

1 A Comprehensive Genome-wide and Phenome-wide Examination of BMI and Obesity in a Northern  
2 Nevadan Cohort

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21 **Abstract**

22 The aggregation of Electronic Health Records (EHR) and personalized genetics leads to powerful  
23 discoveries relevant to population health. Here we perform genome-wide association studies (GWAS)  
24 and accompanying phenome-wide association studies (PheWAS) to validate phenotype-genotype  
25 associations of BMI, and to a greater extent, severe Class 2 obesity, using comprehensive diagnostic  
26 and clinical data from the EHR database of our cohort. Three GWASs of 500,000 variants on the  
27 Illumina platform of 6,645 Healthy Nevada participants identified several published and novel variants  
28 that affect BMI and obesity. Each GWAS was followed with two independent PheWASs to examine  
29 associations between extensive phenotypes (incidence of diagnoses, condition, or disease), significant  
30 SNPs, BMI, and incidence of extreme obesity. The first GWAS excludes DM2-diagnosed individuals  
31 and focuses on associations with BMI exclusively. The second GWAS examines the interplay between  
32 Type 2 Diabetes (DM2) and BMI. The intersection of significant variants of these two studies is  
33 surprising. The third complementary case-control GWAS, with cases defined as extremely obese (Class  
34 2 or 3 obesity), identifies strong associations with extreme obesity, including established variants in the  
35 *FTO* and *NEGR1* genes, as well as loci not yet linked to obesity. The PheWASs validate published  
36 associations between BMI and extreme obesity and incidence of specific diagnoses and conditions, yet  
37 also highlight novel links. This study emphasizes the importance of our extensive longitudinal EHR  
38 database to validate known associations and identify putative novel links with BMI and obesity.  
39

40 **Introduction**

41 The rate of obesity is growing at an alarming rate worldwide – fast enough to call it an epidemic. As  
42 obesity is a risk factor for developing typically related diseases such as Type 2 Diabetes Mellitus  
43 (DM2), cardiovascular disease and some cancers (Wang *et al.* 2011), the situation is becoming a public  
44 health concern. The percentage of obesity is rising nationwide, with current adult obesity rates at close

45 to 40%, up from 32% in 2004 (Ogden *et al.* 2006; TFAHRWJF 2018). In Nevada, the current adult  
46 obesity rate ( $BMI \geq 30$ ) is 27%, an increase from 21% in 2005 (TFAHRWJF 2018). Additionally, since  
47 2016, Nevada witnessed a significant increase in the percentage of adults who are overweight (the  
48 current rate is 66%) (TFAHRWJF 2018). Studies identified several genetic factors that influence the  
49 development of obesity with estimates on the heritability of the disease (40%-75%) (Stunkard *et al.*  
50 1986; 1990; Maes *et al.* 1997; Herrera and Lindgren 2010) and 65-80% (Malis *et al.* 2005).

51

52 High body mass index (BMI) and DM2 are known from many sources to be strongly related both  
53 epidemiologically and genetically (Kopelman 2007; Bays *et al.* 2007; Grarup *et al.* 2014; Cronin *et al.*  
54 2014); however, these two conditions share very few known causative variants (Grarup *et al.* 2014;  
55 Karaderi *et al.* 2015). Although a number of large meta-analyses of multiple genome-wide association  
56 studies (GWASs) have detected possible causative single nucleotide polymorphisms (SNPs) of obesity  
57 and increased BMI (Scuteri *et al.* 2007; Frayling *et al.* 2007; Dina *et al.* 2007; Zeggini *et al.* 2007;  
58 Yanagiya *et al.* 2007; Hinney *et al.* 2007; Hunt *et al.* 2008; Price *et al.* 2008; Grant *et al.* 2008; Hotta *et*  
59 *al.* 2008; Loos *et al.* 2008; Tan *et al.* 2008; Villalobos-Comparán *et al.* 2008; Thorleifsson *et al.* 2008;  
60 Willer *et al.* 2009; Meyre *et al.* 2009; Wing *et al.* 2009; Liu *et al.* 2009; Shimaoka *et al.* 2010; Fawcett  
61 and Barroso 2010; Speliotes *et al.* 2010; Wang *et al.* 2011; Prakash *et al.* 2011; Okada *et al.* 2012; Cha  
62 *et al.* 2012; Berndt *et al.* 2013; Wheeler *et al.* 2013; Graff *et al.* 2013; Olza *et al.* 2013; Boender *et al.*  
63 2014; Qureshi *et al.* 2017; Huđek *et al.* 2018; Gonzalez-Herrera *et al.* 2018), none, to the best of our  
64 knowledge, have included comprehensive GWASs on the quantitative BMI metric and on extreme  
65 obesity case-control simultaneously, as well as investigated phenotypic associations with BMI, obesity,  
66 and significant loci identified by the GWAS.

67

68 Our study begins with the Healthy Nevada Project (HNP), a project centered around a Northern Nevada  
69 cohort formed in 2016 and 2017 by Renown Health and the Desert Research Institute in Reno, NV to

70 investigate factors that may contribute to health outcomes in Northern Nevada. Its first phase provided  
71 10,000 individuals in Northern Nevada with genotyping using the 23andMe platform at no cost.  
72 Renown Health is the only tertiary care health system in the area, and 75% of these 10,000 individuals  
73 are cross-referenced in its extensive electronic health records (EHR) database. The Renown EHR  
74 database contains 86,610 BMI measurements for these 10,000 individuals over twelve years, along  
75 with comprehensive disease diagnoses, (e.g. diabetes or eating disorders) and other general conditions  
76 such as pregnancy, allowing for precise individual phenotypic classifications and thereby leading to  
77 more robust and meaningful phenotype-genotype associations.

78

79 The focus of the comprehensive GWAS-PheWAS examinations of the Healthy Nevada Project (HNP)  
80 cohort and its EHR database is two-fold: the first is to establish infrastructure to perform large-scale  
81 genome-wide and phenome-wide association investigations in alliance with complex electronic health  
82 care records; the second is to validate well-known published variants and associations with BMI and  
83 obesity in this cohort, as well as to identify possibly novel genotypic and phenotypic associations with  
84 BMI and extreme obesity.

85

86 The three GWASs identified several of the "usual suspects" for both BMI and obesity, such as *FTO* and  
87 *NEGR1*, that were shown to have a role in weight regulation (Scuteri *et al.* 2007; Frayling *et al.* 2007;  
88 Dina *et al.* 2007; Zeggini *et al.* 2007; Hinney *et al.* 2007; Hunt *et al.* 2008; Price *et al.* 2008; Grant *et  
89 al.* 2008; Hotta *et al.* 2008; Loos *et al.* 2008; Tan *et al.* 2008; Villalobos-Comparán *et al.* 2008;  
90 Thorleifsson *et al.* 2008; Willer *et al.* 2009; Meyre *et al.* 2009; Wing *et al.* 2009; Shimaoka *et al.* 2010;  
91 Fawcett and Barroso 2010; Speliotes *et al.* 2010; Herrera and Lindgren 2010; Wang *et al.* 2011;  
92 Prakash *et al.* 2011; Okada *et al.* 2012; Berndt *et al.* 2013; Wheeler *et al.* 2013; Graff *et al.* 2013; Olza  
93 *et al.* 2013; Boender *et al.* 2014; Qureshi *et al.* 2017; Gonzalez-Herrera *et al.* 2018). However, this

94 study also identified a number of novel BMI and obesity associations to genes which are differentially  
95 expressed in obese patients (Jiao *et al.* 2008; Pietiläinen *et al.* 2008; Nakajima *et al.* 2016).  
96 Additionally, using linked EHR, the PheWASs examined the pleiotropy of HNP BMI and obesity  
97 associated SNPs: whether these variants are linked with other endocrine or metabolic diagnoses or  
98 conditions of other nature. A second PheWAS identified many known phenotypes related to BMI and  
99 obesity, especially to DM2, abnormal glucose levels, hypertension, hyperlipidemia, sleep apnea,  
100 asthma and other less-studied BMI-related diagnoses.

101  
102

## 103 Materials and Methods

104 *The Renown EHR Database*

105 The Renown Health EHR system was instated in 2007 on the EPIC system (EPIC System Corporation,  
106 Verona, Wisconsin, USA), and currently contains lab results, diagnosis codes (ICD9 and ICD10) and  
107 demographics of more than one million patients seen in the hospital system since 2005.

108

109 *Sample Collection*

110 Saliva as a source of DNA was collected from 10,000 adults in Northern Nevada as the first phase of  
111 the Healthy Nevada Project to contribute to comprehensive population health studies in Nevada. The  
112 personal genetics company 23andMe, Inc. was used to genotype these individuals using the Oragene  
113 DX OGD-500.001 saliva kit [DNA Genotek, Ontario, Canada]. Genotypes are based on the Illumina  
114 Human OmniExpress-24 BeadChip platform [San Diego, CA, USA], that include approximately  
115 570,000 SNPs.

116

117 *IRB and ethics statement*

118 The study was reviewed and approved by the University of Nevada, Reno Institutional Review Board  
119 (IRB, project 956068-12). Participants in the Healthy Nevada Project undergo written and informed  
120 consent to having genetic information associated with electronic health information in a deidentified  
121 manner. All participants were eighteen years of age or older. Neither researchers nor participants have  
122 access to the complete EHR data and cannot map participants to patient identifiers. Patient identifiers  
123 are not incorporated into the EHR; rather, EHR and genetic data are linked in a separate environment  
124 via a unique identifier as approved by the IRB.

125

126 *Processing of EHR data*

127 Most cohort participants had multiple BMI recordings across the thirteen years of EHR; the mean  
128 number of BMI records across the individuals was 12.2 records, with 215 the maximum number of  
129 records for the cohort. For the 5,811 individuals with more than one recorded BMI measure, a simple  
130 quality control step was first performed before computing the average BMI value. More specifically,  
131 the coefficient of variation (CV) across the multiple BMI records for each participant was computed;  
132 for those with CV in the 90<sup>th</sup> quantile, any outlying BMI measure more than 1.5 standard deviations  
133 away from the mean BMI measure was excluded. Only 10% of participants' computed coefficients of  
134 variation was less than 0.10, indicating little variation exists across multiple records in most  
135 individuals. The additional quality control step excluded one or more outlying BMI records in 106  
136 individuals; these 701 BMI records included values such as "2823.42" and values less than 10.  
137 Examples of outliers include 158.38 in an individual's set of values with mean 25.3 and "2874" in an  
138 individual with mean BMI measure of 22.4. Additionally, this quality control step allowed the study to  
139 include pregnant women: of the 464 pregnant women with BMI recorded for pregnant and non-  
140 pregnant phases, outlying pregnancy-related BMI records were easily identified and removed. The raw  
141 BMI values and quality-controlled average BMI values are presented in Supplementary Figure S1.

142

143 *Genotyping and Quality Control*

144 Genotyping was performed by 23andMe using the Illumina Infimum DNA Human OmniExpress-24  
145 BeadChip V4 (Illumina, San Diego, CA). This genotyping platform consists of approximately 570,000  
146 SNPs. DNA extraction and genotyping were performed on saliva samples by the National Genetics  
147 Institute (NG1), a CLIA licensed clinical laboratory and a subsidiary of the Laboratory Corporation of  
148 America.

149

150 Raw genotype data were processed through a standard quality control process (Anderson *et al.* 2010;  
151 Verma *et al.* 2016; Schlauch *et al.* 2016; Verma *et al.* 2018; Schlauch *et al.* 2018). SNPs with a minor  
152 allele frequency (MAF) less than 0.005 were removed. SNPs that were out of Hardy Weinberg  
153 equilibrium (*p*-value < 1x10<sup>-6</sup>) were also excluded. Any SNP with a call rate less than 95% was  
154 removed; any individual with a call rate less than 95% was also excluded from further study. There was  
155 an observable bias within the African American sub-cohort, thus 89 African American participants  
156 were excluded from this study. Additionally, 107 patients with Type I Diabetes were removed, as were  
157 29 participants with eating disorders recorded into their health record. After quality control, this left  
158 500,508 high-quality SNPs and 6,645 participants in the BMI cohort with mean autosomal  
159 heterozygosity of 0.318. The same process yielded 5,994 participants when all individuals with DM2  
160 diagnoses were removed. Within the extreme obesity study, participants with BMI values between  
161 18.5 and 25 were considered as controls, while any participant with BMI at least 35 kg/m<sup>2</sup> was  
162 considered a case subject. Again, any individual with Type I Diabetes and recorded eating disorders  
163 was removed. This resulted in a cohort size of 2,994 participants with 984 extreme obese cases and  
164 2,012 lean controls with a mean autosomal heterozygosity of 0.316.

165

166 A standard principal component analysis (PCA) was performed on the genotype data to identify  
167 principal components to correct for population substructure. Genotype data were pruned to exclude

168 SNPs with high linkage disequilibrium using *PLINK* v1.9 (Purcell *et al.* 2007) and standard pruning  
169 parameters of 50 SNPs per sliding window; window size of five SNPs;  $r^2=0.5$  (Anderson *et al.* 2010).  
170 Regression models were adjusted by the first four principal components, decreasing the genomic  
171 inflation factor of all obesity and BMI traits to  $\lambda \leq 1.06$ .

172

173 *Genome-Wide Association Studies*

174 Using *PLINK*, we first performed a simple linear regression of BMI vs. genotype using the additive  
175 model (number of copies of the minor allele) including age, gender and the first four principal  
176 components as covariates to correct for any bias generated by these variables. In the first BMI study,  
177 participants with DM2 were excluded. The second BMI study included DM2-diagnosed participants  
178 and included DM2 as a covariate in the statistical model. To test associations between obesity and  
179 genotype, a standard case-control logistic regression was applied, adjusting for age, gender and the first  
180 four principal components. Total phenotypic variance explained by the SNPs was calculated by first  
181 producing a genetic relationship matrix of all SNPs on autosomal chromosomes in *PLINK*.  
182 Subsequently, a restricted maximum likelihood analysis was conducted using GTCA (Yang *et al.* 2011)  
183 on the relationship matrix to estimate the variance explained by the SNPs.

184

185 *Analysis of Variance*

186 The mean BMI values across genotypes presented in Supplementary Tables S1 and S2 correlate with  
187 negative and positive effect sizes: SNPs showing a negative effect size have a decrease in mean BMI  
188 values across the genotypes from left to right (homozygous in major allele, heterozygous, homozygous  
189 in minor allele). The 6,645 log-transformed quality-controlled and averaged BMI measures were nearly  
190 normally distributed. As one-way ANOVA computations are robust against even moderate deviations  
191 of normality (Blanca *et al.* 2017), parametric ANOVA methods were used to make comparisons across  
192 the genotypes. All ANOVA F-test *p*-values of the significant SNPs identified in the two BMI studies

193 are statistically significant at the alpha=0.05 level, even after a simple Bonferroni correction (.05/27  
194 =0.0019, and .05/20=0.0025, respectively). Supplementary Table S3 presents the proportion of obese  
195 cases across each genotype. A simple test of equal proportions (Pearson's chi-square test) is performed  
196 across these proportions. All *p*-values associated with the test of equal proportions in Supplementary  
197 Table S3 are also statistically significant at the 0.05 significance level upon a conservative Bonferroni  
198 multiple testing adjustment (.05/34=-.0015).

199 *Power of GWAS*

200 The software program QUANTO (Gauderman 2002) was used to calculate sample sizes to detect effect  
201 sizes in the range [0.5,1] and odds ratios in [1,1.5] with at least 80% power under the additive model, at  
202 a two-sided Type I error level of 5%. Using the rate of extreme obesity ( $BMI \geq 35 \text{ kg/m}^2$ ) as 14.5%  
203 from Ogden et al. (Ogden *et al.* 2006), the case-control GWAS study of approximately 1,000 cases and  
204 2,000 controls has sufficient statistical power ( $\geq 80\%$ ) with MAFs of 16% or greater to detect odds  
205 ratios of 1.225 or greater. As the MAF increases, the power to detect smaller odds ratios increases: for  
206 example, with a MAF of 25%, our sample size was adequate to detect odds ratios of size 1.18 or  
207 higher. With a small MAF of 8%, the power was also at least 80% to detect effect sizes as small as 0.58  
208 in the BMI GWAS cohort of 6,645. With MAF of 17%, power was at least 80% to detect effect sizes as  
209 small as 0.425. Larger MAFs clearly can detect larger effect sizes with the same sample size. Specific  
210 effect sizes and MAFs can be seen in Table 2.

211

212 *Phenome-Wide Association Study*

213 The **R** package PheWAS (Carroll *et al.* 2014) was used to perform two independent PheWAS analyses  
214 for each of our studies. The first examined associations between statistically significant SNPs identified  
215 in the respective GWASs and EHR phenotypes based on ICD codes. The second PheWAS identified  
216 associations between BMI levels or incidence of obesity, respectively, and ICD-based diagnoses. ICD9

217 and ICD10 codes for each individual in the cohort recorded in the Renown EHR were aggregated via a  
218 mapping from the Center for Medicare and Medicaid services  
219 (<https://www.cms.gov/Medicare/Coding/ICD10/2018-ICD-10-CM-and-GEMs.html>). A total of 22,693  
220 individual diagnoses mapped to 4,769 documented ICD9 codes. ICD9 codes were aggregated and  
221 converted into 1,814 individual phenotype groups (“phecodes”) using the PheWAS package as  
222 described in Carroll and Denny (Denny *et al.* 2013; Carroll *et al.* 2014). Of these, only the phecodes  
223 that included at least 20 cases were used for downstream analyses, following Carroll’s protocol (Carroll  
224 *et al.* 2014). Age and gender were standard covariates included in the PheWAS models. The first type  
225 of PheWAS detected associations between statistically significant SNPs ( $p < 1 \times 10^{-5}$ ) identified in each  
226 of the three GWASs above and case/control status of EHR phenotypes represented by ICD codes.  
227 Specifically, a logistic regression between the incidence (number of cases) of each phenotype group  
228 (phecode) and the additive genotypes of each statistically significant SNP was performed, including  
229 age and gender as covariates. Possible associations of the phecodes with at least 20 individuals with  
230 each previously detected SNP were assessed. Two levels of significance were computed: the first, on  
231 which the reported results are based, was generated by first calculating the adjusted  $p$ -values for the  
232 multiple hypothesis tests using the Benjamini-Hochberg false discovery rate (FDR) (Benjamini and  
233 Hochberg 1995) and selecting the raw  $p$ -value corresponding to the FDR = 0.1 significance level,  
234 following Denny’s protocol (Denny *et al.* 2013). This level is represented by a red line in PheWAS  
235 images. The second, more conservative, significance level was computed as a Bonferroni correction for  
236 all possible associations made in this analysis:  $p = 0.05 / N_{ps}$ , where  $N_{ps}$  is the sum of the number of  
237 phecodes tested for each individual SNP, across all identified SNPs. This significance level is  
238 represented by a blue line in PheWAS images.  
239  
240 A second PheWAS, as outlined in Carroll et al. (2014) (Carroll *et al.* 2014), was performed to examine  
241 associations between BMI, and secondarily, obesity, and the phecodes. Specifically, a linear regression

242 between the BMI measures and the case/control status of a phecode was performed (with age and  
243 gender as covariates) for each phecode including at least 20 individuals. Significance levels  
244 corresponded to the FDR value of 0.1 and are not shown in either figure due to space constraints. The  
245 Bonferroni corrections for the BMI study and obesity study were  $3.3 \times 10^{-5}$  and  $3.7 \times 10^{-5}$ , respectively,  
246 notably less conservative than the  $1 \times 10^{-15}$  significance level represented in the images.

247

## 248 **Results**

### 249 *Characteristics of cohort*

250 Our study consisted of 6,870 genotyped participants which had measures for age at consent, gender,  
251 ethnicity and BMI. From those genotyped individuals, we removed 107 participants who had Type I  
252 Diabetes, as well as 29 individuals who had any type of eating disorder. Preliminary quality control of  
253 the genotype data demonstrated a strong genetic bias within the African American subpopulation, and  
254 thus 89 African American participants were excluded prior to association analysis. Our final cohort  
255 characteristics of 6,645 individuals are described in Table 1, which illustrates the makeup of the cohort  
256 with respect to gender, age, ethnic origin, and standardized value of BMI after removal of outliers  
257 using a custom algorithm. For the extreme obesity (Class 2 and 3) case vs. control study, the normal  
258 (healthy) control range consisted of BMI values between 18.5 and 25 kg/m<sup>2</sup>, while the case obese  
259 values were any BMI  $\geq 35$  kg/m<sup>2</sup> (Hruby and Hu 2014). The number of participants in each range is  
260 displayed in column two of Table 1.

### 261 **Table 1. Cohort Characteristics**

	Association with BMI Measures	Association with Obesity
Cohort Size	6,645	2,996
Age (years)	$50.91 \pm 15.97$	$48.91 \pm 15.96$
Male (%)	2145 (32.28)	747 (24.93)
African American (%)	0 (0)	0 (0)
Asian (%)	157 (2.36)	84 (2.80)

Caucasian (%)	5,945 (89.47)	2653 (88.55)
Latino (%)	173 (2.60)	81 (2.70)
Native American (%)	40 (0.60)	18 (0.60)
Pacific Islander (%)	13 (0.20)	4 (0.13)
Unknown (%)	317 (4.77)	156 (5.21)
DM2	651 (9.8)	246 (8.2)
Quality-Controlled BMI Range	$28.58 \pm 6.41$	$22.57 \pm 1.64$ (Control)
		$40.15 \pm 4.99$ (Cases)

262 Table of cohort characteristics. Continuous variables are presented as mean  $\pm$  SD; categorical variables  
263 are presented as counts and percentages. All values were standardized to using a custom algorithm to  
264 remove outliers. BMI has the units of kg/m<sup>2</sup>.  
265

266 *GWAS of BMI in the Healthy Nevada Cohort*

267 Using quality-controlled BMI values, two separate GWASs were performed to find genotypic  
268 associations with BMI using *PLINK*. In the first association study, all individuals diagnosed with Type  
269 2 Diabetes (DM2) were excluded to focus on the association between the genotype and BMI under the  
270 additive model with adjustments for gender, age and the first four principal components (PC1-PC4).  
271 The second association analysis included all DM2-diagnosed individuals and added DM2 as a covariate  
272 to the model, again using the additive model with adjustments for gender, age, diabetes status, and  
273 PC1-PC4. Genomic inflation coefficients (lambda) were computed for the two separate cohorts: 1.06  
274 for the association without DM as a covariate, and 1.06 for the association where DM is a covariate.  
275 Any SNP with association *p*-value of  $p < 1 \times 10^{-5}$  was considered a statistically significant association,  
276 based on the standard of the NHGRI-EBI Catalog of published genome-wide association studies  
277 [<https://www.ebi.ac.uk/gwas/docs/methods/criteria>], as well as obesity studies performed by Frayling et  
278 al. (Frayling *et al.* 2007). Genetic variance in the BMI study with DM2 cases removed was 15.78%;  
279 genetic variance was 17.49% in the BMI study with DM2 cases included.  
280 The first GWAS was performed on 5,994 total participants without DM2 and identified 20 SNPs across  
281 seven chromosomes at statistical significance defined by  $p < 1 \times 10^{-5}$  (Table 2). The majority of these

282 mapped to the *FTO* gene on chromosome 16, while two SNPs mapped to *TDH* on chromosome 8  
283 (Supplementary Figure S2). Of the 20 SNPs, 15 were shown to be associated with BMI in previous  
284 publications (Scuteri *et al.* 2007; Frayling *et al.* 2007; Dina *et al.* 2007; Zeggini *et al.* 2007; Yanagiya  
285 *et al.* 2007; Hinney *et al.* 2007; Hunt *et al.* 2008; Price *et al.* 2008; Grant *et al.* 2008; Hotta *et al.* 2008;  
286 Loos *et al.* 2008; Tan *et al.* 2008; Villalobos-Comparán *et al.* 2008; Thorleifsson *et al.* 2008; Willer *et*  
287 *al.* 2009; Meyre *et al.* 2009; Wing *et al.* 2009; Liu *et al.* 2009; Shimaoka *et al.* 2010; Fawcett and  
288 Barroso 2010; Speliotes *et al.* 2010; Wang *et al.* 2011; Prakash *et al.* 2011; Okada *et al.* 2012; Cha *et*  
289 *al.* 2012; Berndt *et al.* 2013; Wheeler *et al.* 2013; Graff *et al.* 2013; Olza *et al.* 2013; Boender *et al.*  
290 2014; Qureshi *et al.* 2017; Huđek *et al.* 2018; Gonzalez-Herrera *et al.* 2018). A large majority of the  
291 SNPs (17/20) lie within noncoding regions of genes and are intronic in nature. It is interesting to note  
292 that our strongest associations lie within the *FTO* gene ( $p < 3.5 \times 10^{-6}$ ). Results are presented in Table 2:  
293 **BMI without DM2** lists the significant associations of our cohort that exclude all DM2 diagnoses.  
294 **BMI with DM2** presents significant associations with BMI in the cohort that includes participants with  
295 a DM2 diagnosis. Effect sizes (and their standard deviations) are presented as change in BMI per each  
296 copy of the minor allele. Raw per-SNP  $p$ -values are presented.

297

**Table 2. Statistically Significant BMI GWAS SNPs**

rsID	Chrom	Cyto Region	Associated Gene	Minor Allele	MAF	$\beta$	(SE)	GWAS p-value	Mutation Classification
<b>BMI without DM2</b>									
rs1620977	chr1	p31.1	NEGR1	A	27.29	0.5819	0.125	3.30x10 <sup>-6</sup>	intron
rs871122	chr5	p15.32	ADAMTS16	T	16.61	0.6837	0.1528	7.78x10 <sup>-6</sup>	intron
rs4839813	chr6	q16.1	FUT9	T	9.69	0.8755	0.1876	3.13x10 <sup>-6</sup>	intron
rs11774673	chr8	p23.1	NA	C	48.21	0.5083	0.1119	5.69x10 <sup>-6</sup>	unknown
rs2060457	chr8	p23.1	TDH	T	48.58	0.5584	0.1119	6.24x10 <sup>-7</sup>	intron
rs2293859	chr8	p23.1	TDH	G	48.73	0.5506	0.1119	8.80x10 <sup>-7</sup>	ncRNA
rs10733990	chr10	p12.1	NA	A	30.53	0.5857	0.1199	1.06x10 <sup>-6</sup>	unknown
rs10875969	chr12	q13.12	NCKAP5L	A	42.38	0.505	0.1133	8.41x10 <sup>-6</sup>	intron
rs9937053	chr16	q12.2	FTO	A	40.76	0.5193	0.1133	4.69x10 <sup>-6</sup>	intron
rs9930333	chr16	q12.2	FTO	G	40.78	0.5161	0.113	5.08x10 <sup>-6</sup>	intron
rs9940128	chr16	q12.2	FTO	A	40.75	0.5272	0.1131	3.19x10 <sup>-6</sup>	intron
rs1421085	chr16	q12.2	FTO	C	38.43	0.5999	0.1147	1.75x10 <sup>-7</sup>	intron
rs1558902	chr16	q12.2	FTO	A	38.46	0.6007	0.1147	1.67x10 <sup>-7</sup>	intron
rs1121980	chr16	q12.2	FTO	A	40.85	0.5272	0.1128	3.03x10 <sup>-6</sup>	intron
rs17817449	chr16	q12.2	FTO	G	37.93	0.5562	0.1143	1.17x10 <sup>-6</sup>	intron
rs8043757	chr16	q12.2	FTO	T	37.98	0.5612	0.1143	9.29x10 <sup>-7</sup>	intron
rs8050136	chr16	q12.2	FTO	A	37.94	0.5636	0.1143	8.34x10 <sup>-7</sup>	intron
rs3751812	chr16	q12.2	FTO	T	37.52	0.548	0.1149	1.90x10 <sup>-6</sup>	intron
rs9939609	chr16	q12.2	FTO	A	38.06	0.5504	0.1143	1.50x10 <sup>-6</sup>	intron
rs12149832	chr16	q12.2	FTO	A	39.1	0.534	0.1142	3.01x10 <sup>-6</sup>	intron
<b>BMI with DM2</b>									
rs1776012	chr1	p31.1	NEGR1	G	47.74	-0.4848	0.1092	9.13x10 <sup>-6</sup>	intron
rs11774673	chr8	p23.1	NA	C	48.21	0.5149	0.1086	2.14x10 <sup>-6</sup>	unknown
rs1435277	chr8	p23.1	NA	C	44.39	-0.4944	0.1112	8.90x10 <sup>-6</sup>	near-gene-5
rs11250129	chr8	p23.1	TDH	A	48.12	0.5115	0.1089	2.67x10 <sup>-6</sup>	intron
rs2060457	chr8	p23.1	TDH	T	48.58	0.5802	0.1085	9.30x10 <sup>-8</sup>	intron
rs2293859	chr8	p23.1	TDH	G	48.73	0.5722	0.1085	1.37x10 <sup>-7</sup>	ncRNA
rs2246606	chr8	p23.1	TDH	G	42.95	-0.539	0.1115	1.37x10 <sup>-6</sup>	intron
rs2736280	chr8	p23.1	TDH	C	48.22	-0.5308	0.1098	1.36x10 <sup>-6</sup>	intron
rs2572386	chr8	p23.1	FAM167A	G	42.27	-0.5308	0.1115	1.96x10 <sup>-6</sup>	intron
rs2948300	chr8	p23.1	NA	T	49.03	0.5034	0.1099	4.70x10 <sup>-6</sup>	unknown
rs12412241	chr10	p14	NA	A	29.62	-0.5474	0.1171	3.01x10 <sup>-6</sup>	unknown
rs11041833	chr11	p15.4	LMO1	A	41.01	-0.4841	0.1082	7.77x10 <sup>-6</sup>	intron
rs9937053	chr16	q12.2	FTO	A	40.76	0.5002	0.1105	6.04x10 <sup>-6</sup>	intron
rs9930333	chr16	q12.2	FTO	G	40.78	0.4989	0.1102	6.10x10 <sup>-6</sup>	intron
rs9940128	chr16	q12.2	FTO	A	40.75	0.5078	0.1102	4.18x10 <sup>-6</sup>	intron

rs1421085	chr16	q12.2	FTO	C	38.43	0.5616	0.1117	5.04x10 <sup>-7</sup>	intron
rs1558902	chr16	q12.2	FTO	A	38.46	0.5625	0.1116	4.80x10 <sup>-7</sup>	intron
rs1121980	chr16	q12.2	FTO	A	40.85	0.5063	0.11	4.27x10 <sup>-6</sup>	intron
rs17817449	chr16	q12.2	FTO	G	37.93	0.5206	0.1113	2.94x10 <sup>-6</sup>	intron
rs8043757	chr16	q12.2	FTO	T	37.98	0.525	0.1112	2.41x10 <sup>-6</sup>	intron
rs8050136	chr16	q12.2	FTO	A	37.94	0.5244	0.1112	2.47x10 <sup>-6</sup>	intron
rs3751812	chr16	q12.2	FTO	T	37.52	0.5119	0.1118	4.74x10 <sup>-6</sup>	intron
rs9939609	chr16	q12.2	FTO	A	38.06	0.513	0.1112	4.06x10 <sup>-6</sup>	intron
rs12149832	chr16	q12.2	FTO	A	39.1	0.5034	0.1111	5.98x10 <sup>-6</sup>	intron
rs11651343	chr17	p13.3	RTN4RL1	T	8.17	0.9039	0.1974	4.75x10 <sup>-6</sup>	intron
rs750456	chr19	q13.33	CABP5	C	26.19	-0.5486	0.1221	7.15x10 <sup>-6</sup>	intron
rs8105198	chr19	q13.33	CABP5	G	17.18	-0.6466	0.1417	5.14x10 <sup>-6</sup>	coding-synon

298 This table represents statistically significant associations with BMI in our cohort.

299

300 Statistical analysis with *PLINK* demonstrated that DM2 is a significant predictor of BMI, with the *p*-  
301 value of its coefficient consistently less than  $p < 2 \times 10^{-16}$  in each per-SNP linear regression. The entire  
302 cohort includes 6,645 participants: of those, 651 have a diagnosis of DM2 in their twelve-year medical  
303 history. A GWAS applied to this larger cohort identified 27 statistically significant SNPs across seven  
304 chromosomes associated with BMI at  $p < 1 \times 10^{-5}$  (Figure 1). In comparison to the original GWAS  
305 (without DM2 individuals), 75% of the SNPs (15/20) were also found to be associated with BMI in this  
306 association. Furthermore, 77% of the SNPs in this second GWAS (21/27) were previously associated  
307 with BMI in previous research studies (Scuteri *et al.* 2007; Frayling *et al.* 2007; Dina *et al.* 2007;  
308 Zeggini *et al.* 2007; Yanagiya *et al.* 2007; Hinney *et al.* 2007; Hunt *et al.* 2008; Price *et al.* 2008; Grant  
309 *et al.* 2008; Hotta *et al.* 2008; Loos *et al.* 2008; Tan *et al.* 2008; Villalobos-Comparán *et al.* 2008;  
310 Thorleifsson *et al.* 2008; Willer *et al.* 2009; Meyre *et al.* 2009; Wing *et al.* 2009; Liu *et al.* 2009;  
311 Shimaoka *et al.* 2010; Fawcett and Barroso 2010; Speliotes *et al.* 2010; Wang *et al.* 2011; Prakash *et  
312 al.* 2011; Okada *et al.* 2012; Cha *et al.* 2012; Berndt *et al.* 2013; Wheeler *et al.* 2013; Graff *et al.* 2013;  
313 Olza *et al.* 2013; Boender *et al.* 2014; Christensen *et al.* 2015; Nakajima *et al.* 2016; Thomsen *et al.*  
314 2016; Qureshi *et al.* 2017; Huđek *et al.* 2018; Gonzalez-Herrera *et al.* 2018). With the addition of DM2

315 as a covariate, the GWAS identified several additional SNPs on chromosome 8, as well as SNPs on  
316 chromosomes 17 and 19. These additional SNPs were previously linked to BMI and obesity in other  
317 studies (Christensen *et al.* 2015; Nakajima *et al.* 2016; Thomsen *et al.* 2016). Manhattan plots for the  
318 two BMI GWAS studies are presented in Figure 1 and Supplementary Figure S2, with the linear  
319 associations results presented in Table 2.  
320 The SNP on chromosome 17 is of particular interest, as it has the largest effect of any SNP identified in  
321 our study ( $\beta=0.90$ ). It is also the rarest SNP tested in our cohort with minor allele frequency (MAF)  
322 8.17%. The median MAF across the strongest associative SNPs in both studies is 40%, which  
323 demonstrates that most of the SNPs are common and thus result in relatively moderate individual effect  
324 sizes. Most of the SNPs lie within noncoding intronic regions. While these SNPs would not alter the  
325 amino acid coding sequence of the translated protein, several previous studies articulated that  
326 polymorphisms within introns can affect intron splicing as well as transcriptional and translational  
327 efficiency, and therefore may be linked to disease (Lalonde *et al.* 2011).

328  
329 *Case-Control GWAS of Extreme Obesity in the Healthy Nevada Cohort*

330 A complementary GWAS was performed to identify genotype-phenotype links in extreme obesity  
331 ( $BMI \geq 35$ ) versus non-obese ( $BMI$  between 18.5 and  $25 \text{ kg/m}^2$ ) in our cohort. This study incorporated  
332 2996 participants (984 extreme obese cases, 2012 non-obese controls), and under the log-additive  
333 model with adjustments for gender, age and the first four principal components, identified 26 SNPs  
334 across six chromosomes that were associated with obesity at  $p < 1 \times 10^{-5}$ , with approximately 70%  
335 associated with obesity and BMI in prior studies (Figure 2). The percentage of phenotypic variance  
336 attributed to genetic variation was 15.7%. The genomic inflation coefficient (lambda) for the obesity  
337 cohort was computed as 1.05. We also include eight SNPs found slightly above the significance  
338 threshold in the *FTO* gene that are reported in several studies as obesity-related (Ehrlich and  
339 Friedenberg 2016; West *et al.* 2018). In comparison to the two quantitative-trait BMI GWASs, this

340 study identified several more associations around the *NEGR1* gene on chromosome 1. We also  
341 identified SNPs in two genes, *PFKFB3* and *CABP5*, which are associated with obesity in other studies  
342 (Scuteri *et al.* 2007; Jiao *et al.* 2008; Nakajima *et al.* 2016). Note that all the mutations in the *FTO* gene  
343 increase the odds of obesity risk. Table 3 lists the strongest SNPs associated in our extreme obese vs.  
344 non-obese GWAS. Effect sizes and their standard deviations are presented as odds ratios. Raw *p*-values  
345 generated by the GWAS are also presented.

346 ***Table 3. Statistically Significant Obesity GWAS SNPs***

347

rsID	Chrom	Cyto Region	Associated Gene	Minor Allele	MAF	Odds Ratio	(SE)	GWAS p-value	Mutation Classification
rs1776012	chr1	p31.1	<i>NEGRI</i>	G	47.36	0.7365	0.05775	1.19x10 <sup>-7</sup>	intron
rs9424977	chr1	p31.1	<i>NEGRI</i>	C	47.09	0.7411	0.05745	1.83x10 <sup>-7</sup>	intron
rs1620977	chr1	p31.1	<i>NEGRI</i>	A	27.31	1.369	0.06213	4.21x10 <sup>-7</sup>	intron
rs1870676	chr1	p31.1	<i>NEGRI</i>	T	46.97	0.7403	0.05773	1.90x10 <sup>-7</sup>	intron
rs3101336	chr1	p31.1	NA	T	35.93	0.7678	0.0597	9.60x10 <sup>-6</sup>	unknown
rs2568958	chr1	p31.1	NA	G	35.94	0.767	0.05967	8.79x10 <sup>-6</sup>	unknown
rs2815752	chr1	p31.1	NA	G	35.94	0.767	0.05967	8.79x10 <sup>-6</sup>	unknown
rs2173676	chr5	q14.1	NA	C	26.52	0.7433	0.0647	4.56x10 <sup>-6</sup>	unknown
rs11774673	chr8	p23.1	NA	C	48.25	1.304	0.05778	4.25x10 <sup>-6</sup>	unknown
rs1435277	chr8	p23.1	NA	C	44.28	0.7419	0.05898	4.15x10 <sup>-7</sup>	near-gene-5
rs11250129	chr8	p23.1	<i>TDH</i>	A	48.26	1.314	0.05796	2.49x10 <sup>-6</sup>	intron
rs2060457	chr8	p23.1	<i>TDH</i>	T	48.76	1.379	0.05797	2.88x10 <sup>-8</sup>	intron
rs2293859	chr8	p23.1	<i>TDH</i>	G	48.81	1.375	0.05798	4.06x10 <sup>-8</sup>	ncRNA
rs2246606	chr8	p23.1	<i>TDH</i>	G	42.76	0.7357	0.05905	2.01x10 <sup>-7</sup>	intron
rs2736280	chr8	p23.1	<i>TDH</i>	C	48.23	0.727	0.05863	5.41x10 <sup>-8</sup>	intron
rs2572386	chr8	p23.1	<i>FAMI67A</i>	G	41.91	0.7378	0.05941	3.07x10 <sup>-7</sup>	intron
rs1435282	chr8	p23.1	<i>FAMI67A</i>	A	45.57	0.7631	0.05786	2.95x10 <sup>-6</sup>	intron
rs2948300	chr8	p23.1	NA	T	49.06	1.325	0.05809	1.30x10 <sup>-6</sup>	unknown
rs2953802	chr8	p23.1	NA	A	42	1.318	0.05734	1.47x10 <sup>-6</sup>	unknown
rs435581	chr8	p23.1	NA	A	45.19	0.7609	0.05802	2.50x10 <sup>-6</sup>	unknown
rs680951	chr10	p15.1	<i>PFKFB3</i>	G	33.83	0.7614	0.06116	8.26x10 <sup>-6</sup>	intron
rs666595	chr10	p15.1	<i>PFKFB3</i>	A	31.47	0.7537	0.06256	6.22x10 <sup>-6</sup>	intron
rs2058426	chr12	p13.31	NA	A	49.67	0.7798	0.05614	9.40x10 <sup>-6</sup>	near-gene-3
rs2241005	chr12	p13.31	<i>KLRB1</i>	C	49.98	0.7768	0.05608	6.65x10 <sup>-6</sup>	intron
rs1421085	chr16	q12.2	<i>FTO</i>	C	38.18	1.28	0.05766	1.90x10 <sup>-5</sup>	intron
rs1558902	chr16	q12.2	<i>FTO</i>	A	38.21	1.281	0.0576	1.72x10 <sup>-5</sup>	intron
rs17817449	chr16	q12.2	<i>FTO</i>	G	37.63	1.261	0.05732	5.15x10 <sup>-5</sup>	intron
rs8043757	chr16	q12.2	<i>FTO</i>	T	37.66	1.264	0.05733	4.42x10 <sup>-5</sup>	intron
rs8050136	chr16	q12.2	<i>FTO</i>	A	37.66	1.263	0.05733	4.72x10 <sup>-5</sup>	intron
rs3751812	chr16	q12.2	<i>FTO</i>	T	37.27	1.26	0.05764	6.13x10 <sup>-5</sup>	intron
rs9939609	chr16	q12.2	<i>FTO</i>	A	37.83	1.254	0.05737	7.79x10 <sup>-5</sup>	intron
rs2267770	chr16	p13.2	<i>GRIN2A</i>	T	41.01	0.7859	0.0575	2.79x10 <sup>-5</sup>	intron
rs750456	chr19	q13.33	<i>CABP5</i>	C	26.1	0.7405	0.06615	5.58x10 <sup>-6</sup>	intron
rs8105198	chr19	q13.33	<i>CABP5</i>	G	16.77	0.7026	0.07836	6.67x10 <sup>-6</sup>	coding-synon

348

349

350 This table presents statistically significant associations with extreme obesity in the case-control study.  
351 From our three separate GWASs, we identified fifteen different chromosomal cytoband regions across  
352 ten chromosomes associated with at least one BMI or obesity-related trait. All but three of those  
353 cytoband regions contained a gene, while the remaining cytoband hits were in noncoding regions of the  
354 genome. Approximately 70% of the SNPs identified in this study were linked to BMI and obesity in  
355 prior studies (Tables 2 and 3), validating our methods (Scuteri *et al.* 2007; Frayling *et al.* 2007; Dina *et*  
356 *al.* 2007; Yanagiya *et al.* 2007; Hinney *et al.* 2007; Jiao *et al.* 2008; Pietiläinen *et al.* 2008; Grant *et al.*  
357 2008; Hotta *et al.* 2008; Thorleifsson *et al.* 2008; Joe *et al.* 2009; Nakajima *et al.* 2016; Thomsen *et al.*  
358 2016; Justice *et al.* 2017). The functions of the genes which lie within the cytoband regions are outlined  
359 in Table 4.

360 **Table 4. Table Presenting Gene Functions**

Gene	Gene Description	Region	Trait	Function	Reference
NEGR1	Neuronal Growth Regulator 1	p31.1	BMI No DM2 BMI w DM2 obesity	Cell-adhesion molecule and regulator of cellular processes as neurite outgrowth and synapse formation	(Hashimoto <i>et al.</i> 2008; Boender <i>et al.</i> 2014)
ADAMTS16	ADAM Metallopeptidase with Thrombospondin Type 1 Motif 16	p15.32	BMI No DM2	May be a secreted proteinase	(Surridge <i>et al.</i> 2009)
FUT9	Fucosyltransferase 9	q16.1	BMI No DM2	Plays a role in the biosynthesis of Lewis X (LeX) antigen precursor polysaccharides	(Gouveia <i>et al.</i> 2012)
TDH	L-Threonine Dehydrogenase	p23.1	BMI No DM2 BMI w DM2 obesity	Likely pseudogene that cannot produce functional protein	(Yanagiya <i>et al.</i> 2007)
NCKAP5L	NCK Associated Protein 5 Like	q13.12	BMI No DM2	Regulates microtubule organization and stabilization	(Mori <i>et al.</i> 2015)
FTO	Fat Mass and Obesity Associated	q12.2	BMI No DM2 BMI w DM2 obesity	Mediates oxidative demethylation of different RNA species. Acts as a regulator of fat and energy homeostasis	(Frayling <i>et al.</i> 2007; Jia <i>et al.</i> 2011; Wei <i>et al.</i> 2018)
FAM167A	Family with Sequence Similarity 167 Member A	p23.1	BMI w DM2 obesity	Unknown function. May have a role in autoimmune diseases	(Chen <i>et al.</i> 2015)
LMO1	LIM domain only 1	p15.4	BMI w DM2	Transcriptional regulator through binding of transcription factors	(Wang <i>et al.</i> 2010)

RTN4RL1	Reticulon 4 Receptor Like 1	p13.3	BMI w DM2	Cell surface receptor	(Pignot <i>et al.</i> 2003)
CABP5	Calcium Binding Protein 5	q13.33	BMI w DM2 obesity	Plays a role in calcium mediated cellular signal transduction	(Haeseler <i>et al.</i> 2002)
PFKFB3	6-Phosphofructo-2-kinase/Fructose-2,6-biphosphatase 3	p15.1	obesity	Potent regulator of glycolysis	(Nelson and Cox 2005; De Bock <i>et al.</i> 2013)
KLRB1	Killer Cell Lectin-like Receptor Subfamily B, Member 1	p13.31	obesity	Possible regular of natural killer cells	(Rother <i>et al.</i> 2015)
GRIN2A	Glutamate Ionotropic Receptor NMDA Type Subunit 2A	p13.2	obesity	Possibly has a role in learning and long-term memory	(Micu <i>et al.</i> 2006)

361 This table presents functions of genes associated to all SNPs found significantly associated to one or  
362 more BMI and obesity traits in the GWASs.  
363

364 *Analysis of Variance*

365 The mean BMI values across genotypes presented in Supplementary Tables S1 and S2 correlate with  
366 negative and positive effect sizes: SNPs showing a negative effect size have a decrease in mean BMI  
367 values across the genotypes from left to right (homozygous in major allele, heterozygous, homozygous  
368 in minor allele). Note that BMI levels increase with the increase of the number of minor alleles, which  
369 is typical of variants in *FTO* (Frayling *et al.* 2007). All ANOVA F-test *p*-values of the significant SNPs  
370 identified in the two BMI studies are statistically significant at the alpha=0.05 level, even after a simple  
371 Bonferroni correction (.05/27 =0.0019, and .05/20=0.0025, respectively). Supplementary Table S3  
372 presents the proportion of extremely obese cases across each genotype. A box and whisker figure of  
373 ANOVA results for one of the strongest associations (rs9939609) is shown in Supplementary Figure  
374 S3.

375

376 *PheWAS of BMI and Obesity*

377 Beyond the GWASs, we present here two comprehensive PheWAS studies that follow each GWAS.  
378 The first examines pleiotropy, i.e., whether additional phenotypic associations exist between the  
379 statistically significant SNPs associated with BMI or obesity in our cohort. The second investigates

380 which EHR phenotype groups are associated with BMI; more specifically, the analysis identifies  
381 whether the number of individuals in an EHR phenotype group is a predictor of BMI and/or extreme  
382 obesity.  
383 A PheWAS tested the 20 statistically significant SNPs identified in the first BMI GWAS (the sub-  
384 cohort with no DM2-diagnoses) for association with 562 EHR phenotypes and resulted in no  
385 statistically significant associations at the false discovery rate of 0.1 (data not shown). The top two  
386 associations showed that a locus on *FUT9* (rs4839813) associated with obesity, and rs1620977 on  
387 *NEGR1* associated with morbid obesity with raw *p*-values  $p=2.3\times 10^{-5}$  and  $2.8\times 10^{-5}$ , respectively. The  
388 second PheWAS identified 179 EHR (phenotypic) associations of BMI (DM2-diagnosed participants  
389 excluded) with  $p<2\times 10^{-2}$ , that associates to an adjusted *p*-value of 0.1 (see Materials and Methods).  
390 Included in the strongest phenotypic associations are obesity, morbid obesity, and overweight ( $p<1\times 10^{-80}$ ),  
391 sleep apnea ( $p<1\times 10^{-45}$ ), hypertension ( $p<1\times 10^{-40}$ ), abnormal glucose ( $p<1\times 10^{-25}$ ), hyperlipidemia,  
392 asthma, GERD, osteoporosis, and others. (Data not shown).  
393  
394 The PheWAS of the second BMI GWAS (DM2-diagnosed individuals included) examined whether  
395 633 EHR phenotype groups containing at least 20 participants are dependent on the genotypes of the 27  
396 statistically significant SNPs associated with BMI in our cohort (Figure 3). Results of this PheWAS  
397 indicate that *TDH* and *FAMA167-ASI* are strongly associated with DM2. Variants in the *FTO* gene  
398 associate with obesity and overweight phenotypes. An association with hypertension and essential  
399 hypertension and the locus rs12412241 on chromosome 10 was detected ( $p<1\times 10^{-4}$ ). Several strong  
400 associations with *FTO* loci and the prostate-specific antigen (PSA) were also found. Variants in the  
401 *FTO* gene also associated with obesity at the  $p=3\times 10^{-4}$  level, and with hypercholesterolemia with  
402  $p=1\times 10^{-3}$  level. Significant associations ( $p<1.02\times 10^{-4}$ , associated to an adjusted *p*-value of 0.1, see  
403 Materials and Methods) are included in Table 5 and illustrated in Figure 3, where the blue line  
404 represents the Bonferroni correction of  $p=3\times 10^{-6}$ . The second PheWAS examined links between BMI

405 and the 1,523 EHR phenotype groups containing at least 20 individuals in this cohort and showed that  
406 301 such clinical phenotypes groups associated with significance  $p < 1.96 \times 10^{-2}$ . (This significance level  
407 is associated to an adjusted  $p$ -value of 0.1, as described in the Materials and Methods) These are shown  
408 in Figure 4. Included in the highest associations are obesity, morbid obesity, and overweight ( $p < 1 \times 10^{-100}$ )  
409 DM2 ( $p < 1 \times 10^{-87}$ ), hypertension ( $p < 1 \times 10^{-82}$ ), sleep apnea ( $p < 1 \times 10^{-80}$ ), abnormal glucose ( $p < 1 \times 10^{-53}$ ),  
410 hyperlipidemia, asthma and other respiratory disorders, GERD, edema, liver disease, mood  
411 disorders, polycystic ovaries, and others. Significant associations are presented in Supplementary Table  
412 S4 and Figure 4. Only associations at  $p < 1 \times 10^{-15}$  are annotated in the image for ease of viewing. Note  
413 that a single-SNP Bonferroni correction results in a significance level of  $3.3 \times 10^{-5}$ .

414

415 We performed the same two PheWASs on the case-control GWAS. The first PheWAS identified  
416 possible associations between 34 SNPs and 372 phenotype groups with at least 20 individuals (Figure  
417 5). The significance level corresponding to an FDR of 0.1 was  $p = 3.85 \times 10^{-4}$ , resulting in 50 significant  
418 associations. The Bonferroni correction  $p$ -value is  $4 \times 10^{-6}$  and shown in blue (see Materials and  
419 Methods). The strongest associations occurred between the *NEGR1* and *KLRB1* gene and obesity and  
420 morbid obesity and the *NEGR1* gene and abnormal glucose. The *NEGR1* gene, along with *TDH* and  
421 *FAM167A-AS1*, also associated with impaired fasting glucose and diabetes, respectively, at a slightly  
422 lower, yet still significant  $p$ -values. The locus rs2948300 on chromosome 8 associated with essential  
423 hypertension. The SNP rs1620977 on *NEGR1* is linked with incidence of bronchitis. Additionally,  
424 several strong associations of irritable bowel syndrome (IBS) and digestive disorders with loci in  
425 *CABP5* are shown. Results are pictured in Figure 5 and included in Table 6.

426 The second PheWAS in this case-control study identified possible links between 1,362 EHR phenotype  
427 groups with at least 20 individuals and the incidence of extreme obesity (Figure 6). The significant  
428 threshold of  $p = 1.4 \times 10^{-2}$  enabled an FDR of 0.1. At this significance level, 191 significant associations  
429 were identified, including obesity ( $p < 1 \times 10^{-134}$ ), hypertension ( $p < 1 \times 10^{-65}$ ), sleep apnea ( $p < 1 \times 10^{-43}$ ),

430 abnormal glucose ( $p < 1 \times 10^{-40}$ ), hyperlipidemia, asthma, and GERD. The high level of association with  
431 obesity validates our methods. These associations are shown in Figure 6, with phenotypes annotated  
432 above a significance level of  $1 \times 10^{-15}$  for ease of viewing. A line is drawn at the significance level  $1 \times 10^{-$   
433  $^{15}$  as guidance. Results are included in Supplementary Table S5. Note that a single-SNP Bonferroni  
434 correction results in a significance level of  $3.7 \times 10^{-5}$ .

435 **Table 5. Statistically Significant BMI with DM2 PheWAS SNPs**

Phenotype	Description	SNP	Gene	$\beta$	(SE)	Odds Ratio	PheWAS <i>p</i> -value	N	Cases	Controls
250.2	Type 2 Diabetes	rs2246606	TDH	-0.331	0.073	0.718	$6.32 \times 10^{-6}$	5566	459	5107
796	Elevated prostate specific antigen [PSA]	rs9937053	FTO	0.882	0.197	2.416	$7.76 \times 10^{-6}$	6293	71	6222
796	Elevated prostate specific antigen [PSA]	rs9930333	FTO	0.881	0.197	2.413	$7.88 \times 10^{-6}$	6297	71	6226
796	Elevated prostate specific antigen [PSA]	rs9940128	FTO	0.875	0.197	2.398	$8.66 \times 10^{-6}$	6297	71	6226
796	Elevated prostate specific antigen [PSA]	rs1121980	FTO	0.872	0.197	2.391	$9.60 \times 10^{-6}$	6298	71	6227
250	Diabetes Mellitus	rs2246606	TDH	-0.318	0.072	0.728	$1.14 \times 10^{-5}$	5577	470	5107
796	Elevated prostate specific antigen [PSA]	rs1421085	FTO	0.855	0.197	2.351	$1.41 \times 10^{-5}$	6298	71	6227
796	Elevated prostate specific antigen [PSA]	rs1558902	FTO	0.854	0.197	2.350	$1.43 \times 10^{-5}$	6298	71	6227
278.1	Obesity	rs2948300	N/A	0.199	0.047	1.221	$1.90 \times 10^{-5}$	5805	1172	4633
796	Elevated prostate specific antigen [PSA]	rs17817449	FTO	0.823	0.196	2.277	$2.75 \times 10^{-5}$	6298	71	6227
796	Elevated prostate specific antigen [PSA]	rs8043757	FTO	0.822	0.196	2.275	$2.82 \times 10^{-5}$	6298	71	6227
250.2	Type 2 Diabetes	rs2572386	FAM167A	-0.303	0.073	0.739	$3.57 \times 10^{-5}$	5564	459	5105
278	Obesity, Overweight	rs2948300	N/A	0.182	0.044	1.200	$3.71 \times 10^{-5}$	5980	1347	4633
796	Elevated prostate specific antigen [PSA]	rs8050136	FTO	0.788	0.195	2.198	$5.55 \times 10^{-5}$	6298	71	6227
796	Elevated prostate specific antigen [PSA]	rs9939609	FTO	0.787	0.196	2.197	$5.71 \times 10^{-5}$	6298	71	6227
250.2	Type 2 Diabetes	rs1435277	N/A	-0.292	0.073	0.747	$5.94 \times 10^{-5}$	5554	458	5096

401	Hypertension	rs12412241	N/A	-0.221	0.055	0.802	$6.49 \times 10^{-5}$	6057	1385	4672
796	Elevated prostate specific antigen [PSA]	rs3751812	FTO	0.772	0.195	2.163	$7.87 \times 10^{-5}$	6297	71	6226
250	Diabetes Mellitus	rs2572386	FAM167	-0.284	0.072	0.753	$8.44 \times 10^{-5}$	5575	470	5105
401.1	Essential Hypertension	rs12412241	N/A	-0.216	0.056	0.806	$1.02 \times 10^{-4}$	6037	1365	4672

436

437 **Table 6. Statistically Significant Obesity PheWAS SNPs**

Phenotype	Description	SNP	Gene	$\beta$	(SE)	Odds Ratio	PheWAS <i>p</i> -value	N	Cases	Controls
278	Overweight, Obesity	rs1620977	NEGR1	0.409	0.070	1.506	4.09x10 <sup>-9</sup>	2819	640	2179
278.1	Obesity	rs1620977	NEGR1	0.405	0.071	1.499	1.45x10 <sup>-8</sup>	2779	600	2179
278.11	Morbid Obesity	rs1620977	NEGR1	0.432	0.080	1.541	5.96x10 <sup>-8</sup>	2624	445	2179
278.1	Obesity	rs1776012	NEGR1	-0.326	0.067	0.722	1.18x10 <sup>-6</sup>	2762	594	2168
278.1	Obesity	rs1870676	NEGR1	-0.324	0.067	0.723	1.40x10 <sup>-6</sup>	2757	593	2164
278.1	Obesity	rs9424977	NEGR1	-0.321	0.067	0.725	1.46x10 <sup>-6</sup>	2776	599	2177
278	Overweight, Obesity	rs1776012	NEGR1	-0.308	0.065	0.735	2.23x10 <sup>-6</sup>	2802	634	2168
278	Overweight, Obesity	rs1870676	NEGR1	-0.305	0.065	0.737	2.84x10 <sup>-6</sup>	2797	633	2164
278	Overweight, Obesity	rs9424977	NEGR1	-0.303	0.065	0.739	2.87x10 <sup>-6</sup>	2816	639	2177
250.4	Abnormal glucose	rs1776012	NEGR1	-0.444	0.095	0.642	3.20x10 <sup>-6</sup>	2664	272	2392
250.4	Abnormal glucose	rs1870676	NEGR1	-0.444	0.096	0.642	3.58x10 <sup>-6</sup>	2655	270	2385
250.4	Abnormal glucose	rs9424977	NEGR1	-0.424	0.095	0.655	7.81x10 <sup>-6</sup>	2674	273	2401
564	Functional digestive disorders	rs750456	CABP5	0.989	0.229	2.689	1.55x10 <sup>-5</sup>	2518	40	2478
278.11	Morbid Obesity	rs2241005	KLRB1	-0.321	0.075	0.725	1.64x10 <sup>-5</sup>	2625	445	2180
278.11	Morbid Obesity	rs2058426	N/A	-0.322	0.075	0.725	1.66x10 <sup>-5</sup>	2621	445	2176
278.1	Obesity	rs2568958	N/A	-0.294	0.070	0.745	2.89x10 <sup>-5</sup>	2781	601	2180
278.1	Obesity	rs2815752	N/A	-0.294	0.070	0.745	2.89x10 <sup>-5</sup>	2781	601	2180
564.1	Irritable Bowel Syndrome	rs750456	CABP5	0.992	0.238	2.697	2.98x10 <sup>-5</sup>	2515	37	2478
278.11	Morbid Obesity	rs1870676	NEGR1	-0.315	0.075	0.730	3.06x10 <sup>-5</sup>	2605	441	2164
278.1	Obesity	rs3101336	N/A	-0.293	0.070	0.746	3.08x10 <sup>-5</sup>	2781	601	2180
278.11	Morbid Obesity	rs1776012	NEGR1	-0.315	0.076	0.730	3.14x10 <sup>-5</sup>	2606	438	2168
250.2	Type 2 Diabetes	rs2246606	TDH	-0.468	0.115	0.626	4.98x10 <sup>-5</sup>	2591	187	2404
278.1	Obesity	rs2953802	N/A	0.266	0.066	1.305	5.13x10 <sup>-5</sup>	2781	601	2180

278.11	Morbid Obesity	rs9424977	NEGR1	-0.304	0.075	0.738	$5.21 \times 10^{-5}$	2620	443	2177
278	Overweight, Obesity	rs2568958	N/A	-0.274	0.068	0.760	$5.88 \times 10^{-5}$	2821	641	2180
278	Overweight, Obesity	rs2815752	N/A	-0.274	0.068	0.760	$5.88 \times 10^{-5}$	2821	641	2180
278	Overweight, Obesity	rs3101336	N/A	-0.273	0.068	0.761	$6.27 \times 10^{-5}$	2821	641	2180
250.2	Type 2 Diabetes	rs2736280	TDH	-0.452	0.114	0.636	$7.48 \times 10^{-5}$	2588	187	2401
250	Diabetes Mellitus	rs2246606	TDH	-0.447	0.114	0.639	$8.11 \times 10^{-5}$	2596	192	2404
250.2	Type 2 Diabetes	rs2953802	N/A	0.431	0.110	1.539	$8.90 \times 10^{-5}$	2591	187	2404
250.4	Abnormal glucose	rs2568958	N/A	-0.389	0.100	0.678	$1.08 \times 10^{-4}$	2678	274	2404
250.4	Abnormal glucose	rs2815752	N/A	-0.389	0.100	0.678	$1.08 \times 10^{-4}$	2678	274	2404
278	Overweight, Obesity	rs2241005	KLRB1	-0.247	0.064	0.781	$1.11 \times 10^{-4}$	2821	641	2180
250.4	Abnormal glucose	rs3101336	N/A	-0.388	0.101	0.678	$1.12 \times 10^{-4}$	2678	274	2404
278.1	Obesity	rs2241005	KLRB1	-0.253	0.066	0.776	$1.14 \times 10^{-4}$	2781	601	2180
278.1	Obesity	rs2058426	N/A	-0.250	0.066	0.779	$1.43 \times 10^{-4}$	2777	601	2176
278	Overweight, Obesity	rs2058426	N/A	-0.243	0.064	0.784	$1.46 \times 10^{-4}$	2817	641	2176
250	Diabetes Mellitus	rs2736280	TDH	-0.426	0.112	0.653	$1.48 \times 10^{-4}$	2593	192	2401
250.41	Impaired fasting glucose	rs1870676	NEGR1	-0.471	0.125	0.625	$1.59 \times 10^{-4}$	2535	150	2385
278.11	Morbid Obesity	rs2953802	N/A	0.278	0.074	1.321	$1.76 \times 10^{-4}$	2625	445	2180
250	Diabetes Mellitus	rs2953802	N/A	0.407	0.109	1.502	$1.77 \times 10^{-4}$	2596	192	2404
250.41	Impaired fasting glucose	rs1776012	NEGR1	-0.464	0.124	0.629	$1.80 \times 10^{-4}$	2543	151	2392
401.1	Essential Hypertension	rs2948300	N/A	0.282	0.078	1.326	$3.02 \times 10^{-4}$	2737	521	2216
278	Overweight, Obesity	rs2953802	N/A	0.231	0.064	1.260	$3.06 \times 10^{-4}$	2821	641	2180
250.2	Type 2 Diabetes	rs2572386	FAM167A	-0.415	0.115	0.660	$3.15 \times 10^{-4}$	2590	187	2403
497	Bronchitis	rs1620977	NEGR1	0.673	0.188	1.960	$3.47 \times 10^{-4}$	2118	60	2058
250.41	Impaired fasting glucose	rs9424977	NEGR1	-0.441	0.124	0.644	$3.60 \times 10^{-4}$	2552	151	2401

278.11	Morbid Obesity	rs2568958	N/A	-0.283	0.079	0.753	$3.66 \times 10^{-4}$	2625	445	2180
278.11	Morbid Obesity	rs2815752	N/A	-0.283	0.079	0.753	$3.66 \times 10^{-4}$	2625	445	2180
278.11	Morbid Obesity	rs3101336	N/A	-0.282	0.080	0.754	$3.85 \times 10^{-4}$	2625	445	2180

438

## 439 Discussion

### 440 GWAS of Healthy Nevada BMI and Obesity

441 Here we present three GWASs on participants in the Healthy Nevada Project. The first investigates  
442 associations with BMI on subjects that do not have DM2 diagnoses. The second identifies associations  
443 with BMI in which participants with DM2 are included as a comorbidity. The third GWAS is a case-  
444 control study of extreme obesity that complements outcomes of the first two quantitative trait studies.  
445 Each GWAS is followed with two independent PheWASs to examine pleiotropy and additional  
446 phenotypic associations with quantitative BMI levels and incidence of obesity.

447

448 The first GWAS tested the association between genotype and BMI without DM2, to remove DM2  
449 effects on BMI. As expected, the majority of the resulting associations were found in SNPs that lie  
450 within the *FTO* gene. This gene has been associated to BMI and obesity in several studies and is a  
451 major focal point in obesity-related research (Scuteri *et al.* 2007; Frayling *et al.* 2007; Dina *et al.* 2007;  
452 Zeggini *et al.* 2007; Hinney *et al.* 2007; Hunt *et al.* 2008; Price *et al.* 2008; Grant *et al.* 2008; Hotta *et  
al.* 2008; Loos *et al.* 2008; Tan *et al.* 2008; Villalobos-Comparán *et al.* 2008; Thorleifsson *et al.* 2008;  
453 Willer *et al.* 2009; Meyre *et al.* 2009; Wing *et al.* 2009; Shimaoka *et al.* 2010; Fawcett and Barroso  
454 2010; Wang *et al.* 2011; Prakash *et al.* 2011; Okada *et al.* 2012; Berndt *et al.* 2013; Wheeler *et al.*  
455 2013; Graff *et al.* 2013; Olza *et al.* 2013; Qureshi *et al.* 2017; Gonzalez-Herrera *et al.* 2018). Moreover,  
456 the two strongest associations in the GWAS were from SNPs in *FTO* (rs1558902/ rs1421085,  $p =$   
457  $1.67 \times 10^{-7}/1.75 \times 10^{-7}$ ), highlighting the overall importance of this gene in relation to obesity. Frayling  
458 suggests that the association of *FTO* SNPs with DM2 is mediated through BMI (Frayling *et al.* 2007).

460 The exact mechanism by which the *FTO* gene affects BMI is not understood; however, it has been  
461 discovered that the gene product of *FTO* mediates oxidative demethylation of several different RNA  
462 species, such as mRNA, snRNA and tRNA (Jia *et al.* 2011; Wei *et al.* 2018). This indicates that protein  
463 produced from *FTO* likely operates as a RNA regulatory molecule, which can affect both gene  
464 expression as well as translation initiation and elongation (Wei *et al.* 2018).

465

466 Two SNPs within *TDH* were found to be strongly associated to BMI. This gene codes for a  
467 nonfunctional L-threonine dehydrogenase, lacking most of the C-terminus found in other species, and  
468 is thus characterized as a putative pseudogene. Previous research has identified this gene as a possible  
469 susceptibility gene for obesity (Yanagiya *et al.* 2007); however, relatively little is known about any  
470 functional consequences of SNPs within this pseudogene. We also observed a strongly associated locus  
471 in *NEGR1*, one of the first genes shown to have variants associated to BMI (Thorleifsson *et al.* 2008;  
472 Willer *et al.* 2009; Speliotes *et al.* 2010; Boender *et al.* 2014). This gene codes for a cell adhesion  
473 molecule, although its function in relation to BMI is still unknown. Previous research in mice  
474 determined that deletions of *NEGR1* cause a decrease in weight and a change in the regulation of  
475 energy balance, implying that *NEGR1* most likely functions to control the regulation of energy balance  
476 (Lee *et al.* 2012; Boender *et al.* 2014).

477

478 We hypothesized that including DM2 participants (and thus DM2 as a covariate in the genetic model)  
479 would produce a more parsimonious fit, as many studies show a relationship between diabetes and  
480 BMI. We discovered that diabetes was indeed an important predictor of BMI for all 500,000  
481 regressions performed ( $p < 2 \times 10^{-16}$  for every SNP), regardless of age, gender or genotype. Furthermore,  
482 adding DM2 as a predictor in the additive model increases the significance of associations between  
483 SNPs in the *TDH* gene and BMI; five out the top eight most significant associations fall within this  
484 gene (Table 2). It is clear that incidence of DM2 in our cohort affects the genetic association of BMI.

485 Specifically, when DM2 patients are excluded in our cohort, there are two associations within *TDH*.  
486 When DM2 participants are included, we observe five associations with the *TDH* gene. This indicates,  
487 along with the BMI PheWAS results, that not only does *TDH* influence BMI measurements, it also has  
488 an association with DM2.

489

490 It is rare for SNPs to be effectors of two separate diseases, even those as intertwined as BMI and DM2  
491 (Grarup *et al.* 2014). A possible explanation why *TDH* has not been previously associated with DM2 is  
492 due to a lack of statistical power to observe the small risk increases *TDH* may impose on DM2 (Grarup  
493 *et al.* 2014). Nonetheless, the increased rate of DM2 diagnosis worldwide makes this an interesting  
494 candidate gene. How the SNPs in the *TDH* pseudogene may influence either BMI or DM2 is unknown,  
495 as evidence supporting the association between *TDH* and BMI/DM2 is scant; however, previous  
496 research has discovered that not all pseudogenes are "junk" DNA. Some of these genes can be actively  
497 transcribed to produce short interfering RNAs (siRNAs), which can regulate gene expression (Pink *et*  
498 *al.* 2011). In certain cases, they can even competitively bind micro-RNAs (miRNAs), which can  
499 attenuate repression of cellular mRNA (An *et al.* 2016). Additionally, the expression of pseudogene  
500 transcripts tends to be tissue-specific. Given that the greatest expression of *TDH* transcripts is found in  
501 the pancreas (Fagerberg *et al.* 2014), one might speculate that *TDH* affects the production of insulin  
502 and/or digestive enzymes. If true, this may account for our observation where *TDH* influences BMI  
503 measurements, and is associated with DM2. Given the strong associations between *TDH* SNPs and  
504 DM2, as well as potential regulatory functions of pseudogenes, we believe it is essential that future  
505 studies focus on determining the function of the *TDH* pseudogene in a tissue-specific context. Although  
506 studies using genetically modified mice with a *TDH* polymorphism and proteomics analysis of their  
507 pancreatic tissue would be straight-forward, to the best of our knowledge, no such studies have been  
508 reported. We see this as a possible future direction.

509

510 Adding DM2 as a covariate into the statistical model also identified two additional genes that may  
511 influence BMI: *RTN4RL1* and *CABP5*. *RTN4RL1* is a gene that codes for a cell surface receptor and  
512 was previously found to be upregulated approximately 2-fold when exposed to bone morphogenetic  
513 protein 4 (BMP4), a protein that is increased in diabetic animals and may reduce insulin secretion  
514 (Christensen *et al.* 2015). This implies that the effects of *RTN4RL1* on BMI may be secondary to its  
515 main effect on diabetes. Moreover, this gene has also been listed as a potential candidate gene for DM2  
516 in previous GWAS (Thomsen *et al.* 2016). The gene *CABP5*, which codes for a calcium binding  
517 protein that has role in calcium mediated cellular signal transduction (Haeseler *et al.* 2002) may have  
518 a more direct effect on BMI. It was previously discovered as part of a group of several genes that were  
519 upregulated in obese individuals, although its exact function relative to obesity is still unknown  
520 (Nakajima *et al.* 2016).

521  
522 A case-control association study examining the effects between genetics and the risk of extreme obesity  
523 ( $\text{BMI} \geq 35 \text{ kg/m}^2$ ) was the final GWAS we conducted. It has been determined by the World Health  
524 Organization (WHO) that more than 1.9 billion adults are overweight and over 650 million are obese.  
525 Moreover, obesity is associated with several other chronic diseases, such as cardiovascular disease,  
526 DM2 and cancer, all of which could lead to premature death (Kopelman 2007). Overall, our obesity  
527 results consist of many of the same SNPs and genes found to be associated with BMI. However, the  
528 obesity results did demonstrate an increase in the genetic associations at the significance level of  
529  $p < 1 \times 10^{-5}$  in and very close to the *NEGR1* gene compared to previous BMI associations. Previous  
530 studies using genetically modified mice with *NEGR1* deficiency or *NEGR1*-loss-of-function support a  
531 role for *NEGR1* in the control of body weight; however, the mechanism of its involvement is not clear  
532 (Lee *et al.* 2012). Contradictory with anticipated results, these mutant mice display a small but steady  
533 reduction of body mass. Notwithstanding, these studies do suggest that loss of *NEGR1* function in the  
534 mouse models has a negative effect on body mass as well as lean mass, supporting the possibility that

535 *NEGR1* may contribute to a change body mass. It is also important to note that animal models are a  
536 representation of human physiology but not necessarily a precise depiction.

537

538 Our extreme obesity vs. non-obese study also identified two new genes, *PFKFB3* and *KLRB1*, that are  
539 not yet found to be significantly associated to BMI. The odds ratios associated with all SNPs found in  
540 these genes were less than one, indicating that they decreased the odds of extreme obesity risk. These  
541 results are potentially supported by work conducted by Huo et al., who reported that mice  
542 transgenically modified to selectively overexpress *PFKFB3* in adipocytes show increases fat deposition  
543 in their adipose tissue (Huo *et al.* 2012). In contrast, an earlier study reported that transgenic mice with  
544 reduced *PFKFB3* expression show exacerbated diet-induced insulin resistance (Huo *et al.* 2010).  
545 Moreover, a recent study of hypertrophic white adipose tissue morphology, Kerr and coworkers  
546 showed that inhibition of *PFKFB3* mRNA impairs basal and insulin stimulated lipogenesis and  
547 furtherer proposed that gene knockdown may of *PFKFB3* inhibit adipocyte lipid storage (Kerr *et al.*  
548 2019). These studies support a hypothesis whereby polymorphisms that lead to a decrease in *PFKFB3*  
549 may be protective from the development of obesity; however, tissue-specific transcriptional studies in  
550 humans would be required to fully support this assertion.

551

552 We also observed polymorphisms in *KLRB1* to associate with a decrease of odds in extreme obesity  
553 risk. *KLRB1* expression produces a type II transmembrane glycoprotein also known as CD161; a  
554 member of the C-type lectin superfamily. CD161 is expressed on the surface of most natural killer  
555 (NK) cells and natural killer T (NKT) but also on subsets of peripheral T cells and CD3<sup>+</sup> thymocytes.  
556 While the biological function of CD161 is not firmly established, it was suggested that it serves either  
557 as a stimulatory receptor or to inhibit NK cell-mediated cytotoxicity and cytokine production (Lanier *et*  
558 *al.* 1994). Indeed, NK cells were shown to be upregulated in the fat of obese twins (Pietiläinen *et al.*  
559 2008); moreover, BMI and *KLRB1* expression may be correlated in that *KLRB1 transcription has been*

560 reported to increase as BMI increases (Rai *et al.* 2014). Additionally, CD161<sup>bright</sup> CD8<sup>+</sup> mucosal  
561 associated invariant T (MAIT) cells play a central role in maintaining mucosal immunity and therefore,  
562 changes in CD161 expression on these cells may lead to alterations in mucosal immunity and gut  
563 microbiota homeostasis. These changes may in turn manifest as alterations of dietary metabolism. It is  
564 noteworthy that increases in MAIT cells are associated with Juvenile Type 1 Diabetes and  
565 polymorphisms in *KLRB1* have been associated with ischemic heart disease (Makeeva *et al.* 2015), and  
566 differential transcription of *KLRB1* has been reported in DM2 and coronary artery disease (Gong *et al.*  
567 2017). Furthermore, another gene, *GRIN2A*, is a gene that is part of the family of genes {*GRIN1*,  
568 *GRIN2A*, *GRIN2B*, *GRIN2C*, *GRIN2D*, *GRIN3A*, and *GRIN3B*}, which encode proteins that form a  
569 receptor in charge of sending chemical messages between neurons in the brain. The gene *GRIN2B* was  
570 found to be associated to obesity in adult women defined as metabolically healthy in Schlauch et. al  
571 ( $p=1.7\times 10^{-5}$ ). (Schlauch *et al.* 2019).

572  
573 Associations between *FTO* and obesity were just under genome-wide significance levels. This is a  
574 possible indication that *FTO* polymorphisms cause small changes in BMI, rather than the wide range  
575 differences observed between extreme obese cases and controls. Nonetheless, previous research has  
576 demonstrated that a combination of several *FTO* mutations will increase the likelihood of a participant  
577 being classified as obese (Li *et al.* 2009). Speakman *et al.* stated that Frayling showed the *FTO* was  
578 significantly associated with diabetes only through its association with BMI (Speakman *et al.* 2018).

579  
580 The comprehensive series of GWASs presented here validates associations of obesity and BMI found  
581 in previous studies, such as the *FTO* and *NEGR1* loci (Willer *et al.* 2009; Speliotes *et al.* 2010; Okada  
582 *et al.* 2012; Locke *et al.* 2015). Many larger studies identify associative loci in *MC4R* (Willer *et al.*  
583 2009; Speliotes *et al.* 2010; Okada *et al.* 2012). While our studies did not detect SNPs in *MC4R* with  
584 genome-wide significance, they did identify associations at  $p=1\times 10^{-4}$  and  $p=1.7\times 10^{-4}$  of SNPs

585 rs17782313 and rs571312 (Willer *et al.* 2009; Speliotes *et al.* 2010). A number of obesity case-control  
586 studies have found variations in the *MC4R* gene (Xi *et al.* 2012; Evans *et al.* 2014). Our case-control  
587 study does reveal that rs17782313 in *MC4R* associates with obesity at  $p=3\times 10^{-4}$ . Our cohort is a  
588 controlled, regional population. The next two stages of the Healthy Nevada Project will add between  
589 40,000 (2019) and 150,000 (late 2020) more Nevadans to the current cohort. With these much larger  
590 cohort sizes, it is our hope that a stronger associate link with *MC4R* will be identified.

591

592 *PheWAS of Healthy Nevada BMI and Obesity*

593 To the best of our knowledge, this is the first dual-PheWAS targeted at BMI and obesity. Cronin *et al.*  
594 present a comprehensive PheWAS targeted at *FTO* variants, which also show strong associations with  
595 overweight and obesity phenotypes, hypertension and hyperlipidemia (Cronin *et al.* 2014). Milliard *et*  
596 *al.* perform a large PheWAS study to examine phenotypic associations with BMI that focus on the  
597 nervousness phenotypes: the study identified known associations such as diabetes and hypertension  
598 (Millard *et al.* 2019).

599 The PheWAS performed on the SNP associations in this study's BMI cohort identified strong  
600 associations of elevated PSA levels with variants in *FTO*, and indicates that the number of minor  
601 alleles of these variants is predictive of elevated PSA. This finding is in contradiction to the reports  
602 (Bañez *et al.* 2007; Oh *et al.* 2013; Zhang *et al.* 2016; Bonn *et al.* 2016) indicating an inverse  
603 relationship between PSA levels and BMI. However, serum levels of PSA may be elevated due to  
604 reasons other than prostatic malignancy. Benign prostatic hyperplasia (BPH), prostatitis (Nadler *et al.*  
605 1995), ejaculation (Herschman *et al.* 1997), or manipulation of the prostate gland (Chybowski *et al.*  
606 1992; Crawford *et al.* 1992; Tarhan *et al.* 2005) may cause elevated levels of serum PSA. Our study did  
607 not control for such parameters. Our sample includes only 71 individuals with ICD codes indicating  
608 high PSA levels, of which a number are morbidly obese. Increased BMI is often associated with  
609 increased age and our study population was significantly older than the general median age of the U.S.

610 population. Thus, it is possible that older age contributed to increased likelihood of BPH and,  
611 subsequently, elevated serum PSA, negating the reported inverse effect of BMI on PSA levels and  
612 possibly exposing a novel association with variants in *FTO*.  
613 Many of the clinical associations observed in the PheWASs of the HNP in relation to various degrees  
614 of increased BMI and the presence of obesity are recognizable of the cluster of clinical conditions  
615 associated with metabolic syndrome (Alberti *et al.* 2009). Obesity is a risk factor for respiratory  
616 conditions such as chronic obstructive pulmonary disease (COPD), asthma, obstructive sleep apnea and  
617 obesity hypoventilation syndrome, and may influence the development and presentation of these  
618 diseases (Poulain *et al.* 2006). Accumulation of fat tissue impairs ventilatory function in adults  
619 (Lazarus *et al.* 1997) and increased BMI is associated with a reduction in forced expiratory volume in  
620 one second (FEV1), forced vital capacity (FVC), total lung capacity, functional residual capacity and  
621 expiratory reserve volume (Rubinstein *et al.* 1990; Chinn *et al.* 1996; Lazarus *et al.* 1997; Biring *et al.*  
622 1999). Peripheral edema has long been recognized as associated with extreme obesity (Alexander *et al.*  
623 1962). In the U.K. Community Nursing Services study, obesity was found as an independent risk factor  
624 for chronic edema (Moffatt *et al.* 2019).

625

626

## 627 Data Availability Statement

628 *EHR Data*

629 EHR data for the Healthy Nevada cohort are subject to HIPAA and other privacy and compliance  
630 restrictions. Mean quality-controlled BMI values for the 6,645 component values for each individual  
631 are available in Supplementary Table S6.

632

633 *GWAS Results*

634 To reduce the possibility of a privacy breach, 23andMe requires that the statistics for only 10,000 SNPs  
635 be made publicly available. This is the amount of data considered by 23andMe to be insufficient to  
636 enable a reidentification attack. The statistical summary results of the top 10,000 SNPs for the  
637 23andMe data are available here: [www.dri.edu/HealthyNVProjectGenetics](http://www.dri.edu/HealthyNVProjectGenetics). All column definitions are  
638 listed in Table 7.

639

640 **Table 7. Column Identifiers for GWAS Results.**

Column name	Definition
<b>CHR</b>	Chromosome
<b>SNP</b>	Individual SNP identifier
<b>BP</b>	Location of SNP on relative chromosome
<b>A1</b>	Alternative Allele
<b>TEST</b>	Selected statistical test – ADD represents the additive effect
<b>NMISS</b>	Indicates the number of observations – non-missing genotypes
<b>BETA</b>	The effect size for this variant, defined per copy of the A1 allele
<b>SE</b>	The standard error of the effect size
<b>LE</b>	Lower end of the 95% confidence interval for the effect size
<b>UE</b>	Upper end of the 95% confidence interval for the effect size
<b>STAT</b>	The value of the test statistic
<b>P</b>	The p-value for the association test

641 Table describing the column headers for the results file of our genome wide associations. This  
642 summary results file only lists the top 10,000 SNPs in order to prevent a re-identification attack.  
643

644 *PheWAS Results*

645 Summarized counts of each ICD classification (ICD-9 and ICD-10) and phenotype group (phecode) are  
646 presented in Supplementary Table S7.

647

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655

## 656 **Competing interest**

657 Members of the 23andMe Research Team are employees of 23andMe and hold stock or stock options  
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1329

1330 **Figure Legends**

1331 **Figure 1: Manhattan plot of GWAS results of BMI including DM2-diagnosed individuals**

1332    Genome-wide association study results for BMI. This study includes DM2-diagnosed individuals and  
1333    the statistical model includes DM2 as a bimodal covariate. The *x*-axis represents the genomic position  
1334    of 500,508 SNPs. The *y*-axis represents -log<sub>10</sub>-transformed raw *p*-values of each genotypic association.  
1335    The red horizontal line indicates the significance level 1x10<sup>-5</sup>.

1336

1337    **Figure 2: Obesity Case-Control GWAS Manhattan Plot**

1338    Genome-wide association study results for the case-control obesity study. This cohort includes DM2-  
1339    diagnosed individuals. The *x*-axis represents the genomic position of 500,508 SNPs. The *y*-axis  
1340    represents -log<sub>10</sub>-transformed raw *p*-values of each genotypic association. The red horizontal line  
1341    indicates the significance level 1x10<sup>-5</sup>.

1342

1343    **Figure 3: PheWAS results between BMI-significant SNPs and EHR Phenotypes**

1344    This figure shows the results of individual logistic regressions between incidence of 633 phenotype  
1345    groups (phecodes) and the genotypes of 27 SNPs found to have statistically significant associations  
1346    with BMI in a cohort with DM2 patients. Each point represents the *p*-value of one SNP and one of 633  
1347    phecodes with at least 20 cases assigned to it. The horizontal red line represents the significance level  
1348     $p=1.02 \times 10^{-4}$ , and the blue line represents the Bonferroni correction of  $p=3 \times 10^{-6}$ .

1349

1350    **Figure 4: PheWAS results between BMI and EHR Phenotypes**

1351    This figure illustrates the results of individual linear regression between incidence of phenotype groups  
1352    (phecodes) and the continuous BMI metric of all 6,645 individuals. Each of the 301 points represents  
1353    the *p*-value of the association between one of 1,523 phecodes with at least 20 cases assigned to it, and  
1354    BMI. Statistical significance was assessed by using the False Discovery Rate of 0.1, corresponding to a  
1355    raw *p*-value of 1.96x10<sup>-2</sup>. Only associations with  $p < 1 \times 10^{-15}$  are annotated for ease of viewing,  
1356    represented by a horizontal line at 15 on the *y*-axis.

1357

1358 **Figure 5: PheWAS results between obesity-significant SNPs and EHR Phenotypes**

1359 This figure presents results of individual logistic regressions between incidence of 372 phenotype  
1360 groups (phecodes) and the genotypes of 34 SNPs found to be associated with extreme obesity. Each  
1361 point represents the *p*-value of one SNP and one of 372 phecodes with at least 20 cases assigned to it.  
1362 The horizontal red line represents the significance level  $p=3.85\times 10^{-4}$ , and the blue line represents the  
1363 Bonferroni correction of  $p=4\times 10^{-6}$ .

1364

1365 **Figure 6: PheWAS results between extreme obesity and EHR Phenotypes**

1366 This figure illustrates the results of individual linear regression between incidence of phenotype groups  
1367 (phecodes) and the incidence of extreme obesity in 2,996 individuals. Each of the 191 points  
1368 represents the *p*-value of the association between one of 1,362 phecodes with at least 20 cases assigned  
1369 to it, and extreme obesity. Statistical significance was assessed by using the False Discovery Rate of  
1370 0.1, corresponding to a raw *p*-value of  $1.4\times 10^{-2}$ . Only associations with  $p < 1\times 10^{-15}$  are annotated for  
1371 ease of viewing, represented by a horizontal line at 15 on the y-axis.

1372

1373 **Supplemental Figure and Table Legends**

1374 **Supplementary Table S1: GWAS results for BMI in a cohort with no DM2-diagnosed  
1375 participants**

1376 This table lists the 20 statistically significant SNPs associated with BMI in our cohort without DM2-  
1377 diagnosed individuals. General information about the SNP such as chromosome location, GWAS *p*-  
1378 value, power, genotype, cytoband, and ANOVA are listed.

1379

1380 **Supplementary Table S2: GWAS results for BMI in a cohort with DM2-diagnosed participants**

1381 This table lists the 27 statistically significant SNPs associated with the BMI in our cohort that includes  
1382 all individuals with DM2. General information about the SNP such as chromosome location, GWAS *p*-  
1383 value, power, genotype, cytoband, and ANOVA are listed.

1384

1385 **Supplementary Table S3: GWAS results for the extreme obesity case-control study**

1386 This table lists the 26 statistically significant SNPs associated with extreme obesity. General  
1387 information about the SNP such as chromosome location, GWAS *p*-value, power, genotype, cytoband,  
1388 and ANOVA are listed.

1389

1390 **Supplementary Table S4: Significant EHR phenotypic associations with BMI**

1391 This is a table of the 301 phenotype groups (phecodes) reaching statistical significance ( $p < 1.96 \times 10^{-2}$ )  
1392 when associated to BMI in our cohort including DM2-diagnosed individuals. Phecodes and their  
1393 description, effect sizes ( $\beta$ ) of the regression, standard error (SE), and *p*-values are included. Each  
1394 phecode group contains at least 20 cases.

1395

1396 **Supplementary Table S5: Significant EHR phenotypic associations with extreme obesity**

1397 This is a table of the 191 phenotype groups (phecodes) reaching statistical significance ( $p < 1.4 \times 10^{-2}$ )  
1398 when associated to extreme obesity in our cohort. Phecodes and their description, effect sizes ( $\beta$ ) of the  
1399 regression, standard error (SE), and *p*-values are included. Each phecode group contains at least 20  
1400 cases.

1401

1402 **Supplementary Table S6: Quality-controlled and averaged BMI participant values**

1403 This table includes the quality-controlled average BMI value across multiple records for each  
1404 individual, as well as age at Jan 2019 and gender. Due to the length of this table, it can be found at:

1405 [www.dri.edu/HealthyNVProjectGenetics](http://www.dri.edu/HealthyNVProjectGenetics)

1406

1407 **Supplementary Table S7: Counts of each phecode group**

1408 This table presents the mapping between ICD codes (ICD-9 and ICD-10) and phecodes as presented in  
1409 Carroll and the R package PheWAS (Carroll *et al.* 2014) tested in our study, and the number of  
1410 incidences from the BMI cohort in each phecode group. The column labeled Count is derived by the  
1411 aggregation of ICD-9 and ICD-10 codes.

1412

1413 **Supplementary Figure S1: Quality-controlled average BMI values of participants**

1414 This figure illustrates both the raw and normalized BMI values. Due to certain extreme BMI values,  
1415 there is a break in the x-axis of the raw BMI values shown in the first panel.

1416

1417 **Supplementary Figure S2: Manhattan plot of GWAS results of BMI with DM2-diagnosed  
1418 individuals removed**

1419 Genome-wide association study results for BMI. This study excludes DM2-diagnosed individuals. The  
1420 x-axis represents the genomic position of 500,508 SNPs. The y-axis represents -log<sub>10</sub>-transformed raw  
1421 p-values of each genotypic association. The red horizontal line indicates the significance level 1x10<sup>-5</sup>.

1422

1423 **Supplementary Fig S3: ANOVA results for rs9939609**

1424 A box and whisker figure of ANOVA results for one of the strongest associations (rs9939609) with  
1425 BMI is shown in Supplementary Figure S3. Note that BMI levels increase with the increase of the  
1426 number of minor alleles, which is typical of variants in FTO (Frayling *et al.* 2007).

1427 **Author Contributions**

1428 KAS and RWR conducted genetic and clinical data analysis and wrote the manuscript. GE, ADS and  
1429 KAS contributed to the clinical discussion. VCL contributed to the molecular biology discussion. WJM  
1430 extracted participants and their clinical health data from the Renown EHR. The 23andMe research team  
1431 provided participant genotype data and edited the manuscript. JJG obtained funds to conduct this  
1432 experiment, provided leadership in all aspects of the research, and edited the manuscript. All authors  
1433 reviewed, edited and approved the final version of the manuscript.











