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Genome-wide association replicates the association of Duffy antigen receptor for chemokines (*DARC*) polymorphisms with serum monocyte chemoattractant protein-1 (MCP-1) levels in Hispanic children

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ABSTRACT

Obesity is associated with a chronic low inflammatory state characterized by elevated levels of chemokines. Monocyte chemoattractant protein-1 (MCP-1) is a member of the cysteine–cysteine (CC) chemokine family and is increased in obesity. The purpose of this study was to identify loci regulating serum MCP-1 in obese Hispanic children from the Viva La Familia Study. A genome-wide association (GWA) analysis was performed in 815 children, ages 4–19 years, using genotypes assayed with the Illumina HumanOmni1-Quad v1.0 BeadChips. All analyses were performed in SOLAR using a linear regression-based test under an additive model of allelic effect, while accounting for the relatedness of family members via a kinship variance component. The strongest association for MCP-1 levels was found with a non-synonymous single nucleotide polymorphism (SNP), rs12075, resulting in an amino acid substitution (Asp42Gly) in the Duffy antigen receptor for chemokines (*DARC*) gene product (minor allele frequency = 43.6%, $p = 1.3 \times 10^{-21}$) on chromosome 1. Four other *DARC* SNPs were also significantly associated with MCP-1 levels ($p < 10^{-16}$–10^{-6}). The Asp42Gly variant was associated with higher levels of MCP-1 and accounted for approximately 10% of its variability. In addition, MCP-1 levels were significantly associated with SNPs in chemokine receptor 3 (*CCR3*) and caspase recruitment domain family, member 9 (*CARD9*). In summary, the association of the *DARC* Asp42Gly variant with MCP-1 levels replicates previous GWA results substantiating a potential role for *DARC* in the regulation of pro-inflammatory cytokines.

1. Introduction

Obesity is a major contributor to and precedes several metabolic disorders such as insulin resistance, dyslipidemia, hyperglycemia and hypertension [1]. Obesity is characterized as a chronic low inflammatory state [2]. Chemokines and proinflammatory cytokines and hormones released by adipose tissue underlie the chronic inflammatory profile associated with obesity [3]. Chemokines and their receptors play a crucial role in directing the movement of leukocytes throughout the body contributing to adaptive immune response and the pathogenesis of a variety of diseases including atherosclerosis, diabetes and obesity [1,4]. The largest family of chemokines consists of cysteine–cysteine (CC) chemokines that attract mononuclear cells to sites of chronic inflammation. Monocyte chemoattractant protein 1 (MCP-1) or chemokine CC ligand 2 (CCL2) is the most thoroughly characterized CC chemokine and is central to the inflammatory process [5,6]. MCP-1 is produced mainly in endothelial and smooth cells. MCP-1 is involved in the recruitment of leukocytes to sites of inflammation, promotion of angiogenesis and maturation of immune cells. Its levels are elevated in a broad spectrum of chronic inflammatory diseases including obesity, insulin resistance, sepsis, cancer, autoimmune

Abbreviations: GWA, genome-wide association; SNP, single nucleotide polymorphism; *DARC*, Duffy antigen receptor for chemokines; *CARD9*, caspase recruitment domain family, member 9; MCP-1, monocyte chemoattractant protein-1; *TNFRSF8*, tumor necrosis factor super family member 8; *GBP1*, interferon-induced guanylate-binding protein 1; *EFNA1*, ephrin A1; *FCER1A*, Fc Fragment of IgE, high affinity receptor 1 alpha subunit; *OR10J1, 3 and 5*, olfactory receptor, family 10, subfamily J, member 1, 3 and 5; *OLFML2B*, olfactomedin-like 2B; *LMX1A*, LIM homeobox transcription factor 1, alpha; *CD247*, T-cell surface glycoprotein CD3 zeta chain; *CHRM3*, cholinergic receptor, Muscarinic 3.

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diseases and atherosclerosis. Other CC chemokines include macrophage inflammatory protein (MIP)-1 (CCL3), MIP-1 (CCL4), and RANTES (CCL5). A second family of chemokines consists of CXC chemokines such as interleukin-8 (CXCL8) which attracts polymorphonuclear leukocytes to sites of acute inflammation. The third family is the CX3C family; fractalkine (CX3CL1) is the only member.

Chemokines activate surface receptors that are seven-transmembrane domain G-protein coupled receptors which in turn activate signaling cascades that result in the rearrangement, change of shape, and cell movement of actin. The chemokine receptor cysteine–cysteine (CC) receptor 2 (CCR2) is known to influence inflammation associated with obesity [7]. Expression of CCR2 in adipose tissue was observed to be increased in obese individuals and associated with systemic inflammation [8]. However, CC2R is not the only receptor for MCP-1; another receptor, Duffy antigen receptor for chemokines (DARC), mediates the interactions of MCP-1 with erythrocytes and endothelial cells [9]. In circulation, MCP-1 is bound to erythrocyte DARC that acts as a chemokine receptor/reservoir of proinflammatory cytokines. DARC regulates free chemokine activity and presumably releases chemokines to local environments when needed.

The Viva La Familia Study was designed to investigate genetic and environmental factors affecting obesity and its comorbidities in Hispanic children. Previously, we reported that genetic factors associated with obesity also were associated with increased fasting serum levels of MCP-1 [10]. We conducted a genome-wide scan to identify chromosomal or quantitative trait loci (QTL) affecting the variation in cytokines in these children. Significant heritability was observed for serum MCP-1 ($h^2 = 0.62$) [10]. The univariate genome-wide scan showed suggestive evidence for QTLs influencing the variation in MCP-1 levels on chromosomes 11 (logarithm of odds (LOD) score = 2.2) and 1 (LOD = 2.0) [10]. To further pursue these findings, we conducted a genome-wide association study (GWAS) and here report our results for MCP-1 levels in Hispanic children of the Viva La Familia Study.

2. Material and methods

2.1. Study design and participants

The Viva La Familia Study represents a family-based cohort highly enriched for obesity. Families were selected based on an obese proband between the ages of 4 and 19 years. Accordingly, 51% of the children were classified as obese (>95th BMI percentile) with BMI z-scores ranging from 2.3 to 4.5 [11]. The majority of the parents were either overweight (34%) or obese (57%). Subjects ($n = 934$) were from 319 Hispanic families enrolled in the Viva La Familia Study in 2000–2004. Anthropometric and body composition measurements were performed on parents and children. Fasting blood samples were drawn for biochemistry profiling of the children, and genotyping of children and parents. Subjects and study procedures are described in detail in a previous publication ([11]. All children and their parents gave written informed consent or assent. The protocol was approved by the Institutional Review Board for Human Subject Research for Baylor College of Medicine and Affiliated Hospitals and the Texas Biomedical Research Institute.

2.2. Phenotyping

A blood sample was drawn in the morning after a 12-h fast. Serum samples were obtained from whole blood after clotting. Fasting serum concentration of MCP-1 was measured by enzyme-linked immunosorbent assay (ELISA) (interassay CV 5%) (R&D Systems, Minneapolis, MN).

2.3. Genome-wide association analysis (GWA analysis) in Hispanic children

A GWA analysis for 815 children was conducted using single nucleotide polymorphism (SNP) assays included on the Illumina HumanOmni1-Quad v1.0 BeadChips (Illumina, San Diego, CA). Genotype calls were obtained after scanning on the Illumina BeadStation 500GX and analysis using the GenomeStudio software. Our genotyping error rate (based on duplicates) was 2 per 100,000 genotypes. The average call rate per individual sample was 97%. Specific SNPs were removed from analysis if they had call rates of <95% ($n = 6596$) or deviated from Hardy–Weinberg equilibrium at a 5% FDR ($n = 0$). SNP genotypes were checked for Mendelian consistency using the program SimWalk2 [12]. The evidence for population stratification was assessed and estimates of the allele frequencies and their standard errors were obtained using SOLAR [13].

2.4. Measured genotype analysis

GWA analyses were performed using the SOLAR program [13]. Each SNP genotype was converted in SOLAR to a covariate measure equal to 0, 1, or 2 copies of the minor allele (or, for missing genotypes, the weighted covariate based on imputation). These covariates were included in the variance-components mixed models for measured genotype analyses [14] versus null models that incorporated the random effect of kinship and fixed effects such as age, sex, their interaction and higher order terms. For the initial GWA screen, we tested each SNP covariate independently as a 1 degree of freedom likelihood ratio test. A derived alpha value, from simulations using the family data, of 1×10^{-7} or less was considered significant.

3. Results

The mean serum MCP-1 levels were 317 ± 117 and 309 ± 117 pg/ml for boys and girls, respectively. The strongest association for MCP-1 levels was found with a non-synonymous single nucleotide polymorphism (SNP), rs12075 (Asp42Gly) in the Duffy antigen receptor for chemokines (*DARC*) gene (minor allele frequency = 43.6%, $p = 1.3 \times 10^{-21}$). MCP-1 levels also were associated with five other SNPs in *DARC* ($p < 10^{-16}$–10^{-6}) (Table 1). The minor alleles of all *DARC* SNPs were significantly associated with higher levels of MCP-1. SNP rs12075 (Asp42Gly) accounted for approximately 10% of the variability in MCP-1 levels. Together, these six SNPs accounted for 34.1% of the variability in MCP-1 levels.

Suggestive evidence of associations of MCP-1 levels with SNPs in other regions of chromosome 1 were found in ephrin A1(*EFNA1*), Guanylate-binding protein-1 (*GBP1*), Fc Fragment of IgE, high affinity receptor 1 alpha subunit (*FCER1A*), olfactory receptor, family 10, subfamily J, member 1, 3 and 5 (*OR10J1*, *OR10J3*, *OR10J5*), olfactomedin-like 2B (*OLFML2B*), Fc receptor-like protein B (*FCRLB*), LIM homeobox transcription factor 1, alpha (*LMX1A*) and cholinergic receptor, Muscarinic 3 (*CHRM3*), with *p*-values ranging between 4.9×10^{-6} and 7.0×10^{-5} (Fig. 1).

The GWAS (Fig. 2) revealed other highly significant associations for MCP-1 levels: snp2-1167588 on chromosome 2 ($p = 6.5 \times 10^{-8}$); rs7652290, rs7645716 and rs12636651 in chemokine receptor 3 (*CCR3*) ($p = 3.3 \times 10^{-7}$, 9.6×10^{-8} and 6.6×10^{-9}, respectively) on chromosome 3; rs34971035 in caspase recruitment domain family, member 9 (*CARD9*) ($p = 4.6 \times 10^{-8}$) on chromosome 9; and snp10-4307576 ($p = 4.6 \times 10^{-8}$) on chromosome