Genetic Variant of AMD1 Is Associated with Obesity in Urban Indian Children

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Abstract

Background: Hyperhomocysteinemia is regarded as a risk factor for cardiovascular diseases, diabetes and obesity. Manifestation of these chronic metabolic disorders starts in early life marked by increase in body mass index (BMI). We hypothesized that perturbations in homocysteine metabolism in early life could be a link between childhood obesity and adulthood metabolic disorders. Thus here we investigated association of common variants from homocysteine metabolism pathway genes with obesity in 3,168 urban Indian children.

Methodology/Principal Findings: We genotyped 90 common variants from 18 genes in 1,325 children comprising of 862 normal-weight (NW) and 463 over-weight/obese (OW/OB) children in stage 1. The top signal obtained was replicated in an independent sample set of 1843 children (1,399 NW and 444 OW/OB) in stage 2. Stage 1 association analysis revealed association between seven variants and childhood obesity at \( P < 0.05 \), but association of only rs2796749 in AMD1 \([OR = 1.41, P = 1.5 \times 10^{-4}]\) remained significant after multiple testing correction. Association of rs2796749 with childhood obesity was validated in stage 2 \([OR = 1.28, P = 4.2 \times 10^{-3}]\) and meta-analysis \([OR = 1.35, P = 1.9 \times 10^{-9}]\). AMD1 variant rs2796749 was also associated with quantitative measures of adiposity and plasma leptin levels that was also replicated and corroborated in combined analysis.

Conclusions/Significance: Our study provides first evidence for the association of AMD1 variant with obesity and plasma leptin levels in children. Further studies to confirm this association, its functional significance and mechanism of action need to be undertaken.

Introduction

Childhood obesity is a growing public health issue worldwide [1]. Prevalence of overweight/obesity has increased from 16% in 2002 to 24% in 2006 in urban school children in Delhi, India [2]. Both genetic and environmental factors play an important role in the development of obesity. Studies investigating the genetic component of obesity have primarily focused on adult obesity and around 32 obesity susceptibility genes have been identified through large scale genome wide association studies (GWAS) [3]. The search for genetic risk factors for childhood obesity and related phenotypes are mainly limited to the replication of variants identified through genome wide association studies (GWAS) in adults [3].

Childhood obesity is one of the major determinants of many chronic diseases in adulthood such as type 2 diabetes, hypertension and cardio-vascular diseases [4]. Manifestation of these chronic metabolic disorders, which have become pandemic in India [5–7], starts in early life [8]. Indian subjects with impaired glucose tolerance or diabetes are shown to have typically low BMI up to the age of two years, followed by increasing BMI at the age of 12 years [8]. This suggests that molecular events leading to obesity in childhood predispose individuals to chronic metabolic disorders in later life. This makes identification of factors linking childhood obesity and adult chronic disorders very imperative.

Elevated level of homocysteine, termed as hyperhomocysteinemia, is regarded as a potential risk factor for cardiovascular diseases, diabetes, hypertension and number of other pathologies [9–12]. Homocysteine is a thiol containing amino acid which plays an important role in cell metabolism. The metabolic traffic of homocysteine occurs either via remethylation to methionine or through irreversible trans-sulfuration to cysteine. Homocysteine metabolism involves a series of enzymatic reactions that produce variety of metabolic intermediates which are important for cellular processes such as transmethylation, transulfuration and polyamine biosynthesis (Figure 1) [13]. Perturbations in the activities of
enzymes involved in these processes such as methylene tetrahydrofolate reductase (MTHFR), methylene tetrahydrofolate dehydrogenase (MTHFD), methionine synthase reductase (MTRR), cystathionine synthase (CBS) may result in altered levels of homocysteine and thus metabolic disorders.

Contribution of variants in homocysteine pathway genes to susceptibility of diabetes, obesity and vascular diseases has been suggested by previous studies [14–16]. Genetic variants of MTHFR, MTR and MTRR have been shown to be associated with obesity [17]. The C677T polymorphism of MTHFR is reported to be associated with hyperhomocysteinemia, type 2 diabetes and related complications [18,19]. However there is no report of association of homocysteine pathway genes with childhood obesity. Here we hypothesized that genetic variant in homocysteine metabolism pathway genes leading to perturbation in homocysteine metabolism might be a link between childhood obesity and adult metabolic disorders. To test this, we designed the present two stage case-control study to investigate association of 90 common variants from homocysteine metabolism pathway genes with obesity in 3,168 urban Indian children.

Materials and Methods

Ethics Statement

The study was carried out in accordance with the principles of Helsinki Declaration and was approved by Human Ethics Committee of Institute of Genomics and Integrative Biology (CSIR) and All India Institute of Medical Sciences Research Ethics Committee. Informed written consent was obtained from all the participants. Prior informed written consent was obtained from parents/guardians of the children while verbal consent from children themselves was taken.

Study Population

A total of 3,168 children (aged 11–17 years) were recruited from school health survey from four different zones of Delhi (India) after obtaining prior informed consent from school authorities, parents/guardians and verbal consent from children themselves. For stage 1, we selected 1,325 children [862 NW and 463 OW/OB] to identify SNPs showing association with obesity. This was followed by further selection of 1,843 children (1,399 NW and 444 OW/OB) in stage 2 for replication analysis. Subjects were classified as normal-weight (NW) and over-weight/obese (OW/OB) based on age and sex specific BMI cutoff provided by Cole et al [20], that gives BMI cut offs for overweight and obesity by sex for children between 2 and 18 years corresponding to the cut off points of 25 and 30 kg/m² for adults.

Anthropometric and Clinical Measurements

Anthropometric measurements including height, weight, waist circumference (WC) and hip circumference (HC) were taken using standard methods. BMI and waist-to-hip ratio (WHR) were calculated. Plasma levels of glucose, insulin and C-peptide were measured as described earlier [21] and HOMA-IR was calculated as described by Matthews et al. [22]. Plasma levels of leptin, resistin and adiponectin were measured using commercial ELISA kits (R&D Systems, Minneapolis, MN). Anthropometric and clinical characteristics of subjects are provided in Table 1.

Genes and SNPs Selection

We selected 16 genes which have central role in the homocysteine metabolism pathway (Figure 1) [13,23] and 2 genes (ACE and NOS3) reported to be associated with plasma homocysteine levels [24,25]. Common variants (MAF >0.05) of the selected genes were prioritized based on previous reports of association with metabolic disorders, tagging and linkage disequilibrium (LD) pattern information, and spacing between the SNPs to cover the gene. Finally a set of 90 SNPs were selected for genotyping in stage 1 (Table S1).

Genotyping

Genotyping in stage 1 was performed using Illumina GoldenGate assay (Illumina Inc., San Diego, CA, USA). Stringent quality control (QC) were applied to the genotyping data that included genotype confidence score >0.25, SNP call rate >0.9, GenTrans score >0.6, cluster separation score >0.4, Hardy Weinberg Equilibrium (HWE) [P>0.01 in NW, OW/OB and all subjects], minor allele frequency (MAF) >0.05. After excluding 35 SNPs based on QC criteria (Table S1), the successfully genotyped
SNPs had an average call rate of 98% and concordance rate >99% based on 5% duplication.

In stage 2, variant showing strongest association with childhood obesity and standing multiple testing correction was validated by genotyping in an independent sample set of 1843 children (1,399 NW and 444 OW/OB) using iPLEX (Sequenom, San Deigo, CA, USA). Genotyping success rate was 94% with 5% duplicates.

**Statistical Analysis**

Hardy Weinberg Equilibrium (HWE) was tested using \( \chi^2 \). Pairwise LD between the SNPs and haplotype blocks were determined using Haplovew 4.0. Continuous variables were inverse normal transformed and were converted to age and sex specific internal Z-scores by dividing differences of mean value of study population and individual values by standard deviation. Logistic and linear regression analyses were performed to assess associations of variants with obesity and quantitative traits respectively under additive model adjusting for age, sex and Z-

**Table 1. Clinical characteristics of subjects.**

| Character | Stage 1 | Stage 2 | \( p^* \) | \( p^* \) |
|----------|---------|---------|----------|----------|
|          | NW children | OW/OB children | NW children | OW/OB children |
| N (M/F)  | 830 (370/464) | 453 (173/279) | 1399 (420/979) | 444 (132/312) |
| Age (years) | 14.00 (12.50–15.00) | 13.00 (12.00–14.50) | 13.00 (12.00–15.00) | 13.2 (12.0–15.0) |
| Height (m) | 1.54 (1.48–1.61) | 1.56 (1.50–1.62) | 1.52 (1.46–1.58) | 1.56 (1.51–1.62) |
| Z-Height* | -0.11 ± 0.99 | 0.27 ± 0.89 | -0.11 ± 1.00 | 0.35 ± 0.93 |
| Weight (kg) | 42.30 (37.00–48.84) | 64.00 (55.45–73.90) | 42.00 (36.00–48.40) | 63.5 (56.0–71.0) |
| Z-Weight* | -0.51 ± 0.75 | 1.20 ± 0.54 | -0.40 ± 0.70 | 1.17 ± 0.55 |
| BMI (kg/m²) | 17.58 (15.86–19.44) | 25.85 (23.97–28.97) | 17.91 (16.26–19.78) | 25.6 (24.0–28.0) |
| Z-BMI* | -0.56 ± 0.76 | 1.27 ± 0.49 | -0.40 ± 0.65 | 1.18 ± 0.50 |
| WC (m) | 0.66 (0.61–0.72) | 0.84 (0.77–0.90) | 0.65 (0.60–0.71) | 0.85 (0.78–0.90) |
| Z-WC* | -0.42 ± 0.77 | 1.11 ± 0.59 | -0.40 ± 0.79 | 1.11 ± 0.63 |
| HC (m) | 0.81 (0.76–0.86) | 0.97 (0.92–1.04) | 0.80 (0.75–0.86) | 0.97 (0.91–1.02) |
| Z-HC* | -0.42 ± 0.76 | 1.23 ± 0.63 | -0.41 ± 0.71 | 1.11 ± 0.62 |
| WHR | 0.82 (0.78–0.87) | 0.86 (0.81–0.90) | 0.82 (0.78–0.86) | 0.87 (0.83–0.92) |
| Z-WHR* | -0.22 ± 0.89 | 0.41 ± 0.93 | -0.20 ± 0.98 | 0.62 ± 0.96 |
| Glucose (mmol/L) | 5.04 (4.72–5.33) | 5.00 (4.72–5.33) | 4.77 (4.44–5.11) | 4.60 (4.22–4.90) |
| Z-Glucose* | -0.50 ± 0.76 | 1.27 ± 0.49 | -0.40 ± 0.65 | 1.18 ± 0.50 |
| WC (m) | 0.66 (0.61–0.72) | 0.84 (0.77–0.90) | 0.65 (0.60–0.71) | 0.85 (0.78–0.90) |
| Z-WC* | -0.42 ± 0.77 | 1.11 ± 0.59 | -0.40 ± 0.79 | 1.11 ± 0.63 |
| HC (m) | 0.81 (0.76–0.86) | 0.97 (0.92–1.04) | 0.80 (0.75–0.86) | 0.97 (0.91–1.02) |
| Z-HC* | -0.42 ± 0.76 | 1.23 ± 0.63 | -0.41 ± 0.71 | 1.11 ± 0.62 |
| WHR | 0.82 (0.78–0.87) | 0.86 (0.81–0.90) | 0.82 (0.78–0.86) | 0.87 (0.83–0.92) |
| Z-WHR* | -0.22 ± 0.89 | 0.41 ± 0.93 | -0.20 ± 0.98 | 0.62 ± 0.96 |
| Glucose (mmol/L) | 5.04 (4.72–5.33) | 5.00 (4.72–5.33) | 4.77 (4.44–5.11) | 4.60 (4.22–4.90) |
| Z-Glucose* | -0.50 ± 0.76 | 1.27 ± 0.49 | -0.40 ± 0.65 | 1.18 ± 0.50 |
| WC (m) | 0.66 (0.61–0.72) | 0.84 (0.77–0.90) | 0.65 (0.60–0.71) | 0.85 (0.78–0.90) |
| Z-WC* | -0.42 ± 0.77 | 1.11 ± 0.59 | -0.40 ± 0.79 | 1.11 ± 0.63 |
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| Z-HC* | -0.42 ± 0.76 | 1.23 ± 0.63 | -0.41 ± 0.71 | 1.11 ± 0.62 |

Data are presented as median with interquartile ranges.

Statistical power of the study was determined using Quanto software (http://hydra.usc.edu/gxe/) assuming log additive model of inheritance and 24% prevalence of overweight/obesity (1) at \( \alpha = 0.05 \). Sample size in stage 1 and combined analysis provided 44–76% and 76–97% power respectively to detect association of variant with allele frequency of 0.20 and effect size of 1.20–1.30. 

Results

Association analysis in stage 1 suggested association of seven variants- rs2796749 (AMD1), rs7768897 (AMD1), rs1985908 (MTHFD1L), rs9397028 (MTHFD1L), rs1021737 (CTH), rs6693082 (CTH) and rs1801133 (MTHFR) with childhood obesity at \( P<0.05 \) (Tables 2 and 3). The association of rs2796749 in AMD1 \[ OR = 1.41, P=1.5 \times 10^{-4} \] remained significant even after multiple testing correction. Linear regression analysis to assess the association of variants with BMI as continuous trait also revealed association of rs2796749 with BMI \[ \beta = 0.16, Z-score unit, P=4.2 \times 10^{-4} \] (Table 3). AMD1 variant rs2796749 was also associated with other quantitative measures of adiposity (Table 4). With increase in per copy of risk allele G of AMD1 variant rs2796749, significant increase in height and weight by 0.10 Z-score units \( P=0.02 \) and 0.17 \( P=1.3 \times 10^{-5} \) respectively was observed (Table 4). Similar trend in increase in WC and HC was found with the increase in risk allele of rs2796749 \[ \beta = 0.18, P=3.2 \times 10^{-5} \] for WC and \[ \beta = 0.18, P=4.4 \times 10^{-5} \] for HC. The
| Gene ID     | Gene name                                      | SNP ID     | Position in gene | Base change | AA change | MAF | NW | MAF OW/ OB | OR (95%CI) | P       |
|------------|------------------------------------------------|------------|------------------|-------------|-----------|-----|----|------------|------------|---------|
| MTHFR      | Methylene tetrahydrofolate reductase          | rs3737965  | Intron 1         | G/A         | 0.09      | 0.08 |    | 0.86       | 0.63–1.18  | 0.36    |
| rs9651118  |                                                 |            |                  |             |           |      |    |            |            |         |
| rs1801133  |                                                 |            |                  | A/C         | 0.38      | 0.41 |    | 1.12       | 0.95–1.32  | 0.19    |
| rs1801131  |                                                 |            |                  | E429A       | 0.38      | 0.41 |    | 1.12       | 0.95–1.32  | 0.19    |
| CTH        | Cystathionase                                  | rs663465   | 5’ flank         | C/T         | 0.43      | 0.45 |    | 1.10       | 0.93–1.30  | 0.27    |
| rs663649   |                                                 |            |                  | T/C         | 0.18      | 0.19 |    | 1.04       | 0.84–1.29  | 0.07    |
| rs1021737  |                                                 |            |                  | G/T         | 0.25      | 0.29 |    | 1.26       | 1.05–1.52  | 0.01    |
| rs6693082  |                                                 |            |                  | T/G         | 0.25      | 0.30 |    | 1.26       | 1.05–1.51  | 0.01    |
| MTR        | S-methylene tetrahydrofolate-homocysteine methyltransferase | rs946403   | Intron 13        | A/G         | 0.37      | 0.41 |    | 1.16       | 0.98–1.38  | 0.09    |
| rs1770449  |                                                 |            |                  | A/G         | 0.30      | 0.28 |    | 0.91       | 0.76–1.08  | 0.27    |
| rs1805087  |                                                 |            |                  | T/C         | 0.31      | 0.39 |    | 0.95       | 0.79–1.13  | 0.55    |
| rs16834521 |                                                 |            |                  | A/G         | 0.31      | 0.34 |    | 1.15       | 0.97–1.37  | 0.12    |
| rs2229276  |                                                 |            |                  | A/G         | 0.31      | 0.34 |    | 1.16       | 0.97–1.38  | 0.10    |
| rs1050993  |                                                 |            |                  | C/T         | 0.29      | 0.28 |    | 0.91       | 0.76–1.09  | 0.31    |
| CHDH       | Choline dehydrogenase                          | rs6445607  | Intron 1         | T/G         | 0.25      | 0.27 |    | 1.14       | 0.94–1.39  | 0.17    |
| rs2241808  |                                                 |            |                  | A/G         | 0.41      | 0.43 |    | 1.09       | 0.92–1.28  | 0.34    |
| MTRR       | Methionine synthase reductase                  | rs1801394  | Exon 2           | G/A         | 0.45      | 0.50 |    | 1.18       | 1.00–1.40  | 0.05    |
| rs162036   |                                                 |            |                  | T/C         | 0.14      | 0.16 |    | 1.14       | 0.90–1.43  | 0.27    |
| rs10380    |                                                 |            |                  | G/A         | 0.14      | 0.15 |    | 1.10       | 0.87–1.39  | 0.42    |
| BHMT       | Betaine-homocysteine S-methyl transferase      | rs492842   | Intron 1         | T/C         | 0.43      | 0.40 |    | 0.87       | 0.74–1.03  | 0.10    |
| rs3733890  |                                                 |            |                  | C/T         | 0.28      | 0.27 |    | 0.95       | 0.78–1.14  | 0.56    |
| rs585800   |                                                 |            |                  | A/T         | 0.14      | 0.13 |    | 0.87       | 0.68–1.12  | 0.27    |
| AMD1       | Adenosylmethionine decarboxylase 1             | rs2796749  | 5’ flank         | C/G         | 0.34      | 0.27 |    | 0.71       | 0.59–0.85  | 1.5×10-4 |
| rs1007274  |                                                 |            |                  | G/A         | 0.24      | 0.27 |    | 1.10       | 0.91–1.33  | 0.31    |
| rs7768897  |                                                 |            |                  | C/T         | 0.25      | 0.20 |    | 0.77       | 0.63–0.93  | 7.5×10-3 |
| NOS3       | Nitric oxide synthase 3                        | rs1799983  | Exon 7           | G/T         | 0.17      | 0.19 |    | 1.14       | 0.92–1.41  | 0.22    |
| rs2566514  |                                                 |            |                  | G/C         | 0.35      | 0.34 |    | 0.94       | 0.80–1.12  | 0.50    |
| MAT1A      | Methionine adenosyltransferase I               | rs17677908 | 5’ flank         | T/C         | 0.26      | 0.29 |    | 1.16       | 0.96–1.39  | 0.12    |
| rs2282367  |                                                 |            |                  | G/A         | 0.15      | 0.14 |    | 0.94       | 0.75–1.19  | 0.63    |
| rs10788546 |                                                 |            |                  | C/T         | 0.16      | 0.15 |    | 0.97       | 0.77–1.22  | 0.79    |
| rs2993763  |                                                 |            |                  | A/G         | 0.38      | 0.35 |    | 0.88       | 0.74–1.04  | 0.14    |
| rs1985908  |                                                 |            |                  | T/C         | 0.48      | 0.42 |    | 0.78       | 0.66–0.92  | 4.0×10-3 |
| FOLH1      | Folate hydrolase 1                             | rs202719   | Intron 11        | T/C         | 0.11      | 0.13 |    | 1.24       | 0.97–1.59  | 0.086   |
| rs6485965  |                                                 |            |                  | G/A         | 0.38      | 0.37 |    | 0.96       | 0.80–1.15  | 0.67    |
| MTHFD1     | Methylene tetrahydrofolate dehydrogenase 1     | rs8006686  | 3’ flank         | G/A         | 0.17      | 0.17 |    | 0.99       | 0.79–1.23  | 0.90    |
| rs1950902  |                                                 |            |                  | G/A         | 0.08      | 0.07 |    | 1.00       | 0.72–1.38  | 0.99    |
| rs8016556  |                                                 |            |                  | A/G         | 0.26      | 0.24 |    | 0.90       | 0.74–1.09  | 0.27    |
| rs2236225  |                                                 |            |                  | T/C         | 0.50      | 0.49 |    | 0.93       | 0.78–1.11  | 0.41    |
| rs2281603  |                                                 |            |                  | T/C         | 0.21      | 0.20 |    | 0.95       | 0.78–1.17  | 0.64    |
| MTHFD1L    | Methylene tetrahydrofolate dehydrogenase 1-like| rs9397027  | 5’ flank         | G/A         | 0.42      | 0.37 |    | 0.80       | 0.67–0.95  | 0.01    |
| rs2073063  |                                                 |            |                  | A/G         | 0.43      | 0.39 |    | 0.84       | 0.71–1.00  | 0.05    |
| rs1551179  |                                                 |            |                  | C/T         | 0.24      | 0.23 |    | 0.95       | 0.79–1.15  | 0.61    |
| rs509474   |                                                 |            |                  | G/C         | 0.33      | 0.32 |    | 0.98       | 0.81–1.17  | 0.78    |
rs2796749 was also associated with plasma leptin level ($\beta = 0.14$, $P = 4.2 \times 10^{-3}$).

Because of strong association of $AMD1$ variant rs2796749 with childhood obesity and measures of adiposity, we attempted to replicate the signal obtained at rs2796749 in additional sample set of 1843 children in stage 2. Association of rs2796749 with childhood obesity was validated in stage 2 ($\text{OR} = 1.28$, $P = 6.8 \times 10^{-3}$) and was confirmed by meta-analysis ($\text{OR} = 1.35$, $P = 6.8 \times 10^{-3}$) (Table 3). Similar results were obtained in combined analysis by pooling genotype data from two stages ($\text{OR} = 1.35$, $P = 6.8 \times 10^{-3}$). The meta-analysis and combined analysis corroborated the association of rs2796749 ($\beta = 0.12$, $P = 6.5 \times 10^{-6}$ in meta-analysis and $\beta = 0.13$, $P = 2.5 \times 10^{-3}$ in combined analysis). Association of rs2796749 with measures of adiposity and plasma leptin levels was also replicated in stage 2 and was corroborated in combined analysis (Figure 2). No association of rs2796749 with other biochemical traits investigated could be detected (Table 4).

Stage 1 analysis also suggested association of $MATI\alpha$ variant rs1985908 with childhood obesity ($\text{OR} = 1.28$, $P = 4.0 \times 10^{-3}$), BMI ($\beta = 0.14$, $P = 1.5 \times 10^{-3}$) and leptin levels ($\beta = -0.12$, $P = 2.7 \times 10^{-3}$). $CTH$ variant rs6693082 which showed nominal association with obesity, was also found to be associated with BMI ($P = 9.1 \times 10^{-3}$), WC ($P = 4.0 \times 10^{-3}$), HC ($P = 6.3 \times 10^{-4}$) and adiponectin levels ($P = 4.5 \times 10^{-3}$) (Table S2). However the associations of rs1985908 and rs6693082 with obesity and other traits did not remain significant after correcting for multiple comparisons.

Further, we investigated the association of all the SNPs genotyped in stage 1 with quantitative traits related to obesity that included measures of glucose homeostasis, lipid metabolism and adipokines. We observed suggestive association of some variants with quantitative traits (Table S2). $CTH$ variant rs1021737 showed association with total cholesterol ($P = 6.8 \times 10^{-7}$) while $MATI\alpha$ variant rs17677908 and $MTR$ variant rs16834521 were found to be associated with TG ($P = 6.8 \times 10^{-3}$ and $3.56 \times 10^{-3}$ respectively). Besides $AMD1$ and $MATI\alpha$, levels of leptin were also associated with $MTRR$ variant rs1801394 ($P = 6.8 \times 10^{-3}$). However, none of the associations remained significant after accounting for multiple tests. We also performed haplotype association analysis that suggested association of haplotypes of variants SNPs in $AMD1$, $CTH$, $MTHFR$ and $MTRR$ (Table S3). None of the haplotype associations remained significant after 10,000 permutations.

**Discussion**

Several GWAS and large-scale meta-analysis for adult BMI have identified susceptibility loci for increased BMI, explaining only a small proportion of variation in BMI [3]. Investigation of genetic risk factors for childhood obesity has been restricted mainly to the replication of GWAS findings [3]. Though modern genotyping arrays capture most of the common variations, there...
### Table 3. Association of homocysteine metabolism pathway genes with obesity in urban Indian children.

| SNP         | Gene  | RAF NW children | RAF OW/OB children | Obesity | BMI |
|-------------|-------|-----------------|--------------------|---------|-----|
|             |       | OR (95%CI)      | P                  | β       | SE  | P   |
| Stage 1     |       |                 |                    |         |     |     |
| rs2796749   | AMD1  | 0.657           | 0.733              | 1.41 (1.18–1.69) | 1.5×10⁻⁶ | 0.16 | 0.05 | 4.2×10⁻⁶ |
| rs7768897   | AMD1  | 0.745           | 0.795              | 1.30 (1.07–1.59) | 7.5×10⁻³ | 0.11 | 0.05 | 0.03  |
| rs1985908   | MAT1A | 0.524           | 0.581              | 1.28 (1.09–1.51) | 4.0×10⁻³ | 0.14 | 0.04 | 1.5×10⁻³ |
| rs9397028   | MTHFD1L| 0.577           | 0.627              | 1.25 (1.05–1.49) | 0.01   | 0.15 | 0.05 | 1.3×10⁻³ |
| rs1021737   | CTH   | 0.250           | 0.292              | 1.26 (1.05–1.52) | 0.01   | 0.16 | 0.05 | 1.6×10⁻³ |
| rs6693082   | CTH   | 0.250           | 0.300              | 1.26 (1.05–1.51) | 0.01   | 0.16 | 0.05 | 9.1×10⁻⁶ |
| rs1801133   | MTHFR | 0.169           | 0.203              | 1.23 (1.01–1.52) | 0.04   | 0.12 | 0.06 | 0.02  |
| Stage 2     |       |                 |                    |         |     |     |
| rs2796749   | AMD1  | 0.668           | 0.723              | 1.28 (1.09–1.54) | 4.2×10⁻³ | 0.10 | 0.03 | 2.6×10⁻³ |
| Combined    |       |                 |                    |         |     |     |
| rs2796749   | AMD1  | 0.664           | 0.728              | 1.35 (1.19–1.52) | 1.9×10⁻⁶ | 0.13 | 0.03 | 2.5×10⁻⁶ |
| Meta-analysis* |     |                 |                    |         |     |     |
| rs2796749   | AMD1  | 1.35            | 2.7×10⁻⁶           | 0.12    | 6.5×10⁻⁶ |     |     |     |

RAF: risk allele frequency; OR: odds ratio. Odds ratio are presented with respect to risk allele that are highlighted as underlined text. β: change in Z score of BMI per increase in risk allele; SE: standard error. Combined analysis was performed by combining raw genotype data from stage 1 and stage 2. Meta-analysis was performed by combining the summary estimates (OR/β, 95%CI and SE). No heterogeneity in two study population was observed (P value for Cochrane’s Q statistic = 0.48 and I² heterogeneity index = 0.0). *OR/β and P value for fixed effect meta-analysis.

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### Table 4. Association of AMD1 variant rs2796749 with clinical traits in urban Indian children.

| Trait                  | Stage 1 | Stage 2 | Combined | Mean |
|------------------------|---------|---------|----------|------|
|                        | β       | P       | β       | P    | β    | P    | CC  | CG  | GG  |
| Height¹     | 0.10    | 0.02    | 0.09    | 0.01 | 0.09 | 6.7×10⁻⁴ | −0.20 | 0.01 | 0.04 |
| Weight¹     | 0.17    | 1.3×10⁻⁴ | 0.12    | 2.8×10⁻⁴ | 0.14 | 9.1×10⁻⁸ | −0.17 | −0.05 | 0.10 |
| BMI¹        | 0.16    | 4.2×10⁻⁴ | 0.10    | 2.6×10⁻³ | 0.13 | 2.5×10⁻⁶ | −0.11 | −0.06 | 0.10 |
| WC¹         | 0.18    | 3.2×10⁻⁵ | 0.11    | 2.5×10⁻³ | 0.14 | 3.2×10⁻⁷ | −0.16 | −0.06 | 0.10 |
| HC¹         | 0.18    | 4.4×10⁻⁵ | 0.12    | 3.3×10⁻⁴ | 0.16 | 3.7×10⁻⁸ | −0.17 | −0.05 | 0.12 |
| WHR¹        | 0.07    | 0.07    | 0.04    | 0.27  | 0.06 | 0.05  | −0.07 | −0.04 | 0.03 |
| Adiponectin¹ | −0.03   | 0.49    | −0.01   | 0.71  | −0.02 | 0.41  | −0.08 | 0.04  | 0.00 |
| Leptin¹     | 0.14    | 4.2×10⁻⁴ | 0.09    | 0.02  | 0.12 | 2.2×10⁻⁵ | −0.01 | −0.01 | 0.06 |
| Resistin¹   | 0.01    | 0.82    | 0.02    | 0.60  | 0.01 | 0.74  | −0.01 | 0.01  | 0.01 |
| hsCRP¹      | 0.08    | 0.08    | 0.06    | 0.11  | 0.07 | 0.01  | 0.03  | −0.03 | 0.02 |
| Total Cholesterol² | −0.009 | 0.84 | 0.07 | 0.03 | 0.04 | 0.12 | 0.01 | −0.05 | −0.003 |
| HDL²        | −0.07   | 0.05    | 0.09    | 0.81  | −0.03 | 0.35  | −0.003 | −0.001 | −0.01 |
| LDL²        | 0.02    | 0.65    | 0.08    | 0.02  | 0.06 | 0.04  | −0.01 | −0.02 | 0.03 |
| TG²         | 0.06    | 0.10    | −0.03   | 0.45  | 0.01 | 0.73  | 0.07  | 0.05  | 0.05 |
| FPG²        | −0.03   | 0.47    | 0.002   | 0.94  | 0.07 | 0.01  | 0.02  | 0.05  | 0.001 |
| FPI²        | 0.06    | 0.15    | 0.07    | 0.06  | −0.01 | 0.67  | −0.08 | 0.03  | −0.005 |
| C-peptide¹  | 0.08    | 0.05    | 0.03    | 0.36  | 0.06 | 0.04  | −0.09 | 0.06  | −0.01 |
| HOMA-IR¹    | 0.05    | 0.27    | 0.07    | 0.05  | 0.07 | 0.02  | −0.09 | 0.04  | −0.01 |

β represents change in Z score per increase in risk allele. The mean values presented were obtained by ANCOVA analysis adjusted for appropriate covariates.

¹Analysis performed after adjusting for age and sex.
²Analysis performed after adjusting for age, sex and BMI.

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remain substantial additional contributions to phenotypic variation from the variants not well captured by these arrays [26]. Investigation of variants prioritized based on their functional and positional significance provides an opportunity to identify those not detected by GWAS. Employing functional candidate analysis, we recently identified association of common variants of **IL6**, **LEPR**, and **PBEF1** with increased susceptibility to obesity and measures of adiposity in Indian children [27]. Homocysteine metabolism pathway seems to be a promising candidate pathway by virtue of its involvement in important cellular processes. Thus, here we comprehensively examined association of common variants of homocysteine metabolism pathway genes with obesity in children.

The study reveals association of **AMD1** variant rs2796749 with obesity, measures of adiposity and plasma leptin levels in urban Indian children. To the best of our knowledge, this is the first study demonstrating the association of **AMD1** variant with obesity. In our previous study of association of homocysteine metabolism pathway genes with type 2 diabetes and adult obesity [28], rs2796749 did not show association with adult obesity or BMI but was directionally consistent. Further, we investigated effect of this variant on adult BMI using the association data from GIANT consortium GWA meta-analysis which included 249,796 individuals of European ancestry [29]. In this recent meta-analysis also, rs2796749 showed consistency in direction of association with BMI, though could not reach statistical significance ($P = 0.19$). This suggests that **AMD1** variant rs2796749 might have influence on adult BMI also, but with smaller effect size.

**AMD1** codes for an important regulatory enzyme adenosylmethionine decarboxylase 1 (AdoMetDC) which catalyzes the decarboxylation of S-adenosyl methionine (SAM), a methyl-group donor in transmethylation reactions. The imbalances in decarboxylation of SAM might result in disruption of DNA methylation process leading to abnormal gene expressions [30], which is implicated in various metabolic disorders including obesity and diabetes [31]. In addition to its role in transmethylation reaction, AdoMetDC is also a regulatory enzyme in polyamine biosynthesis. SAM is committed to polyamine bio-synthesis after decarboxylation and serves as an aminopropyl donor in spermidine and spermine synthesis [32]. The polyamines are ubiquitous and are

Figure 2. Association of rs2796749 with clinical traits in urban Indian children. Mean Z-scores adjusted for age and sex are plotted on the y-axis for the corresponding genotypes on x-axis for all the traits except leptin for which the mean Z-scores are adjusted for age, sex and Z-BMI. $P$ values presented were determined by linear regression analysis for Z-score change per risk allele of the respective trait.

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implicated in cellular growth and differentiation, regulation of adipocyte formation and glucose metabolism [33].

A recent study reported increased levels of polyamines in childhood obesity and their correlation with biomarkers of oxidative stress, inflammation and leptin [34]. The study supported the role of polyamines on growth and development on adipose tissue. On similar lines, our study demonstrated association of rs2796749 in 5′ flank region of AMD1 with obesity in Indian children. The same variant also showed association with quantitative measures of adiposity including weight, BMI, hip and waist circumferences and plasma levels of leptin. Moreover, AMD1 mRNA is shown to increase in response to insulin via insulin response element in 5′ flank region of AMD1 [35]. The variant rs2796749 might also have role in influencing the transcription of AMD1 in response to insulin, which needs to be investigated.

MAT1A codes for methionine adenosyltransferase (MAT), which catalyzes the conversion of methionine to Sadenosylmethionine (SAM). MAT1A variants have been shown to be associated with hypertension and stroke [36]. Our study also suggested association of MAT1A variant rs1985908 with childhood obesity. MAT1A variants also showed association with the plasma levels of leptin, adiponectin and triglycerides. But these associations could not stand multiple testing corrections, hence further replication in larger sample set and in other ethnic groups are required to substantiate the observations in the present study.

Although the present study design limits the potential to identify the causal relationships, based on previous studies, we can speculate that AMD1 variant might influence the susceptibility to obesity by modulating either the polyamines metabolism or DNA methylation. Dietary habits can affect these processes as nutrients such as vitamin B12 and folate are required as important cofactors in these enzymatic reactions (Figure 1). The plasma levels of folate and vitamin B12 as well as the dietary intake of these are inversely correlated with the plasma homocysteine levels [37]. Moreover, individuals consuming only vegetarian diet tend to have low vitamin B12 and elevated homocysteine levels [38,39]. Majority of Indian population adheres to a vegetarian diet that predisposes them to vitamin B12 deficiency. Thus diet which can influence the availability of vitamins may modulate the impact of genetic variants on obesity phenotypes. Previous studies including investigation in Indian populations suggested the possibility of interaction between diet and genetic variants of homocysteine pathway genes in influencing the homocysteine levels [40], however no study provided strong evidence for this interaction and its impact on obesity. Unfortunately, lack of data on the levels of folate and vitamin B12 and the dietary habits of our study subjects limited further investigation of the interaction of these factors with the genetic variants and their combined effect on obesity phenotypes.

In case-control association studies, limited statistical power and population stratification can lead to spurious association results. Though the sample size of our study is considerable, there is a likelihood of false negative observations for variants with small effect sizes, as the present study is sufficiently powered to capture only large effects (OR > 1.3) of the common variants with frequencies more than 0.20. Further, to minimize the effect of population stratification, we have collected samples from a small geographical region that forms a homogenous cluster as reported by the Indian Genome Variation Consortium [41]. Moreover, the multidimensional scaling (MDS) analysis using the genotype data for 595 unlinked markers (r²<0.20) for stage 1 samples shows that the study population belongs to one cluster (Figure S1).

In conclusion, we demonstrate here, for the first time, association of AMD1 variant with susceptibility to obesity and measures of adiposity in Indian children. Further studies to confirm the association of AMD1 variant with childhood, its functional significance and molecular mechanism need to be undertaken. Moreover, our study provides a lead for future investigations towards understanding the genetic predisposition of obesity in childhood and exploring therapeutic options for prevention of childhood obesity.

### Supporting Information

#### Figure S1 Multidimensional scaling for the study population in stage 1. The 595 unlinked markers (r²<0.20) were used to obtain the positions on the first and second dimensions using PLINK.

#### Table S1 SNPs selected for the study and their association with obesity in urban Indian children AA: Amino acid; HWE: Hardy Weinberg Equilibrium; MAF: minor allele frequency; QC: quality control; OR: odds ratio; CE: confidence interval. OR and P values presented were calculated with respect to minor allele using logistic regression analysis under additive model with age and sex as covariates.

#### Table S2 Association of analyzed SNPs with biochemical parameters in urban Indian children β, L95 and U95 represent change in Z-score units of the parameters with per increase in risk allele with 95% confidence interval. β values are presented with respect to the minor alleles. Analysis for height, weight, BMI, WC and HC were adjusted for age and sex whereas analyses for all other parameters were adjusted for age, sex and Z-BMI.

#### Table S3 Association of haplotypes with obesity in urban Indian children OR: odds ratio; NW: normal-weight; OW/OB: overweight and obese.

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### Author Contributions

Conceived and designed the experiments: RT NT DB. Performed the experiments: OPD GC. Analyzed the data: RT AJ OPD GC SG. Contributed reagents/materials/analysis tools: RKM. Wrote the paper: RT AJ DB.

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