Investigating Interactions among Genetic and Environmental Risk Factors in Longitudinal Family Studies with Application to the Quebec Newborn Twin Study

Cheng Wang

A thesis submitted to the
Faculty of Graduate and Postdoctoral Studies
in partial fulfillment of the requirements for the
MSc degree in Epidemiology

School of Epidemiology and Public Health
Faculty of Medicine
University of Ottawa

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Abstract

Introduction: Gene-environment (GE) interactions involving the IGF pathway may affect childhood obesity. Detecting such interactions using longitudinal family studies requires accounting for individual and familial correlations.

Methods: Simulations were performed to study three methods to test for GE interactions in longitudinal family data using repeated outcomes (linear mixed model) or individual outcome averages as summary statistics (twin model, partition based score I test). Interactions between the IGF pathway genes (IGF-1, IGFALS) and environmental factors (physical activity, daycare attendance and sleep duration) were tested using the Quebec Newborn Twin Study data.

Results: The twin model yielded the best performance. Results from the QNTS analysis showed suggestive association for an IGF-1 variant at position 102791894 of chromosome 12 interacting with physical activity. However, this association was not statistically significant after multiple testing correction.

Conclusion: More robust methods and studies are needed to better understand the IGF pathway's role in childhood obesity.

Acknowledgements

I would like to express my sincerest gratitude to my mentors, friends and family, who have given me the invaluable support needed to complete this thesis. To me personally, this work and my study here at University of Ottawa has been a truly transformative experience, and I owe this to everyone.

Firstly, I would to like to thank my supervisor Dr. Marie-Hélène Roy-Gagnon for your mentorship and support. Thank you for patiently guiding me through this process, and encouraged me to explore new ideas. Not only have I learned epidemiology and genetics under you, but I will always be in debt to you for teaching me how to do rewarding research that combines both abstract theory and concrete application. I also would like to thank my co-supervisor, Dr. Kelly Burkett. Your guidance and advice have been vital to my thesis. I am fortunate to have you introducing me to the generalized linear model and the mindset for engaging statistics. My future endeavor will forever benefit from your advices. I am grateful to Dr. Lise Dubois for providing counsel on my thesis advisory committee and granting me the use of data for this project. Whenever I encountered a question, your timely response was always there to help me push forward. I also want to express my gratitude to Jean-François Lefebvre, who has given me tremendous support on all technical fronts. The parallel computing analysis would not be possible without your help. Heartfelt thanks must be extended to my lab mates and classmates. Thank you for the encouragement and support, whether it is during conference presentations, biostatistics exam, snack break “counselling” and many more. I am truly lucky to

have met all of you. Also I must wholeheartedly thank my friends in Toronto. Thank you for accommodating my erratic schedule, and shared with me your prudent advices as well as moments of happiness. Spending time with you guys has been a great source of strength and calm for me during the course of this project. Last but not least, I offer my deepest gratitude to my family for being the foundation of my life. Whether from China, California or Toronto, your unwavering support has given me the courage to chase after my dream and confront whatever challenges along the way. It is said that a journey of thousand miles starts with the first step. Thank you, mom and dad, for lifting me up during my initial stumbles. I dedicate this thesis to you.

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Legend

BMI: body mass index

DZ twins: dizygotic twins

GE interaction: gene-environment interaction

GH: growth hormone

GHRH: growth hormone releasing hormone

GT interaction: gene-time interaction

GTE interaction: gene-time-environment interaction

GWAS: genome-wide association studies

HWE: Hardy-Weinberg Equilibrium

IGF: insulin-like growth factor

IGF-1: insulin-like growth factor-1

IGFALS: insulin-like growth factor-binding protein, acid-labile subunit

MAPK: mitogen-activated protein kinase

MC4R: melanocortin 4 receptor

MZ twins: monozygotic twins

PBI test: partition based score I test

QNTS: Quebec Newborn Twin Study

SNP: single nucleotide polymorphism

XOR interaction: exclusive OR interaction

1. Introduction

1.1 Childhood Obesity and its Health Impact

Obesity is considered a complex trait involving many pathological processes in the body. The primary symptom is an excess buildup of bodily adipose tissues due to an imbalance of energy consumption and expenditure. The cause of this imbalance is complex, and is thought to be jointly-influenced by genes and environment. Clinically, obesity is most often diagnosed using body mass index (BMI). BMI is defined as the ratio of weight (in kg) over squared height (in m) (Güngör, 2014). In childhood obesity, the BMI diagnostic cutoffs are based on percentile figures for specific age and gender groups (Güngör, 2014; Han, Lawlor, & Kimm, 2010).

As a complex disease, childhood obesity impacts almost all body systems (Han et al., 2010). Obesity is also potentially chronic as childhood body fat level has shown correlation with adult adiposity (Freedman et al., 2005). The disease leads to various co-morbidities including type 2 diabetes, hypertension, cancer and psychosocial complications (Chesi & Grant, 2015; Han et al., 2010). Individuals characterized as obese have higher risk of developing those co-morbidities as well as higher adulthood mortality (Bjørge, Engeland, Tverdal, & Smith, 2008; Han et al., 2010; Pulgarón, 2013). Economically, studies suggested that health complications accompanying childhood obesity can translate into higher healthcare burden on the society (John, Wolfenstetter, & Wenig, 2012). A Canadian study by Kuhle et al. (2011) has shown that childhood obesity and overweight were associated with higher utilization of both primary and

specialized health services. The study also estimated that obesity and overweight accounted for 1.3% to 2.1% of the adolescent healthcare expenditure in its study population (Kuhle et al., 2011).

As a public health concern, childhood obesity is a growing problem worldwide. In Canada, the prevalence of obese and overweight children reached 31% in 2012-2013 (Statistics Canada, 2014). Prevalence for obesity stands at 15% and 11% in young males and females respectively (Statistics Canada, 2014). In United States, about 20% of the children and the adolescent populations now have obesity, representing a threefold increase over the last 4 decades (Centers for Disease Control and Prevention, 2017). Worldwide, from 1990 to 2010, the proportion of children classified as overweight or obese increased from 4.2% to 6.7% (Güngör, 2014). Given the large impact and the growing trend of childhood obesity worldwide, there is an increasing need to understand the disease's etiology so appropriate public health interventions can be applied.

1.2 The Genetic Basis of Childhood Obesity

Genetics plays an important role in obesity etiology. Studies have estimated that 40% to 70% of the obesity variation observed in the populations can be attributed to genetic influences (Manco & Dallapiccola, 2012; Waalen, 2014). There is evidence for genetic contribution to obesity through different biological mechanisms. Genome-wide association studies (GWAS) have uncovered associations between body adiposity measures and common genetic variants

such as those in the melanocortin 4 receptor (MC4R) gene (Han et al., 2010; Xi, Chandak, Shen, Wang, & Zhou, 2012). Other mechanisms include rare variants and epigenetic processes such as DNA methylation (Güngör, 2014; Han et al., 2010). However due to the complexity of the disease, current evidence on genomic level variations in population cannot fully explain the predicted genetic contribution to obesity. This gap in our understanding may be due to the lack of accounting for the interactive nature of obesity etiology, particularly the interplay between genetic and environmental factors.

1.2.1 Childhood Obesity in the Context of the Missing Heritability Problem

Since obesity is a complex trait or phenotype, our understanding of the full genetic basis for the disease is limited. For complex traits such as height and obesity, the current evidence, based mostly on GWAS, is unable to account for the majority of the phenotype's genetically attributed variation as predicted by the heritability estimates (Manolio et al., 2009). This gap in the evidence base for linking population-level phenotype variations to specific effects due to genetic variants, has been termed the so-called "missing heritability problem". Although heritability estimates suggest that 40% to 70% of the body weight variation is under genetic influence, currently identified genetic associations could only explain 1.45% of this variance (Waalen, 2014). The gene locus with the largest effect size accounts for only 0.34% of the trait variation (Waalen, 2014). On average, an at-risk variant is associated with approximately 0.17 increase in BMI (Manco & Dallapiccola, 2012).

Many explanations have been proposed to fill in the gap in our understanding of complex trait genetics. One hypothesis speculates that the heritability for complex phenotypes was over-estimated due to assuming that all of the genetic effects are additive. Interaction effects between gene and environment violates the assumption of purely additive genetic effects and leads to overestimation of heritability. The inflated heritability estimates or so-called "the phantom heritability" over predict the proportion of phenotype variance due to genetic factors (Fu, O'Connor, & Akey, 2013; Marian, 2012). Therefore, studying potential joint effect of genes and environments can offer a more complete explanation of the population variation in body weight. Evidence ranging from animal models to studies on how alcohol interacts with human genetic risk for breast cancer have pointed to the potential explanatory power of genetic interactions (Fu et al., 2013).

1.2.2 An Interactive Pathogenesis Pathway for Childhood Obesity

In addition to the significant genetic contributions to childhood obesity, diverse environmental factors are involved in the disease pathogenesis as well. On a small scale, other conditions, such the presence of endocrine diseases, can elevate the person's obesity risk (Han et al., 2010). At the family and community level, factors that influence an individual's acquired lifestyle can affect obesity development. For example, a supportive family environment can encourage children to pursue physical activities which are protective against childhood obesity (Ebbeling, Pawlak, & Ludwig, 2002). Lastly, macro scale social dynamic and economic trends also have an impact on the obesity risks across many communities. One such trend is

globalization and the spread of mass-produced food products worldwide. One of the mass-produced food products that saw a global increase in its consumption is the sweetened drinks, which is a risk factor for obesity (Ebbeling et al., 2002; Lobstein et al., 2015).

Given the importance of both genetic and environmental contributions to childhood obesity, it is reasonable to suggest that gene-environment (GE) interactions could play an important role in its etiology. The overall genetic influence on body weight variations can change under different environment conditions. In one study, the proportion of BMI variation due to genetic effect was higher among individuals whose parents had higher education levels. Decreased BMI heritability was also observed among individuals performing increased level of physical activity (Kaprio, 2012). When examining specific genetic variants associated with obesity risk, the increase in BMI due to consuming soft drinks and deep fried food were higher if the individual had more at-risk gene variants (Narimatsu, 2017). The effects of risk-increasing gene variants on BMI were weakened with increasing level of physical activity (Qi et al., 2012).

In childhood obesity, GE interactions are also complicated by the potential temporal dependence of the effect (Manco & Dallapiccola, 2012). At any point during childhood, the relative contribution from genetic and environmental factors could change (Hebebrand, Volckmar, Knoll, & Hinney, 2010; Manco & Dallapiccola, 2012). Given the current limitations in our understanding of obesity pathogenesis, it is important to explore GE interactions to help us elucidate the genetic basis of the disease. This need is even more urgent for childhood obesity

as the etiology is more complex, and the research lags behind its adult counterpart (Dubois et al., 2007).

1.3 The Role of the IGF Gene Pathway in Childhood Obesity

The insulin-like growth factor (IGF) pathway is a biological signaling cascade that is involved in body growth and immune response. The IGF pathway genes like insulin-like growth factor-1 (IGF-1) and insulin-like growth factor-binding protein, acid-labile subunit (IGFALS), are intimately involved in body fat regulation. At its most upstream point, the IGF pathway is regulated by the hypothalamus through growth hormone (GH) signals. GH is secreted by the anterior pituitary somatotroph cells under stimulation from hypothalamic neurosecretory cells (Garten, Schuster, & Kiess, 2012). Under growth hormone releasing hormone (GHRH) and Ghrelin signaling from the hypothalamus, GH release leads to up-regulation of IGF-1 and IGFALS gene expressions (Garten et al., 2012; Suwanichkul, Boisclair, Olney, Durham, & Powell, 2000). GH-induced production of the IGF-1 proteins from adipose and liver tissues enact downstream physiological changes including growth and differentiation (Horacio M Domené et al., 2009; Garten et al., 2012). Playing a more ancillary role, increased production of IGFALS contributes to the formation of stabilizing ternary complex with IGF-1 (Suwanichkul et al., 2000). Figure 1 presents a simplified schematic of the IGF pathway with details discussed in the next two sections (1.3.1 and 1.3.2).

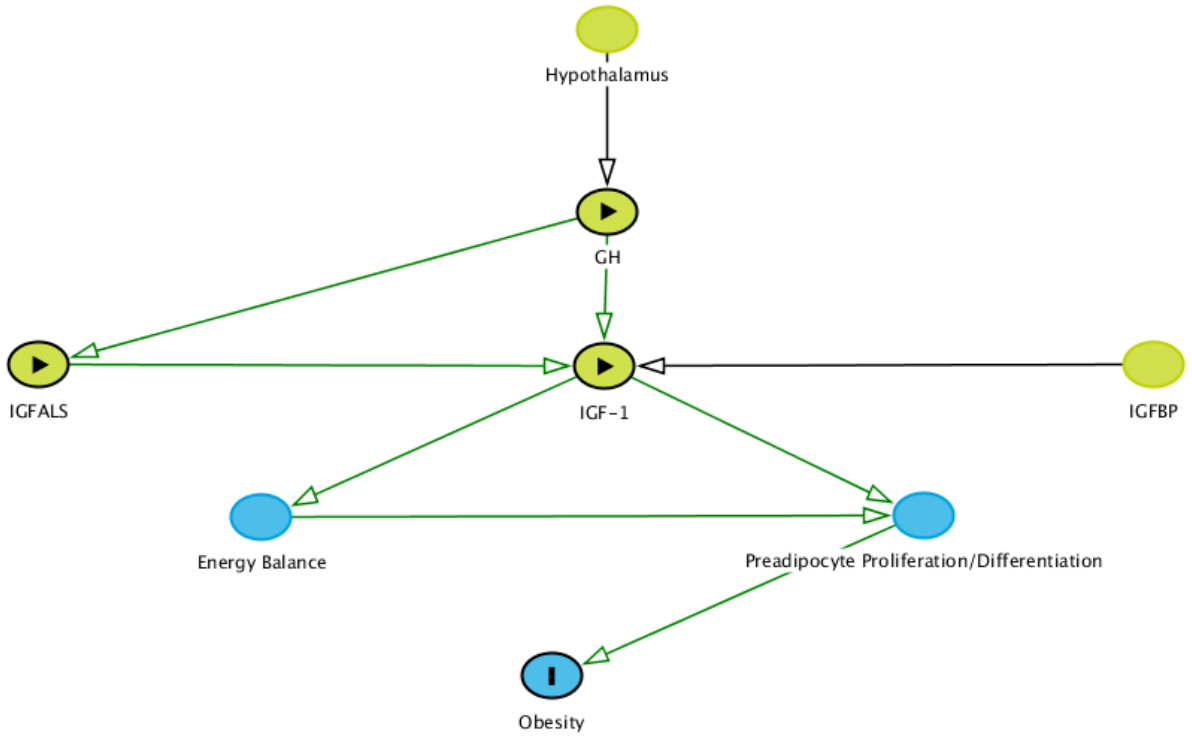

Figure 1. The insulin-like growth factor (IGF) pathway and its effect on obesity development

The IGF pathway genes and their upstream regulators are in green. The outcome (obesity) and its directly related pathogenesis processes are in blue. Under signals from the hypothalamus, the growth hormone (GH) stimulates both insulin-like growth factor 1 (IGF-1) and IGF acid-labile subunit (IGFALS) gene expressions. IGFALS and IGF binding proteins (IGFBP) interact with IGF-1 to prolong its half-life. IGF-1 promotes preadipocyte growth and differentiation as well as regulating body energy metabolism. Diagram was constructed using DAGitty (Textor, Hardt, & Knüppel, 2011).

1.3.1 The Insulin-like Growth Factor-1 (IGF-1) Gene

The IGF-1 gene is mapped to human chromosome 12q23.2 and includes 6 exons. Depending on the specific tissue sites and endocrine signals, 4 of the 6 IGF-1 exons are alternatively spliced (Hamosh & McKusick, 2016). The gene plays a regulatory role in processes directly related to obesity. An increase in IGF-1 levels can modulate carbohydrate metabolism through stimulating glucose uptake and ensuring the survival of insulin secreting beta-cells (Horacio M Domené et al., 2009). IGF-1 is also a proliferation, differentiation and survival signal to the adipose tissue. Preadipocyte differentiation is regulated by IGF-1 probably via the PI3-kinase/AKT pathway. Whereas IGF-1 promotes preadipocyte growth through stimulating proliferation signaling from the mitogen-activated protein kinase (MAPK) pathway (Garten et al., 2012).

Evidence from population studies has supported these molecular mechanisms linking obesity to IGF-1. While there are studies with negative findings, several other studies reported higher IGF-1 levels among children with obesity (Juul, 2003). A longitudinal study had found an association linking an IGF-1 genetic variant to increased BMI among individuals followed from 8 to 14 years of age (Voorhoeve et al., 2006).

1.3.2 The Insulin-like Growth Factor-Binding Protein, Acid-Labile Subunit (IGFALS) Gene

In contrast with IGF-1's direct effect on obesity, the influence of IGFALS is more subtle, acting primarily through its effect on IGF-1 half-life. The IGFALS gene is located on human chromosome 16p13.3, and contains only two exons and one intron totaling 3.3 kb in length (Suwanichkul et al., 2000). Its biological function is to form ternary complex with insulin-like growth factors (IGF-1, IGF-2) and IGF binding proteins (IGFBP-3, IGFBP-5) (H M Domené, Bengolea, Jasper, & Boisclair, 2005). The ternary complex concentrates the IGF-1 proteins in the circulation (H M Domené et al., 2005). More IGF-1 proteins are reserved since the complex offers protection from proteolytic degradation, and prevents blood vessel endothelia cells from transporting IGF-1 out of the circulation (Horacio M Domené et al., 2009; Payet, Firth, & Baxter, 2004). The overall result is that when complexed with IGFALS and IGFBP, 75% to 90% of the serum IGF proteins can be stably stored (Janosi, Firth, Bond, Baxter, & Delhanty, 1999; Twigg & Baxter, 1998). Because of its role in the ternary complex formation, IGFALS is important to IGF-1 bioavailability regulation. Mutation in the IGFALS gene was shown to impair complex formation with the IGF and IGFBP proteins (Firth, Yan, & Baxter, 2011).

Through its regulatory effect on circulating IGF-1 level, IGFALS may influence obesity and growth in general. Animal IGFALS knockout studies have demonstrated parallel decreases in growth rate and IGF-1 level (H M Domené et al., 2005; Ueki et al., 2000). Human patients with mutations in the IGFALS gene also showed impaired growth and low serum IGF-1 concentration (H M Domené et al., 2005; Horacio M Domené et al., 2007; Heath et al., 2008; Hess et al., 2013). Conversely, in a study on central precocious puberty patients with increased growth rate, higher level of IGFALS was found (Cisternino et al., 2002). In human patient studies, the

majority of the IGFALS mutations leading to inactivation were found in the leucine-rich repeat regions responsible for protein-protein interaction, highlighting the importance of IGFALS in IGF-1 complex binding (Horacio M Domené et al., 2009; Kobe & Kajava, 2001). Drop in serum IGF-1 proteins was also observed alongside with disruption in ternary complex formation (H M Domené et al., 2005; Horacio M Domené et al., 2007). In addition to the positive correlation between body growth and IGFALS levels, high IGFALS protein level was also linked to obesity in population studies. Higher levels of IGFALS were found in obesity cases compared to controls, though results were not statistically significant (Barrios et al., 2001; Rasmussen, Juul, Kjems, & Hilsted, 2006). In a study comparing patients with anorexia nervosa to controls, significant positive association between IGFALS concentration and BMI was observed (Fukuda et al., 1999).

1.4 Potential Environmental Modifications of the IGF Pathway

Obesity is the product of complex interactions between genetic and environmental factors. Within the IGF pathway, there are numerous points where environment can modulate IGF signaling on carbohydrate metabolism and adipose tissue growth. Hence, modification of IGF signaling levels through mechanisms such as gene transcription regulation can ultimately affect changes in obesity-related processes. At the population level, this can manifest as GE interaction effects on the disease trait. In this study, we hypothesize three potential GE interaction scenarios between the IGF pathway genes (IGF-1, IGFALS) and environmental exposures (physical activity, daycare attendance and sleep duration). The IGF gene pathway and our hypothesized environmental interactions are summarized in Figure 2.

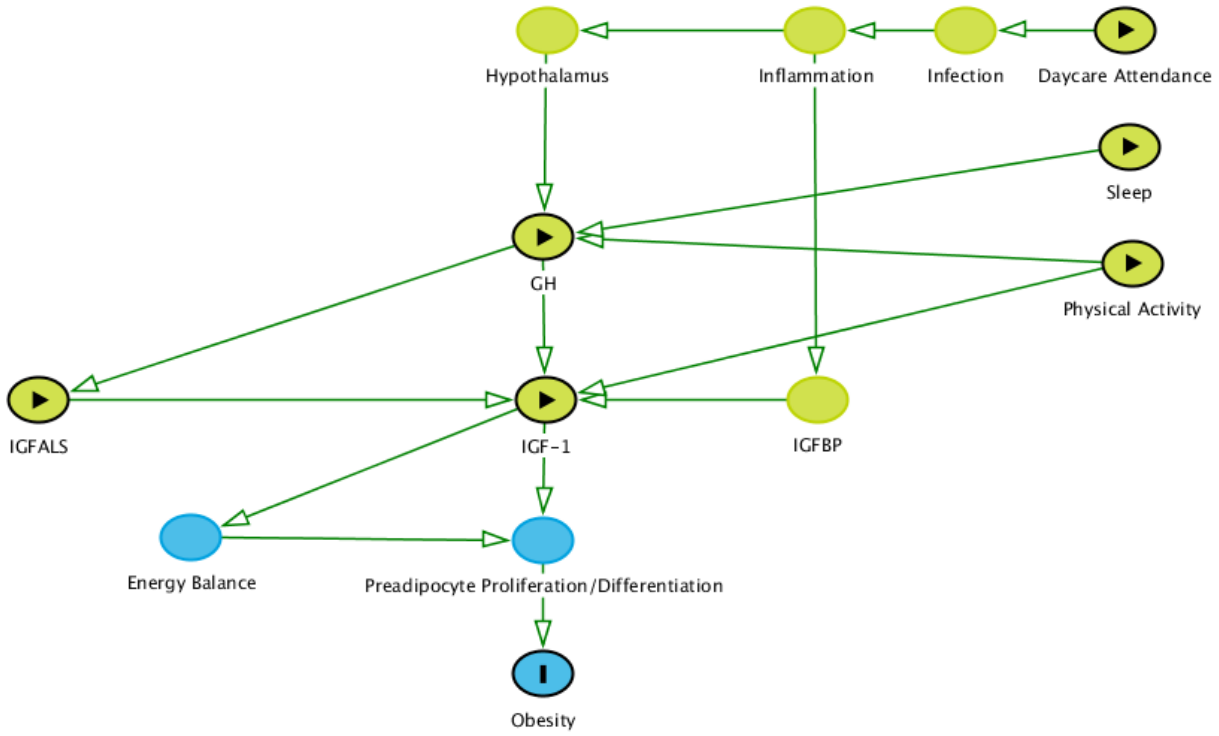

Figure 2. Possible mechanisms of the IGF gene pathway's association with obesity

Hypothesized exposures and intermediate exposures are in green. The outcome (obesity) and its directly related pathogenesis processes are in blue. Growth hormone (GH) stimulates both insulin-like growth factor 1 (IGF-1) and IGF acid-labile subunit (IGFALS) gene expression. IGFALS and IGF binding proteins (IGFBP) interact with IGF-1 to prolong its half-life. IGF-1 promotes preadipocyte growth and differentiation as well as regulating body energy metabolism. The environment factors can interact with the IGF pathway at various points (details discussed in section 1.4). Physical activity and sleep can potentially impact the IGF pathway directly. Daycare attendance could affect the GH/IGF axis via inflammation responses. Diagram was constructed using DAGitty (Textor et al., 2011).

1.4.1 Physical Activity and the IGF Pathway

Physical activity is a factor intimately involved in the development of childhood obesity, making it a good candidate to test possible GE interactions with the IGF pathway. The protective benefit of active lifestyle against obesity among children and adolescents is well-established as demonstrated by a recent systematic review (Jiménez-Pavón, Kelly, & Reilly, 2010). Furthermore, physical activity is able to modify obesity risks associated with genetic variants. This was evidenced by the different heritability estimates for fat mass among individuals with varying levels of physical activity (Waalen, 2014). Hence we hypothesize that the IGF pathway may interact similarly with physical activity as part of the factor's complex relationship with obesity.

One documented interaction between physical activity and the IGF pathway is its effect on GH signaling. Physical fitness has been shown to be positively correlated with GH, the mediator of hypothalamic stimulation on the IGF pathway (Berryman, Glad, List, & Johannsson, 2013). This stimulating effect on GH release appears to be threshold-dependent and correlates with the intensity of exercise (Sherlock & Toogood, 2007; Stokes, 2003). Specifically, 10-20 minutes of aerobic exercise could stably elevate serum GH level for 2 hours (Sherlock & Toogood, 2007). High GH level was also observed either during the latter part of activity regimen or after prolonged exercise above anaerobic threshold (Sherlock & Toogood, 2007; Stokes, 2003). The oxygen supply-to-demand ratio and the circulating hydrogen ions concentration showed concurrent variation with changing GH levels in response to physical activity. It is hypothesized

that serum hydrogen ion or muscle oxygen metabolism regulation may mediate the effect of exercise on GH release (Stokes, 2003).

In addition to GH, physical activity may influence IGF-1 regulation directly. Increase in IGF-1 level among GH-deficient patients following exercise and lack of association with GH-peaks suggested a GH-independent mechanism for the interaction between IGF-1 and physical activity. Transient (10-15 minutes) IGF-1 spike may be attributed to increased release from existing storage rather than up-regulated production. Compared with GH, the evidence for association between physical activity and IGF-1 level are more inconsistent; positive, negative and no relationships have all been reported (Nindl & Pierce, 2010).

1.4.2 Sleep and the IGF Pathway

Another potential modulator of the IGF pathway activity is sleep duration. Shorter sleep duration is a possible risk factor for childhood obesity (Han et al., 2010). This may be due to its effect on the hypothalamus and GH, which also regulate the IGF-1 and IGFALS genes. Consequently the potential effect on GH provides an opportunity for sleep to modify the IGF pathway signaling on obesity-related processes. Though mechanistically still unclear, sleep may increase GH release directly or it could be the result of shared physiological pathways in the hypothalamus (Sherlock & Toogood, 2007). Nevertheless, 60-70% of the daily GH secretion in men and less than 50% of that in women occur during early sleep. The quantity of GH produced correlates with the length of the slow wave phase of sleep (Van Cauter et al., 2004). It is

possible that aging-related decrease in GH signals is in part due to deteriorations in sleep time and quality (Sherlock & Toogood, 2007). In addition to correlation studies, there is also experimental evidence for sleep influencing GH secretion. Pharmacological stimulation of slow wave sleep, even at minimal dose, had led to 200% increase in GH production due to higher secretory intensity and duration. Individuals with GH deficiency often suffer poor sleep characterized by sleep fragmentation and reduced duration (Van Cauter et al., 2004).

1.4.3 Daycare Attendance and the IGF Pathway

While physical activity and sleep duration may interact with the IGF pathway genes closely, more indirect effect modification involving extended intermediate processes is also possible. The additional regulatory role of the IGF pathway in the inflammatory response may mediate such indirect interaction. Experimental evidence has shown that the IGF binding proteins (IGFBP) are valid substrate for neutrophil protease proteins, cathepsin G and elastase both in vitro and in vivo. This possible regulation of IGFBP by immune system cells suggests that protease enzyme regulation of IGFBP and its associated IGF-1 can be influenced by the inflammatory response (Gibson & Cohen, 1999). Another possible site of effect for inflammation and the IGF pathway is at the hypothalamus. Studies have shown that the long-term low-level inflammation in the hypothalamus during obesity can lead to increased appetite and energy intake (Gregor & Hotamisligil, 2011). This effect on feeding may involve Ghrelin as it regulates both eating behaviour and IGF signaling.

On the other hand, a well-known environmental influence on inflammation is daycare attendance. Daycare facilities have been associated with increased infection risk for a variety of communicable diseases such as upper respiratory tract infection and bacterial meningitis. This is reflected by the antibiotic usage, which is 2.4 to 3.6 times higher for children attending daycare centres compared to staying at home (Brady, 2005). In areas of high population concentration such as daycare facilities, infectious diseases can spread quickly through common routes like sneezing and coughing or other contacts such as sharing toys (Brady, 2005). Thus frequent and even chronic infection with its associated inflammatory response could be expected among children attending daycare. This could lead to interaction with the IGF pathway through inflammation and ultimately influence obesity pathogenesis.

1.5 Detecting Gene-Environment Interaction Using Longitudinal Family Studies

One way to evaluate potential GE interactions is through conducting longitudinal family studies. This involves recruiting and collecting information on related individuals, as usually done in traditional genetic studies. The longitudinal component of the design refers to data being repeatedly collected on the same individual over time. Hence, qualitative or quantitative traits such as BMI will be assessed at multiple time points (Ruzong Fan et al., 2012). In this thesis, we used longitudinal family data from the Quebec Newborn Twin Study (QNTS) to test our hypotheses on the joint effect of the IGF pathway and environment in relation to childhood obesity. The background of QNTS and the challenges of data analysis in longitudinal family studies are discussed in this section.

1.5.1 The Quebec Newborn Twin Study

QNTS is a longitudinal family study that follows twin pairs recruited from Montreal, Canada (Boivin et al., 2013). Six hundred and sixty-two twin pairs (67% participation rate) born between 1995 and 1998 were included in the study and the data collection is still ongoing (Boivin et al., 2013; Dubois et al., 2013). The studied population includes twins who were free of major illness at birth (Dubois et al., 2013). Those who passed away before 5 months of age were also excluded (Dubois et al., 2012). Zygosity was determined using a multitude of evidence including chorionicity data, physical similarity as well as genotype data (Boivin et al., 2013; Dubois et al., 2013). Of the 662 twins, 375 pairs provided blood and saliva samples for genotyping with consent given by their parents (Boivin et al., 2013). As part of the study, QNTS collects data on a variety of social, biological and psychological measures. The data were gathered through medical records, interviews, and laboratory assessments. The ongoing follow-up assessment initially started around 5-6 months of age and has continued throughout early childhood, kindergarten, elementary and secondary school (Boivin et al., 2013; Dubois et al., 2012). Separate data collection personnel were assigned for each individual within a twin pair to control for bias due to knowledge of zygosity status (Boivin et al., 2013). The overall structure of QNTS data consists of repeated measures for individuals nested within families (twin pairs).

1.5.2 The Advantages of Longitudinal Family Study Designs

The advantage of longitudinal family studies is that they provide benefits from having both repeated measures and family sampling. For its longitudinal aspect, the design offers potentially higher power and the ability to study temporal effect on gene-phenotype association (Wu, Hu, & Melton, 2014). Quantitative traits such as BMI are inherently time varying, so it is important to study them in consideration with time. The longitudinal measures allow us to assess the change and the stability of a phenotype over time (Barnholtz-Sloan, Poisson, Coon, Chase, & Rybicki, 2003). These questions can be genetically meaningful since they can potentially clarify the timing and the relative contribution of genetic factors at different lifetime stages (Ruzong Fan et al., 2012). Additionally, since outcome measures are repeated many times, more reliable data are available, thereby increasing the potential detection power without incurring additional genotyping expenses from recruiting more participants (Wu et al., 2014). Similarly, family studies are important since genetic epidemiology is interested in traits that segregate among related individuals. By sampling families rich in potentially associated genetic variants, we could improve statistical power (Chen, Malzahn, Balliu, Li, & Bailey, 2014). Knowledge of the family structure among sampled individuals can also help to impute data for missing genotypes or determining the segregation pattern of an allele (Chen et al., 2014). Thus, conducting longitudinal family studies offers potential improvement in statistical power as well as the flexibility to test temporally or family-related hypotheses. These advantages can aid our effort in uncovering GE interactions.

1.5.3 Accounting for Complex Correlation Structure in Longitudinal Family Studies

Although longitudinal family studies offer many advantages, its data structure is more complex compared to cross sectional studies. Analyzing GE interactions in longitudinal family studies requires properly accounting for the 2-layer correlation structure in the data. When outcomes are measured repeatedly on the same individual, non-independent data points arise for each individual across time. One analytical approach to account for the correlation within individual is the hierarchical linear mixed model incorporating individual-level random effects (Barnholtz-Sloan et al., 2003). This allows direct and custom specification of the correlation structure. Another approach is to simplify this correlation by using summary statistics of the individual outcomes. Typical examples of summary statistics include mean over time or regression parameters from the outcomes fitted against time, such as the slope or the intercept. The advantage with using summary statistics is that it allows data analysis with any of the standard cross sectional family study methods such as the classic twin study model (Burkett et al., 2015).

In addition to longitudinal correlation, outcomes among individuals are also correlated according to their familial relationships. Similar to modeling repeated outcomes, a random effect can be assigned to family groupings in the linear mixed model to account for familial correlation. However, this would simplify the family structure if the random effect cannot distinguish between different familial relationships. For example in twin studies, the correlation between individuals from a monozygotic (MZ) twin pair and a dizygotic (DZ) twin pair are

different (Burkett et al., 2015). DZ twins on average share half of the genomic DNA compared to MZ individuals who are genetically identical. Furthermore, the presence of both longitudinal and familial correlations in the data can be modeled using nested levels or custom variance structure for the random effect (Barnholtz-Sloan et al., 2003; Sung et al., 2014). In addition to the regression-based methods, nonparametric tests have also been adapted for family data. Permutation testing used for nonparametric estimation of p-values can account for familial relationships by restricting the random shuffling of outcome-predictor data to within each family (Satten, Biswas, Papachristou, Turkmen, & König, 2014).

1.5.4 Challenges of Detecting Different GE Interaction Relationships

Biologically, an interaction effect can be defined by its hypothesized mechanism as demonstrated by the proposed IGF pathway GE interactions discussed in section 1.4. The hypothesis specifies any physical or chemical reactions between risk factors and/or their associated intermediates. For example, we speculated that the GE interaction between physical activity and the IGF pathway could occur via their joint effect on GH signaling. In order to model such biological interactions using mathematical frameworks, a statistical concept for interaction effect is needed. We define statistical interactions as the degree of effect modification observed in the data. Effect modification occurs when the effect size of one risk factor varies across the levels of other risk factors (Rothman, Greenland, & Lash, 2008). Thus, the GE interaction effect is statistically modeled as the deviation from a so-called additive effect pattern, where genetic and environmental factors exert independent effects on the outcome.

Using the example discussed above, the joint effect of physical activity and the IGF pathway on BMI would be statistically assessed as any residual effect found in addition to the main independent effects from the two predictors.

In the case of GE interactions, the effect modification relationship can be complex as the causative pathways for diseases such as childhood obesity often involve intricate and multilateral interplays between the disease risk factors. One source of complexity is that the type of interacting environmental factors can be diverse. For example, genetic variants conferring breast cancer susceptibility are known to interact with different exposures such as the number of births and alcohol consumption (Fu et al., 2013). On the other hand, environmental effect can also dramatically shift the effect of genes. In the case of innate immunity up-regulation, genetic variations that provide beneficial resistance to infection could contribute to autoimmune causes of diabetes (Sadee et al., 2014). The benefit and harm of those immune system genetic variants would depend on specific environment. Overall, due to the complexities of the GE interaction pathways, the relationship between genetic and environmental factors can take on many forms.

Here we give four possible relationships of GE interactions, of which three are non-linear, and we explain the plausible biological basis for each. The effect patterns under each interaction scenario are different, as shown in Figure 3. The linear interaction is the common interaction assumed by the multiplicative product term in a regression model. One way to interpret the product term is treating it as a component of the overall genetic effect:

$$Genetic\ Effect = Main\ Effect + GE\ Interaction\ Effect \times Environment\ Level$$

This gives an intuitive interpretation where the main effect is the level of genetic influence at some baseline environmental exposure. Any subsequent increase in the environmental exposure will contribute to the genetic effect as specified by the product term parameter. This type of interaction is often observed between environmental carcinogens and cancer susceptibility genes. In these cases, the environmental modification of the genetic effect is monotonic and dosage-dependent. A second type of GE interaction called exclusive OR (XOR) relationship can be interpreted as two opposing patterns of genetic effect under different environmental conditions. For example in comparison with the general population, individuals with two copies of the sickle cell anemia allele have reduced survival. But having one copy of the disease allele will increase their relative fitness in malaria-infested regions. In addition to varying linearly or in a XOR fashion, many biological responses to the environment do not increase indefinitely and show a plateauing effect at some level. In GE interactions, this could be caused by a third type of relationship which has a conditional dominance pattern of genetic effect, where the influence of an at-risk allele would be enhanced under some environmental condition and eventually plateau. The end result is that without environmental exposure, there is an additive pattern of genetic effect. But under some environmental condition, the genetic effect is enhanced but plateaus when there are two at-risk alleles producing a dominant pattern of inheritance. The fourth type of interactive relationships considered in this thesis is the small marginal effect interaction. This GE interaction is the most difficult to detect, because

modest genetic effect (e.g. effects produced by recessive pattern of inheritance) will be triggered only when an environmental exposure is present.

In addition to jointly influencing the population average of a trait, gene-time-environment (GTE) interactions are also possible for each of the aforementioned scenarios. They are important during childhood, when relative genetic and environmental contributions to a phenotype are temporally dependent (Hebebrand et al., 2010; Manco & Dallapiccola, 2012).

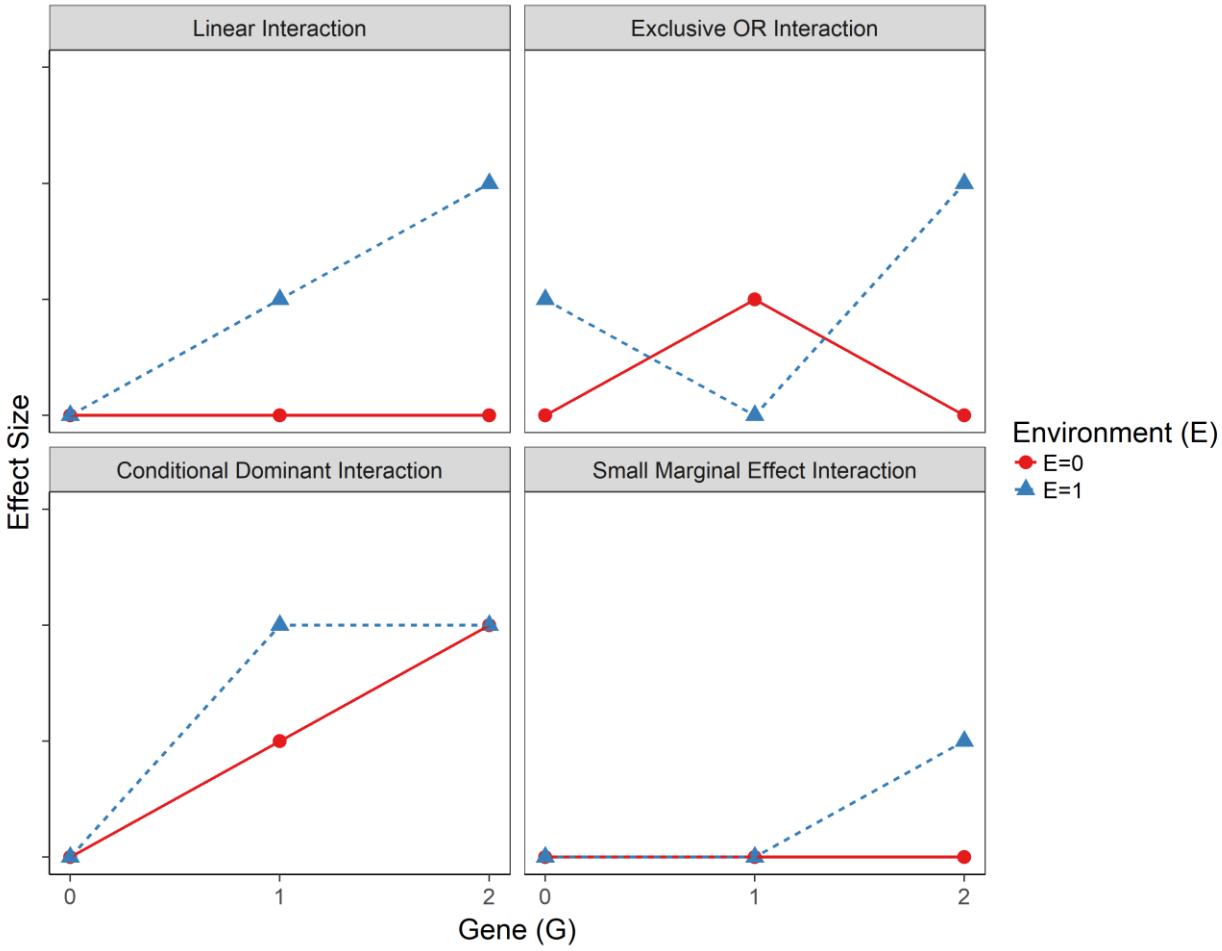

Figure 3. Effect patterns for various gene-environment interaction scenarios

Genetic (G) factor coded as 0, 1, and 2 for the number of minor alleles. Environment (E) factor coded as 1 or 0 for presence or absence of the environmental exposure.

Because of the complex interactive relationships between genetic and environmental factors, modeling GE interactions requires either making simplifying assumptions or allowing model flexibility. For regression-based methods such as the twin model and the linear mixed model, interaction is typically modeled as a product term between the genetic and environmental factors as shown below (Ruixue Fan et al., 2014).

$$\mu = \beta_0 + \beta_G G + \beta_E E + \beta_{GE} G \times E$$

In the above model, μ is the outcome average, while G and E terms are the genetic and environmental factors. β_G, β_E and β_{GE} denote the effects due to gene, environment and their interaction, respectively. As shown below, by rearranging the terms in the model, genetic effect can be shown to be a linear combination of the main effect (β_G) and the interaction effect depending on the specific environmental factor level ($\beta_{GE} E$). The interaction term represents the additional increase in the mean due to the genetic factor under some environmental condition, as discussed above.

$$\mu = \beta_0 + (\beta_G + \beta_{GE} E) \times G + \beta_E E$$

Thus, by using multiplicative product terms to model interactions in regression models, we implicitly assume that the interaction relationship is linear in nature. Figure 3 shows the linear pattern of effect specified using a multiplicative term in contrast to scenarios not well-fitted by such interaction model.

The assumption of linear relationship simplifies the interaction effect pattern and may not be suitable for non-linear scenarios. Hence, nonparametric approaches have been developed to relax this assumption. For example, the partition based score I (PBI) test evaluates hypothesized interactions between categorical factors by comparing datasets partitioned using different combinations of predictor variables (Ruixue Fan et al., 2014; Satten et al., 2014). The significance of interactions is assessed by comparing the outcome explanatory powers of different partitioning variable combinations, and so the test does not assume specific interaction relationships between the factors (Ruixue Fan et al., 2014; Satten et al., 2014). This method was shown to be able to identify more GE interactions than the linear regression approach in the analysis of a real dataset (Ruixue Fan et al., 2014).

1.6 Study Objectives

As discussed above, childhood obesity is an important public health issue owing to its growing worldwide impact and the disease complexity. Experimental and small sample clinical studies have suggested potential interactions between the IGF pathway genes and environment in relation to obesity pathogenesis. Thus we propose using longitudinal family studies to better assess the roles that the IGF pathway and environment play in childhood obesity etiology.

At the same time, our analysis strategy for longitudinal family studies requires accounting for complex correlation structures and interactive relationships. To address these issues properly,

we need a systematic comparison of the currently available methods for longitudinal family data. This will allow us to make informed analysis decisions when studying GE interactions involving the IGF pathway.

Therefore, in this study, we sought to address these knowledge gaps by achieving the following two objectives:

I. Systematically evaluate the performance of the considered analytic methods for detecting genetic interactions in longitudinal family data, specifically in two areas:
    a. Robustness to non-linear interaction
    b. Effect of simplifying correlation structures

II. Apply insights gained from objective (1) to evaluate the effect of interactions involving IGF pathway genes (IGF-1 and IGFALS) and environmental factors (physical activity, daycare attendance and sleep duration) on body mass index measured longitudinally in the Quebec Newborn Twin Study (QNTS).

2. Materials and Methods

2.1 Data Collection

2.1.1 Sequencing Data Collection

Sequencing data on the IGF-1 and IGFALS genes were obtained with HiSeq Illumina platform at McGill University and Génome Québec Innovation Centre. Molecular inversion probe method was used to construct DNA libraries for which deep sequencing was carried out. Initially, there were 257 and 272 polymorphic sites for the IGF-1 and IGFALS genes respectively. After selection for only single nucleotide polymorphism (SNP) loci, controlling for sequencing quality (above 20), and excluding SNPs with > 10% missing data, 180 and 140 SNP loci remained for the IGF-1 and IGFALS genes. We also excluded rare variants whose minor allelic frequencies (MAF) were less than 0.05. SNPs that failed the Hardy-Weinberg Equilibrium (HWE) test (p-value < 0.01) were also excluded. A sample of unrelated individuals was used to estimate allele frequencies for the MAF and HWE filtering steps; the sample was obtained by randomly sampling an individual from each family. Two SNPs from IGF-1 and 3 SNPs from IGFALS were judged to be in linkage disequilibrium ($R^2 > 0.8$) and thus constituted 2 haplotypes. One SNP with the highest MAF was selected from each haplotype for analysis. The final analysis was performed on 7 SNPs from the IGF-1 gene and 2 SNPs from the IGFALS gene.

2.1.2 Outcome Data Collection

BMI data based on weight (in kg) and height (in m) were obtained for each individual at 6 time points during their early childhood (from birth to around 6 years of age). At birth, BMI was recorded from medical records (Dubois et al., 2012). Laboratory measurement of BMI was

performed at the 6 month and 62 month follow-up points (Dubois et al., 2012). Weight and height data were obtained via interview survey for the other follow-up points, and BMI was determined based on these data. 99 individuals were excluded for missing more than 4 out of the 6 BMI measurements. Through comparison with the overall BMI data distribution as well as among individual trajectories, 6 BMI measures from 6 individuals were judged to be unrealistic and likely recording errors (Appendix Figure S1). Consequently, they were excluded from the analysis.

2.1.3 Environmental Data Collection

Interviews were conducted at the follow-up time points to obtain information on participant's environmental exposures. Physical activity level was assessed at two follow-up time points (32 and 50 months of age). Parents were asked to rate the physical activity level of the study participants when comparing to his/her peers of same age and sex. The response was coded as a categorical variable in the original questionnaire with 5 levels ("a lot more", "more", "equal", "less", "a lot less"). Information on participant's daycare attendance was obtained through parental interview at 4 time points (6, 20, 32, 50 months of age). Attendance status was assessed by asking the parents whether the participant was using daycare or other babysitting services including care by relative at the time of interview. Responses were binary ("yes" or "no"). Individual sleep time was measured by two parental interview questions on day and night sleep times. Parents were asked to describe the amount of time the participants slept during day and night time in terms of broad categories. For daytime sleep, the categories were

"less than 1 hr", "1-2 hr", "2-3 hr", "3-4 hr" and "more than 4 hr". For night sleep, the response levels consisted of "less than 4 hr", "4-5 hr", "5-6 hr"… and so on till "9-10 hr", "more than 10 hr" and "more than 8 hr".

2.1.4 Ethnicity Data Collection

We used individual's reported race as a measure of their ethnic background. 8% of the responses did not consider themselves to be Caucasian ("white"). Thus we excluded those individuals from our analysis in order to control for confounding due to ethnicity (population stratification). 47 individuals with non-Caucasian ethnicity were not included in the final analysis sample.

2.1.5 Quality Control and the Final Sample Used for Analyses

The initial study sample before any imputations and exclusions (including those based on ethnicity and missing BMI data) contained 558 individuals with both sequencing and environmental data. Figure 4 shows the subsequent quality control steps to obtain the final study sample. Out of the initial study sample, 33 and 57 individuals were excluded for having >10% missing rate for the IGF-1 and IGFALS sequencing data, respectively. MZ individuals (195 for IGF-1 and 190 for IGFALS) without sequencing data were later imputed based on their siblings' genotypes assuming MZ twins share identical DNA. After merging the imputed sequencing data with environment data, 682 individuals had data on both genetic and

environment variables. 99 individuals were then excluded for having more than 4 missing entries for BMI as mentioned in section 2.1.2. To control for population stratification, 47 individuals with non-Caucasian ethnicity were excluded as discussed in section 2.1.4. The final sample used for analyses consisted of 536 individuals from 292 QNTS families. 244 of the 292 families had data on both twin individuals. 48 families had data on only 1 individual. 143 families were MZ twins, and 149 families were DZ twins.

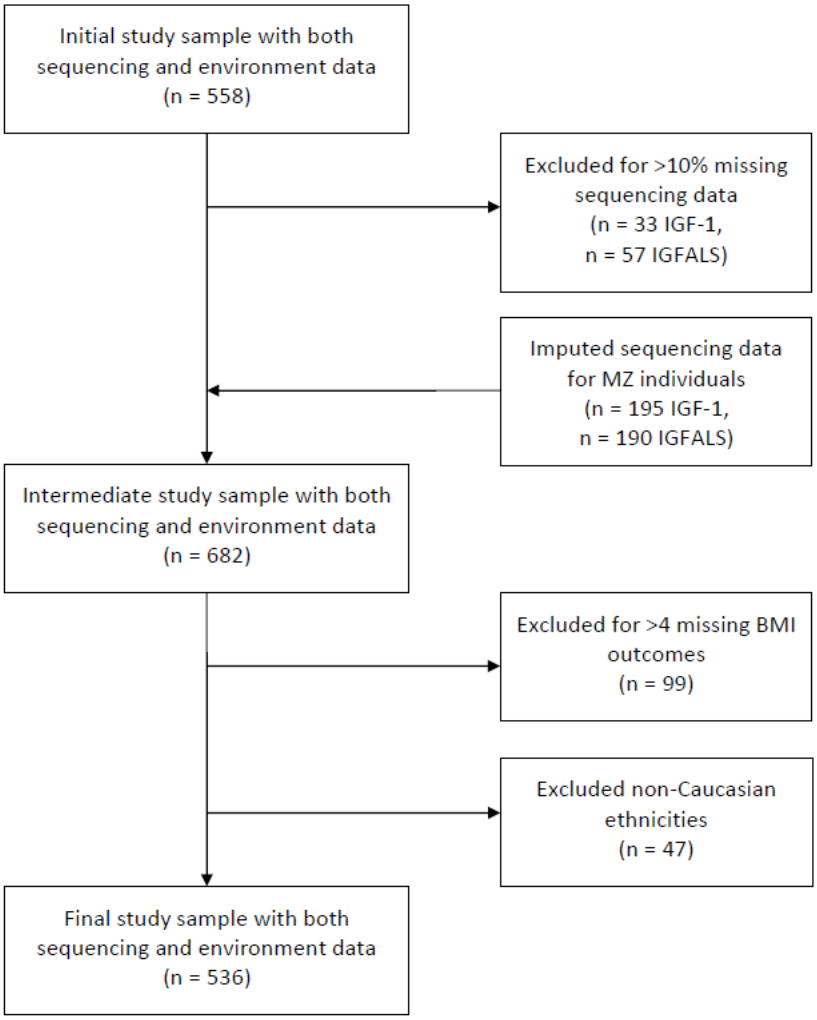

Figure 4. Data quality control steps to obtain the final sample for analyses

Initial study sample consisted of individuals with both sequencing and environment data. An intermediate sample was obtained after filtering out individuals with >10% missing sequencing data and imputing data for monozygotic (MZ) twin individuals. Final study sample was selected after excluding individuals with >4 missing BMI measures and/or those whose ethnicity was not Caucasian.

2.2 Simulation Study

The first objective of this thesis is to conduct simulation analysis to investigate the performance of the selected longitudinal family methods under different interaction scenarios. This would inform our subsequent analysis of the QNTS data for GE interactions of the IGF pathway. To simulate the desired scenarios, we used a modified linear mixed model to generate hypothetical BMI outcomes, with input data partially based on actual participants from QNTS. Different interaction relationships were specified by varying the patterns of effect in relation to different genetic and environmental exposure levels. The simulations are now described in detail.

2.2.1 Simulation Model

Longitudinal BMI data trajectories were simulated using a modified linear mixed model. The model equation is formulated as follow:

$$Y_{ijk} = \left(\beta_0 + C_{0ij}\right) + \beta_S S_{ij} + (\beta_T + C_{Tij})T_{ijk} + \beta_{T'} T'_{ijk} + \beta_G G_{ij} + \beta_{GT} G_{ij} \times T_{ijk} + \beta_E E_{ij} + \theta_{ijk} + \varepsilon$$

Y_{ijk} is the BMI trait value for individual j of the i^{th} twin pair at time point k. $\beta's$ with the corresponding subscripts represents fixed effects for age in months (T_{ijk}), sex (S_{ij}), the genetic factor (G_{ij}) and the environmental factor (E_{ij}). β_{GT} specifies the linear interaction effect

between the genetic factor (G_{ij}) and time (T_{ij}). $\beta_{T'}T'_{ijk}$ is used to modify the rate of change in BMI over time after the second time point. This allows simulation of a segmented longitudinal trend for BMI that resembles the actual trajectories found in QNTS. Specifically, the T'_{ijk} value is assigned as follow:

$$T'_{ijk} = \begin{cases} 0, & if\ k \leq 2 \\ T_{ijk} - 6, & otherwise \end{cases}$$

Based on this specification, an additional time effect from $\beta_{T'}$ will be applied after 6 months of age (time point, $k > 2$). This will simulate the initial phases of fast growth before 6 months and the subsequent plateauing of BMI afterwards as observed in QNTS data.

C_{oij} and $C_{Tij}$are the random effects on the intercept and the rate of change of BMI over time, respectively, for each individual. The effects are simulated as components of a bivariate normal random vector for each twin pair. The covariance structure of the random vector is set up to reflect the difference in familial correlation between MZ and DZ twins due to background additive genetic effect.

$$C_{0ij} \sim N(0, V_0), V_0 = \begin{bmatrix} \sigma_A^2 + \sigma_C^2 & \begin{cases} \sigma_A^2 + \sigma_C^2\ if\ MZ \\ \frac{1}{2}\sigma_A^2 + \sigma_C^2\ if\ DZ \end{cases} \\ \begin{cases} \sigma_A^2 + \sigma_C^2\ if\ MZ \\ \frac{1}{2}\sigma_A^2 + \sigma_C^2\ if\ DZ \end{cases} & \sigma_A^2 + \sigma_C^2 \end{bmatrix}$$

$$C_{Tij} \sim N(0, \mathrm{V}_T), \mathrm{V}_T = \begin{bmatrix} \tau_A^2 + \tau_C^2 & \begin{cases} \tau_A^2 + \tau_C^2 \text{ if MZ} \\ \frac{1}{2}\tau_A^2 + \tau_C^2 \text{ if DZ} \end{cases} \\ \begin{cases} \tau_A^2 + \tau_C^2 \text{ if MZ} \\ \frac{1}{2}\tau_A^2 + \tau_C^2 \text{ if DZ} \end{cases} & \tau_A^2 + \tau_C^2 \end{bmatrix}$$

Parameters σ_A^2 and τ_A^2 represent the additive genetic effect on BMI correlation while σ_C^2 and τ_A^2 specify the common environmental effect on the correlation.

θ_{ijk} determines the simulated GE interaction effect on the outcome for each individual at each time point. The effect is specified using an interaction matrix describing the combined interactive effect of the genetic factor and the environmental factor. Specific effect matrices for simulated interaction scenarios will be discussed in section 2.2.3. An example is given here:

	Environment = 0	Environment = 1
Gene = 0	0	0
Gene = 1	0	β_{GE}/β_{GTE}
Gene = 2	0	$2\beta_{GE}/2\beta_{GTE}$

β_{GE} and β_{GTE} represent the interaction effect between gene and environment (GE) and gene-time-environment (GTE) respectively. The above example specifies the effect pattern for linear interaction.

Simulation model parameters for genetic and environmental factors were chosen to discern potential power differences between the analytic models and are described in section 2.2.3. Parameter values for other variables were based on actual QNTS data.

2.2.2 Input Predictor Data for the Simulation Model

Simulated BMI trajectories for 788 individuals were generated with input predictors that were partially based on their corresponding QNTS variables. Actual QNTS data on the individual's family structure, zygosity, sex and age were used for the simulation input. There were 394 families (twin pairs) in total, of which 226 were DZ twins and 168 were MZ twins. Missing data on age were imputed by randomly sampling the age distribution of the whole sample at the missing time point. To ensure that age always increased, the sampled age was discarded and re-drawn if the age value was either younger than the preceding observation or older than the subsequent observation.

Hypothetical genetic and environmental factor data were generated using probability models for input into the simulation model. The environmental factor was coded as either present (1) or absent (0), and was sampled from a Bernoulli random distribution independently for each individual. The environmental exposure frequency was set to 0.3. The genetic factor was coded to reflect additive genetic effect (0, 1, and 2 for the number of minor alleles). Hypothetical parental genotypes for each individual were generated as the sum of two independent Bernoulli random variables, thus assuming random mating. The minor allele frequency was set

to 0.3. Individual genotype data was then obtained from parental genotypes using probabilities according to Mendelian inheritance pattern.

2.2.3 Simulation Scenarios

A variety of simulation scenarios with different interaction relationships were examined. For each relationship, we varied the effect sizes for the interaction between the genetic and the environmental factors. When simulating scenarios with no interaction effect, we examined different levels of genetic and environmental main effect. The simulation parameter specifications are summarized in Table 1.

Table 1. Parameter configuration for simulation scenarios

Parameter[2]	Gene-environment interaction effect modeled[1]					
	No Effect		Effect on Average		Effect on Change	
	Non-Segmented[3]	Segmented[3]	Linear Interaction	Non-linear Interaction[4]	Linear Interaction	Non-linear Interaction[4]
β_0	11	11	11	11	11	11
β_S	0.5	0.5	0.5	0.5	0.5	0.5
β_T	0.04	0.8	0.04	0.04	0.8	0.8
$\beta_{T'}$	0	-0.8	0	0	-0.8	-0.8
β_G	0 to 1	0	0.25	0	0	0
β_{GT}	0	0 to 0.03	0	0	0.01	0
β_E	0 to 2	0 to 1.2	0.5	0	0.5	0
σ^2_A	4.3	3	4.3	4.3	3	3
σ^2_C	2.25	1.5	2.25	2.25	1.5	1.5
τ^2_A	0	0.001	0	0	0.001	0.001
τ^2_C	0	0.001	0	0	0.001	0.001
σ^2_E	2.25	1.5	2.25	2.25	1.5	1.5
β_{GE}	0	0	0.1 to 1	0.1 to 1	0	0
β_{GTE}	0	0	0	0	0.001 to 0.03	0.001 to 0.03

[1]A range of values was simulated for some parameters, effect sizes varied at 0.1 increments for β_{GE} when the interaction effect was on the average. For interaction effect on the rate of change scenarios, β_{GTE} varied at an increment of 0.003 before reaching 0.01 and then by 0.005 afterwards. Under no interaction effect scenarios, β_G varied at the same increments as β_{GE} (non-segmented trend), and β_{GT} varied at the same increments as β_{GTE} (segmented trend). β_E varied at 0.2 increments (non-segmented trend) or was inflated by 40 times relative to its corresponding β_{GT} value (segmented trend).

[2] β_0: average BMI at baseline predictor level; β_S: sex effect; β_T, $\beta_{T'}$: time effect; β_G: genetic effect; β_{GT}: gene-time (GT) interaction effect; β_E: environmental effect; σ^2_A: additive genetic effect on random intercept correlation; σ^2_C: common environmental effect on random intercept correlation; τ^2_A: additive genetic effect on random slope correlation; τ^2_C: common environmental effect on random slope correlation; σ^2_E: common effect on BMI variance; β_{GE}: gene-environment (GE) interaction effect; β_{GTE}: gene-time-environment (GTE) interaction effect

[3] For no interaction effect scenarios, non-segmented or segmented refer to the longitudinal trend in the simulated outcome data

[4] Non-linear interaction scenarios included exclusive OR (XOR) interaction, conditional dominant interaction and small marginal effect interaction

The scenarios were broadly categorized into models with no interaction effect, interaction effect on the outcome average and interaction effect on the outcome rate of change over time. For the no interaction effect scenarios, we varied the independent effect from the genetic and environmental factors (β_G and β_E). For scenarios with a GE interaction effect on the outcome average, the segmented time trend was not simulated. Hence, the additional time effect parameter in the simulation model was set to zero ($\beta_T, = 0$). In scenarios with a GTE interaction effect on the rate of change in BMI over time, the segmented time trend was generated by attenuating the time effect on BMI after the second time point. The additional time effect parameter was negative ($\beta_T, = -0.8$). Scenarios with varying interaction effect sizes were studied as described in Table 1.

In addition to varying the effect sizes, we also simulated scenarios corresponding to the four different interaction relationships discussed in section 1.5.3. The interaction scenarios included a linear pattern that corresponds to the relationship modeled by multiplicative terms in regression models. Three other non-linear relationships were also simulated. They were chosen to reflect the diversity of plausible interactions in the real world. The patterns of the interaction effect in the simulated relationships are shown in Figure 5.

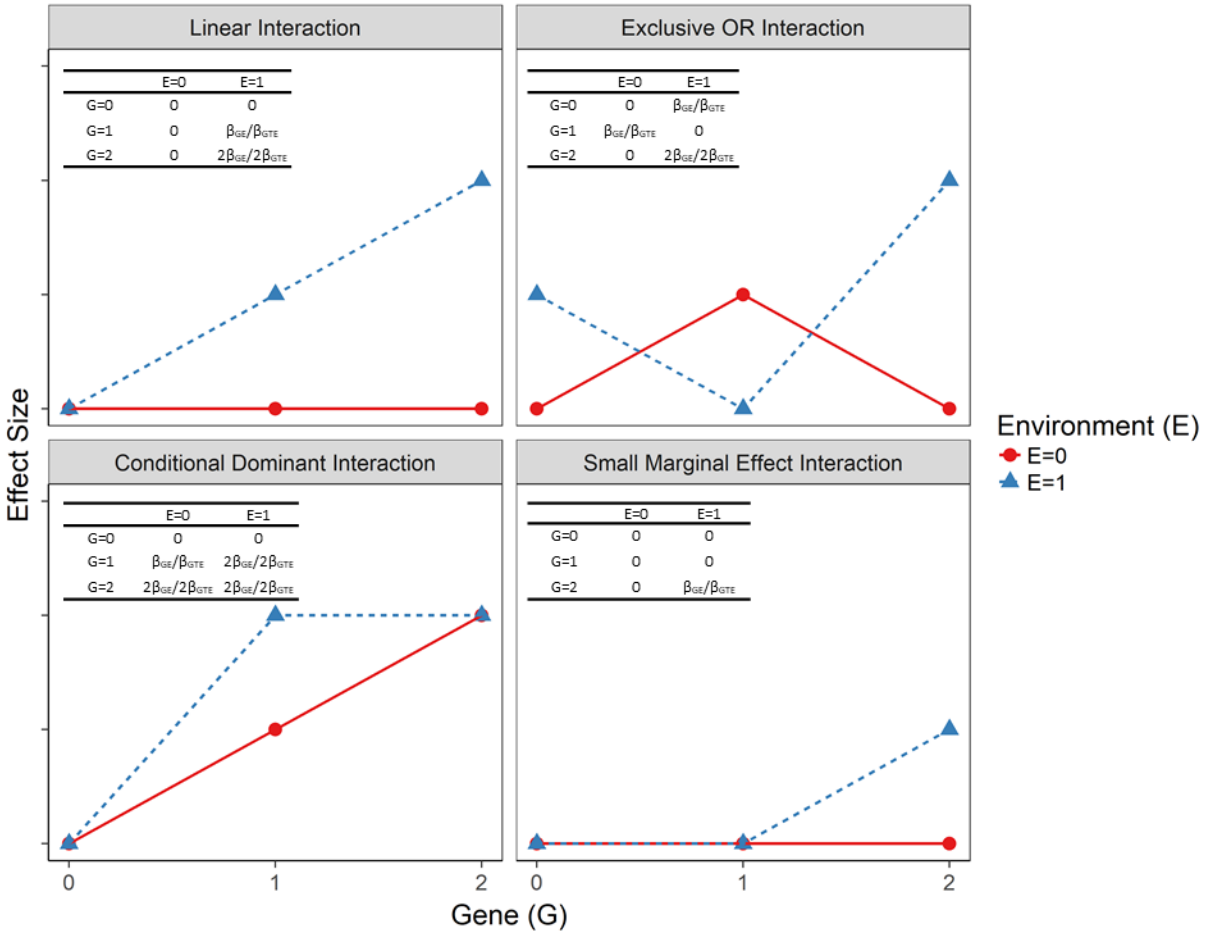

Figure 5. Effect specification for various interaction scenarios

β_{GE} and β_{GTE} are interaction effect parameters for gene-environment and gene-time-environment interactions respectively. Genetic (G) factor coded as 0, 1, and 2 for the number of minor alleles. Environment (E) factor coded as 1 or 0 for presence or absence of the environmental exposure.

2.3 Statistical Analysis

2.3.1 Analytical Models

This study investigated three different analytical methods for longitudinal family studies. Each method uses a combination of different approaches for modeling the correlation structure and interaction relationships, as outlined in the introduction. All analyses were performed using R (R Core Team, 2016).

2.3.1.1 Twin Model

The twin model refers to the path model used in classical twin studies (Neale & Maes, 2004). The model equation is formulated as follow:

$$Y_{ij} = \boldsymbol{x_{ij}\beta} + aA_{ij} + cC_{ij} + eE_{ij}$$

Y_{ij} represents the BMI outcome for twin pair i, individual j. $\boldsymbol{x_{ij}\beta}$ is the systematic component of the model, which includes the effects due to genetic and environmental factors. $\boldsymbol{x_{ij}}$ is a vector of predictor values, while $\boldsymbol{\beta}$ is a vector of fixed effects for the corresponding predictor variables. The random variation in the outcome is modeled by the A_{ij}, C_{ij}, and E_{ij} terms, which are mutually independent standard normal random variables. The a, c, e terms are the pathway coefficients that reflect the partitioning of the outcome variance due to background additive

genetic effect (a), common environment influence (c) and random environment variation (e). Each of the variance components is equal to its path coefficient squared. We used the twinlm function from the mets R package to fit the model (Holst & Scheike, 2015).

In order to analyze repeated measures using the twin model, we summarized the outcome measures (BMI) for each individual by their arithmetic means across the time points. The variance partitioning setup of the model is able to accurately account for the difference in correlation due to different familial relationships. DZ twins have half of the variance due to background additive genetic effect in comparison to MZ twins. Since the systematic component of the twin model is formulated as a typical regression equation, it will assume a linear interaction relationship by using a multiplicative term for interaction effect.

2.3.1.2 Linear Mixed Model

The linear mixed model approach considered in this study was implemented using the lmekin function from the coxme R package (Therneau, 2015). The model equation uses typical formulation:

$$Y_{ijk} = \boldsymbol{x_{ijk}\beta} + \gamma_{ij} + \varepsilon_{ijk}$$

Y_{ijk} is the trait (BMI) value for individual j of twin pair i at time k. The systematic effect of the predictors at each time point for the individual is specified by $\boldsymbol{x_{ijk}\beta}$. $\boldsymbol{x_{ijk}}$ is a vector of predictor

values, while $\boldsymbol{\beta}$ is a vector of fixed effects for the corresponding predictor variables. This systematic component will include the effects due to time, the genetic and the environmental factors. γ_{ij} is the random intercept for individual j of twin pair i. ε_{ijk} represents the random error for the outcome.

This linear mixed model utilizes all of the repeated measures and models longitudinal correlation structure through the random effect term γ_{ij} (Laird & Ware, 1982). Each individual receives a random intercept to induce correlation among his/her repeated outcomes. The correlation due to different family structure is accounted for by using a kinship matrix to specify the covariance structure for the random effect distribution. The entries in the kinship matrix are kinship coefficients that reflect genetic relatedness between individuals. These kinship coefficients will specify the covariance of individual random effect with self and with others.

Given n individuals, we construct a $n \times n$ kinship matrix with kinship coefficient entries denoted by φ_{ij}, where $i = 1,2 \dots n$ and $j = 1,2 \dots n$. For individuals with him/herself (ie. diagonal entries, $i = j$), the kinship coefficient φ_{ij} will equal 0.5. If two individuals are from the same MZ twin pair, then $\varphi_{ij} = 0.5$, whereas for DZ twin individuals $\varphi_{ij} = 0.25$. When two individuals come from separate twin pair families, they are assumed to be unrelated so $\varphi_{ij} = 0$. An example matrix is as follow:

	DZ twin 1	DZ twin 2	MZ twin 1	MZ twin 2
DZ twin 1	0.5	0.25	0	0
DZ twin 2	0.25	0.5	0	0
MZ twin 1	0	0	0.5	0.5
MZ twin 2	0	0	0.5	0.5

The kinship coefficients are now described in more detail. Mathematically, the kinship coefficient represents the probability of obtaining identical alleles when one allele at a given genetic locus is randomly sampled from two individuals. Thus, when randomly sampling alleles from a single individual, the probability of obtaining the same allele twice is 0.5. Since MZ twins share identical genomic DNA, the kinship coefficient between the pair is also 0.5. DZ twins on average share half of their genomic DNA from the same ancestor, so the probability of sampling the same allele between DZ individuals is 0.25. Assuming all twin pairs coming from independent families, the probability of sampling the same allele between any individuals from two families is 0.

To facilitate model fitting, a close approximation of the kinship matrix was obtained to ensure the matrix was positive definite. In this approximated matrix, the kinship coefficient between the two individuals of a MZ twin pair was 0.49999999 instead of 0.5, while the coefficients for all other relationships remained the same. As linear mixed models use linear regression equation, modeling the interaction effect with a multiplicative term will again implicitly assume a linear interactive relationship.

2.3.1.3 Partition Based Score I Test

The partition based score I (PBI) test is a nonparametric approach for assessing interactions by contrasting the outcome variations explained under different ways of partitioning the dataset, with each induced by considering different subsets of the categorical predictor variables (Ruixue Fan et al., 2014). For a given partition scheme, the dataset is split into sub-datasets according to the levels of the considered predictor variables. Thus, a portion of the variations in the outcome will be explained by the predictor variables that specify the partitioning. A dispersion statistic that measures the amount of the explained outcome variation by a set of partitioning variables is estimated as follow:

$$I = \sum_{i=1}^{k} \frac{n_i}{n} \cdot \frac{(\overline{y_i} - \overline{y})^2}{\frac{s_y^2}{n_i}}$$

The sub-datasets generated from the partitioning process is denoted by i $(i = 1,2 \dots k)$. $\overline{y_i}$ is the mean outcome for the i^{th} partitioned sub-dataset. n_i is the sample size for the i^{th} sub-dataset. $\bar{y}$ and S_y^2 are overall outcome mean and sample variance respectively. n is the overall sample size.

The PBI test statistic is estimated as the difference between the dispersion statistics obtained from partitioning using both interacting variables and the maximum of the dispersion statistics estimated when partitioning by each variable alone. It is formularized as below:

$$I_T = I_{GE} - \max(I_{G,}I_E)$$

To evaluate a potential GE interaction, we estimate the dispersion statistics for a dataset that is partitioned by both genetic and environmental variables (I_{GE}) and by each variable separately (I_G, I_E). The test statistic (I_T) in this case is the difference between I_{GE} and the maximum of I_G, I_E. The p-value of the test is estimated using permutation. The permutation procedure accounts for familial correlation by constraining the permutation step to within each family. In this study, the test p-value was estimated with 10000 permutation replications.

The PBI test is not designed to utilize repeated outcome measures. In order to analyze longitudinal data using the PBI test, we use the average BMI over time as a summary measure for each individual. To allow division of dataset, the predictors specifying a given partition scheme must be categorical. Any continuous predictors would need to be categorized before applying the test. Since the PBI test is nonparametric and does not involve regression model equations, the test does not assume linear interaction relationship.

2.3.1.4 Summary of the Approaches Used by the Analytical Models

Table 2 summarizes the methodological features of the analytical models as discussed above in section 2.3.1. Each method utilizes a combination of different approaches for modeling the correlation structure and the interaction relationship.

Table 2. Summary of the approaches used by the twin model, the linear mixed model and the partition based score I test for analyzing gene-environment interaction in longitudinal family data

	Twin Model	Linear Mixed Model (LMM)	Partition Based Score I (PBI) Test
Correlation within individual	Summary statistic (mean)	Random effects - repeated outcomes	Summary statistic (mean)
Correlation within family	Random effects - kinship matrix	Random effects - kinship matrix	Permutation within family
Assumption of interactive relationship	Assume linear interaction	Assume linear interaction	No assumption of interaction relationship

2.3.2 Simulation Study Analysis

Simulation datasets were created for the scenarios described in section 2.2.3. We generated 2000 simulation replicates under each scenario to ensure good precision in the type 1 error and the power estimates. For each scenario, we applied the analytical models described in section 2.3.1 to the simulated datasets. The power of each analysis method to detect GE interactions was estimated as the proportion of significant results (p-value < 0.05) over the 2000 replicates. Significance tests were performed on the interaction terms (β_{GE} and β_{GTE}) for

regression-based models and on the PBI test statistic. For null interaction scenarios, the proportion of significant results (p-value < 0.05) was used to estimate the type 1 error rate. For the twin and the linear mixed models, we included sex, age, the genetic and the environmental factors as predictors in the systematic component of the model.

2.3.3 QNTS Data Analysis

In addition to the simulation analysis investigating method performance, we also evaluated our hypothesized IGF pathway GE interactions using the three methods discussed above (section 2.3.1). BMI outcome and genetic factors were considered as continuous variables. Because of the gaps in follow-up assessment, environment data were converted to time-invariant measures. Multiple testing corrections were applied to the tests of GE interactions. Sensitivity analyses were performed to evaluate data exclusion decisions.

2.3.3.1 Outcome and Predictor Definition

BMI outcome data was treated as a continuous variable without any recoding. Zygosity status was coded dichotomously as either 0 (DZ twins) or 1 (MZ twins). Individual age was recorded as number of months allowing decimals to account for partial month. We did not differentiate between different gestational ages among individuals, and set all ages to be zero at birth. Sex was coded as binary with 0 being female and 1 being male. Sequencing data was coded according to the additive genetic effect model where the number of the minor alleles is

counted (0, 1, and 2). Since the environmental exposure data was not available for all of the time points, we collapsed them into a single summary measure per individual, as described below.

There were only two assessments of physical activity, and the majority of responses remained unchanged between assessment points at 32 and 50 month follow-up. Thus we only used the data obtained at the 32 month follow-up in our analysis as it is the midpoint of the follow-up period. The variable was coded as a 3-level categorical variable (0, 1, and 2 for being “more”, “equal or “less” physically active compared to peers). The extreme categories (“a lot more” and “a lot less”) were combined with “more” and “less” categories respectively due to the low counts in the extreme categories.

Daycare attendance was assessed at 4 time points (6, 20, 32, 50 months of age). We summarized the overall daycare attendance for an individual by the proportion of his/her follow-up time where the individual attended daycare service. We assumed that the participants attended daycare service for the full duration of time till the next follow-up point, if the response was “yes”. For a given time period between two adjacent follow-up points to be included in the calculation, daycare attendance status must be available. The time period was also excluded if we could not calculate the time between the two follow-up points due to missing data for age. The analysis variable was a continuous proportion ranging from 0 to 1.

Coding of the sleep duration variable in this study was the most complex as sleep needs vary significantly for babies and children as they get older. We first converted categorical responses to numerical values. We excluded two categories “does not sleep 5 hr straight” and “sleep 5 hr straight” from our analysis since they only appeared in the first follow-up point (6 month), and were not meaningful for conversion into numerical values. For all other responses, we converted time categories into continuous hour data by selecting the median at each level. For example, “1-2 hr” was coded as 1.5 hours of sleep. For categories such as “less than 1 hr” or “more than 8hr”, we coded using the implied upper and lower bound (1 and 8 hours) respectively.

An individual’s overall sleep time at each time point was calculated as the sum of his/her day and night sleep durations in hours. For each time point, we scored whether the subject’s total sleep time met the minimum recommended level from the American Academy of Sleep Medicine and endorsed by the American Academy of Pediatrics (Table 3) (Jenco, 2016; Paruthi et al., 2016). There were no recommendations for children below 4 months of age so we treated all of the total sleep times before 4 months as satisfying the recommendation. Finally, since sleep time data were available for 4 follow-up time points (6, 20, 32, 50 months of age), we summarized individual sleep duration status as the proportion of follow-up time where the subject’s total sleep time met the minimum recommended level. Follow-up proportion was calculated similarly as daycare service attendance, and the summary variable was a continuous proportion ranging from 0 to 1.

Table 3. Minimum recommended sleep time by the American Academy of Sleep Medicine

Age (month)	Minimum Sleep Time (hours)
4-11	12
12-35	11
36-71	10
72-155	9

2.3.3.2 Exploratory and GE Interaction Analysis Strategies

Univariate analyses were conducted to obtain sample distributions for the variables and compared the final analysis sample with the initial sample. Depending on the nature of the variable, Chi-squared test or ANOVA test were applied to BMI, age, zygosity, sex, physical activity, daycare attendance, sleep duration and race. The MAF for the IGF-1 and IGFALS SNPs were calculated for both Caucasian and non-Caucasian individuals. The twin model and the linear mixed model were fit to assess potential GE interactions between each SNP-environmental factor pair. The interaction was modeled with a multiplicative term in the regression equation. Both the twin model and the linear mixed model adjusted for sex in addition to genetic and environmental predictors. The time effect was modeled as a segmented trend in the linear mixed model with a knot placed at age = 6 months (approximately the 2nd follow-up time point). The PBI test was performed to evaluate interaction effects between each SNP-environmental factor pair. Continuous environmental exposures were categorized by their quartile levels (0, 1, 2 and 3 for 1^{st}, 2^{nd}, 3^{rd} and 4^{th} quartiles) to allow dataset partitioning with the PBI test. For each method, Bonferroni multiple comparison adjustment was made for GE interaction tests involving the same environmental exposure (number of tests = 9). Population stratification was controlled by excluding non-Caucasian individuals. To assess the potential

effect of excluding impossible BMI values and non-Caucasian individuals, sensitivity analyses were conducted by repeating the analysis on datasets that did not exclude those individuals or outcome measures.

3. Results

In this chapter, we present the results from the two sets of analyses that were performed. In section 3.1 and 3.2, we describe the performance of the three methods when applied to data simulated under the four interaction schemes previously described. In section 3.3, we describe the results from applying the three methods to the QNTS data in order to evaluate the evidence for GE interaction in the IGF pathway.

3.1 Simulation Analysis Results

We sampled one simulated dataset from each GE interaction scenario, and compared with the actual QNTS data (Figure 6 and 7). Longitudinal trajectories of BMI averaged for different groupings of individuals (by gene-environment for simulated data and by zygosity for QNTS data) were plotted. Simulated interaction effects on the average or on the rate of change in BMI over time are shown in Figure 6 and 7 respectively. In scenarios where the interaction effect was on the average, we simulated a constant time main effect. For scenarios where the interaction effect was on the rate of change over time, segmented trajectories were generated

by modifying the time main effect after 6 months. Compared to the actual QNTS trajectories, the simulated trajectories for the scenarios having an interaction effect on average BMI had similar BMI values at the baseline and at the end of follow-up. Some groups from the interaction effect on the rate of change scenarios had higher BMI values compared to QNTS. Those groups had less frequent genetic and/or environmental exposure categories and consequently fewer individuals. The segmented BMI trend also reflected the initial rapid rise in BMI and its subsequent plateauing as observed in the actual QNTS data. Differences in the averages or the rate of changes between the simulated trajectories for each gene-environment factor level demonstrated the intended interaction effect for each simulation scenario.

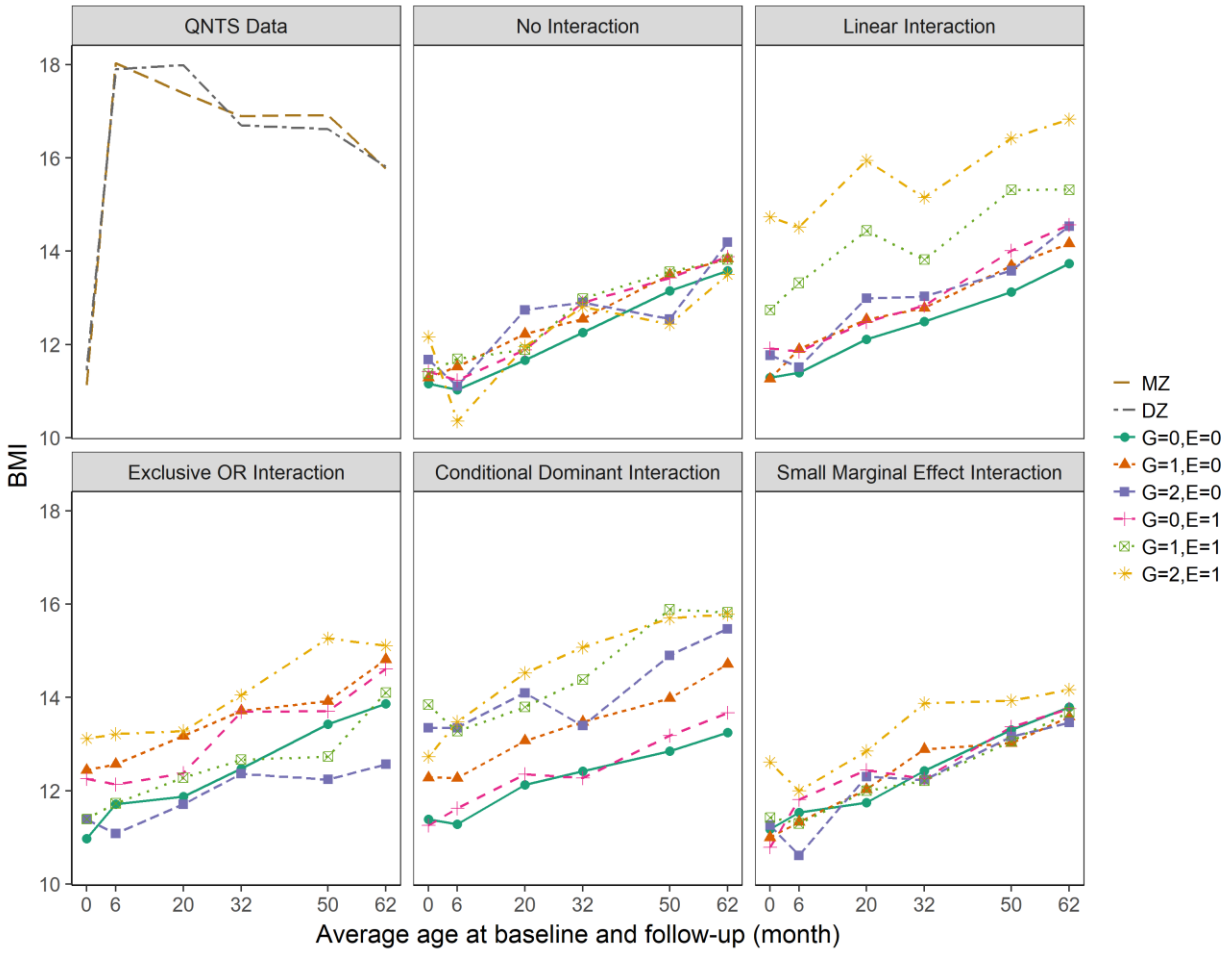

Figure 6. Trajectories of average BMI over time for actual QNTS data and non-segmented over time trend simulation scenarios

Example datasets from each simulation scenarios are compared to the actual QNTS data. Trajectories of average BMI at each time point based on actual QNTS data are grouped by zygosity status; MZ (monozygotic twin) and DZ (dizygotic twin). Averages of simulated BMI at each time point and their trajectories are grouped by genetic (G) and environmental (E) factor levels. Genetic factor coded as 0, 1, and 2 for the number of minor alleles. Environment factor coded as 1 or 0 for presence or absence environmental exposure. Simulated data generated for both interaction effect on the average scenarios and no interaction effect null scenarios.

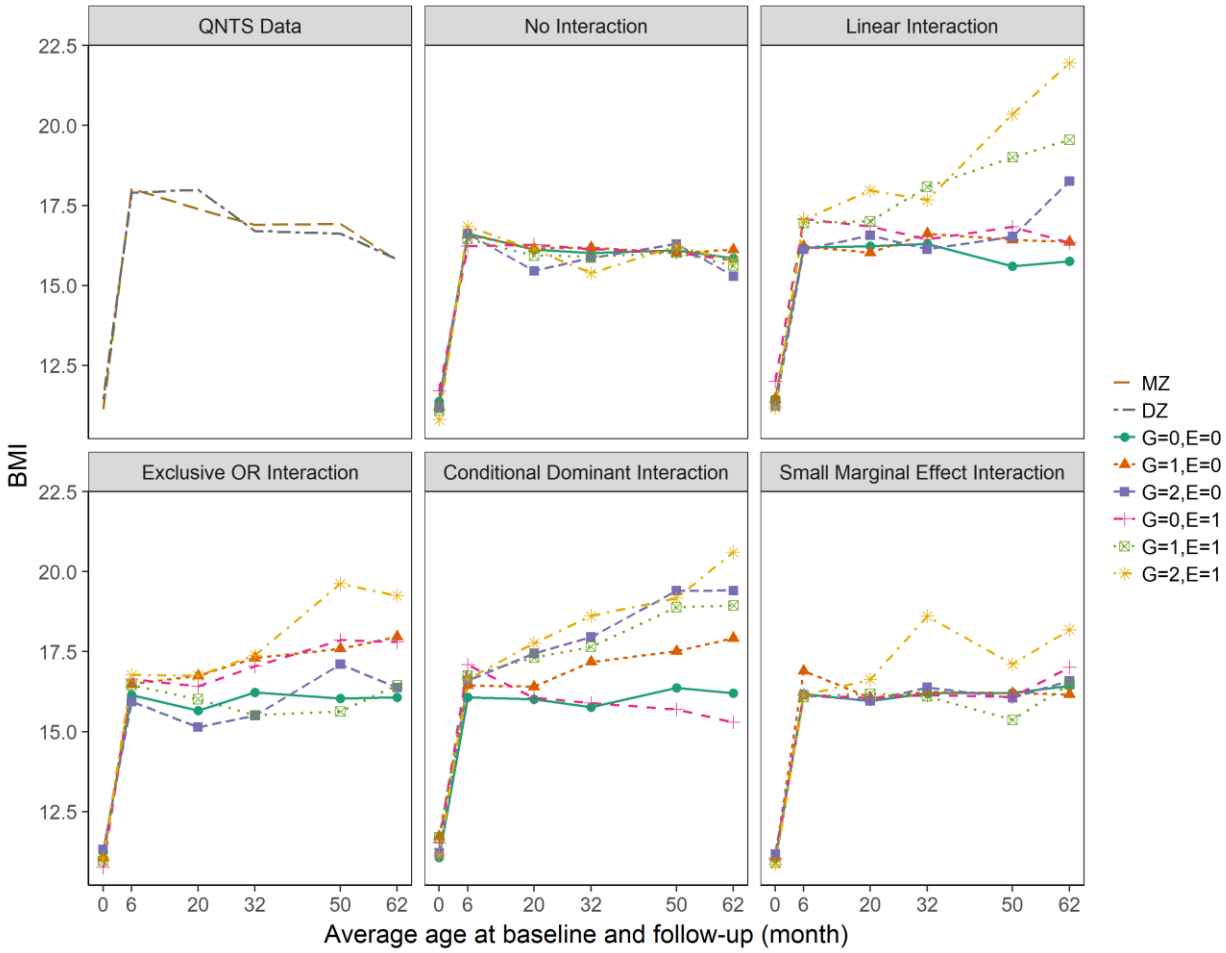

Figure 7. Trajectories of average BMI over time for actual QNTS data and segmented over time trend simulation scenarios

Example datasets from each simulation scenarios are compared to the actual QNTS data. Trajectories of average BMI at each time point based on actual QNTS data are grouped by zygosity status; MZ (monozygotic twin) and DZ (dizygotic twin). Averages of simulated BMI at each time point and their trajectories are grouped by genetic (G) and environmental (E) factor levels. Genetic factor coded as 0, 1, and 2 for the number of minor alleles. Environment factor coded as 1 or 0 for presence or absence environmental exposure. Simulated data generated for

both interaction effect on the rate of change over time scenarios and no interaction effect null scenario.

Figure 8 shows the estimated type 1 error rates for simulation scenarios without GE interaction effects. Both the twin and linear mixed models controlled the type 1 error rate well (estimated type 1 error rate < 0.05). The linear mixed model appeared to be more conservative in comparison to the other tests. The PBI test met the 0.05 threshold only when there was no main effect simulated. As the magnitude of genetic and environmental main effects increased in the non-segmented scenario, the PBI test type 1 error rate exceeded 0.05 briefly (β_G from 0.1 to 0.4 and $\beta_E$from 0.2 to 0.8). At higher main effect sizes (β_G > 0.4 and β_E > 0.8), the false positive rate decreased, and the test became more conservative in those scenarios. For the segmented trend scenario, the PBI test's estimated type 1 error rate increased as the GT interaction and the environmental factor effects increased. This increase in false positives plateaued when β_{GT} = 0.015 and β_E = 0.6, and stabilized at a false positive rate of around 0.5.

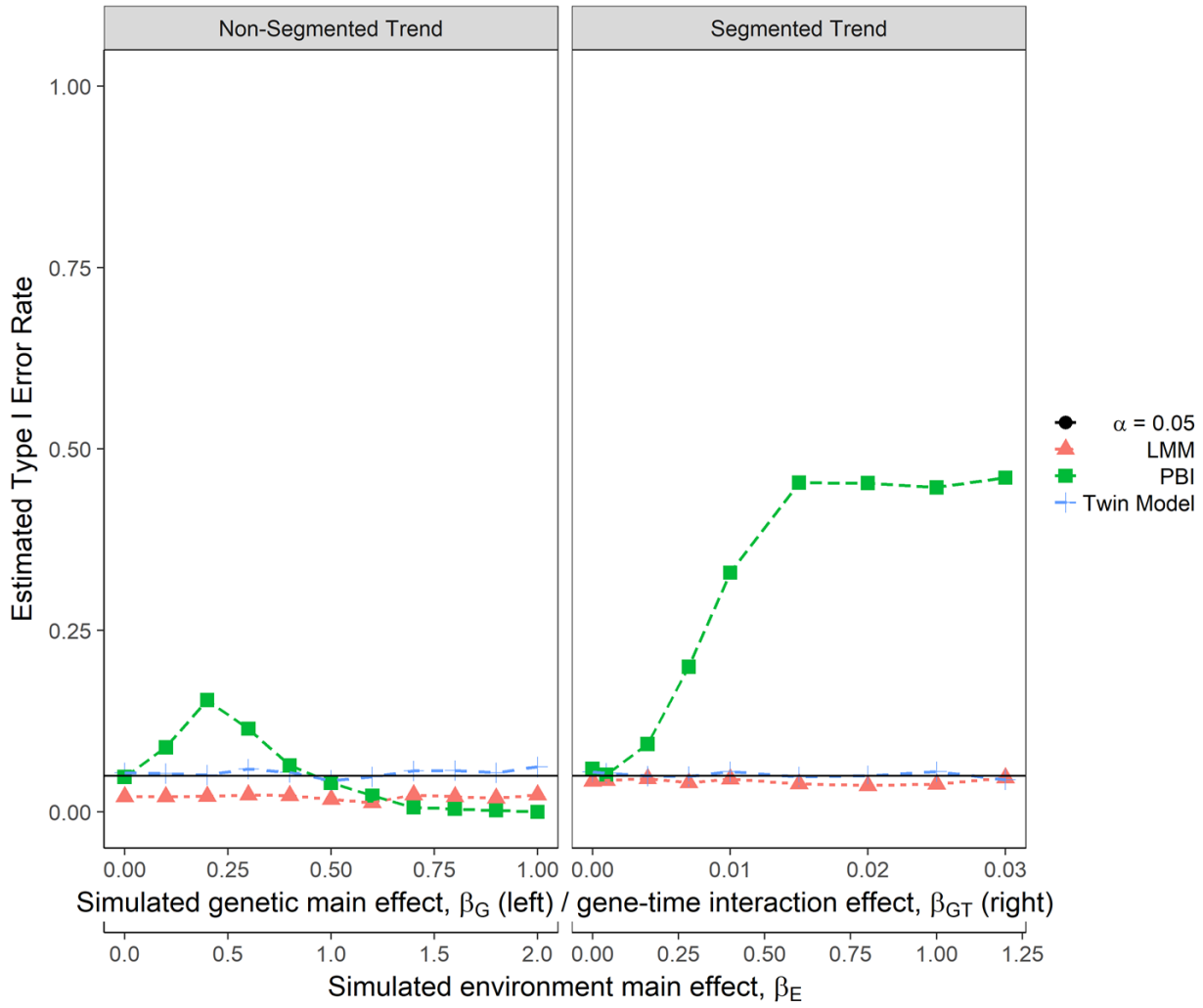

Figure 8. Estimated type 1 error rates for the compared analytical approaches

Type 1 error rates were estimated for each analytical approach as the proportion of false positive results (p-value < 0.05) calculated over 2000 simulation replicates with no interaction effect and either a segmented or non-segmented linear trend. The environmental main effect (β_E) was varied in both segmented and non-segmented scenarios. The genetic main effect (β_G) and gene-time interaction effect (β_{GT}) were varied for the non-segmented and segmented trend scenarios, respectively. LMM = linear mixed model; PBI = partition based score I test.

For simulated scenarios incorporating GE interaction effect on the BMI average, the twin model was more powerful compared to the linear mixed model and the PBI test, except when the interactive relationship was extremely non-linear (Exclusive OR interaction or XOR) (Figure 9). When the interactive relationship was XOR, the PBI test had the best performance. For linear interaction scenarios, regression-based models (twin and linear mixed models) performed well, while the PBI test had almost no power even at larger interaction effect sizes. All methods had lower power under small marginal effect and conditional dominant interaction scenarios, when compared with their respective best case scenarios with highest power.

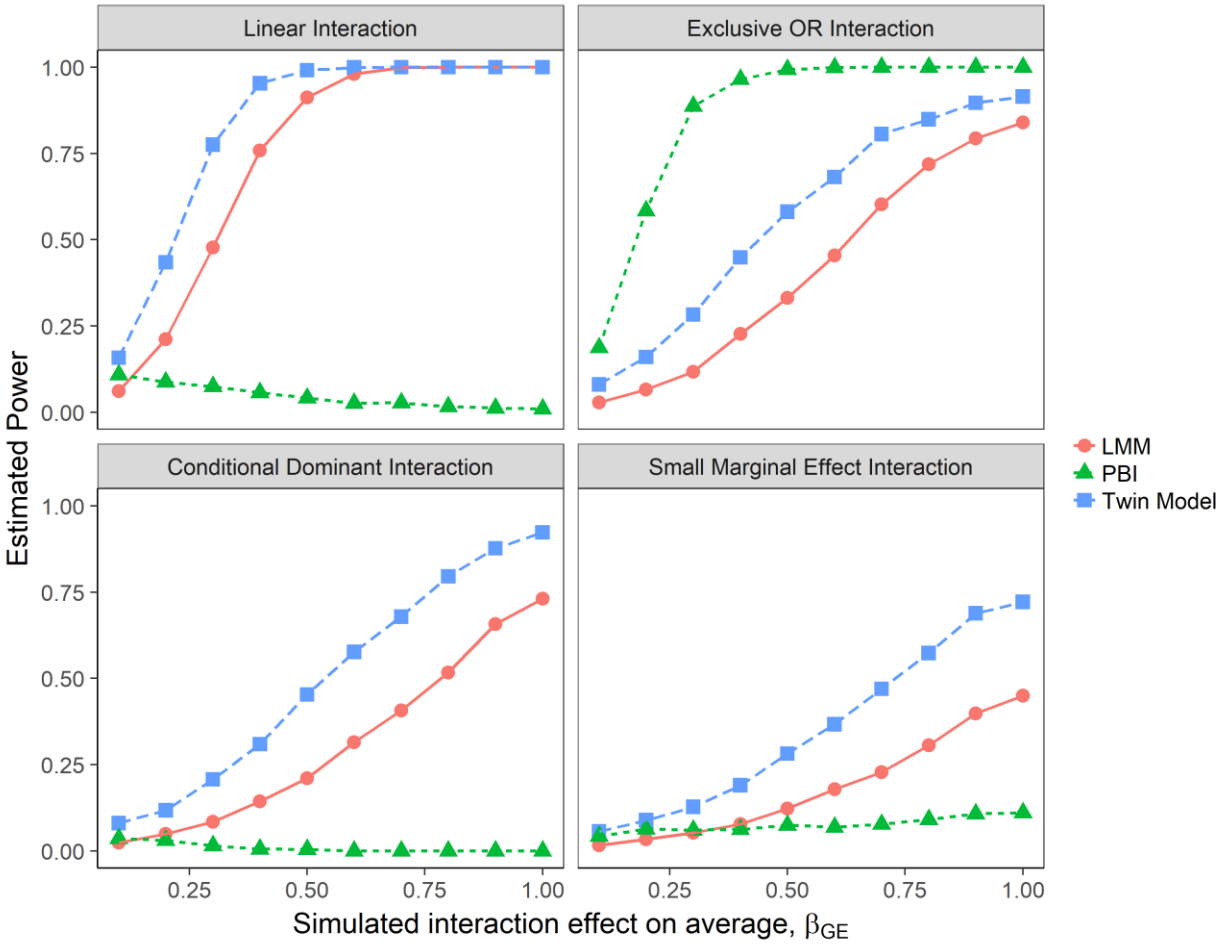

Figure 9. Estimated power to detect GE interaction effect on the average scenarios

Power was estimated for each analytical approach as the proportion of true positive result (p-value < 0.05) over 2000 simulation replicates for the considered interaction scenarios. LMM = linear mixed model; PBI = partition based score I test.

The power analysis results for scenarios where the interaction effect acted on the rate of change were similar to the scenarios where the interaction effect acted on the average (Figure 10). Regression-based methods (twin and linear mixed models) performed well for linear interaction situations (top right panel), but were less powerful for non-linear scenarios (other panels). The PBI test was powerful only when the relationship was significantly non-linear (XOR). The twin model had higher power compared to the PBI test and linear mixed model except when the interaction was XOR. For the conditional dominant and small marginal effect interactions scenarios, all three methods had more difficulty detecting the true effects when contrasted with their respective best case scenarios.

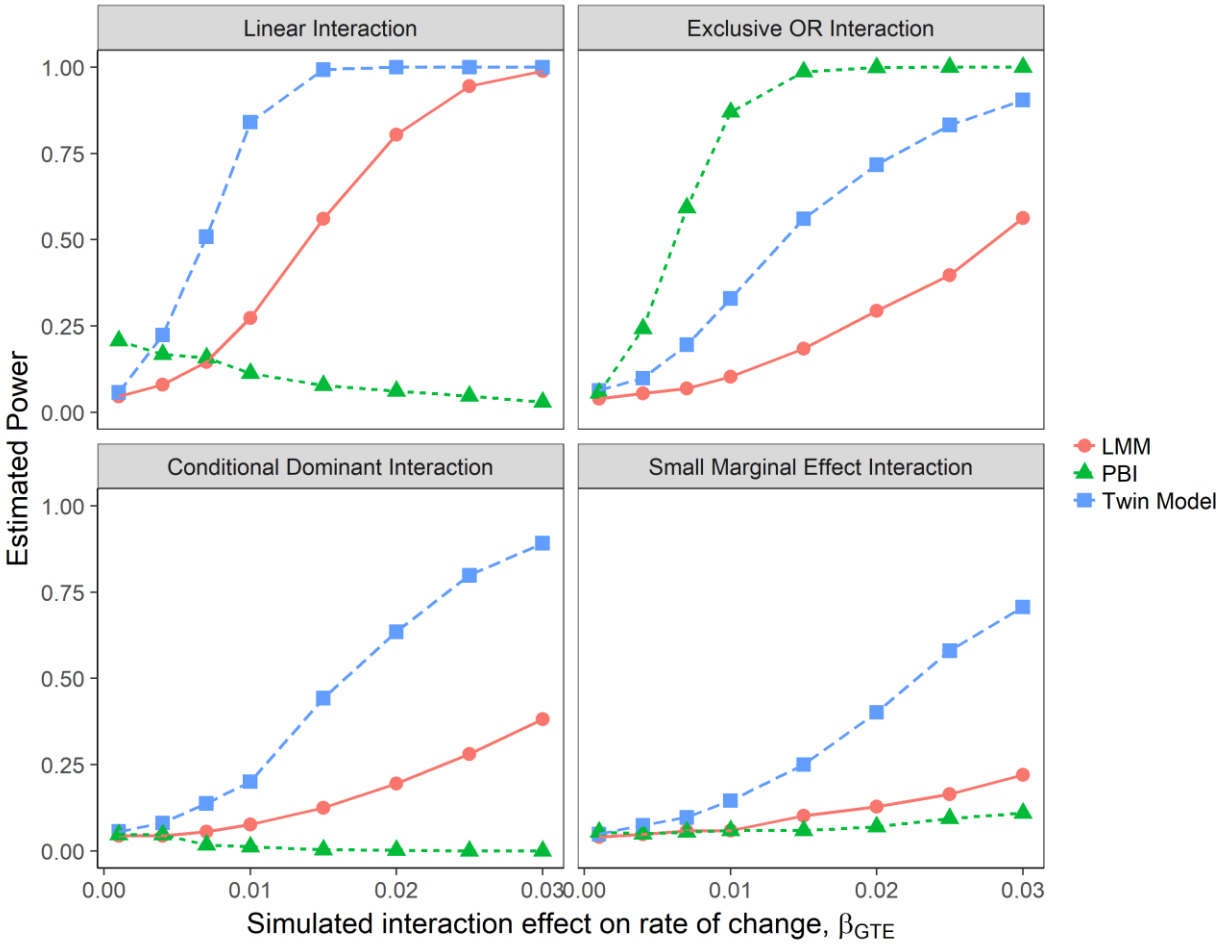

Figure 10. Estimated power to detect GTE interaction effect on the rate of change over time scenarios

Power was estimated for each analytical approach as the proportion of true positive result (p-value < 0.05) over 2000 simulation replicates for the considered interaction scenarios. LMM = linear mixed model; PBI = partition based score I test.

3.2 Behaviour of the PBI Test during Simulation Analysis

From our simulation analysis, we observed unusual behavior for the PBI test. The test was either too conservative or very prone to false positives under different scenarios. Since the PBI test statistic is dependent on the dispersion statistic values (I_{GE}, I_E and E_G) calculated for different ways of partitioning the dataset, we monitored the values of those statistics for different simulation parameter values. For the no interaction effect scenarios (Figure 11), the test statistic PBI decreased steadily as the simulated main effects increased under the non-segmented trend model. The decrease in the test statistic was due to higher dispersion statistic values when partitioning data by the environmental factor alone, compared to partitioning by both gene-environment factors. This meant that partitioning based on environmental factor level alone appeared to explain more BMI variations. However, in the segmented trend scenario with no interaction effect, the PBI test statistic remained close to zero regardless of the changes in the effect sizes for GT interaction and the environmental factor. In this case, the dispersion statistic values from gene-environment partitioning and environment alone partitioning were similar. So partitioning data by environmental factor alone or gene-environmental factors yielded similar level of outcome explanatory power.

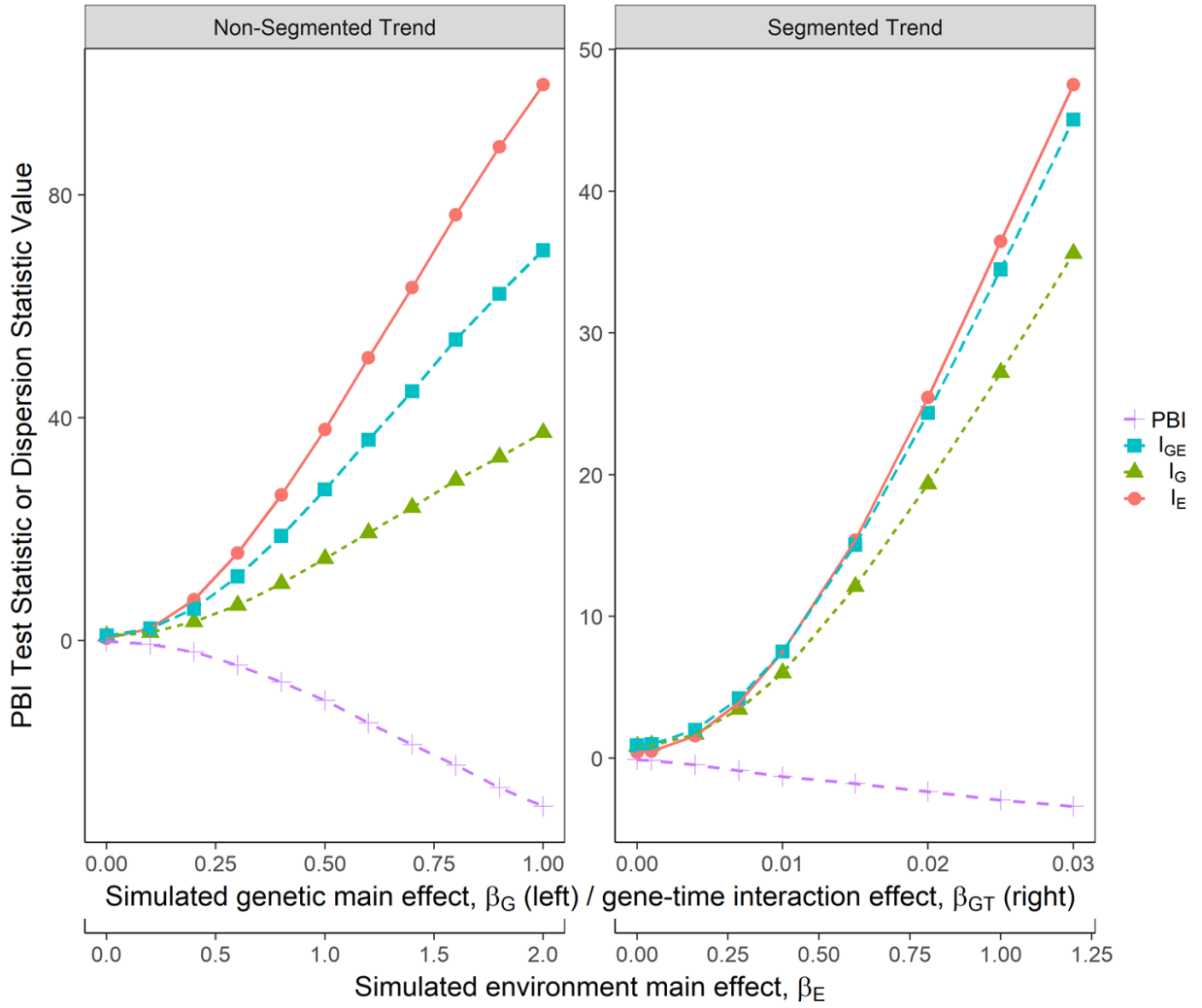

Figure 11. Behavior of partition based score I (PBI) test statistics and dispersion statistics for no interaction effect scenarios

Average value over 2000 replicates for PBI test statistics (PBI) and dispersion statistics for gene-environment (I_{GE}), gene only (I_G) and environment only (I_E) dataset partitioning schemes are plotted. PBI test statistics is equal to the difference between I_{GE} and the maximum of I_G or I_E. The environmental main effect (β_E) was varied in both segmented and non-segmented scenarios. The genetic main effect (β_G) and gene-time interaction effect (β_{GT}) were varied for the non-segmented and segmented trend scenarios, respectively.

Similar behaviour for the PBI test statistics and the dispersion statistics were also observed for simulated GE interaction scenarios (Figure 12). For linear and conditional dominant scenarios where the PBI test performed poorly, the PBI test statistics decreased as the GE interaction effect sizes increased. In those scenarios, partitioning based on environmental or genetic factor alone appeared to explain the outcome variation well. This was demonstrated by the higher dispersion statistic values for those partition schemes. For XOR interaction, gene-environment factor partitioning of the dataset consistently explained the outcome better than partitioning based on any single factor. This was reflected by the increasing trend in the test statistic value as well as higher dispersion statistic values from gene-environment partitioning. In the case of a small marginal effect interaction, it was similar to the no interaction effect scenario with the segmented time trend. The explanatory powers from partitioning data by gene and by gene-environment factors were similar and so the PBI test statistic remained fairly close to zero. For GTE interaction scenarios where the GE effect was on the rate of change, similar patterns in PBI test statistics and the dispersion statistics were observed (Appendix Figure S2).

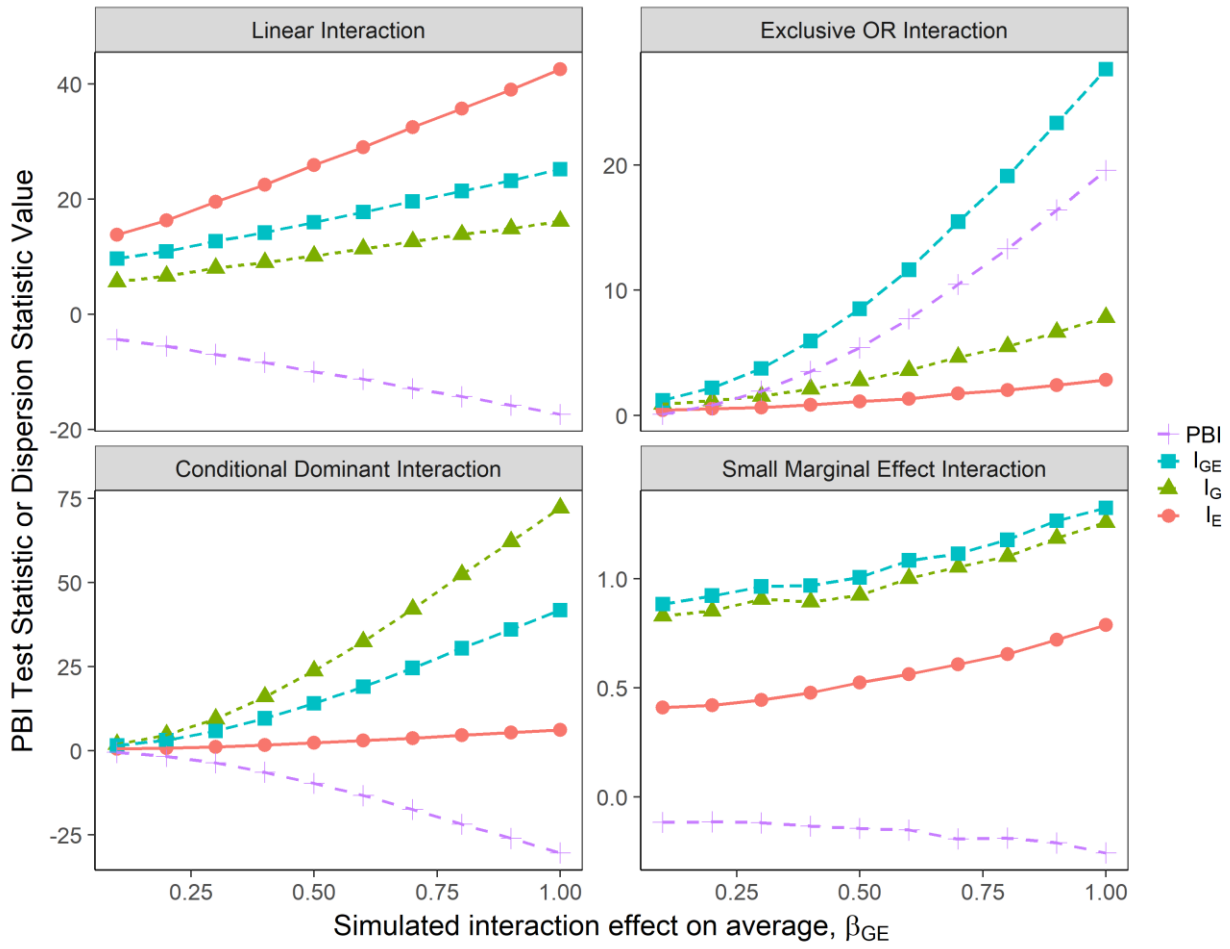

Figure 12. Behavior of partition based score I (PBI) test statistics and dispersion statistics for interaction effect on average scenarios

Average value over 2000 replicates for PBI test statistics (PBI) and dispersion statistics for gene-environment (I_{GE}), gene only (I_G) and environment only (I_E) partitioning schemes are plotted. PBI test statistics is equal to the difference between I_{GE} and the maximum of I_G or I_E. Effect sizes for gene-environment interaction (β_{GE}) were varied under each interaction relationship.

3.3 QNTS Analysis Results

Figure 13 shows the distributions and average BMI of all QNTS individuals included in our analysis at each time point. The trend demonstrated a phase of rapid increase before the first follow-up point (average age = 6 months). This was followed by a plateauing phase of moderate decline in average BMI. When stratified by genetic and environmental variables, some subgroups showed large deviation from the aforementioned overall pattern (eg. IGFALS rs3751893 and sleep level, Appendix Figure S3, S4). Distributions of BMI among analyzed individuals at each time point showed slightly left skewed distribution at birth, and right skewed distributions at the subsequent follow-up points.

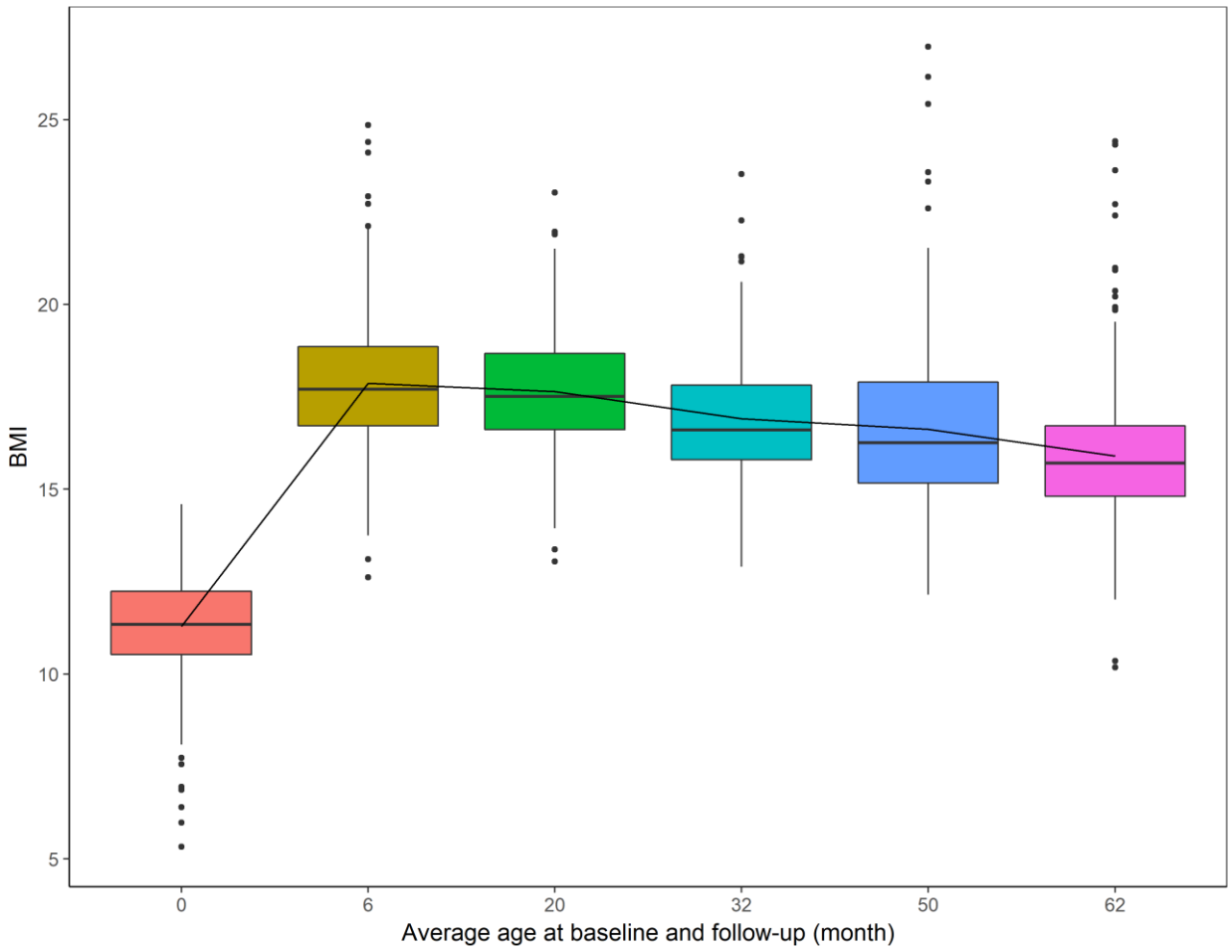

Figure 13. Distributions of BMI and trajectories of average BMI for analyzed QNTS participants

The trajectory of average BMI at each follow-up time point based on the entire analysis sample is highlighted by the black line. Boxplots represent the quartile ranges of BMI at each follow-up time point. Outliers are denoted by black points.

Table 4 compares the distributions of variables in the final sample used for analyses with those from the whole QNTS data. Overall, the two samples were similar with no significant difference except individual BMI average, age at follow-up point 4 (average age = 50 months) and race. The individual BMI averages were higher in the analysis sample (mean (SD): 15.09 (1.59) vs. 14.8 (2.06)). Mean age at follow-up point 4 was lower for the analysis sample (49.85 (1.82) vs. 50.08 (1.89)). Difference in ethnic composition was expected since we excluded all non-Caucasian individuals from the analysis. Comparison of MAF for the analyzed genetic loci between Caucasian individuals and non-Caucasian individuals are summarized in Table 5. The allelic distributions at several loci were significantly different (IGF-1: rs6219, 12:102791894, rs6214 and IGFALS: rs17559, rs3751893). Hence, we adjusted for potential confounding effect due to population stratification by excluding non-Caucasian individuals.

Table 4. Distribution characteristics of analysis sample and available data

	Analyzed individuals		Available QNTS individuals		
	No. of twins or individuals	Proportion or Mean (SD)	No. of twins or individuals	Proportion or Mean (SD)	
Individuals	536		810		
BMI					
At birth	499	11.3 (1.37)	698	11.25 (1.41)	
Follow-up #1	344	17.86 (2.04)	436	17.96 (2)	
Follow-up #2	182	18.05 (3.17)	234	18.06 (2.89)	
Follow-up #3	120	16.91 (1.91)	172	16.87 (1.86)	
Follow-up #4	117	16.63 (2.63)	156	16.64 (2.5)	
Follow-up #5	453	15.901 (1.72)	599	15.899 (1.7)	
Individual average BMI	536	15.09 (1.59)	795	14.8 (2.06)	*
Age					
At birth	536	0 (0)	810	0 (0)	
Follow-up #1	536	6.28 (0.74)	802	6.29 (0.74)	
Follow-up #2	531	19.49 (0.75)	782	19.53 (0.81)	
Follow-up #3	497	31.77 (0.96)	734	31.85 (1.02)	
Follow-up #4	435	49.85 (1.82)	610	50.08 (1.89)	*
Follow-up #5	478	62.25 (3.23)	638	61.98 (3.12)	
Zygosity					
DZ twin	149	0.51	197	0.49	
MZ twin	143	0.49	208	0.51	
Sex					
Female-female twin	122	0.42	164	0.405	
Male-male twin	102	0.35	168	0.415	
Female-male twin	68	0.23	73	0.18	
Physical Activity					
More	156	0.37	254	0.40	
Equal	253	0.59	354	0.57	
Less	17	0.04	21	0.03	
Individual proportion of follow-up attending daycare facility	530	0.42 (0.37)	782	0.41 (0.38)	
Individual proportion of follow-up with sufficient sleep	524	0.57 (0.31)	764	0.54 (0.32)	
Race					*
Caucasian	518	1	704	0.91	
Other	0	0	72	0.09	

*Significant difference $p<0.05$ with ANOVA test or Chi-squared test where appropriate

Table 5. Description of analyzed genetic loci and their allele frequencies

SNP	Genomic Position (chr:bp)	Gene	Allele	MAF		
				Race		
			(Other : Minor)	Caucasian	Other	
rs6219	12:102396414	IGF-1	C:T	0.07	0.17	*
rs1140655	12:102397002	IGF-1	T:A	0.30	0.37	
rs207473318	12:102791864	IGF-1	T:A	0.10	0.11	
12:102791894**	12:102791894	IGF-1	C:A	0.06	0.02	*
rs6214	12:102399791	IGF-1	C:T	0.46	0.35	*
rs6220	12:102400737	IGF-1	A:G	0.27	0.33	
rs28399924	12:102402246	IGF-1	T:A	0.17	0.15	
rs17559	16:1791032	IGFALS	G:A	0.10	0.23	*
rs3751893	16:1792208	IGFALS	G:A	0.18	0.31	*

MAF: minor allelic frequency; chr: chromosome; bp: basepair

*Significant difference $p<0.05$ with Fisher's exact test (with simulation approximated p-value)

**SNP not identified in dbSNP (build 148)

We examined the significance of potential GE interactions as well as each factor's main effect using the twin model, the linear mixed model and the PBI test. The PBI test evaluated interaction effects only. As shown in Figure 14, none of the effects were significant after correcting for multiple comparisons among SNPs interacting with the same environmental factor (number of tests = 9). Interactions between physical activity and IGF-1 12:102791894 SNP or between daycare attendance and IGFALS rs17559 SNP were significant before adjustment. In this case, physical activity and IGF-1 12:102791894 interaction was detected by the twin model, while daycare attendance and IGFALS rs17559 interaction was captured by the linear mixed model. Residual analysis for the twin and the linear mixed model showed adequate fit with the data (Appendix Figure S5, S6). Sensitivity analysis testing the influence of excluding impossible BMI values and excluding non-Caucasian individuals reported no significant change to our conclusion (Appendix Figure S7, S8).

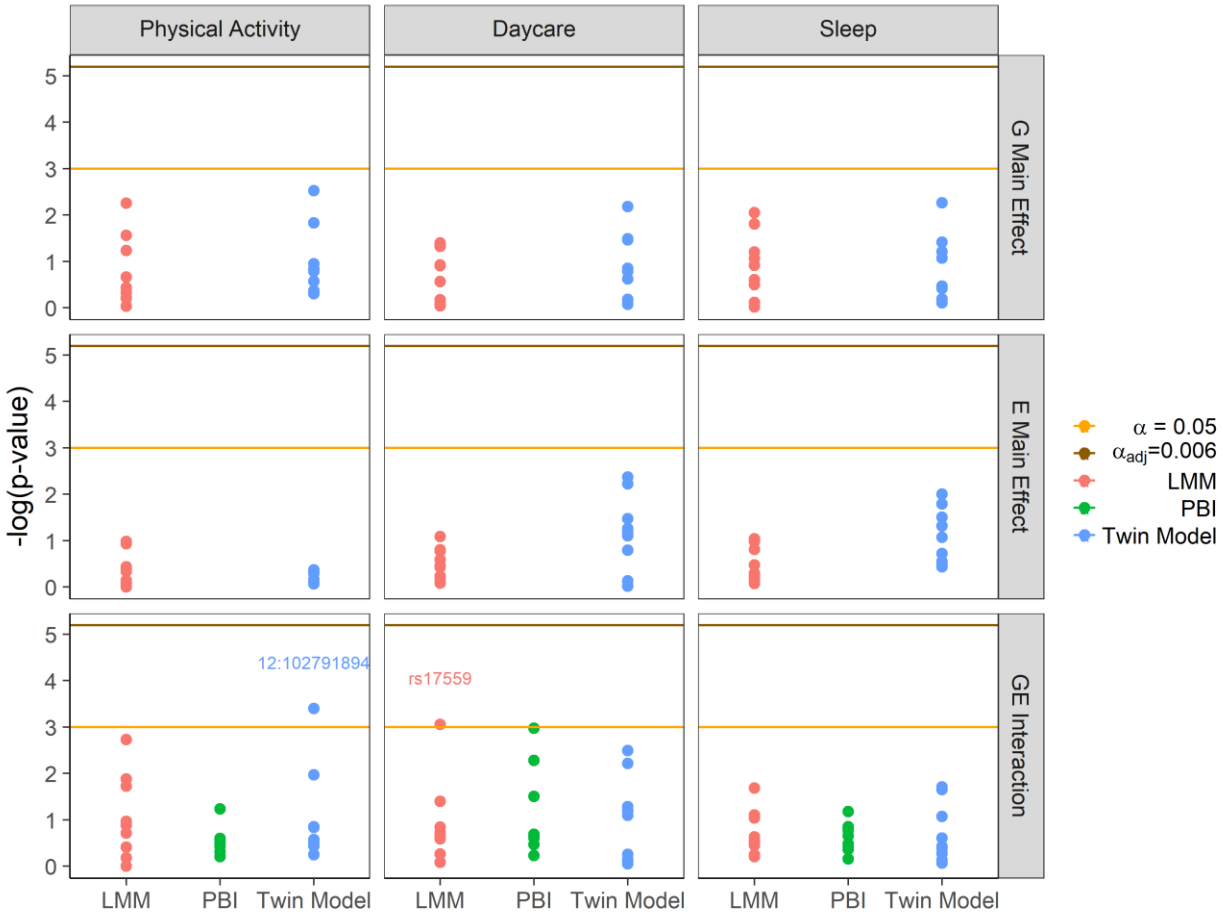

Figure 14. Significance of interactions between the IGF pathway genes (IGF-1, IGFALS) and environmental factors (physical activities, daycare attendance, sleep duration)

Negative natural log transformed p-values from the twin model, the linear mixed model and the PBI test were compared with unadjusted significance level (α = 0.05) and Bonferroni-adjusted level (α_{adj} = 0.006). Genetic and environmental main effects were evaluated by the twin and linear mixed models. All methods estimated significance of gene-environment interaction effects. Any effect with significance per unadjusted level (p-value < 0.05) is labeled for the involved IGF-1 or IGFALS SNP. LMM = linear mixed model; PBI = partition based score I test.

4. Discussion

In this study, we sought to evaluate potential interactions between the IGF pathway genes (IGF-1, IGFALS) and various environmental factors (physical activity, daycare attendance and sleep duration). Both the simulation analysis and the QNTS data analysis have produced results highlighting the challenges when modeling complex correlation and interaction relationships.

We first examined the performances of the twin model, the linear mixed model and the PBI test under simulated interaction scenarios. The data was generated to mimic the longitudinal family data from QNTS. The pattern of dependence between the methods' performances and their methodological features suggested that good performance is contingent on building a parsimonious model without imposing unreasonable simplifying assumptions. Specifically, when reasonable approximation was achieved, simplification of the longitudinal data structure by averaging the outcome at different time points did not negatively impact the performance. The analytical models had different detection powers under different GE interaction scenarios. This pattern of performances was indicative of how well the modeling assumptions on the interaction relationship matched with the underlying data. Thus similar to correlation modeling, how well an interaction model resembles the underlying data trend is also important to the method's performance.

The analysis of the QNTS data did not reveal any statistically significant GE interactions for the IGF pathway genes after correction for multiple testing. A previously unidentified SNP (based on dbSNP build 148) situated near the IGF-1 gene was found to be suggestively significant when interacting with physical activity. Since GE interaction pathways are inherently complex, we speculate on a number of possible mechanisms that could mask the true signal of our hypothesized GE interactions.

To our knowledge, this study was the first to assess the performances of various longitudinal family methods under different GE interaction relationships. Although our evaluation of the IGF pathway GE interactions was limited by generalizability and environment variable measurement issues, the result was robust to potential population stratification and selection bias. We believe our assessment represented conservative estimates of the hypothesized GE interaction effects.

4.1 Modeling Correlation Structure in Longitudinal Family Studies

There are various approaches for modeling the correlation structure within longitudinal family data. The methodological challenge of modeling complex correlation patterns revolves around the conflicting needs for an accurate but also parsimonious model. Methods with more accurate representation of the correlation structure have better control of type 1 errors as demonstrated by the conservative performance of the linear mixed model. However, improving accuracy often requires building more complex models which lead to spending more degrees of

freedom. This can result in lower detection power comparing to simpler methods that sacrifice some of their accuracy. Consequently, an optimal model should balance both accuracy and parsimony for ideal performance. A useful framework to consider this balance when analyzing longitudinal family data is to evaluate how much information from the dataset can be captured by a given model.

4.1.1 Modeling Longitudinal Correlation Structures

Longitudinal correlation was fully modeled by the linear mixed model as it used all of the available data points from each individual. However, we found that summarizing the repeated outcomes by their mean and applying the cross sectional twin study model had higher power to detect the GE interaction. In terms of controlling for false positives, both the twin model and the linear mixed model controlled for type 1 error (estimated error rate at or below 0.05). The linear mixed model had below 0.05 false positive rate suggesting that the method may be too conservative. However, the PBI test had type 1 error rate above the 0.05 limit. The primary cause for inflated type 1 error was likely due to the PBI test's susceptibility to interference from genetic or environmental main effects. Together, these results suggested that simplifying longitudinal correlation with mean statistics was able to achieve good performance.

Another method for simplifying longitudinal data involves carrying out cross sectional analysis on only one of the repeated outcomes at a time. The difference in the performance of these

alternative simplification approaches can be revealing on how the averaging method was able to achieve high detection power. In addition to our results, other studies have also demonstrated good detection power for methods that summarize each individuals longitudinal outcomes with their averages (Burkett et al., 2015; Hu, Hui, & Sun, 2014). However, when only one of the repeated measures was used instead of the averages, the detection power suffered (Basson et al., 2016; Hu et al., 2014; Sung et al., 2014). This difference in performance between the two simplification approaches could be due to the fact that when compared to a single time point, using the mean over time was able to preserve more information from the original longitudinal measures. Theoretically, the mean statistic can retain more information due to its accounting of the effect from each repeated measure, since it is formulated as a weighted sum of data points. Hence using either mean statistics or single outcome measure will simplify the longitudinal correlation structure, but more information is retained from the original dataset when the outcomes are averaged. In this way, averaging individual over time data can be an optimal compromise to obtain parsimonious representation of longitudinal data pattern with sufficient accuracy.

4.1.1 Modeling Familial Correlation Structures

Similar to longitudinal correlation, familial correlation was also modeled differently by the methods considered in this study. The twin model and the linear mixed model used random effects and the kinship matrix to accurately represent the difference in correlations within MZ and DZ twin pairs. In the PBI test, however, the family structure was simplified by the localized

permutation approach, which assumed constant correlation regardless of family relationships. For the twin and linear mixed model with correctly specified family structure, the type 1 error was controlled. The PBI test was not able to maintain adequate type 1 error control, but this may be due to interference from main effects rather than simplification of familial relationships.

Though accurate modeling of familial correlation is desirable, simplification in some cases would improve detection power by reducing the degrees of freedom used. Simple family studies with uniform family structures may have constant correlation. However, larger studies will involve different types of kinship relations ranging from no correlation among spouses to high correlation between parent and child (Basson et al., 2016). The twin pair relationships examined in our study represents one of the simplest cases for which the constant correlation assumption begins to unravel, since correlation between DZ and MZ individuals are different. In our study, due to the potential interference of main effect on the PBI test, we could not attribute the test's poor performance to its simplification of familial relationships. In the literature, studies have reported either no difference or improvement in performance when the differences in correlations among familial relationships were modeled accurately (Barnholtz-Sloan et al., 2003; Burkett et al., 2015).

4.2 Modeling GE Interaction Complexity in Longitudinal Family Studies

Because of GE interaction's potentially complex nature, its analysis also involves balancing between model parsimony and accuracy. Specifically, we should consider how much

information would be lost if a simple interaction model is imposed on data from a more complex gene-environment relationship. Regression equations can capture the main effects of individual factors well using single, 1-dimensional terms. However, GE interactions represent multi-dimensional relationships between several factors. The multiplicative term commonly used in the regression models to represent interactions will not be reflective of all the possible relationships, since it only accurately portrays linear trends. In our analysis, the performance of regression approaches (twin model and linear mixed model) dropped whenever the interaction scenario was not linear. This suggested that the multiplicative term's assumption of linear relationship was not reasonable, and may result in performance-impacting loss of information when imposed on a non-linear scenario. Illustrated using a theoretical example, if the environmental modification of a genetic factor forms a quadratic relationship, then modeling as a linear interaction will not adequately reflect the actual genetic effects (Yang, 2014). This is because the multiplicative term cannot capture the non-monotonic, parabolic trend of change in a quadratic relationship, resulting in excessive loss of potential information if imposed on the data.

Our overall result suggested that each method worked well only under specific interaction scenarios. Regression approaches performed well under linear interaction relationships, as expected. The PBI test performed well only for extremely non-linear XOR scenario. In addition, all of the methods performed poorly under conditional dominant and small marginal effect interaction scenarios. The specific correspondence between the models and the scenarios may be indicative of the low robustness of our analytical method's assumptions regarding

interaction relationships. Even for the nonparametric PBI test, which does not assume any interaction patterns, its performance in the best case scenario (XOR interaction) differed dramatically to those of the other scenarios.

The patterns of model performance were similar for both GE and GTE interactions. This could be due to the fact that the simulated gene-time relationship in our study was simple and monotonically increasing. This simple relationship may be adequately captured using mean statistics without excessive loss of information. Basson et al. (2016) suggested that a few repeated measures taken in short time frame may be adequately represented with simplified model assuming constant correlation. However if the gene-time aspect of the interaction is more complex, the performance profiles under GTE scenarios may be different from the GE ones.

4.3 Additional Influences from Genetic and Environmental Main Effects on the PBI Test

Under null interaction scenarios, the PBI test was not able to maintain the desired type 1 error rate. Closer examination of the test behaviour revealed that main effects from genetic and environmental factors could potentially interfere with the detection of interaction signals. This is because the test detects GE interactions by relying on the difference in explanatory power between various partitioning schemes. Under XOR interaction, partitioning the dataset according to both the genetic and environmental factors explained significantly higher

proportion of the outcome variance than partitioning based on single factor alone. Consequently, the test statistic was large and detected interactions with high probability.

However, when only main effects or interaction effects with close resemblance to main effect pattern (i.e. conditional dominant scenario) were present, partitioning data based on genetic or environmental factor alone led to either on par or superior explanatory performance over the combined gene-environment scheme. This created situations where the test for interaction reported false positive and false negative findings. When partitioning by gene-environment factors had similar outcome-explaining performance as the single factor partition schemes, the test statistic will be close to the zero. In this case, the permutation test would identify many false positive cases leading to inflated detection rate. This was observed in the null interaction scenario with the segmented time trend, as the type 1 error rate for PBI test was high. On the other hand, if partitioning based on one of the factors alone was significantly better compared to the combined factor approach, the test statistic becomes more negative and the permutation test would identify many false negatives. The false negatives decreased the power of the PBI test to detect true interactions, as shown in the linear and conditional dominant interaction scenarios. The PBI test showed both inflated type 1 or type 2 errors depending on the specific simulation scenario. Consequently, we could not be sure of its actual performance in situations besides the XOR interaction.

4.4 Evaluating Potential IGF Pathway GE Interaction in Childhood Obesity

4.4.1 The Difficulty of Detecting GE Interaction due to its Complexity

In our analysis of the actual QNTS data, no significant interaction between the IGF pathway genes (IGF-1, IGFALS) and the environmental factors (physical activity, daycare attendance and sleep duration) was found after correction for multiple testing. In addition to the possibility that no actual interaction exists, the complexities of GE interactions may also prevent reliable detection at population level. One source of this complexity is the high likelihood of non-linear relationships between the IGF pathway and environmental factors. For example, the mechanism of physical activity's influence on the IGF pathway is hypothesized to involve intermediate steps such as hydrogen ion mediators or muscular response to changing oxygen demand (Stokes, 2003). Those intermediate steps can introduce extra variations into the system facilitating non-linear relationships. From aerobic exercise studies, there is evidence for a non-linear, threshold-dependent pattern of stimulation on GH signaling by physical activity (Sherlock & Toogood, 2007; Stokes, 2003). Consequently, the complex non-linear relationship between physical activity and the IGF pathway may not be adequately captured by our methods.

In addition to non-linear mechanisms, the long distance of action in the interactive pathway can also increase its complexity. In the case of daycare attendance, our hypothesis was that daycare environment affects IGF pathway indirectly through inflammatory mechanisms. The interaction effect over this long pathway of action can be diluted at multiple points. First, higher inflammatory response was speculated as a result of higher infection risk at daycare. This can

be attenuated in many ways. For example, our follow-up ended around 62 months (5 years). At that age, the children may not have extensive contact with toys that would be shared among peers. If so, then a major route for infection transmission will not be available. Even when daycare attendance does lead to increased inflammation, the effect will still need to influence intermediate biological factors such as the neutrophil proteases before impacting the IGF pathway (Gibson & Cohen, 1999). Thus, any GE interaction between daycare attendance and IGF requires a long and complex pathway involving both social and biological factors. This means that the pathway can be more susceptible to disruptive influences that mask the true signal.

A third potential source of complexity for GE interaction is that the effect modification mechanism can be more nuanced than expected. For sleep regulation on the IGF pathway, evidence indicated that the high quality, slow wave phase of sleep increased GH secretion (Sherlock & Toogood, 2007). Among GH deficient patients, symptoms of sleep fragmentation and loss of sleep were reported (Van Cauter et al., 2004). It is possible that the association between sleep and GH release is not just a function of sleep time but is related to other measures of sleep quality as well. This implies that measures more appropriate for testing GE interactions should assess sleep duration while taking into account factors affecting sleep quality such as the degree of sleep fragmentation or the proportion of slow wave sleep. Characteristics of sleep quality such as proportion of the slow wave phase and the degree of sleep fragmentation are difficult to assess using interviewer questionnaires. Hence the

questionnaire results from QNTS may be insufficient to fully evaluate the potential interaction between sleep and the IGF pathway.

4.4.2 Novel SNP near the IGF-1 Gene Needs Further Characterization

Two SNPs were found to be suggestively significant before multiple comparison adjustment. IGFALS SNP rs17559 was shown to interact with daycare attendance in the linear mixed model, while the twin model did not yield a significant interaction association for this SNP. However, our simulation results showed higher power for the twin model compared to the linear mixed model in all of the simulated scenarios. Thus, the significant association reported by the linear mixed model alone may not be indicative of actual interaction, since the detection was not confirmed by the twin model. Additionally, the significance level of this interaction for the linear mixed model was very close to 0.05 ($p = 0.047$). Thus we concluded that it was likely a false positive result.

Another interaction that was significant before adjustment was between a variant in IGF-1 at chromosome 12 position 102791894 and physical activity. It was detected by the twin model ($p = 0.034$) and had near significant p-value under the linear mixed model (0.065). This SNP was not documented in the dbSNP database, and is positioned outside the protein coding region of the IGF-1 gene. We speculate that the variant may influence IGF-1 through gene regulation mechanisms. Further characterization of this variant is required before we can study its role in GE interaction with physical activity more closely.

4.5 Strength and Limitations

Due to design and data constraints, our study had limitations that should be noted when considering the result. For the simulation analysis, several issues could potentially limit the generalizability of our findings. First of all, we have made simplifying assumptions regarding the time effect on genetic and environmental factors when designing the simulation scenarios. For example regarding GT interactions, this study simulated relatively simple temporal pattern (non-segmented or segmented pattern with only 1 knot). In reality, it is possible for more complex longitudinal outcome trajectories. Likewise, we did not consider environment-time interactions assuming the exposure level to be constant over time. This may not be realistic for real world situations. Similar to temporal effect issues, the twin family structure simulated in this study was also fairly simple. There were no multi-generational families, and only two types of relationships (MZ and DZ) were possible. Thus, our simulation result may not readily generalize to other studies with more complex and diverse familial relationships. Lastly, we did not simulate missing data, which could also affect the suitability of our methods for modeling longitudinal correlations.

Analysis of the actual QNTS data was also limited due to several design and data constraints. In terms of participant selection, our analysis only sampled Caucasian individuals. Thus the result may be less generalizable to other ethnic groups. Since the analysis was on individual data from birth to 5 years (62 months) of age, the results should be interpreted with caution for children

in other age groups. The study was also susceptible to potential measurement biases. Environmental exposure data were collected using interview questionnaires. Several questions were subjective in nature such as asking the parents to judge their child's activity level relative to peers. This could introduce measurement error in our result. Reporting bias was possible since responses to some of the question could be influenced by societal norms on proper child rearing. Recall issues may also exist when parents were asked to give numerical estimations of the child's sleep time. Additionally, some of the interview questions might not be able to capture the full extent of the variations required for evaluating GE interaction. For example, daycare attendance was recorded as a binary response of "yes" or "no". BMI outcome was measured using either laboratory or self-reported methods at different follow-up points. This could introduce unnecessary heterogeneity and bias in the outcome data. During the data preparation process, arbitrary decisions were made that could bias the data. For example, when converting sleep duration categories into numerical values, some categories were converted into their minimum possible values instead of median values due to lack of information on the upper bounds of sleep time in those categories. This could lead to underestimation of total sleep time. Arbitrary decisions were also made regarding the cutoffs for filtering by missing data and judgement of erroneous data points. Because of the gaps in the available data and lack of adequate statistical models, we were also not able to assess the environmental effects as time-varying. Collectively, these would affect the precision, the validity and the reliability of the environment exposure data.

Despite limitations, our study also had several strong features. To our knowledge, this investigation was the first to assess how different GE interaction relationships would impact method performance using comprehensive simulations. In the simulation analysis, we had considered several representative and plausible interaction relationships that might be encountered in nature. The result of our study could serve as starting point for future investigation into other possible scenarios. For analysis of childhood obesity GE interactions, we applied a number of arbitrary sample selection criteria such as excluding individuals with 5 or 6 missing BMI measurements. However, the potential for significant selection bias due to those sampling decisions was small, as our analysis sample was not statistically different from the whole QNTS data (Table 4). Population stratification was also controlled via restricting analysis to Caucasian individuals. The sensitivity analysis had given us confidence in adjusting for population stratification, since the inclusion of non-Caucasian individuals did not change the conclusion. Similarly, our data filtering assumptions did not affect our conclusions as the sensitivity analysis revealed no significant difference. Though the precision, the validity and the reliability of our environmental exposures were limited, we believe the result represented a conservative assessment of our hypothesis. Residual analysis also supported the adequacy of our fitted regression models.

5. Conclusion

In summary, this study examined potential GE interactions involving IGF pathway genes and the environmental factors using a longitudinal twin study design. We also investigated

methodological issues that influence the detection of GE interactions in data with complex correlation structures at both individual and familial levels. The performance of the twin model, the linear mixed model and the PBI test were examined under various GE interaction scenarios. We noted that a given method's performance was influenced both by the complexity of the model and its compatibility with the underlying interaction relationships. The simpler average-over-time summary statistics of the repeated outcomes analyzed with the traditional twin study model appeared to perform well. This may be due to the fact that true signals within the longitudinal data were preserved while random noise was reduced when simplified with means. Higher statistical power was achieved when the data was analyzed with this simplified approach. However, methods were not robust to misspecification of the interaction relationship and each method showed poor performance outside their best case scenarios. This suggested that imposing the incorrect interaction model on the data will have large negative impact on the power to detect interaction.

As longitudinal family studies become more prevalent in genetics research, evaluation of the analysis methods for this type of design is needed. Other plausible GE interaction scenarios and correlation structures should be explored using simulation. Characterization of the methods' theoretical performances in different data settings can inform the selection of appropriate analysis strategies for empirical GE interaction studies. At the same time, the pool of available methods for longitudinal family studies is limited. More family-based methods should be extended to analyze repeated outcomes. Methodology development efforts should also be directed towards models that are more robust to different GE interaction relationships. To

understand how robustness can be achieved in longitudinal family studies, we should precisely investigate how well a simplified correlation structure or interaction model fit with the underlying data. A framework to think about this problem is by considering how much information is lost when we represent the original data generating process with simpler models. The quantity of information can be precisely measured using the concept of entropy from information theory. Thus, it would be interesting to study the relationship between the model performance and the entropy measure of information loss for different longitudinal family methods.

Studying interaction between IGF pathway genes and environmental exposures holds great potential for increasing our understanding of childhood obesity etiology. Theoretical analysis suggested that accounting for interaction effects could allow significantly higher proportion of heritability to be explained (Zuk, Hechter, Sunyaev, & Lander, 2012). Future study design should give more considerations to the inherent complexities of GE interactions. We should study more closely related factors in an interaction pathway to minimizing potential noise from intermediate steps. In the IGF pathway, the effect of environment on the more upstream GH gene involves less mediator proteins compare to the IGF-1 and IGFALS genes. More precise and comprehensive assessment of environmental exposure can also improve the detection of interactions involving sleep and daycare attendance. Examples of precise assessment include laboratory evaluation of sleep quality instead of self-reported total sleep time.

Our study found one suggestive association between IGF-1 genetic variant (chromosome 12 position 102791894) and physical activity on childhood obesity using the twin model. However, this association was not significant after multiple testing corrections. The novel SNP found at chromosome 12 position 102791894 near IGF-1 gene may be involved in gene regulation. More studies characterizing the function of this SNP and evaluating its potential interaction with environmental exposures can be fruitful.

6. References

Barnholtz-Sloan, J. S., Poisson, L. M., Coon, S. W., Chase, G. A., & Rybicki, B. A. (2003). Analysis of gene x environment interactions in sibships using mixed models. *BMC Genetics*, *4 Suppl 1*, S18. http://doi.org/10.1186/1471-2156-4-S1-S18

Barrios, V., Argente, J., Muñoz, M. T., Pozo, J., Chowen, J. A., & Hernández, M. (2001). Diagnostic interest of acid-labile subunit measurement in relationship to other components of the IGF system in pediatric patients with growth or eating disorders. *European Journal of Endocrinology / European Federation of Endocrine Societies*, *144*(3), 245–50. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11248743

Basson, J., Sung, Y. J., de las Fuentes, L., Schwander, K. L., Vazquez, A., & Rao, D. C. (2016). Three Approaches to Modeling Gene-Environment Interactions in Longitudinal Family Data: Gene-Smoking Interactions in Blood Pressure. *Genetic Epidemiology*, *40*(1), 73–80. http://doi.org/10.1002/gepi.21941

Berryman, D. E., Glad, C. A. M., List, E. O., & Johannsson, G. (2013). The GH/IGF-1 axis in obesity: pathophysiology and therapeutic considerations. *Nature Reviews. Endocrinology*, *9*(6), 346–56. http://doi.org/10.1038/nrendo.2013.64

Bjørge, T., Engeland, A., Tverdal, A., & Smith, G. D. (2008). Body mass index in adolescence in relation to cause-specific mortality: a follow-up of 230,000 Norwegian adolescents. *American Journal of Epidemiology*, *168*(1), 30–7. http://doi.org/10.1093/aje/kwn096

Boivin, M., Brendgen, M., Dionne, G., Dubois, L., Pérusse, D., Robaey, P., … Vitaro, F. (2013). The Quebec Newborn Twin Study into adolescence: 15 years later. *Twin Research and Human Genetics : The Official Journal of the International Society for Twin Studies*, *16*(1), 64–9.

http://doi.org/10.1017/thg.2012.129

Brady, M. T. (2005). Infectious disease in pediatric out-of-home child care. *American Journal of Infection Control*, *33*(5), 276–85. http://doi.org/10.1016/j.ajic.2004.11.007

Burkett, K. M., Roy-Gagnon, M.-H., Lefebvre, J.-F., Wang, C., Fontaine-Bisson, B., & Dubois, L. (2015). A Comparison of Statistical Methods for the Discovery of Genetic Risk Factors Using Longitudinal Family Study Designs. *Frontiers in Immunology*, *6*, 589. http://doi.org/10.3389/fimmu.2015.00589

Centers for Disease Control and Prevention. (2017). Obesity Facts | Healthy Schools | CDC. Retrieved May 22, 2017, from https://www.cdc.gov/healthyschools/obesity/facts.htm

Chen, H., Malzahn, D., Balliu, B., Li, C., & Bailey, J. N. (2014). Testing Genetic Association With Rare and Common Variants in Family Data. *Genetic Epidemiology*, *38*(S1), S37–S43. http://doi.org/10.1002/gepi.21823

Chesi, A., & Grant, S. F. A. (2015). The Genetics of Pediatric Obesity. *Trends in Endocrinology & Metabolism*, *26*(12), 711–21. http://doi.org/10.1016/j.tem.2015.08.008

Cisternino, M., Draghi, M., Lauriola, S., Scarcella, D., Bernasconi, S., Cavallo, L., … Tatò, L. (2002). The acid-labile subunit of human ternary insulin-like growth factor-binding protein complex in girls with central precocious puberty before and during gonadotropin-releasing hormone analog therapy. *The Journal of Clinical Endocrinology and Metabolism*, *87*(10), 4629–33. http://doi.org/10.1210/jc.2002-020308

Domené, H. M., Bengolea, S. V, Jasper, H. G., & Boisclair, Y. R. (2005). Acid-labile subunit deficiency: phenotypic similarities and differences between human and mouse. *Journal of Endocrinological Investigation*, *28*(5 Suppl), 43–6. Retrieved from

http://www.ncbi.nlm.nih.gov/pubmed/16114275

Domené, H. M., Hwa, V., Argente, J., Wit, J. M., Wit, J. M., Camacho-Hübner, C., … Rosenfeld, R. G. (2009). Human acid-labile subunit deficiency: clinical, endocrine and metabolic consequences. *Hormone Research*, *72*(3), 129–41. http://doi.org/10.1159/000232486

Domené, H. M., Scaglia, P. A., Lteif, A., Mahmud, F. H., Kirmani, S., Frystyk, J., … Jasper, H. G. (2007). Phenotypic effects of null and haploinsufficiency of acid-labile subunit in a family with two novel IGFALS gene mutations. *The Journal of Clinical Endocrinology and Metabolism*, *92*(11), 4444–50. http://doi.org/10.1210/jc.2007-1152

Dubois, L., Diasparra, M., Bédard, B., Kaprio, J., Fontaine-Bisson, B., Pérusse, D., … Boivin, M. (2013). Gene-environment contributions to energy and macronutrient intakes in 9-year-old children: results from the Quebec Newborn Twin Study. *Physiology & Behavior*, *119*, 30–7. http://doi.org/10.1016/j.physbeh.2013.05.039

Dubois, L., Girard, M., Girard, A., Tremblay, R., Boivin, M., & Pérusse, D. (2007). Genetic and environmental influences on body size in early childhood: a twin birth-cohort study. *Twin Research and Human Genetics : The Official Journal of the International Society for Twin Studies*, *10*(3), 479–85. http://doi.org/10.1375/twin.10.3.479

Dubois, L., Ohm Kyvik, K., Girard, M., Tatone-Tokuda, F., Pérusse, D., Hjelmborg, J., … Martin, N. G. (2012). Genetic and environmental contributions to weight, height, and BMI from birth to 19 years of age: an international study of over 12,000 twin pairs. *PloS One*, *7*(2), e30153. http://doi.org/10.1371/journal.pone.0030153

Ebbeling, C. B., Pawlak, D. B., & Ludwig, D. S. (2002). Childhood obesity: public-health crisis, common sense cure. *The Lancet*, *360*(9331), 473–482. http://doi.org/10.1016/S0140-

6736(02)09678-2

Fan, R., Huang, C.-H., Hu, I., Wang, H., Zheng, T., & Lo, S.-H. (2014). A partition-based approach to identify gene-environment interactions in genome wide association studies. *BMC Proceedings*, *8*(Suppl 1 Genetic Analysis Workshop 18Vanessa Olmo), S60. http://doi.org/10.1186/1753-6561-8-S1-S60

Fan, R., Zhang, Y., Albert, P. S., Liu, A., Wang, Y., & Xiong, M. (2012). Longitudinal association analysis of quantitative traits. *Genetic Epidemiology*, *36*(8), 856–69. http://doi.org/10.1002/gepi.21673

Firth, S. M., Yan, X., & Baxter, R. C. (2011). D440N mutation in the acid-labile subunit of insulin-like growth factor complexes inhibits secretion and complex formation. *Molecular Endocrinology (Baltimore, Md.)*, *25*(2), 307–14. http://doi.org/10.1210/me.2010-0295

Freedman, D. S., Khan, L. K., Serdula, M. K., Dietz, W. H., Srinivasan, S. R., & Berenson, G. S. (2005). The relation of childhood BMI to adult adiposity: the Bogalusa Heart Study. *Pediatrics*, *115*(1), 22–7. http://doi.org/10.1542/peds.2004-0220

Fu, W., O'Connor, T. D., & Akey, J. M. (2013). Genetic architecture of quantitative traits and complex diseases. *Current Opinion in Genetics & Development*, *23*(6), 678–83. http://doi.org/10.1016/j.gde.2013.10.008

Fukuda, I., Hotta, M., Hizuka, N., Takano, K., Ishikawa, Y., Asakawa-Yasumoto, K., … Demura, H. (1999). Decreased serum levels of acid-labile subunit in patients with anorexia nervosa. *The Journal of Clinical Endocrinology and Metabolism*, *84*(6), 2034–6. http://doi.org/10.1210/jcem.84.6.5737

Garten, A., Schuster, S., & Kiess, W. (2012). The Insulin-Like Growth Factors in Adipogenesis and

Obesity. *Endocrinology and Metabolism Clinics of North America*, *41*(2), 283–295. http://doi.org/10.1016/j.ecl.2012.04.011

Gibson, T. L., & Cohen, P. (1999). Inflammation-related neutrophil proteases, cathepsin G and elastase, function as insulin-like growth factor binding protein proteases. *Growth Hormone & IGF Research : Official Journal of the Growth Hormone Research Society and the International IGF Research Society*, *9*(4), 241–53. http://doi.org/10.1054/ghir.1999.0115

Gregor, M. F., & Hotamisligil, G. S. (2011). Inflammatory mechanisms in obesity. *Annual Review of Immunology*, *29*, 415–45. http://doi.org/10.1146/annurev-immunol-031210-101322

Güngör, N. K. (2014). Overweight and obesity in children and adolescents. *Journal of Clinical Research in Pediatric Endocrinology*, *6*(3), 129–43. http://doi.org/10.4274/Jcrpe.1471

Hamosh, A., & McKusick, V. A. (2016). OMIM Entry - * 147440 - INSULIN-LIKE GROWTH FACTOR I; IGF1. Retrieved from https://www.omim.org/entry/147440

Han, J. C., Lawlor, D. A., & Kimm, S. Y. S. (2010). Childhood obesity. *Lancet*, *375*(9727), 1737–48. http://doi.org/10.1016/S0140-6736(10)60171-7

Heath, K. E., Argente, J., Barrios, V., Pozo, J., Díaz-González, F., Martos-Moreno, G. A., … Campos-Barros, A. (2008). Primary acid-labile subunit deficiency due to recessive IGFALS mutations results in postnatal growth deficit associated with low circulating insulin growth factor (IGF)-I, IGF binding protein-3 levels, and hyperinsulinemia. *The Journal of Clinical Endocrinology and Metabolism*, *93*(5), 1616–24. http://doi.org/10.1210/jc.2007-2678

Hebebrand, J., Volckmar, A.-L., Knoll, N., & Hinney, A. (2010). Chipping away the “missing heritability”: GIANT steps forward in the molecular elucidation of obesity - but still lots to go. *Obesity Facts*, *3*(5), 294–303. http://doi.org/10.1159/000321537

Hess, O., Khayat, M., Hwa, V., Heath, K. E., Teitler, A., Hritan, Y., … Tenenbaum-Rakover, Y. (2013). A novel mutation in IGFALS, c.380T>C (p.L127P), associated with short stature, delayed puberty, osteopenia and hyperinsulinaemia in two siblings: insights into the roles of insulin growth factor-1 (IGF1). *Clinical Endocrinology*, *79*(6), 838–44. http://doi.org/10.1111/cen.12200

Holst, K. K., & Scheike, T. (2015). mets: Analysis of Multivariate Event Times. R package version 1.1.1. Retrieved from https://cran.r-project.org/package=mets

Hu, Y., Hui, Q., & Sun, Y. V. (2014). Association analysis of whole genome sequencing data accounting for longitudinal and family designs. *BMC Proceedings*, *8*(Suppl 1), S89. http://doi.org/10.1186/1753-6561-8-S1-S89

Janosi, J. B., Firth, S. M., Bond, J. J., Baxter, R. C., & Delhanty, P. J. (1999). N-Linked glycosylation and sialylation of the acid-labile subunit. Role in complex formation with insulin-like growth factor (IGF)-binding protein-3 and the IGFs. *The Journal of Biological Chemistry*, *274*(9), 5292–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10026136

Jenco, M. (2016). AAP endorses new recommendations on sleep times. Retrieved November 8, 2016, from http://www.aappublications.org/news/2016/06/13/Sleep061316

Jiménez-Pavón, D., Kelly, J., & Reilly, J. J. (2010). Associations between objectively measured habitual physical activity and adiposity in children and adolescents: Systematic review. *International Journal of Pediatric Obesity*, *5*(1), 3–18. http://doi.org/10.3109/17477160903067601

John, J., Wolfenstetter, S. B., & Wenig, C. M. (2012). An economic perspective on childhood obesity: recent findings on cost of illness and cost effectiveness of interventions. *Nutrition*

(Burbank, Los Angeles County, Calif.), *28*(9), 829–39. http://doi.org/10.1016/j.nut.2011.11.016

Juul, A. (2003). Serum levels of insulin-like growth factor I and its binding proteins in health and disease. *Growth Hormone & IGF Research : Official Journal of the Growth Hormone Research Society and the International IGF Research Society*, *13*(4), 113–70. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12914749

Kaprio, J. (2012). Twins and the mystery of missing heritability: the contribution of gene-environment interactions. *Journal of Internal Medicine*, *272*(5), 440–448. http://doi.org/10.1111/j.1365-2796.2012.02587.x

Kobe, B., & Kajava, A. V. (2001). The leucine-rich repeat as a protein recognition motif. *Current Opinion in Structural Biology*, *11*(6), 725–32. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11751054

Kuhle, S., Kirk, S., Ohinmaa, A., Yasui, Y., Allen, A. C., & Veugelers, P. J. (2011). Use and cost of health services among overweight and obese Canadian children. *International Journal of Pediatric Obesity : IJPO : An Official Journal of the International Association for the Study of Obesity*, *6*(2), 142–8. http://doi.org/10.3109/17477166.2010.486834

Laird, N., & Ware, J. (1982). Random-effects models for longitudinal data. *Biometrics*, *38*(4), 963–74. Retrieved from http://www.jstor.org/stable/2529876

Lobstein, T., Jackson-Leach, R., Moodie, M. L., Hall, K. D., Gortmaker, S. L., Swinburn, B. A., … McPherson, K. (2015). Child and adolescent obesity: part of a bigger picture. *The Lancet*, *385*(9986), 2510–2520. http://doi.org/10.1016/S0140-6736(14)61746-3

Manco, M., & Dallapiccola, B. (2012). Genetics of pediatric obesity. *Pediatrics*, *130*(1), 123–33.

http://doi.org/10.1542/peds.2011-2717

Manolio, T. A., Collins, F. S., Cox, N. J., Goldstein, D. B., Hindorff, L. A., Hunter, D. J., … Visscher, P. M. (2009). Finding the missing heritability of complex diseases. *Nature*, *461*(7265), 747–53. http://doi.org/10.1038/nature08494

Marian, A. J. (2012). Elements of "missing heritability". *Current Opinion in Cardiology*, *27*(3), 197–201. http://doi.org/10.1097/HCO.0b013e328352707d

Narimatsu, H. (2017). Gene–Environment Interactions in Preventive Medicine: Current Status and Expectations for the Future. *International Journal of Molecular Sciences*, *18*(2), 302. http://doi.org/10.3390/ijms18020302

Neale, M. C., & Maes, H. H. (2004). *Methodology for Genetic Studies of Twins and Families*. Dordrecht, The Netherlands: Kluwer Academic Publishers B.V.

Nindl, B. C., & Pierce, J. R. (2010). Insulin-like growth factor I as a biomarker of health, fitness, and training status. *Medicine and Science in Sports and Exercise*, *42*(1), 39–49. http://doi.org/10.1249/MSS.0b013e3181b07c4d

Paruthi, S., Brooks, L. J., D'Ambrosio, C., Hall, W. A., Kotagal, S., Lloyd, R. M., … Wise, M. S. (2016). Recommended Amount of Sleep for Pediatric Populations: A Consensus Statement of the American Academy of Sleep Medicine. *Journal of Clinical Sleep Medicine : JCSM : Official Publication of the American Academy of Sleep Medicine*, *12*(6), 785–6. http://doi.org/10.5664/jcsm.5866

Payet, L. D., Firth, S. M., & Baxter, R. C. (2004). The Role of the Acid-Labile Subunit in Regulating Insulin-Like Growth Factor Transport across Human Umbilical Vein Endothelial Cell Monolayers. *The Journal of Clinical Endocrinology & Metabolism*, *89*(5), 2382–2389.

http://doi.org/10.1210/jc.2003-031880

Pulgarón, E. R. (2013). Childhood obesity: a review of increased risk for physical and psychological comorbidities. *Clinical Therapeutics*, *35*(1), A18-32. http://doi.org/10.1016/j.clinthera.2012.12.014

Qi, Q., Li, Y., Chomistek, A. K., Kang, J. H., Curhan, G. C., Pasquale, L. R., … Qi, L. (2012). Television Watching, Leisure Time Physical Activity, and the Genetic Predisposition in Relation to Body Mass Index in Women and Men. *Circulation*, *126*(15), 1821–1827. http://doi.org/10.1161/CIRCULATIONAHA.112.098061

R Core Team. (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from https://www.r-project.org/

Rasmussen, M. H., Juul, A., Kjems, L. L., & Hilsted, J. (2006). Effects of short-term caloric restriction on circulating free IGF-I, acid-labile subunit, IGF-binding proteins (IGFBPs)-1-4, and IGFBPs-1-3 protease activity in obese subjects. *European Journal of Endocrinology / European Federation of Endocrine Societies*, *155*(4), 575–81. http://doi.org/10.1530/eje.1.02246

Rothman, K. J., Greenland, S., & Lash, T. L. (2008). *Modern Epidemiology* (3rd ed.). Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins.

Sadee, W., Hartmann, K., Seweryn, M., Pietrzak, M., Handelman, S. K., & Rempala, G. A. (2014). Missing heritability of common diseases and treatments outside the protein-coding exome. *Human Genetics*, *133*(10), 1199–215. http://doi.org/10.1007/s00439-014-1476-7

Satten, G. A., Biswas, S., Papachristou, C., Turkmen, A., & König, I. R. (2014). Population-based association and gene by environment interactions in Genetic Analysis Workshop 18.

Genetic Epidemiology, *38 Suppl 1*, S49-56. http://doi.org/10.1002/gepi.21825

Sherlock, M., & Toogood, A. A. (2007). Aging and the growth hormone/insulin like growth factor-I axis. *Pituitary*, *10*(2), 189–203. http://doi.org/10.1007/s11102-007-0039-5

Statistics Canada. (2014). Body mass index of children and youth, 2012 to 2013. Retrieved November 23, 2015, from http://www.statcan.gc.ca/pub/82-625-x/2014001/article/14105-eng.htm#n1

Stokes, K. (2003). Growth hormone responses to sub-maximal and sprint exercise. *Growth Hormone & IGF Research : Official Journal of the Growth Hormone Research Society and the International IGF Research Society*, *13*(5), 225–38. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12932743

Sung, Y. J., Simino, J., Kume, R., Basson, J., Schwander, K., & Rao, D. C. (2014). Comparison of two methods for analysis of gene-environment interactions in longitudinal family data: the Framingham heart study. *Frontiers in Genetics*, *5*, 9. http://doi.org/10.3389/fgene.2014.00009

Suwanichkul, A., Boisclair, Y. R., Olney, R. C., Durham, S. K., & Powell, D. R. (2000). Conservation of a growth hormone-responsive promoter element in the human and mouse acid-labile subunit genes. *Endocrinology*, *141*(2), 833–8. http://doi.org/10.1210/endo.141.2.7333

Textor, J., Hardt, J., & Knüppel, S. (2011). DAGitty: a graphical tool for analyzing causal diagrams. *Epidemiology (Cambridge, Mass.)*, *22*(5), 745. http://doi.org/10.1097/EDE.0b013e318225c2be

Therneau, T. M. (2015). coxme: Mixed Effects Cox Models. R package version 2.2-5. Retrieved from https://cran.r-project.org/package=coxme

Twigg, S. M., & Baxter, R. C. (1998). Insulin-like growth factor (IGF)-binding protein 5 forms an alternative ternary complex with IGFs and the acid-labile subunit. *The Journal of Biological Chemistry*, *273*(11), 6074–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9497324

Ueki, I., Ooi, G. T., Tremblay, M. L., Hurst, K. R., Bach, L. A., & Boisclair, Y. R. (2000). Inactivation of the acid labile subunit gene in mice results in mild retardation of postnatal growth despite profound disruptions in the circulating insulin-like growth factor system. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(12), 6868–73. http://doi.org/10.1073/pnas.120172697

Van Cauter, E., Latta, F., Nedeltcheva, A., Spiegel, K., Leproult, R., Vandenbril, C., … Copinschi, G. (2004). Reciprocal interactions between the GH axis and sleep. *Growth Hormone & IGF Research : Official Journal of the Growth Hormone Research Society and the International IGF Research Society*, *14 Suppl A*, S10-7. http://doi.org/10.1016/j.ghir.2004.03.006

Voorhoeve, P. G., van Rossum, E. F. C., Te Velde, S. J., Koper, J. W., Kemper, H. C. G., Lamberts, S. W. J., & de Waal, H. A. D. (2006). Association between an IGF-I gene polymorphism and body fatness: differences between generations. *European Journal of Endocrinology / European Federation of Endocrine Societies*, *154*(3), 379–88. http://doi.org/10.1530/eje.1.02101

Waalen, J. (2014). The genetics of human obesity. *Translational Research : The Journal of Laboratory and Clinical Medicine*, *164*(4), 293–301. http://doi.org/10.1016/j.trsl.2014.05.010

Wu, Z., Hu, Y., & Melton, P. E. (2014). Longitudinal data analysis for genetic studies in the

whole-genome sequencing era. *Genetic Epidemiology*, *38 Suppl 1*, S74-80. http://doi.org/10.1002/gepi.21829

Xi, B., Chandak, G. R., Shen, Y., Wang, Q., & Zhou, D. (2012). Association between common polymorphism near the MC4R gene and obesity risk: a systematic review and meta-analysis. *PloS One*, *7*(9), e45731. http://doi.org/10.1371/journal.pone.0045731

Yang, R.-C. (2014). Analysis of linear and non-linear genotype Ã— environment interaction. *Frontiers in Genetics*, *5*, 227. http://doi.org/10.3389/fgene.2014.00227

Zuk, O., Hechter, E., Sunyaev, S. R., & Lander, E. S. (2012). The mystery of missing heritability: Genetic interactions create phantom heritability. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(4), 1193–8. http://doi.org/10.1073/pnas.1119675109

7. Appendix

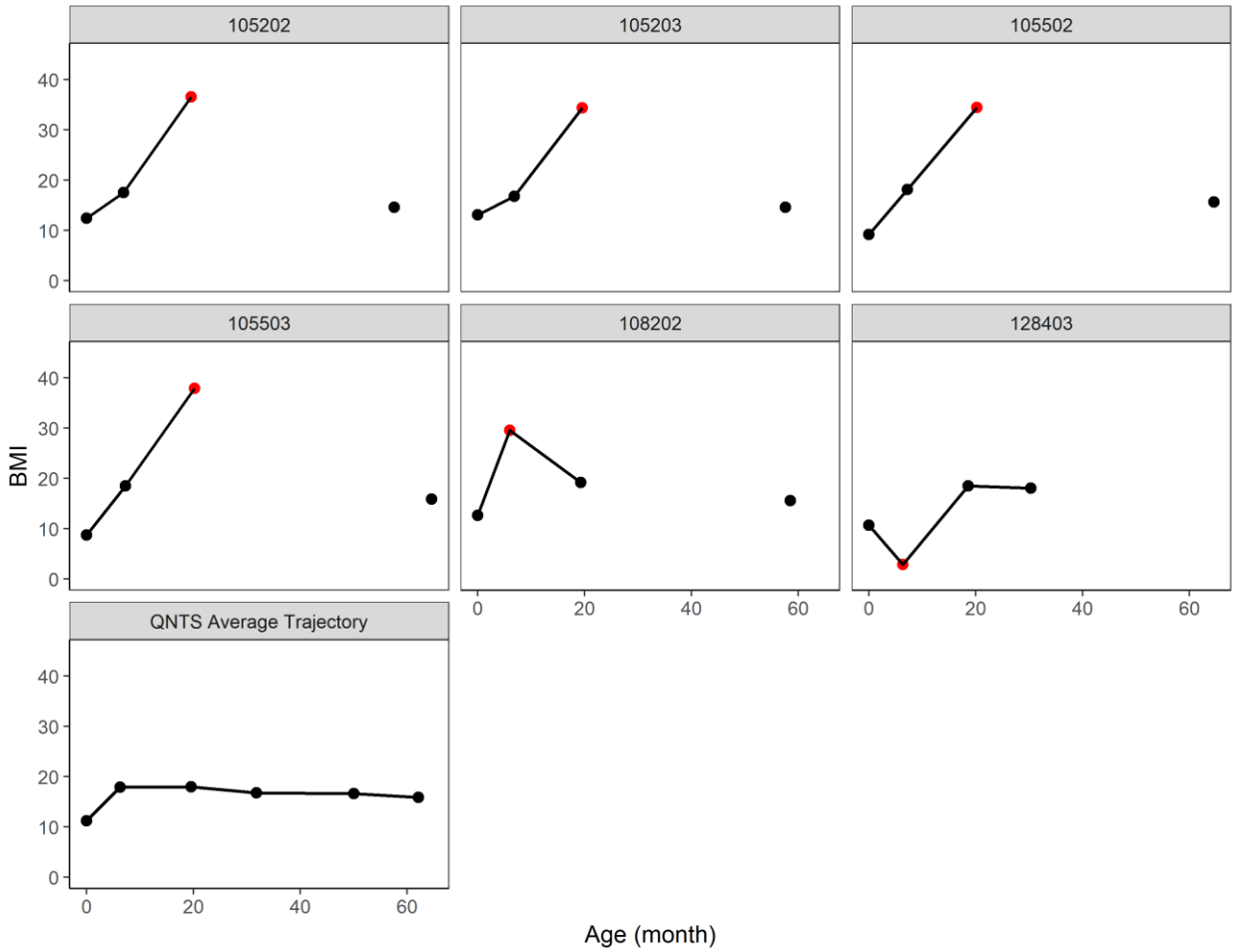

Figure S1. BMI trajectories for individuals with excluded data

Trajectories of BMI over the follow-up period for individuals with excluded data. BMI outcomes were excluded if their values or trends were judged to be impossible and likely to be measurement errors. The first six panel headings correspond to the ID of each individual. Excluded data points are marked red. The red time points in Panels 1 through 5 were excluded due to being improbably high relative to the BMI trend, while the red time point in Panel 6 was excluded for being improbably low. The last panel plots the trajectory of the average BMI from the QNTS data for comparison.

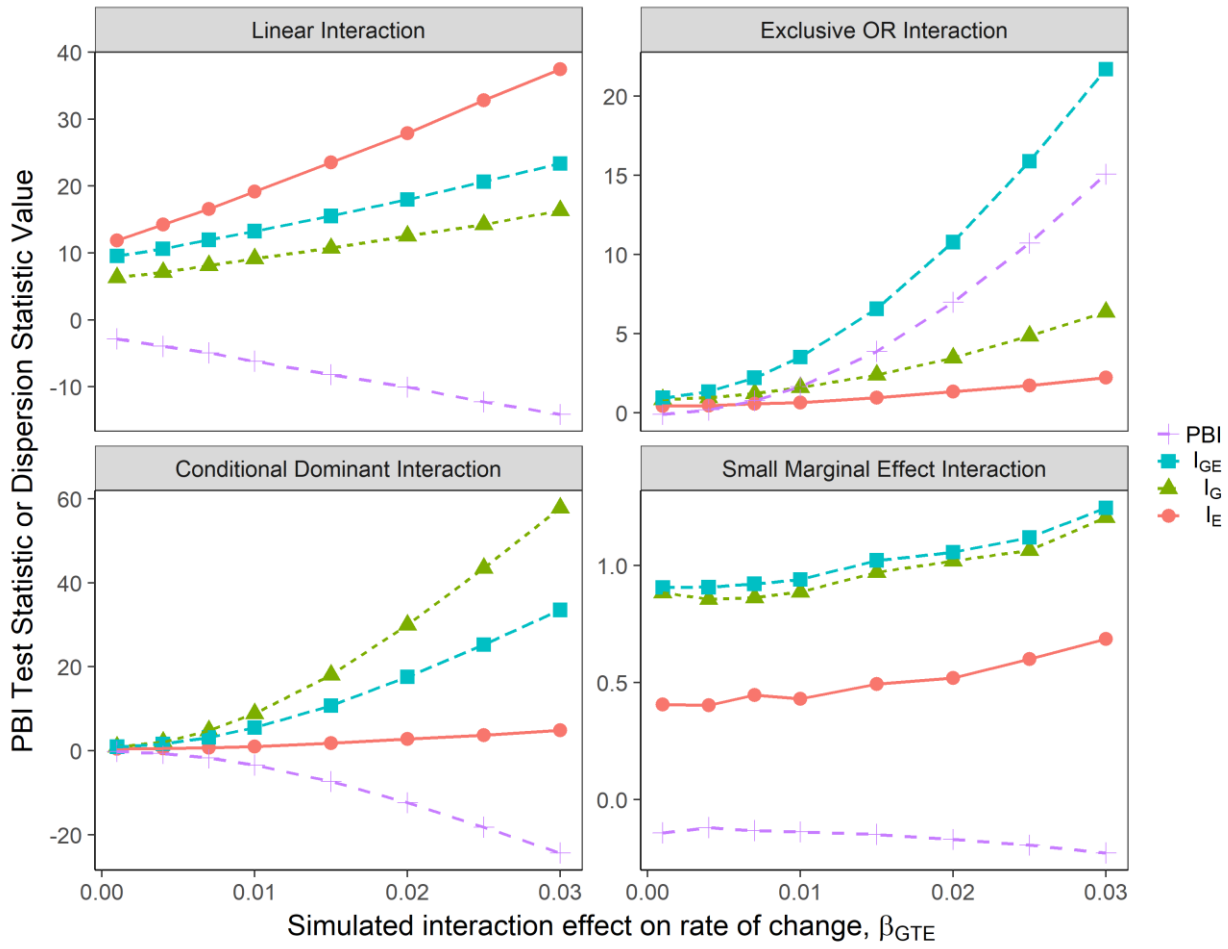

Figure S2. Behavior of partition based score I (PBI) test statistics and dispersion statistics for interaction effect on temporal rate of change scenarios

Average value over 2000 replicates for PBI test statistics (PBI) and dispersion statistics for gene-environment (I_{GE}), gene only (I_G) and environment only (I_E) partitioning schemes are plotted. PBI test statistics is equal to the difference between I_{GE} and the maximum of I_G or I_E. Effect size for gene-time-environment interaction (β_{GTE}) were varied under each interactive relationship.

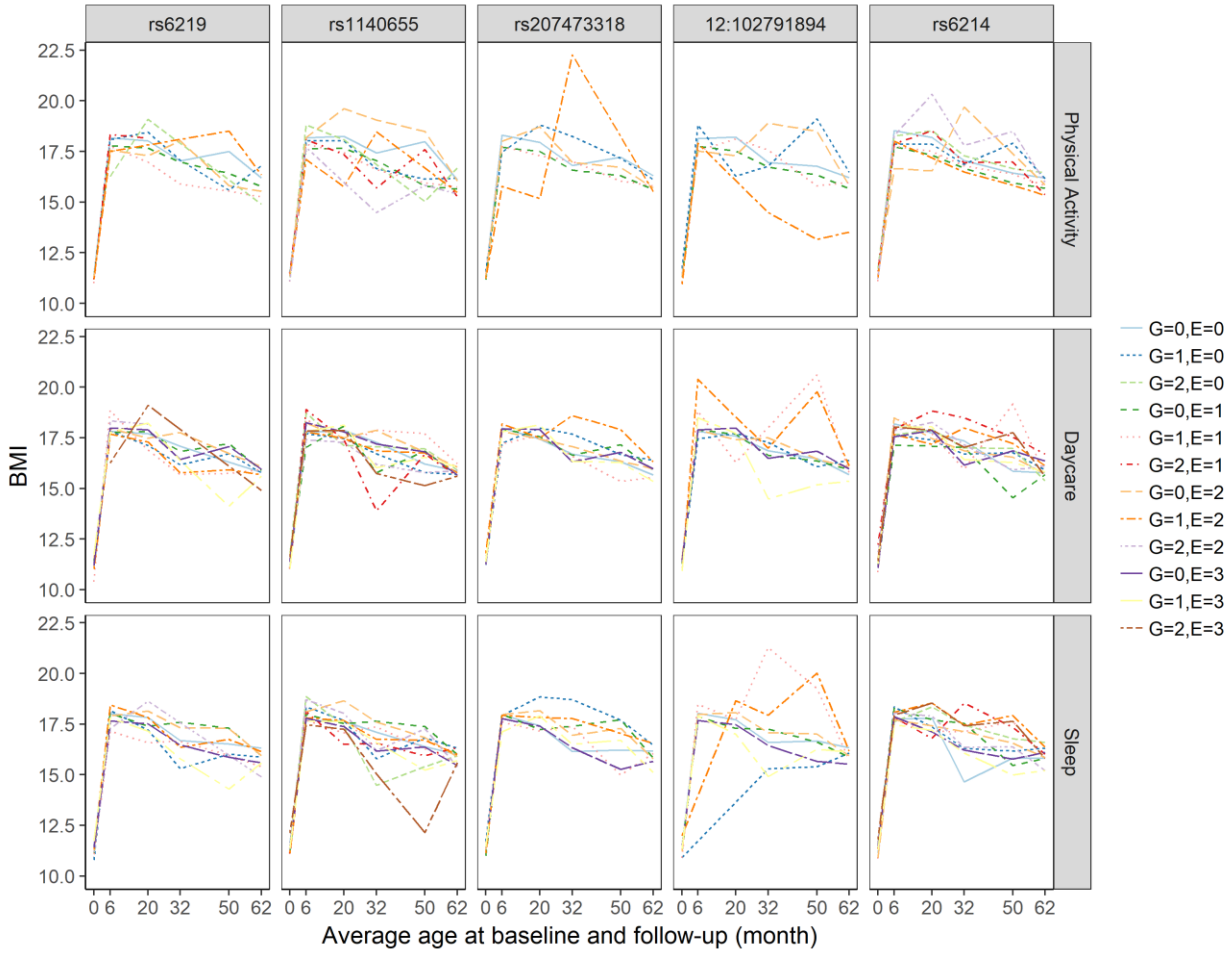

Figure S3. Trajectories of average BMI for analyzed QNTS participants by gene-environment pair

Trajectories of average BMI at each follow-up time point grouped by genetic (G) and environmental (E) factor levels. Panels correspond to the specific pair of the genetic (columns) and the environmental factor (rows). Genetic variables coded as 0, 1 and 2 for the number of minor alleles. Physical activity coded as 0, 1, and 2 being "more", "equal or "less" physically active compare to peers. Daycare attendance and sleep duration coded as 0, 1, 2 and 3 for the 1st, 2nd, 3rd and 4th quartiles of the continuous proportion measures (see section 2.3.3.1 for more detail on daycare and sleep data definition).

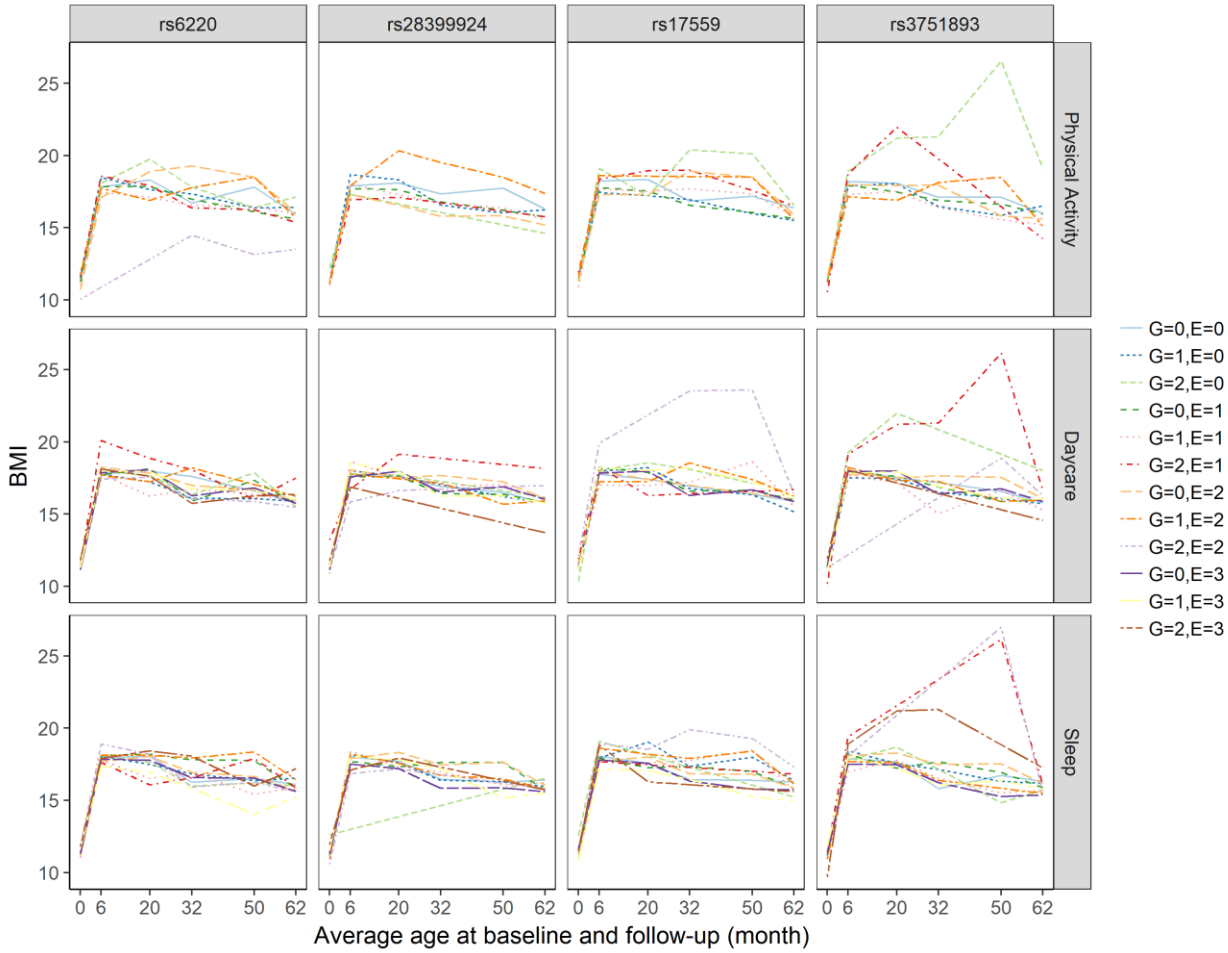

Figure S4. Trajectories of average BMI for analyzed QNTS participants by gene-environment pair (continued)

Trajectories of average BMI at each follow-up time point grouped by genetic (G) and environmental (E) factor levels. Panels correspond to the specific pair of the genetic (columns) and the environmental factor (rows). Genetic variables coded as 0, 1 and 2 for the number of minor alleles. Physical activity coded as 0, 1, and 2 being “more”, “equal or “less” physically active compare to peers. Daycare attendance and sleep duration coded as 0, 1, 2 and 3 for the 1st, 2nd, 3rd and 4th quartiles of the continuous proportion measures (see section 2.3.3.1 for more detail on daycare and sleep data definition).

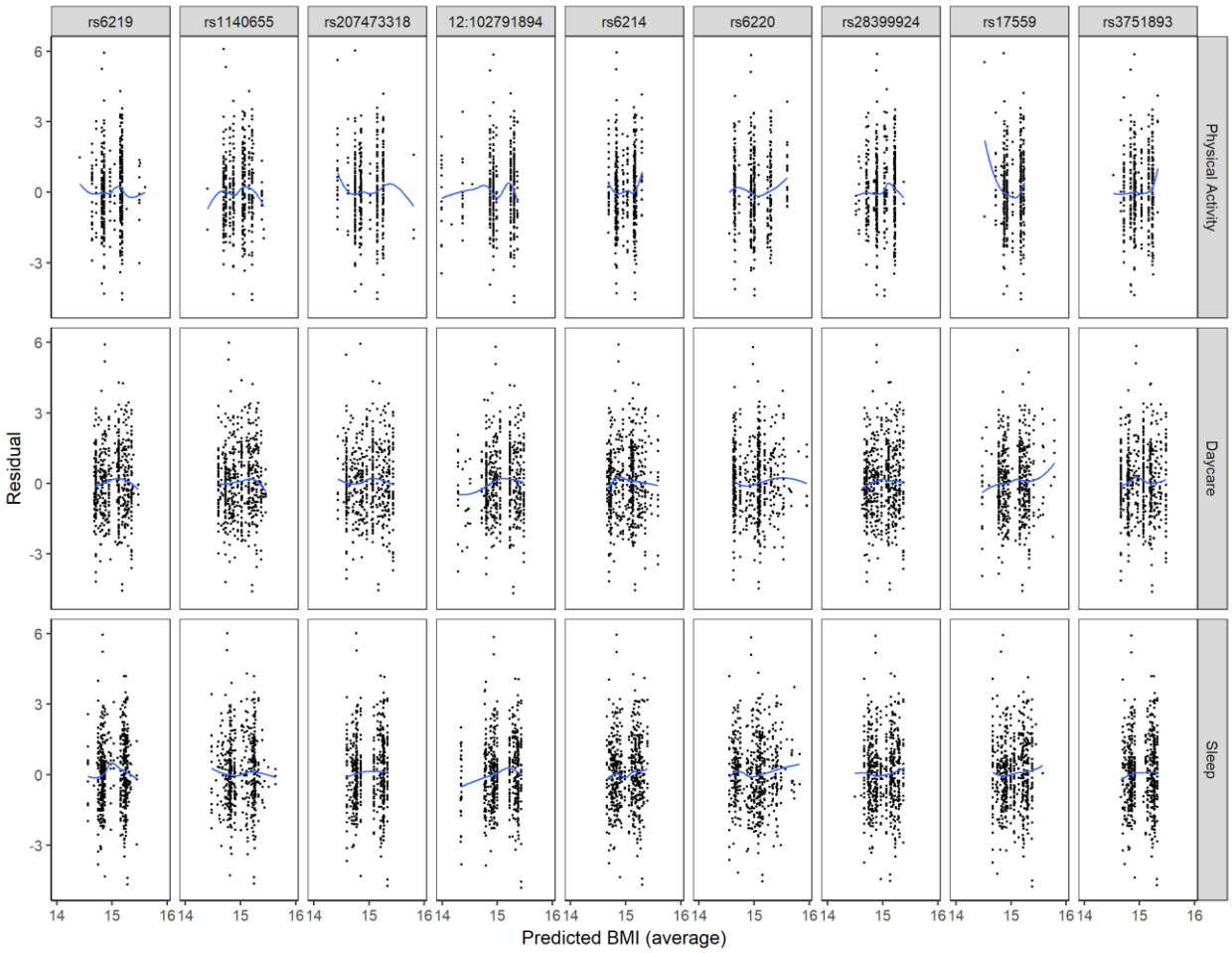

Figure S5. Residual plot for twin model analysis of QNTS sample

Residual statistic values against predicted individual BMI average over repeated measures are plotted for each gene-environment interaction analysis. IGF-1 and IGFALS SNPs are listed by columns, while environmental variables (physical activity, daycare attendance and sleep duration) are listed by rows. Trend in residuals is highlighted by the blue line.

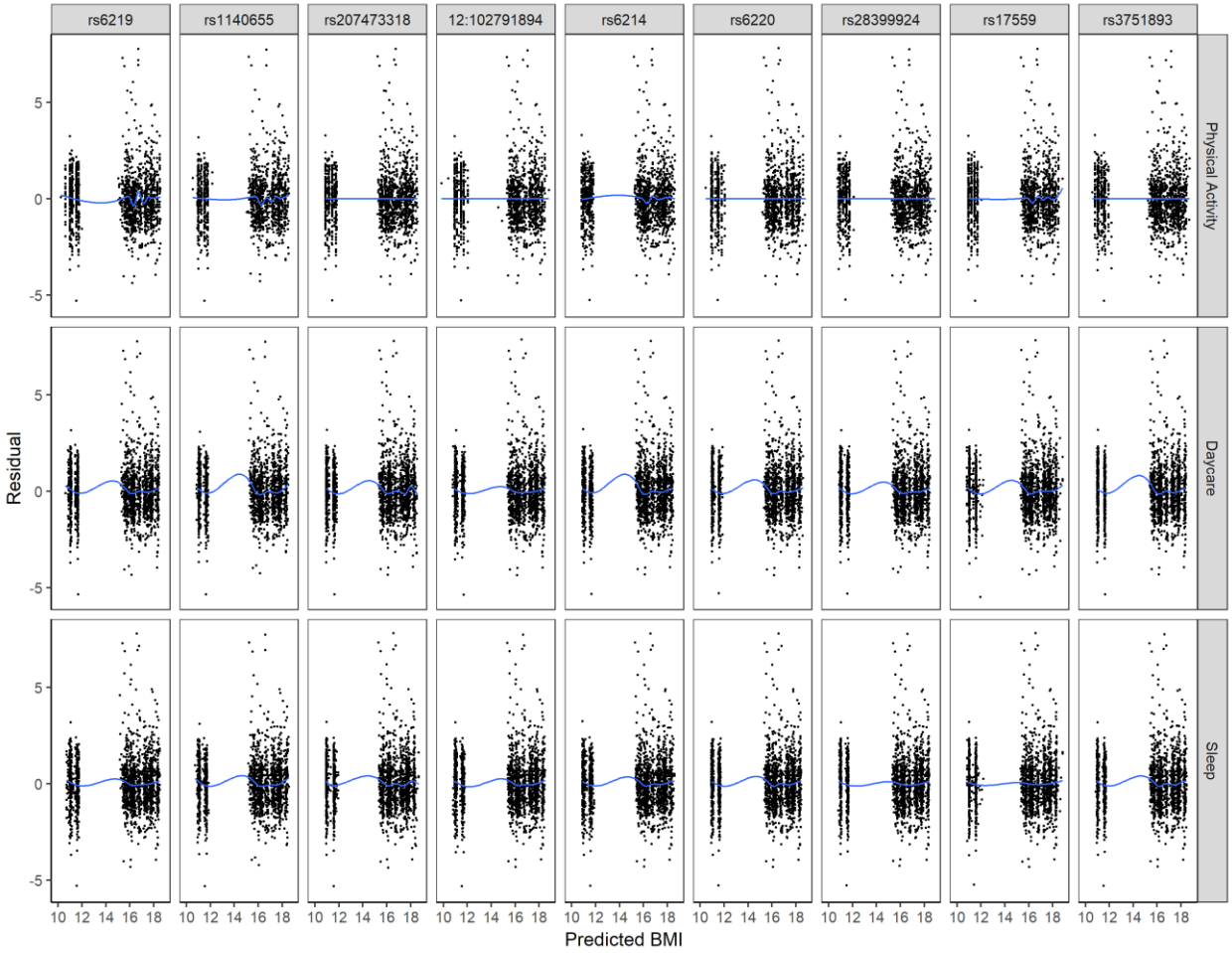

Figure S6. Residual plot for linear mixed model analysis of QNTS sample

Residual statistic values against predicted BMI are plotted for each gene-environment interaction analysis. IGF-1 and IGFALS SNPs are listed by columns, while environmental variables (physical activity, daycare attendance and sleep duration) are listed by rows. Trend in residuals across different predicted BMI values is highlighted by the blue line.

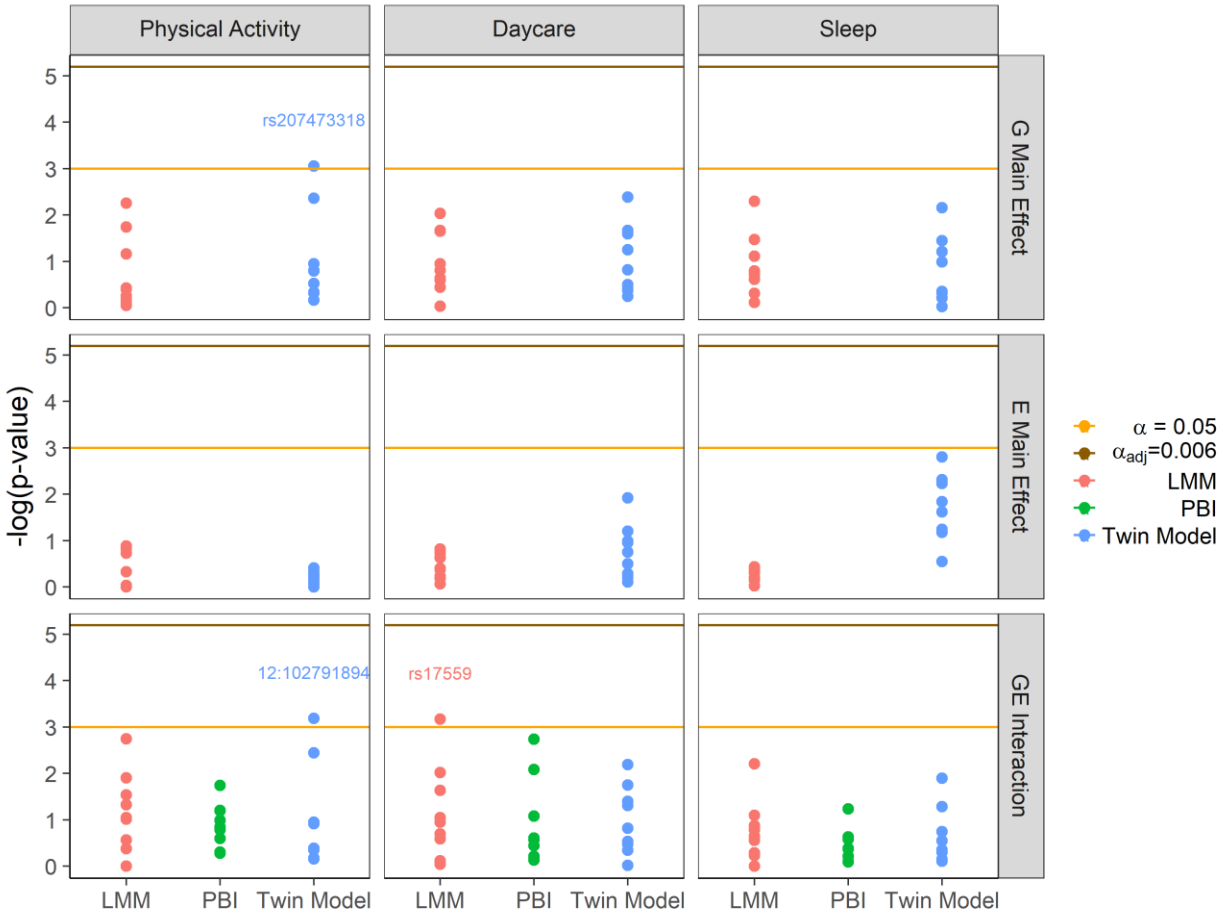

Figure S7. Significance of interactions between the IGF pathway genes (IGF-1, IGFALS) and environmental factors (physical activities, daycare attendance, sleep duration) analyzed using dataset without excluding BMI data points judged to be impossible

Negative natural log transformed p-values from the twin model, linear mixed model and PBI test were compared with unadjusted significance level (α = 0.05) and Bonferroni-adjusted level (α_{adj} = 0.006). Genetic and environmental main effects were evaluated by the twin and linear mixed models. All methods estimated significance of gene-environment interaction effects. Any effect with significance per unadjusted level (p-value < 0.05) is labeled for the involved IGF-1 or IGFALS SNP. LMM = linear mixed model; PBI = partition based score I test.

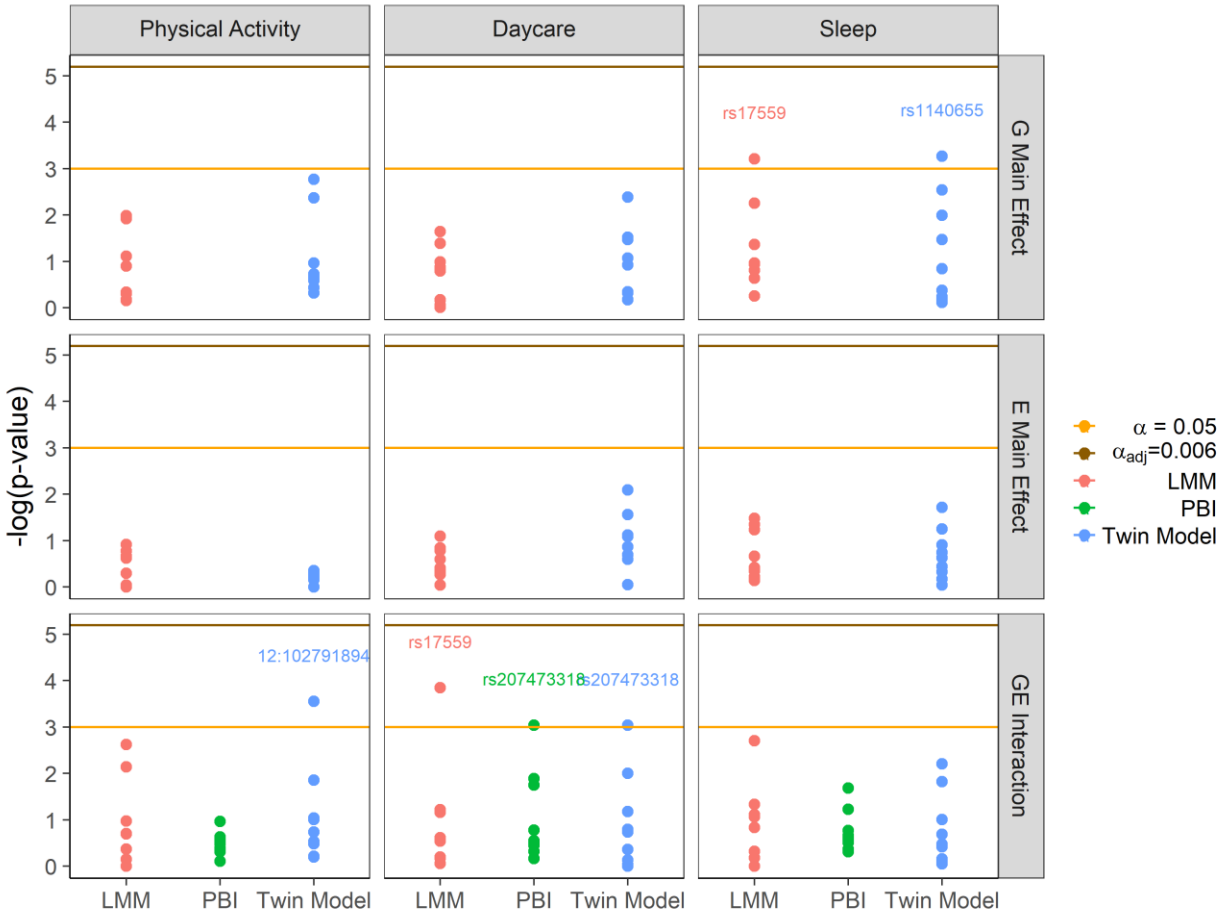

Figure S8. Significance of interactions between the IGF pathway genes (IGF-1, IGFALS) and environmental factors (physical activities, daycare attendance, sleep duration) analyzed using dataset with both Caucasian and non-Caucasian individuals

Negative natural log transformed p-values from the twin model, linear mixed model and PBI test were compared with unadjusted significance level (α = 0.05) and Bonferroni-adjusted level (α_{adj} = 0.006). Genetic and environmental main effects were evaluated by the twin and linear mixed models. All methods estimated significance of gene-environment interaction effects. Any effect with significance per unadjusted level (p-value < 0.05) is labeled for the involved IGF-1 or IGFALS SNP. LMM = linear mixed model; PBI = partition based score I test.