19.98	7.73	0.06	0.08
Immune PC 16	-5.36	6.35	0.84	0.4010	-17.86	7.15	0.06	0.06
Immune PC 1	-4.59	6.41	0.71	0.4770	-17.23	8.05	0.05	0.05
Immune PC 10	-3.97	6.33	0.62	0.5327	-16.44	8.50	0.04	0.03
Immune PC 6	6.73	11.09	0.60	0.5461	-15.13	28.59	0.04	0.02

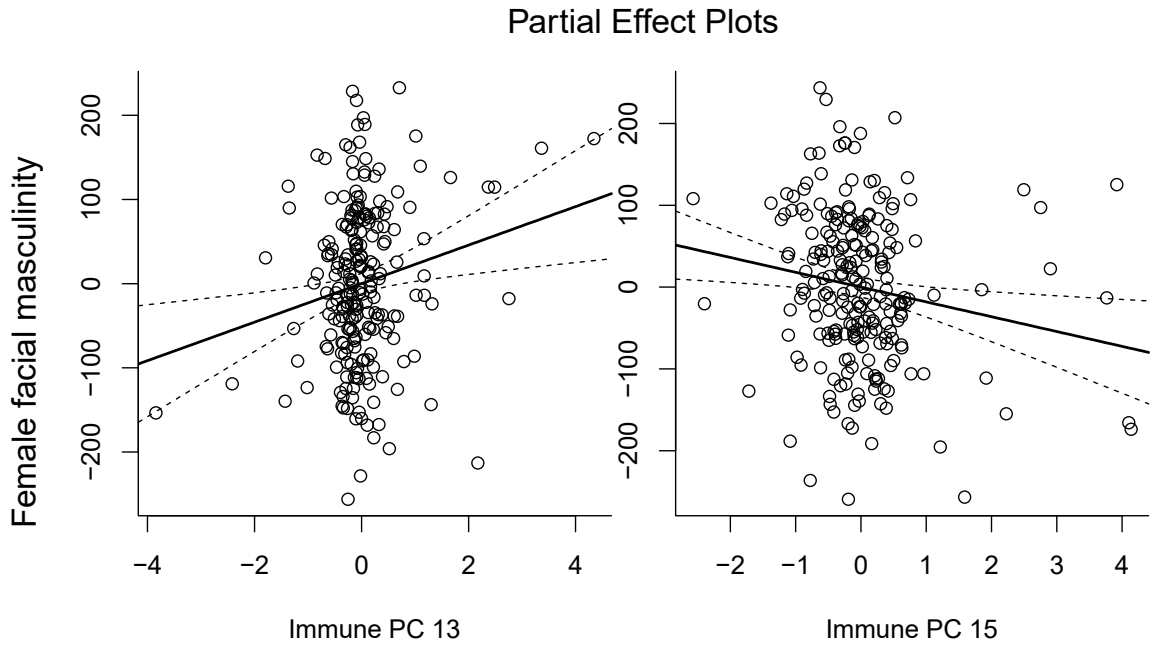

Figure 4. Partial effect plots of the immune PCs that predicted female facial femininity.

We checked the robustness of the model results by looking at whether any of the immune PCs were significantly correlated with the pubertal developmental stage of females. There were two separate Tanner's pubertal developmental stage classifications for female pubertal developmental stage, one based on pubic hair development and the other on breast development. We summarize the two measures into a single PC (eigenvalue > 1) accounting for 67.10% of the total variance. None of the immune PCs were correlated with pubertal developmental stage, indicating that our results were not subject to confounds due to pubertal developmental stage (Supplementary Table 7).

We also checked whether the immune PCs were correlated with the female participants' BMI, weight, and height at 22 years. (Supplementary Table 8). Before Bonferroni corrections, there were several significant correlations, namely immune PC 10 with BMI, height, and weight, PC 12 with height, and PC 13 with BMI. But none of these correlations survived Bonferroni corrections (Supplementary Table 8), indicating that none of them were robust. Given the lack of any significant relationships with BMI, the relationship

between immune function at 14 years and facial femininity at 22 years in women is associated with facial adiposity at 22 years. The lack of a robust relationship with height and weight also indicates that immune function is unlikely to be related with sexual dimorphism in overall body (and face) size.

4. Discussion

The idea that individuals prefer more sexually dimorphic faces because facial sexual dimorphism indicates health, particularly immune function, is one of the most prominent theoretical frameworks in the study of human facial preferences (Little et al., 2011; Rhodes, 2006; Thornhill & Gangestad, 1999). However, this framework has come under intense scrutiny because studies on the relationship between facial sexual dimorphism and current adult immune function have yielded mixed results (Cai et al., 2019; Foo, Simmons, et al., 2017; Gray & Boothroyd, 2012; Phalane et al., 2017; Rantala et al., 2012; Rhodes et al., 2003; Scott et al., 2013; Thornhill & Gangestad, 2006). Here, rather than testing current immune function, we used a longitudinal public health dataset to test the life-history prediction that individuals who had better immune function during adolescence should have more sexually dimorphic face shape as adults. As predicted, we found positive relationships between some aspects of immunity during adolescence and sexually dimorphic 3D face shape in adulthood in both men and women. For men, the immune PCs that positively predicted male facial masculinity included measures related to allergies, antibacterial immunity, and cellular immunity. For women, the immune PCs that positively predicted facial femininity included measures linked to allergic responses and antibacterial immunity. Our results support a fundamental theoretical assumption underlying studies on the evolutionary basis of facial sexual dimorphism preferences in humans: facial sexual dimorphism indicates immune health during development.

Our conclusions were supported by several observations. The evidence ratios derived from the AICc scores indicated that the selected models comprising our final averaged models were 19.9 to 54.1 times more likely than the null for men and 179.5 to 487.8 times for women, suggesting that immune function during early adolescence indeed predicts adult facial sexual dimorphism (based on the criterion of >10x suggested by Burnham & Anderson, 2002). Our conclusions were further corroborated by the effect sizes of our final averaged models, which showed a medium effect size of $r = 0.3$ for both sexes (Cohen, 1988). Our effect sizes were larger than those typically found in evolutionary biology studies, which range from $r = 0.16$ to 0.25 (Møller & Jennions, 2002). Therefore, the relationship between adolescent immune function and adult facial sexual dimorphism in both men and women may indeed be biologically relevant. We note that despite the medium overall model effect sizes, the independent contributions of individual immune PCs that significantly predicted facial sexual dimorphism in either sex were small, ranging from $r = 0.15$ to $r = 0.19$. However, all of them were consistently in the positive direction predicted by theory for both sexes (higher masculinity for men and higher femininity for women – high immunity).

Our results for men match those of Rhodes et al (2003), the only other study that has examined the developmental relationship between health and facial sexual dimorphism in humans. Similar to Rhodes et al (2003), we found that health during adolescence (immune function specifically in our case) positively predicted male facial masculinity. In contrast to their null findings for women, however, we found that immune function during adolescence also positively predicted female facial femininity. One notable methodological difference between the current study and Rhodes et al (2003) lies in the health measures used. Rhodes et al (2003) was based on doctors' ratings of the participants' medical records. Such ratings may reflect not just resistance to infections, but also other aspects of health such as reproductive or even mental health. In contrast, our health measures were based on objective physiological

markers of immunity. Therefore, using more direct measures of potential susceptibility to disease, we found positive relationships between immune function and facial sexual dimorphism in both sexes as predicted by life-history theory.

Our findings for men were consistent with the immunocompetence handicap hypothesis, which suggests that facial masculinity is an honest indicator of men's immune function during development due to the immunosuppressive effects of the male hormone testosterone (Folstad & Karter, 1992; Foo et al., 2017). However, it is unclear how the hypothesis would apply to our female results given that the effect of the female hormone estrogen on female immune function is mixed (Foo et al., 2017). Therefore, the relationship between immune function and facial femininity in women is likely to be explained by other mechanisms. Indeed, some researchers have proposed potential mechanisms mediating the relationship between attractive traits and immune function that do not invoke the involvement of sex hormones, including nutrition-mediated trade-offs (Sheldon & Verhulst, 1996) or trade-offs occurring simply due to increased reproductive investment (Rolff, 2002).

Our findings also stand in contrast to those that have failed to find a relationship between facial masculinity in men and MHC heterozygosity (Lie et al., 2008), including a recent large-scale study based on more than 1000 men (Zaidi et al., 2019). These null findings have led to the argument that facial masculinity in men might not be condition-dependent (Zaidi et al., 2019). However, MHC heterozygosity provides a genetic predictor of variation in one particular aspect of the adaptive immune system: the ability to recognize a diversity of antigens. It does not measure the actual ability of individuals to mount a phenotypic response to pathogens. Here, our direct phenotypic measures of immune function suggests that masculinity is actually dependent on a number of innate and adaptive aspects of the immune system.

The use of a comprehensive set of immune function measures is an important strength of the current study. The immune system is a highly complex collection of mechanisms that work in tandem to protect us against pathogens (Parkin & Cohen, 2001). Given its complexity, single measures are unlikely to represent overall immunity. Indeed, eco-immunologists have recently cautioned against overgeneralizing findings derived from a small number of measures (Blount et al., 2003; Demas & Nelson, 2012; Møller & Petrie, 2002; Norris & Evans, 2000). Here, our findings reiterate the need to include multiple facets of immunity when studying the immune system. For each sex, only two out of a total of 16 immune PCs significantly predicted facial sexual dimorphism.

The finding that some immune measures show links with other life-history traits while others do not is typical of the non-human animal literature (Blount et al., 2003; Demas & Nelson, 2012; Møller & Petrie, 2002; Norris & Evans, 2000). Several potential explanations have been proposed for such mixed results. One possibility is that life-history trade-offs between different branches of the immune system may prevent them from showing an elevated response simultaneously (Blount et al., 2003; Demas & Nelson, 2012; Moller & Petrie, 2002). For instance, multiple studies have demonstrated such trade-offs through negative correlations between different aspects of immunity in non-human animal species (Faivre et al., 2003; Johnsen & Zuk, 1999; Martin et al., 2006). Similarly, in humans, antibacterial/viral immunity vs allergies (i.e. Th1 vs Th2 immune classes) are mutually antagonistic (Kaiko, Horvat, Beagley, & Hansbro, 2008). Indeed, we observe such a trade-off in immune PC2 for women, where measures for antibacterial/viral immunity vs allergies are loaded in opposite directions. To better understand the evolutionary links between immune function and sexually selected traits, it is therefore necessary to employ a range of measures that index different aspects of immunity. We hope that the current study will encourage future studies to do so.

A second possibility is that sexually-selected traits might have evolved to indicate immune defence against certain pathogens encountered during a given species' evolutionary history (Demas & Nelson, 2012; Hamilton & Zuk, 1982; Møller & Petrie, 2002; Norris & Evans, 2000). This explanation may account for the positive relationship between facial sexual dimorphism and allergies that we found for both men and women. Most of the immune measures in this study are linked to important protective processes, such as antibacterial immunity, cellular immunity, and inflammation (Parkin & Cohen, 2001). Allergies, however, are reactions to harmless particles, such as pollen or animal dander (Holgate, Church, Broide, & Martinez, 2012). In fact, they often lead to negative outcomes, ranging from mild irritations (e.g. having a runny nose) to potentially life-threatening reactions such as anaphylaxis (Holgate et al., 2012). So why has facial sexual dimorphism evolved to indicate heightened allergies in both sexes? One influential hypothesis regarding the evolutionary basis of the allergic response is that it represents an evolved mechanism for flushing out parasitic worms through increased production of fluids (weep) and increased muscular contractility (sweep)(Okada, Kuhn, Feillet, & Bach, 2010; Strachan, 1989). However, the eradication of parasitic worms in modern developed societies through better hygiene infrastructure has left this aspect of immune function without its original target, possibly leading it to misdirect its reactions to harmless particles (Okada et al., 2010; Strachan, 1989). Such an explanation may be particularly relevant to Caucasians, whose late-stage evolution occurred in environments in which helminth parasite loads were much lower than those who continued in tropical/subtropical environments (e.g. Africans). Several lines of evidence demonstrate the link between allergic responses and the protective mechanisms against parasitic worms. Both responses are IgE-antibodies-mediated and allergic symptoms are reminiscent of the weep-and-sweep mechanisms for flushing out parasitic worms (hence the runny nose) (Anthony, Rutitzky, Urban, Stadecker, & Gause, 2007; Holgate et al., 2012;

Okada et al., 2010). The proteins in allergens and parasitic worms that trigger an immune response also share structural similarities (Tyagi et al., 2015). Epidemiological studies show that infections by parasitic worms are protective against allergies (Flohr et al., 2006), which is consistent with the hypothesis that allergies are misdirected responses of an idle defence mechanism. Given the links between allergic responses and the protective mechanisms against parasitic worms, it is possible that facial sexual dimorphism has its evolutionary origin in an indicator of protection against parasitic threats.

We note four potential limitations/future directions for our study. First, our results were based on a search through 65535 models with 16 potential immune function PCs. Four PCs for men and two PCs for women were associated with facial sexual dimorphism. Given that an experimental approach to testing these associations would be impossible with human participants, future work should examine whether the relationships we have observed can be replicated with different cohorts of participants. On the other hand, although our dataset contains more than 40 different immune measures, it is by no means an exhaustive list (Parkin & Cohen, 2001). Therefore, future studies may find it fruitful to examine the developmental relationship between immune function and facial sexual dimorphism using other measures of immunity, such as the formation of adaptive responses to novel pathogens following vaccinations. Third, our immune measures were taken from a single time point. The adolescent developmental period can last up to 10 years or more (Sawyer, Azzopardi, Wickremarathne, & Patton, 2018). Therefore, measurements of immunity taken across multiple time points might provide more stable estimates of immunity during adolescence. Therefore, we might expect such aggregate measures from multiple time points to better predict adult facial sexual dimorphism than measures taken from a single time point. Fourth, it would also be interesting, as a future direction, to investigate whether the 3D faces of

individuals in our sample with better immunity are more attractive to the opposite sex than those with poorer immunity, as predicted by the theory of sexual signalling.

In summary, our results showed that some aspects of immune function during early adolescence, the period in which much of our facial sexual dimorphism develops, positively predicted sexually dimorphic 3D face shape in both men and women. They support a relatively neglected yet fundamental assumption of life-history theory: that facial sexual dimorphism is attractive in humans partly because it indicates immune health during development. More broadly, they suggest that there might indeed be a biological basis to our preferences for facial sexual dimorphism in potential mating partners.

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Conflict of interest

The authors declare no conflict of interest.

Data accessibility

The Raine Study holds a rich and detailed collection of data gathered over 30 years for the purpose of health and well-being research. The informed consent provided by each participant does not permit individual-level data to be made available in the public domain (i.e., a public data repository). However de-identified analytic data sets are available to all researchers for original research or auditing of published findings. All data access is managed through established Raine Study procedures which require data handlers to agree to a code of conduct that includes safeguards to protect the identity of participants. Details of the data access processes and code of conduct are available on the Raine Study website (https://rainestudy.org.au/).

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