A novel common variant in DCST2 is associated with length in early life and height in adulthood

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A novel common variant in DCST2 is associated with length in early life and height in adulthood

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#The authors wish it to be known that, in their opinion, J.S., N.J.T., F.R., K.B. and V.W.V.J. should be regarded as joint Lead Senior Authors; these authors jointly directed this work.

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Common genetic variants have been identified for adult height, but not much is known about the genetics of skeletal growth in early life. To identify common genetic variants that influence fetal skeletal growth, we meta-analyzed 22 genome-wide association studies (Stage 1; \(N = 28,459\)). We identified seven independent top single nucleotide polymorphisms (SNPs) \((P < 1 \times 10^{-6})\) for birth length, of which three were novel and four were in or near loci known to be associated with adult height \((\text{LCORL}, \text{PTCH1}, \text{GPR126} \text{and} \text{HMGA2})\). The three novel SNPs were followed-up in nine replication studies (Stage 2; \(N = 11,995\)), with rs905938 in \text{DC-STAMP domain containing 2 (DCST2)} genome-wide significantly associated with birth length in a joint analysis \((\text{Stages 1 + 2}; \beta = 0.046, \text{SE} = 0.008, P = 2.46 \times 10^{-8}, \text{explained variance} = 0.05\%)\). Rs905938 was also associated with infant length \((N = 28,228; P = 5.54 \times 10^{-4})\) and adult height \((N = 127,513; P = 1.45 \times 10^{-9})\). DCST2 is a DC-STAMP-like protein family member and DC-STAMP is an osteoclast cell-fusion regulator. Polygenic scores based on 180 SNPs previously associated with human adult stature explained 0.13% of variance in birth length. The same SNPs explained 2.95% of the variance of infant length. Of the 180 known adult height loci, 11 were genome-wide significantly associated with infant length \((\text{SF3B4}, \text{LCORL}, \text{SPAG17}, \text{C6orf173}, \text{PTCH1}, \text{GDF5}, \text{ZNFX1}, \text{HHIP}, \text{ACAN}, \text{HLA locus and} \text{HMGA2})\). This study highlights that common variation in \text{DCST2} influences variation in early growth and adult height.

INTRODUCTION

Fetal and infancy length growth are important measures of development in early life. Early length growth seems to be associated with height in adulthood (1). It has been shown that fetal and infant growth are independently associated with higher risks of cardiovascular disease, type 2 diabetes and many other complex diseases. Previous findings suggested genetic links between fetal growth and metabolism (2,3). However, these studies mainly focused on birth weight as early growth measure. Skeletal growth is a different measure of development in early life. Skeletal growth during fetal life and infancy is a complex trait with heritability estimates of 26–72% (4). Although correlated with each other, fetal, infant and adult skeletal growth may be influenced by different genetic factors. Many common genetic variants have been identified for adult height (5), but not much is known about the genetics of skeletal growth in early life. Although, several rare genetic defects with large effects on length at birth and during infancy have been found (6,7), common genetic variants that influence normal variation in birth and infant length have not yet been identified. Therefore, we aimed to identify common genetic variants influencing early length growth, also in perspective of their effect on adult stature.
RESULTS

To identify common genetic variants associated with birth length, we examined 2,201,971 million directly genotyped and imputed SNPs with birth length in 22 independent discovery studies with genome-wide association (GWA) or Metabochip data (Stage 1; \(N = 28,459\); Fig. 1). Birth length was measured using standardized procedures (Supplementary Material, Tables S1 and S2). Studies with self-reported measurements were excluded a priori. Birth length was standardized using growth analyzer (http://www.growthanalyser.org), transforming birth length into sex- and age-adjusted standard deviation scores (SDS). We used the North-European 1991 reference panel to compare results between studies. We applied linear regression between number of alleles or dosages obtained from imputations and standardized birth length (full details in Materials and Methods).

Gene identification

In the discovery phase (Stage 1), we found seven independent top SNPs with suggestive evidence of association (\(P < 1 \times 10^{-6}\)) with birth length (Supplementary Material, Figs. S1 and S2, QQ- and Manhattan plot). Four SNPs mapped to loci already known to be associated with adult height (Supplementary Material, Table S3, \(LCORL\), \(PTCH1\), \(GPR126\) and \(HMGA2\))(5). The 3 SNPs reflecting potentially novel associations were taken forward in nine independent replication studies (Stage 2; \(N = 11,995\); Fig. 1). Only one of the three SNPs displayed significant evidence for replication in Stage 2 and reached genome-wide significance in the joint analysis (Stages 1 + 2; \(P < 5 \times 10^{-8}\); Table 1). This novel association arose from SNP rs905938, mapping to chromosome 1q22 in DC-STAMP domain containing 2 (DCST2) (Fig. 2, regional association plot). Each C allele [minor allele frequency (MAF) = 0.24] of rs905938 was associated with an increase (standardized) of 0.046 SDS in birth length (standard error = 0.008, \(P = 2.46 \times 10^{-8}\); explained variance = 0.05%). The genome-wide significantly associated SNP showed low degree of heterogeneity between the discovery studies (\(P = 0.93, I^2 = 0\%\)). Figure 3 shows the forest plot of the associations between rs905938[C] and birth length across all studies. Other suggestive loci in the discovery analysis are shown in Supplementary Material, Table S3 (\(P < 1 \times 10^{-5}\)). Summary statistics of all SNPs are available at http://egg-consortium.org.

Functional analyses

We assessed common variants with deleterious functional implications in linkage disequilibrium (LD, \(r^2 > 0.8\)) with rs905938 using HaploReg (8). There were no non-synonymous variants in LD with rs905938. We found three putative functional intronic variants in high LD with rs905938. Details are depicted in Supplementary Material, Table S4. Subsequently, we assessed whether variants in the identified locus were involved in the

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**Figure 1** Study design.
regulation of messenger RNA expression (eQTLs) in genome-wide expression datasets of lymphoblastoid cell lines (LCLs, N = 1830) (9,10). We found cis eQTLs [false discovery rate (FDR), 1% account for all SNP-probe pairs that were within 1 Mb of each other) for transcripts of PBXIP1, GBA and ADAM15. Yet, rs905938 and the cis eQTL SNPs were not in perfect LD (r² = 0.80, Supplementary Material, Table S5).

Therefore, we cannot exclude that multiple independent effects arise from the same region of association.

**DCST2 and growth phenotypes**

We tested the associations of rs905938[C] with ‘fetal growth’ measures in the 1st, 2nd and 3rd trimester of pregnancy in the

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### Table 1 Summary statistics of the three novel SNPs at P < 1 × 10^{-6} in the discovery analysis and the replication follow-up results

<table>
<thead>
<tr>
<th>Marker</th>
<th>MAF</th>
<th>β</th>
<th>SE</th>
<th>P</th>
<th>n</th>
<th>I²</th>
<th>HetP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discovery (Stage 1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs905938[C] at 1q22 (DCST2)</td>
<td>0.24</td>
<td>0.050</td>
<td>0.010</td>
<td>2.59 × 10^{-7}</td>
<td>28 327</td>
<td>0.0</td>
<td>0.930</td>
</tr>
<tr>
<td>rs12545524[G] at 8q22.1 (near GDF6)</td>
<td>0.14</td>
<td>0.078</td>
<td>0.014</td>
<td>1.54 × 10^{-8}</td>
<td>22 170</td>
<td>6.6</td>
<td>0.376</td>
</tr>
<tr>
<td>rs11037473[A] at 11p11.2 (nearest genes TTC17-HSD17B12)</td>
<td>0.06</td>
<td>-0.109</td>
<td>0.021</td>
<td>2.17 × 10^{-7}</td>
<td>22 259</td>
<td>0.0</td>
<td>0.735</td>
</tr>
<tr>
<td>Replication (Stage 2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs905938[C] at 1q22 (DCST2)</td>
<td>0.23</td>
<td>0.035</td>
<td>0.015</td>
<td>1.99 × 10^{-2}</td>
<td>11 908</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>rs12545524[G] at 8q22.1 (near GDF6)</td>
<td>0.11</td>
<td>-0.012</td>
<td>0.017</td>
<td>4.67 × 10^{-1}</td>
<td>17 614</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>rs11037473[A] at 11p11.2 (nearest genes TTC17-HSD17B12)</td>
<td>0.08</td>
<td>-0.035</td>
<td>0.020</td>
<td>8.06 × 10^{-2}</td>
<td>17 606</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Discovery + replication (Stages 1 + 2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs905938[C] at 1q22 (DCST2)</td>
<td>0.24</td>
<td>0.046</td>
<td>0.008</td>
<td>2.46 × 10^{-8}</td>
<td>40 235</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>rs12545524[G] at 8q22.1 (near GDF6)</td>
<td>0.13</td>
<td>0.042</td>
<td>0.011</td>
<td>9.08 × 10^{-5}</td>
<td>39 784</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>rs11037473[A] at 11p11.2 (nearest genes TTC17-HSD17B12)</td>
<td>0.07</td>
<td>-0.069</td>
<td>0.014</td>
<td>1.49 × 10^{-6}</td>
<td>39 865</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

SNPs markers are identified according to their standard rs numbers (NCBI build 36). Independent novel SNPs with a strong suggestive effect in the discovery analysis on birth length are shown (P < 1 × 10^{-6}). SNPs in loci that are known to be associated with adult height were excluded for replication efforts (adult height loci: LCORL, PTCH1, GPR126 and HMGA2). MAF, minor allele frequency; SE, standard error. P values are obtained from linear regression of each SNP against standardized birth length adjusted for sex and gestational age. We included both GWA and metabochip cohorts in our discovery analysis, rs905938 is on the metabochip, and rs12545524 and rs11037473 are not, this explains the differences in numbers (n). Derived inconsistency statistic I² and HetP values reflect heterogeneity across discovery studies with the use of Cochran’s Q tests.

**Figure 2** Regional association plot of 1q22 in the 22 birth length discovery studies (N = 28 459). SNPs are plotted with their P values (as -log_{10} values; left y-axis) as a function of genomic position (x-axis). Estimated recombination rates (right y-axis) taken from HapMap are plotted to reflect the local LD-structure around the top associated SNP (‘white open diamond’) and the correlated proxies (‘circles’ according to a black-to-gray scale from r² = 0 to 1). The joint analysis P value of discovery and replication studies is reported with the ‘white square’ (N = 40 235).
Known adult height loci in relation to birth and infant length

We also explored whether common genetic variants known to be associated with adult height (5) influenced birth length variation. We found that 17 out of 180 known adult height loci were associated with birth length (FDR < 5%, Supplementary Material, Table S6; Fig. 4, QQ-plot of 180 SNPs and birth length). We then calculated a height-increasing-alleles score of the 180 known height loci (5) to predict birth length in the Generation R Study (N = 2085; Fig. 5). The score composed of variants associated with adult height explained 0.13% of the variance in birth length (P = 0.1), in contrast to the ∼10% of the phenotypic variation in adult height reported in the original manuscript (5).

To evaluate whether different common genetic variants influenced both birth and infant length, we tested 2 193 675 million SNPs for association with infant length in almost the same set of samples used for the analysis of birth length (19 studies, N = 28 238; Supplementary Material, Table S7). We identified genome-wide significant associations at 11 genetic loci (Supplementary Material, Figs S3 and S4, QQ- and Manhattan plot), which all are known to be associated with adult height (Table 3, SNPs in or near SF3B4, LCORL, SPAG17, C6orf173, PTCH1, GDF5, ZNFX1, HHIP, ACAN, HLA locus and HMG2A) (5,13). In addition, we found that variants in 58 of the adult height loci were associated with infant length at an FDR of 5% (Supplementary Material, Table S8; Fig. 4, QQ-plot of 180 SNPs and infant length). Next, we tested in the Generation R Study (N = 2385) how much of the phenotypic variance in infant length was explained by the score composed of height-increasing-alleles. Variants from the 180 known adult height loci together explained 2.95% of the variance in infant length (P = 3.10 × 10⁻¹⁷, Fig. 5).

**DEPICT analysis of birth and infant length**

Finally, we used a pathway analysis tool called DEPICT (Pers et al., unpublished data) to prioritize genes at associated regions, search for reconstituted gene sets that were enriched in genes near associated variants, and identify tissue and cell types in which genes from loci associated with birth and infant length were highly expressed (full details in Materials and Methods). For both traits, we used independent SNPs (r² < 0.05) associated at P < 1 × 10⁻⁵, from 21 birth length and 44 infant length loci. There were no pathways significantly overrepresented in the birth length results. In contrast, for infant length DEPICT significantly prioritized nine genes which were overrepresented (FDR < 5%, Supplementary Material, Table S9), including three known Mendelian human stature genes (ACAN, GDF5 and PTCH1) as well as several relevant reconstituted genes.
and the diagonal lines represent the expected
(rs905938 in DISCUSSION)
particular tissue or cell types for any of the two traits. Material, Table S10). There was no significant enrichment for osteoblast proliferation and WNT signaling, Supplementary gene sets (e.g. abnormal sternum ossification, regulation of development, head circumference and brain structure (12,27). As we
is also associated with type 2 diabetes and ADCY5 with adult blood pressure (2,3). These findings highlight genetic links between fetal growth and metabolism (2,3,26). As we
We found that the SNP effects for birth length of 137 of the 180 established height loci were in the same direction as reported in the GIANT paper (5) (Supplementary Material, Table S6; probability of success $P = 0.761$, Table S8; probability of success $P = 0.900$, $P = 2.20 \times 10^{-11}$).

One hundred sixty-two of the 180 loci were in the same direction or near loci known to be associated with birth weight ($LCORL$), the genetic architecture of adult height seems more similar to the genetic architecture of infant length than birth length [58 shared loci (9)]. Also, there was no significant eQTL of DCST2 in immortalized LCLs (10). However, DCST2 is a DC-STAMP-like protein family member and DC-STAMP is an important regulator of osteoclast cell-fusion in bone homeostasis (14–16). The transcripts of PBPX1P, GBA and ADAM15 were in weak LD with our lead SNP rs905938. The PBPX1P protein is known to regulate estrogen receptor functions (17). Mutations in the GBA gene cause Gaucher disease, and strong associations with Parkinson’s disease and dementia with Lewy bodies have been described (18–21). ADAM15 is prominently expressed in osteoblasts and to a lesser extent in osteoclasts (22). A study in mice showed that ADAM15 is required for normal skeletal homeostasis and that its absence causes increased nuclear translocation of $\beta$-catenin in osteoblasts leading to increased osteoblast proliferation and function, which results in higher trabecular and cortical bone mass (23). The 1q22 locus is a complex region harboring multiple interesting genes that could affect birth length. We emphasize that we could not specifically pinpoint the causal gene(s) as our lead SNP (rs905938) was not in perfect LD with our cis eQTL SNPs.

Although, there is some overlap between adult height loci and birth length, which is illustrated by 17 shared loci, the genetic architecture of adult height seems more similar to the genetic architecture of infant length than birth length [58 shared loci for infant length, based on conservative statistical method (FDR)]. One point of consideration for the interpretation of our findings is the potential of measurement error for birth length (24). This may lead to less power to detect novel genetic variants as standard errors of SNPs could be increased. The estimate of the risk-allele score slope of Figure 5 is not influenced by measurement error and the differences in the slopes suggest that birth and infant length are influenced by distinct genetic variants. We found that the SNP effects for birth length of 137 of the 180 established height loci were in the same direction as reported in the GIANT paper (5) (Supplementary Material, Table S6; probability of success $P = 0.761$, $P = 6.25 \times 10^{-11}$). One hundred sixty-two of the 180 loci were in the same direction for infant length (Supplementary Material, Table S8; probability of success $P = 0.900$, $P = 2.20 \times 10^{-11}$).

Four SNPs associated with birth length ($P < 1 \times 10^{-5}$) are in or near loci known to be associated with birth weight ($LCORL$, $HMGA2$, $ADCY5$ and $ADRB1$). $LCORL$ is associated with birth weight, birth length, infant length and adult height, but we could not find an obvious link between the gene and adult-onset diseases. $HMGA2$ is associated with aortic root size (25), type 2 diabetes (26), and many other traits like tooth size (25), type 2 diabetes (26), and many other traits like tooth connection and function, which results in higher trabecular and cortical bone mass (23).

As we found overlap between genetic variants of birth weight and birth length, we looked-up the effect of rs905938 in $DCST2$ on birth weight in a previous EGG study (3). Rs905938 was associated with birth weight, but weaker as compared with birth length ($\beta = 0.035$ SDS, SE = 0.010, $P = 2.35 \times 10^{-4}$, $N = 26\ 558$).

In conclusion, in the present study we identified one novel locus (rs905938 in $DCST2$ at 1q22) associated with birth length at a genome-wide significant level. This common variant was also associated with infant length and adult height.
A genetic variant was also associated with infant length and adult height, with decreasing magnitude of the associations in later life (0.046 SDS for birth length, 0.035 SDS for infant length and 0.024 SDS for adult height). To our knowledge, no phenotype has been previously associated with the DCST2 gene and while the gene is expressed in osteoclasts, its function should be further studied.

MATERIALS AND METHODS

Stage 1: discovery genome-wide association analyses of birth length

We combined 21 population-based studies with GWA or Metabochip data and birth length available (total N = 28 459 individuals). One of our discovery cohorts had two independent sub-samples within their study leading to a total of 22 independent GWA/Metabochip sub-samples for our analysis: one sub-sample from the Avon Longitudinal Study of Parents and Children (ALSPAC, GWA, n = 4816); Children Allergy, Milieu, Stockholm, Epidemiology [Swedish] (BAMSE, GWA, n = 423); Children’s Hospital Of Philadelphia (CHOP, GWA, n = 432); Copenhagen Study on Asthma in Childhood 2000 (COPSAC-2000, GWA, n = 348); Copenhagen Study on Asthma in Childhood Registry (COPSAC-Registry, GWA, n = 1111); Danish National Birth Cohort (DNBC, GWA, n = 932); Generation R Study (Generation R, GWA, n = 2085); Hyperglycemia and Adverse Pregnancy Outcomes study (HAPO, GWA, n = 1325); Helsinki Birth Cohort Study (HBCS, GWA, n = 1572); Infancia y Medio Ambiente (INMA, GWA, n = 848); Leipzig Childhood Obesity cohort (LEIPZIG, Metabochip, n = 607); Lifestyle Immune System Allergy study (LISA, GWA, n = 552);
of between-study heterogeneity of the effect sizes. The meta-analysis results were obtained for a total of 2,201,971 SNPs. SNPs that crossed the threshold of $P \leq 1 \times 10^{-6}$ were considered to represent strong suggestive evidence of association with birth length. SNPs that were already known to be associated with adult height were excluded for the replication analysis (5). The explained variance of the top SNPs were calculated in one of the largest cohorts, the Generation R Study ($n = 2085$).

Stage 2: replication analysis of top birth length SNPs

In the discovery phase, we found seven independent SNPs with strong suggestive evidence of association ($P < 1 \times 10^{-6}$) with birth length. Four SNPs were already known to be associated with adult height (5). These SNPs were excluded for follow-up analyses. The three remaining novel SNPs were followed-up in replication studies. We included both GWA and Metabochip studies in our discovery analysis. Rs905938 was on our Metabochips, and rs12545524 and rs11037473 were not. This results in differences in numbers for our top SNPs in the discovery and replication analyses. Rs905938 was taken forward in 9 independent replication studies ($N = 11,995$), rs12545524 and rs11037473 in 13 independent replication studies including the four discovery Metabochip studies ($N = 17,679$). Details of the replication studies are presented in Supplementary Material, Table S2. Within the replication studies, we analyzed the association between number of alleles and standardized birth length. Combined effect estimates and heterogeneity between cohorts was calculated using fixed effects meta-analyses in R Version 2.8.1 (The R foundation for Statistical Computing, library rmeta). Top SNPs that crossed the significant threshold of $P$-replication $\leq 0.05$ and the widely accepted genome-wide significance threshold of $P \leq 5 \times 10^{-8}$ for all studies combined were considered to represent robust evidence of association with birth length. The institutional review boards for human studies approved the protocols and written consent was obtained from the participating subjects or their caregivers if required by the institutional review board.

DEPICT analysis

We used the novel Data-driven Expression-Prioritized Integration for Complex Traits (DEPICT) method (Pers et al., unpublished data). DEPICT is designed to systematically identify the most likely causal gene at a given locus, gene sets that are enriched in genetic associations, and tissues and cell types in which genes from associated loci are highly expressed. First, DEPICT assigns genes to associated SNPs using LD $r^2 > 0.5$ distance to define locus boundaries, merges overlapping loci and discards loci mapping within the extended major histocompatibility complex region (chromosome 6, base pairs 25,000–35,000). Next, the DEPICT method prioritizes genes within a given associated locus based on the genes’ functional similarity to genes from other associated loci. Genes that are highly similar to genes from other loci obtain low prioritization $P$ values, and simulated GWAS results are used to adjust for gene length bias as well as other potential confounders. There can be several prioritized genes in a given locus. Next, DEPICT conducts gene set enrichment analysis by testing whether genes in associated loci enrich for reconstituted versions of known pathways, genes
sets as well as protein complexes. Leveraging the guilt by association hypothesis that genes co-expressing with genes from a given gene set are likely to be part of that gene set (Cvijic et al. 35, for details), the gene set reconstitution is accomplished by identifying genes that were co-expressed with genes in a given gene set based on a panel of 77 840 gene expression microarrays. Gene sets from the following repositories were reconstituted: 5984 protein complexes that were derived from 169 810 high-confidence experimentally derived protein–protein interactions 36; 2473 phenotypic gene sets derived from 211 882 gene–phenotype pairs from the Mouse Genetics Initiative 37; 737 Reactome database pathways 38; 184 KEGG database pathways 39; and 5083 Gene Ontology database terms 40. Finally, DEPICT conducts tissue and cell type enrichment analysis, by testing whether genes in associated loci are highly expressed in any of 209 Medical Subject Heading annotations of 37 427 microarrays from the Affymetrix U133 Plus 2.0 Array platform (Wood et al. 41 and Geller et al. 42 for previous applications of DEPICT). In this work, 21 autosomal SNPs for birth length and 44 autosomal SNPs for infant length were used as input to DEPICT resulting in 21 and 41 non-overlapping loci, respectively, that covered a total of 34 genes and 83 genes, respectively. The gene prioritization, gene set enrichment and tissue/cell type enrichment analyses were run using the default settings in DEPICT.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG online.

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