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Serologic, but not genetic, markers are associated with impaired anthropometrics at diagnosis of pediatric Crohn's disease

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Author Contributions:

Sara K. Naramore, MD conducted the study and wrote the first and final drafts of the manuscript. William E. Bennett, Jr., MD, MS assisted with the study design, statistical analyses, and revision of the manuscript. Guanglong Jiang, MS conducted the statistical analyses and revised the manuscript. Subra Kugathasan, MD, Lee A. Denson, MD, and Jeffrey S. Hyams, MD are members of the RISK Stratification Project and assisted with the study design and revision of the manuscript. Steven J. Steiner, MD assisted with the study design and revised and approved the final manuscript. PRO-KIIDS collected and contributed the data for the study.

Abstract:

Objectives: Children with Crohn's disease may present with malnutrition and linear growth impairment which can be secondary to insufficient caloric intake, chronic inflammation, malabsorption, and suppression of growth-promoting hormones. We evaluated clinical, serologic, and genetic data to determine risk factors for impaired anthropometrics in Crohn's disease at diagnosis.

Methods: Our study evaluated 772 children newly diagnosed with Crohn's disease, inflammatory phenotype, enrolled in the RISK Stratification Project to determine the factors associated with anthropometric impairment. Data were collected on demographics, growth parameters, disease location, serologic and immunologic markers, and disease severity. We performed a genome-wide association study of genetic polymorphisms associated with inflammatory bowel disease. Regression analysis determined associations between anthropometrics and clinical, serologic, and genetic variables.

Results: There were 59 (7%) children with height z-score <-2, 126 (14%) with a weight z-score <-2, and 156 (17%) with a BMI z-score <-2. Linear growth impairment was associated with hypoalbuminemia (p=0.0052), elevated granulocyte-macrophage colony stimulating factor auto-antibodies (GM-CSF Ab) (p=0.0110), and elevated CBir antibodies against flagellin (p=0.0117). Poor weight gain was associated with female gender (p=0.0401), hypoalbuminemia (p=0.0162), and thrombocytosis (p=0.0081). Malnutrition was associated with hypoalbuminemia (p=0.0061) and thrombocytosis (p=0.0011). Children with moderate or severe disease had lower weight (p=0.02 and $p=1.16\times10^{-6}$, respectively) and BMI z-scores ($p=2.7\times10^{-3}$ and $p=1.01\times10^{-6}$, respectively) than children with quiescent and mild disease. There was no association between age of diagnosis, Tanner stage, or disease location and having impaired anthropometrics. There was no genome-wide association between the genetic polymorphisms and the serologic variables and anthropometric measurements.

Conclusions: This is the largest study evaluating growth in treatment-naïve children with Crohn's disease, inflammatory phenotype. It is the first study to use genome-wide sequencing to assess for genetic determinants of growth impairment. GM-CSF auto-antibodies and CBir antibodies are more likely to be elevated in children with growth impairment. Future investigations should evaluate the relationship between genetic polymorphisms, pathologic immune responses, and the biological pathways regulating growth.

Keywords: inflammatory bowel disease; children; growth impairment; malnutrition; genetic polymorphism

What is Known:

- Children with Crohn's disease often have impaired linear growth and malnutrition.
- Specific genetic polymorphisms involved with inflammatory bowel disease are associated with the age of onset, disease behavior, location, or severity of the disease.

What is New:

- At time of diagnosis of Crohn's disease, children with linear growth impairment are more likely to have elevated granulocyte-macrophage colony stimulating factor auto-antibodies and elevated CBir antibodies against flagellin.
- No specific genetic sequences were associated with growth impairment or malnutrition.

Introduction

Crohn's disease is a chronic intestinal inflammatory condition which may adversely affect growth and nutrition. Approximately 100-300 per 100,000 people have inflammatory bowel disease (IBD) in North America (1). Among children with Crohn's disease, 13-58% will have poor linear growth; those with pre-pubertal disease onset may develop permanent growth impairment, despite receiving therapy with biologics (2, 3). Previous investigations demonstrated that linear growth impairment is associated with ileal disease, NOD2 mutations, and elevated GM-CSF auto-antibodies (4).

Specific serum biomarkers and cytokines are elevated in patients with active IBD. Chronic inflammation alters the actions of growth-promoting hormones and leads to low levels of insulin-like growth factor (IGF-1) (5). Subsequently, growth hormone (GH) resistance and a slower progression through puberty develop (6). Thayu M, *et al* (7), demonstrated that inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), are increased at diagnosis and decrease with treatment; children with IBD who experienced an improvement in their height z-score over time had an increase in IGF-1 and decrease in IL-6 and TNF-α. Thus, chronic elevation in inflammatory cytokines and immune-mediated GH resistance contribute to growth impairment.

Elucidation of risk factors beyond nutritional status and disease severity are important in understanding growth delay and its management. We performed a cross-sectional study of children with Crohn's disease enrolled in the RISK Stratification Project, which established a national database of clinical, genetic, and histologic information. We hypothesized that multiple variables, including serology, genetics, inflammatory cytokines, disease severity, and pubertal development, would be associated with growth impairment in children with Crohn's disease.

Methods

Study Population

The RISK Stratification Project was funded by Crohn's and Colitis Foundation and conducted by Pediatric Research Organization for Kids with Intestinal Inflammatory Diseases (PRO-KIIDS) to identify baseline biological and microbial factors that determine complicated stricturing and penetrating pediatric Crohn's disease (8). 28 medical centers enrolled 1813 children under 18 years with a new diagnosis of IBD from 2008-2012. Only children with Crohn's disease diagnosed by their primary physician were included, and children with ulcerative colitis or indeterminate IBD were excluded. Children were excluded if they had incomplete data collected on the disease location, were lost to follow-up, or developed stricturing or penetrating disease within 90 days of diagnosis. Ultimately, 913 children with an inflammatory phenotype of Crohn's disease participated in the project, which is the largest multicenter, prospective study of pediatric patients with Crohn's disease. A database and biorepository were created, including DNA, plasma, stool, and mucosal biopsies. For our study, we excluded children who did not have all the serologic, immunologic, and genetic sequencing data collected. Our study evaluated 772 children with non-stricturing and non-penetrating Crohn's disease at the time of diagnosis. Children with stricturing or penetrating disease were excluded as they may have a different clinical phenotype and genotype than children with the inflammatory phenotype. The Indiana University School of Medicine Institutional Review Board and the RISK Ancillary Study Steering Committee approved this study.

Data Collection

Demographic data and each child's height, weight, and body mass index (BMI) were recorded during enrollment into the RISK Stratification Project. Disease severity was

determined using the Pediatric Crohn's Disease Activity Index (PCDAI) and the Physician Global Assessment (PGA). The PCDAI includes growth parameters. Disease location was classified as upper intestinal tract, ileum, or colon. Children were included in all disease location classifications where they had active disease. Laboratory results were recorded for hemoglobin, platelets, eosinophil count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and albumin. Titer levels were obtained using an enzyme-linked immunosorbent assay for anti-*Saccharomyces cerevisiae* antibodies (ASCA), anti-neutrophil cytoplasmic antibodies (ANCA), anti-outer membrane protein C of *E. Coli* antibodies (ompC), antibodies against flagellin of *Clostridium* taxa (CBir), granulocyte-macrophage colony stimulating factor auto-antibodies (GM-CSF Ab), and antibodies to *Pseudomonas fluorescens* (I2**)**. The antibody titers were measured at Cedars-Sinai Hospital, except for the GM-CSF auto-antibodies which were measured at Cincinnati Children's Hospital Medical Center. Serum levels of the cytokines IGF-1, IL-6, and TNF-α were obtained at Cincinnati Children's Hospital Medical Center. Additionally, the Tanner stages of pubertal development were recorded at diagnosis, either through self-report or examination by a physician.

DNA was isolated from peripheral blood samples. Genotyping for the subjects was conducted at Emory University School of Medicine on the Immunochip, an Illumina Infinium High-Density array (9, 10). The Immunochip contains 196,524 loci from 12 autoimmune and inflammatory diseases. Quality control (QC) was conducted on genotype data to exclude single nucleotide polymorphisms (SNPs) with minor allele frequency <3%, missing rate >5%, Hardy-Weinberg Equilibrium test p-value $<10^{-4}$, and individuals with a missing rate >10%.

Statistical Analysis

The height, weight, and BMI z-scores of each child were calculated at enrollment. Clinical variables were analyzed for an association with (1) linear growth impairment, (2) poor weight gain, and (3) malnutrition. Linear growth impairment was defined as a height z-score <-2, and children with poor weight gain had a weight z-score <-2. A BMI z-score <-2 identified children with malnutrition. The demographic variables included were age at diagnosis, gender, and race. Race was self-reported as Caucasian, African-American, Asian, or having a mixed or unknown race. For the laboratory variables, we only included those which had less than 20% of the data missing, which resulted in CRP, IGF-1, IL-6, TNF-α, and I2 being excluded. We defined children in pre- or mid-puberty as Tanner stages I-III. Children near complete pubertal development were classified as Tanner stages IV or V. We conducted a Kruskal-Wallis rank sum test to evaluate for an association between Tanner stage, age at diagnosis, and anthropometric measurements.

Children with early-onset IBD may have a different clinical phenotype and genotype than children and adolescents diagnosed at an older age. We used the Chi-square test to determine if there was an association between linear growth impairment or poor weight gain and any of the clinical variables in children six years old and younger and children older than six years. A p-value less than 0.05 was considered significant.

We analyzed if an association existed between each of the growth parameters and disease severity. The PGA classifies patients as having quiescent, mild, moderate, or severe disease. The PCDAI provides a score from 0-100. Patients have inactive disease if the PCDAI <10, mild disease for 10-27.5, moderate disease for 30-37.5, and severe disease for 40-100 (11). A t-test compared the mean height, weight, and BMI z-scores with the PGA and PCDAI. The z-scores of children with quiescent or mild disease were combined in order to

ensure sufficient power for the analysis. The z-scores of those with quiescent or mild disease were compared to children with moderate disease and to those with severe disease. Children who have a PCDAI ≤30 were analyzed against children who had a PCDAI >30. A multivariate regression analysis evaluated for an association between age, gender, height, weight, and BMI z-scores, and disease severity. A chi-square analysis evaluated if there was an association between disease severity and children ≤6 years and children >6 years old.

Univariate and multivariate logistic regression analyses were performed to determine the association between the clinical variables and the presence of linear growth impairment, poor weight gain, or malnutrition as defined by height, weight, and BMI z-scores. The first study outcome evaluated children with z-scores <-2. A separate analysis assessed height, weight, and BMI z-scores as continuous variables and included the normal clinical reference values for each variable. A p-value < 0.05 was considered significant. These multivariate models were built using the statistical software R v3.4.

A genome-wide association study (GWAS) was conducted using PLINK software to determine if an association exists between growth impairment and genetic polymorphisms known to be involved in IBD (12). Population stratification was performed with Eigenstrat (13). Race was excluded from the analysis as it or the first two principal components from Eigenstrat did not have a significant association with height, weight, and BMI z-scores. After quality control, 121,487 SNPs and 772 subjects were included in the GWAS. 159 of the 163 SNPs associated with IBD were included (14). If the SNPs were in exons, the surrounding genes were reported. A p-value of 4×10^{-7} was considered significant, and a p-value $<5\times10^{-5}$ was used to identify genes with strong evidence of association. A final multivariate regression evaluated if there is an association between the laboratory studies, auto-antibodies, genotype data, and anthropometric z-scores <-2. The regression was also conducted using the z-scores as continuous outcomes in order to identify all significant covariates. Integrity

Pathway Analysis evaluated the common molecular pathways and gene functions of the genes of highest significance (15).

Results

Study population

The characteristics of the 772 children are shown in Table 1. The mean (+/-SD) age of the study population at the time of diagnosis of Crohn's disease was 11.6 +/- 3.1 years. The median age was 12 years with a range of 1 to 17 years. 44 children were 6 years old or younger. 286 (37%) were female. The study was composed of 637 (82%) Caucasian, 46 (6%) African-American or mixed race, 29 (4%) Asian, and 60 (8%) children of unknown race. 446 of the 772 (58%) children had Tanner staging recorded at diagnosis (Table, Supplemental Digital Content 1, http://links.lww.com/MPG/B698, Tanner Staging). 244 children (55%) were in pre-puberty (Tanner stage 1), 104 (23%) were in mid-puberty (Tanner stages II-III), and 98 (22%) were in late puberty (Tanner stages IV-V) (Table 1). Of the 244 children in Tanner stage 1, 17 (7%) had a height z-score <-2, 33 (13.5%) had a weight z-score <-2, and 35 (14%) had a BMI z-score<-2. There was no significant association between Tanner stage and age at diagnosis (p=0.90). Additionally, there was no association between Tanner stage and height (p=0.91), weight (p= 0.74), or BMI (p= 0.7128) z-scores <-2.

Anthropometrics at diagnosis

The study cohort had a mean height z-score of -0.30 +/- 1.13, mean weight z-score of -0.69 +/- 1.28, and mean BMI z-score of -0.80 +/- 1.42. There were 59 (7%) children with height z-score <-2, 126 (14%) children with a weight z-score <-2, and 156 (17%) children with a BMI z-score <-2. There was no significant difference in the anthropometric z-scores in children with very early-onset IBD ages 6 years and younger and children over age 6 years.

Disease severity and location

751 (97%) of the children had disease severity classified according to the PGA and PCDAI. The PCDAI range was 0-82.5. There was no significant difference in disease severity in children ≤6 years old and children >6 years old (p=0.5382 for PGA, p=1 for PCDAI). In a univariate analysis using the PGA, children who had moderate disease (p=0.02) or severe disease ($p=1.16\times10^{-6}$) had lower weight z-scores than children with quiescent or mild disease. The same result was found using the PCDAI (Table, Supplemental Digital Content 2, http://links.lww.com/MPG/B698, growth parameters according to disease severity). Children with PCDAI >30 had lower weight z-scores than those with PCDAI ≤30 ($p=1.81\times10^{-6}$). The BMI z-scores in children with quiescent or mild disease were significantly higher than those who had moderate disease ($p=2.7\times10^{-3}$) and severe disease ($p=1.01\times10^{-6}$). There was no statistically significant association between the disease severity using the PGA or PCDAI and the height z-scores. A multivariate regression including age and gender did not show a significant difference in anthropometrics and disease severity (Table, Supplemental Digital Content 3, http://links.lww.com/MPG/B698, disease severity including confounding variables).

Each disease location, classified as either upper intestinal, ileal, and ileocolonic/colonic disease, was not associated with anthropometric impairment (Table 2). To ensure accuracy of this finding, a t-test was conducted comparing the mean z-scores for height (p=0.29), weight (p=0.53), and BMI (p=0.87) in children with isolated colonic disease and children with ileal or ileocolonic disease.

Clinical variables associated with anthropometrics

Specific laboratory studies and auto-antibodies were associated with height, weight, and BMI z-scores in both regression analyses: using a discrete outcome of z-scores <-2 and

using z-scores as a continuous outcome. The significant clinical variables associated with a height z-score <-2 were hypoalbuminemia and elevated GM-CSF auto-antibodies (Table 3). Children with a weight z-score <-2 were more likely to be female and have hypoalbuminemia and thrombocytosis. Children who had a BMI z-score <-2 were also more likely to have hypoalbuminemia and thrombocytosis. The results of the continuous data analysis are in the Text, Supplemental Digital Content 4, http://links.lww.com/MPG/B698 and Table, Supplemental Digital Content 5, http://links.lww.com/MPG/B698. The laboratory studies and auto-antibodies that were not significantly associated with impaired anthropometrics, included ESR, ASCA, ANCA, and OmpC (Table 2).

Additionally, we assessed for an association between age at diagnosis and the presence of each antibody, without including anthropometrics. There was a significant association between age and the titer levels of ASCA IgA ($p=6.4x10^{-7}$), ASCA IgG ($p=1.2x10^{-38}$), GM-CSF auto-antibodies ($p=4.4x10^{-93}$), I2 ($p=2.1x10^{-7}$), CBir antibodies ($p=2.9x10^{-49}$), and ANCA ($p=1.1x10^{-23}$). The antibodies with a positive correlation with age were ASCA IgA (r=0.12), ASCA IgG (r=0.11), GM-CSF auto-antibodies (r=0.10), and ANCA (r=0.10). I2 (r= -0.08) and CBir antibodies (r= -0.08) had a negative correlation with age.

Outcome of genome-wide association study analysis

There was no genome-wide association between the 121,487 SNPs and anthropometrics. No genome-wide significance was observed for height, weight, or BMI z-scores <-2 or when assessed as a continuous outcome. For a height z-score <-2, the genes containing SNPs which were most closely associated with significance were the following: OR5D14, OR5L1, OR4C6, OR5D13, KCNH8, EFHB, TAP2, PAPOLG, VAV3, and HLA-DOB. These genes are involved with multiple physiological processes, including DNA

synthesis, cell signaling, and antigen presentation (Table 4). The genes with the highest evidence of association with poor weight gain included: RMI2, GLP2R, C8orf87, SKAP2, and ANXA6. These genes are active in DNA repair, nutrient absorption in enterocytes, cell exocytosis, and cell survival. The genes with SNPs most strongly associated with malnutrition included: CCR7, SMARCE1, and TAGAP. These genes are involved with antigen presentation and B and T cell activation and migration. The SNPs located in non-coding genes were not included, since the pathophysiology and clinical significance are unknown. The results of the continuous data analysis are in the Text, Supplemental Digital Content 4 and Table, http://links.lww.com/MPG/B698, Supplemental Digital Content 6, http://links.lww.com/MPG/B698.

Discussion

This is the largest study examining clinical, serologic, and genetic factors associated with anthropometric impairment in children with non-stricturing and non-penetrating Crohn's disease at diagnosis. The mean anthropometrics of our cohort were similar to the much smaller cohort examined by Pfefferkorn M, *et al*, which assessed linear growth outcomes over two years in children with Crohn's disease (3). Thrombocytosis, hypoalbuminemia, elevated GM-CSF auto-antibodies, and elevated CBir antibodies were significant factors associated with linear growth impairment. Thrombocytosis, hypoalbuminemia, and female gender were associated with poor weight gain. Age, including children with early onset IBD, and disease location did not identify children with impaired anthropometrics. No genetic sequences were associated with anthropometric impairment.

Previous investigations demonstrated that serum antibodies are associated with age at diagnosis. ASCA is more common in older children whereas CBir1 is more frequent under age 7. Our study replicated these results and observed significant associations between other

antibodies and age. Thus, there are differences in the immunologic response to microbial antigens based on age (16).

Earlier research has demonstrated there is a correlation between low z-scores for weight and height at the time of diagnosis of Crohn's disease and positive antibodies associated with IBD, particularly ASCA. Higher titers of ASCA antibodies were associated with a lower weight z-score (17). However, in our much larger study, a significant association occurred between elevated GM-CSF auto-antibodies and a height z-score <-2, but not ASCA. CBir antibodies were associated with growth impairment in the continuous data analysis of height z-scores. GM-CSF auto-antibodies have previously been demonstrated to be associated with GH resistance and linear growth impairment, particularly in patients with a *NOD2* mutation (4). This association was modified by small bowel location and weight at diagnosis. In a related murine model, GM-CSF neutralization in NOD2-deficient mice was associated with hepatic growth hormone resistance. Elevated levels of GM-CSF auto-antibodies will lead to activated macrophages, increased intestinal permeability, and a higher likelihood of ileal and stricturing and/or penetrating behavior (18, 19). Additionally, patients with GM-CSF auto-antibodies have impaired phagocytosis by neutrophils which protects the mucosal barrier (19). Dysregulation of GM-CSF likely leads to a poor innate immune response to microbial antigens, and in combination with increased intestinal permeability and bacterial translocation, growth hormone resistance develops.

While our study did not demonstrate any SNPs to be associated with impaired anthropometrics, previous investigations have shown several SNPs involved in IBD are associated with age of onset, disease behavior, location, or severity (20-23). Importantly, there are many associations between clinical factors and genetic sequences, but these do not necessarily indicate a causal relationship. Future investigations should evaluate the biological

pathways of these genes to elucidate the pathogenic mechanisms leading to growth impairment.

Limitations in our study include the following: (1) limitation to only children who had non-stricturing and non-penetrating Crohn's disease, (2) the cross-sectional analysis limited to children who had a single measurement of anthropometrics at diagnosis, before therapy was initiated, and (3) the PCDAI includes anthropometric measurements. The SNPs evaluated were not specifically associated with growth. With improved understanding of the functions of the SNPs associated with IBD, future studies may elicit the SNPs which place children at highest risk for poor growth. However, our study included a very large cohort of children with treatment-naïve Crohn's disease who were evaluated with twenty-one clinical and serologic variables and extensive genetic sequencing. It is the most comprehensive study evaluating growth at the time of diagnosis and establishes a foundation to investigate growth over time.

Currently, a comprehensive evaluation of anthropometrics, lab results, and endoscopic findings remains necessary for diagnosis and management. Future investigations should evaluate pathogenic mechanisms for poor growth and identify factors that support catch-up growth. Our study demonstrated an association between GM-CSF auto-antibodies and CBir antibodies and linear growth impairment; additional research should investigate the relationship between the genetic polymorphisms, the immune response, and hormonal pathways regulating growth in children with Crohn's Disease.

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Abbreviations:

ANCA: anti-neutrophil cytoplasmic antibodies

ASCA: anti-*Saccharomyces cerevisiae* antibodies

BMI: body mass index

CBir: antibodies against flagellin of the *Clostridium* species

CRP: C-reactive protein

ESR: erythrocyte sedimentation rate

GH: growth hormone

GM-CSF: granulocyte-macrophage colony stimulating factor auto-antibodies

GWAS: genome-wide association study

I2: antibodies to *Pseudomonas fluorescens*

IBD: inflammatory bowel disease

IGF-1: insulin-like growth factor

IL-6: interleukin-6

OmpC: anti-outer membrane protein C of *E. Coli* antibodies

PCDAI: Pediatric Crohn's Disease Activity Index

PGA: Physician Global Assessment

PRO-KIIDS: Pediatric Research Organization for Kids with Intestinal Inflammatory Diseases

QC: Quality control

SNP: single nucleotide polymorphism

TNF-α: tumor necrosis factor-α

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Table Legend:

Table 1. Demographics and Tanner Staging of Study Cohort

N	772
Age (years)	
Mean	11.6 (+/- 3.1)
Range	1-17
Median	12
Male	486 (63%)
Race	
Caucasian	637 (82%)
African-American or mixed	46 (6%)
Asian	29 (4%)
Unknown	60 (8%)
Tanner Staging (N=446)	
I	244 (55%)
II	65 (15%)
III	39 (9%)
IV	51 (11%)
V	47 (10%)

Table 2. Univariate regression analysis of clinical variables associated with height, weight, and BMI z-scores <-2

	Height z-score <-2		Weight z-score <-2		BMI z-score <-2	
Clinical Variable (N=772)	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Age at diagnosis	0.95 (0.88-1.04)	0.26	0.98 (0.92-1.04)	0.44	0.95 (0.90-1.01)	0.08
Gender	1.4 (0.82-2.38)	0.21	1.31 (0.89-1.91)	0.17	0.78 (0.54-1.11)	0.17
Upper GI tract (N=429)	1.01 (0.60-1.74)	0.96	1.25 (0.85-1.84)	0.26	1.52 (1.07-2.19)	0.02
Ileum (N=560)	1.01 (0.57-1.85)	0.99	0.79 (0.53-1.18)	0.24	0.99 (0.69-1.47)	1.00
Colon (N=599)	0.8 (0.45-1.49)	0.46	1.26 (0.80-2.05)	0.34	1.82 (1.16-2.96)	0.01
Hemoglobin (g/dL)	1.01 (0.96-1.03)	0.68	1.01 (0.98-1.03)	0.46	1.01 (0.99-1.03)	0.13
Platelets (10^9/L)	1.002(1.0005-1.0037)	**0.01**	1.003 (1.002-1.004)	**3.56×10-6**	1.003 (1.002-1.004)	**5.16×10-9**
Eosinophils (10^9/L)	0.98 (0.82-1.00)	0.61	1 (0.99-1.00)	0.35	1 (1.00-1.01)	0.12
ESR (mm/hr)	1.01 (0.99-1.02)	0.20	1 (0.99-1.01)	0.52	1.01 (1.00-1.02)	0.01
Albumin (g/dL)	0.47 (0.30-0.73)	**0.001**	0.54 (0.40-0.74)	**1.18×10-4**	0.53 (0.39-0.70)	**1.37×10-5**
ASCA IgA (EU/mL) (N=758)	1.01 (0.99-1.02)	0.25	1.01 (1.00-1.01)	0.16	1 (1.00-1.01)	0.37
ASCA IgG (EU/mL) (N=758)	1 (0.99-1.01)	0.51	1 (0.99-1.01)	0.79	1 (1.00-1.01)	0.52
ANCA (EU/mL) (N=758)	1.01 (0.99-1.01)	0.25	1 (0.99-1.01)	0.84	0.99 (0.99-1.00)	0.37
GM-CSF (mcg/ml) (N=734)	1.03 (1.00-1.05)	**0.02**	0.99 (0.96-1.01)	0.49	0.99 (0.96-1.01)	0.26
CBir (EU/mL) (N=758)	1.01 (1.00-1.01)	**0.01**	1 (1.00-1.01)	0.35	0.99 (0.99-1.00)	0.98
OmpC (EU/mL) (N=758)	0.99 (0.97-1.01)	0.83	1 (0.98-1.01)	0.92	1 (0.99-1.01)	0.68

Table 3. Multivariate binary regression analysis of clinical variables associated with height, weight, and BMI z-scores <-2

Height			
Clinical Variable (N=772)	**Odds Ratio**	**Standard Error**	**p-value**
Albumin (g/dL)	0.50	0.25	0.0052
GM-CSF auto-antibodies (mcg/ml) (N=734)	1.03	0.01	0.0110
Weight			
Clinical Variable (N=772)	**Odds Ratio**	**Standard Error**	**p-value**
Female	1.56	0.22	0.0401
Platelets (10^9/L)	1.00	0.001	0.0081
Albumin (g/dL)	0.66	0.17	0.0162
BMI			
Clinical Variable (N=772)	**Odds Ratio**	**Standard Error**	**p-value**
Platelets (10^9/L)	1.00	0.001	0.0011
Albumin (g/dL)	0.64	0.16	0.0061

Table 4. Genetic sequences associated with anthropometric z-scores using discrete outcome analysis

Gene	p-value	Gene Function	Common Pathway
Height z-score <-2			
OR5D14, OR5L1,	3.68×10^{-6}	Olfactory receptor proteins	Cell signaling
OR4C6, OR5D13	4.29×10^{-6}	Olfactory receptor proteins	
KCNH8, EFHB	1.22×10^{-5}	Potassium and calcium cell signaling	
VAV3	5.91×10^{-5}	Cytoskeleton protein synthesis	
PAPOLG	4.91×10^{-5}	DNA synthesis	
TAP2	2.15×10^{-5}	Transport protein for antigen processing	Antigen presentation
HLA-DOB	6.53×10^{-5}	MHC Class II-β cell signaling	
Weight z-score <-2			
SKAP2	4.70×10^{-5}	Protein in Src pathway for cell growth and survival	Cell signaling
ANXA6	7.72×10^{-5}	Cell signaling for exocytosis	
GLP2R	1.29×10^{-5}	Nutrient absorption in enterocytes	
C8orf87	3.91×10^{-5}	Codes for uncharacterized protein	
RMI2	1.59×10^{-5}	DNA repair	
BMI z-score < -2			
TAGAP	7.90×10^{-5}	Rho GTPase-activator protein	Cell signaling
CCR7, SMARCE1	2.25×10^{-5}	B and T cell activation and migration, dendritic cell maturation; chromatin remodeling	Antigen presentation