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Identification of a locus modulating serum C-reactive protein levels on chromosome 5p15

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Abstract

Objective: Individual propensity to chronic, low-grade inflammation – a determinant of atherosclerosis – is in part under the control of genetic factors. To identify genes involved in this modulation, we performed a 10 cM genome screen for linkage with plasma C-reactive protein in 38 extended families including 317 non-diabetic and 177 type 2 diabetic family members (2,547 relative pairs).

Methods and results: In a variance component analysis, heritability of CRP values was significant ($h^2=0.39$, $p<0.0001$). This effect was independent of BMI and was present in both diabetic ($h^2=0.42$, $p=0.003$) and non-diabetic ($h^2=0.34$, $p=0.0015$) relatives. The strongest evidence of linkage with CRP was on chromosome 5p15, where the LOD score reached genome-wide significance (LOD=3.41, genome-wide $p=0.013$). Both diabetic and non-diabetic family members contributed to linkage at this location. Smaller linkage peaks were detected on chromosomes 5q35 (LOD=1.35) and 17p11 (LOD=1.33). When the analysis was restricted to diabetic family members, another peak of moderate intensity (LOD=2.17) was evident at 3p21.

Conclusions: A major gene influencing CRP levels appears to be located on chromosome 5p15, with an effect that is independent of diabetes. Another gene on 3p21 may control CRP variation but only in the presence of a diabetic or insulin-resistant environment.

Keywords

CRP; genome-wide scan; diabetes; insulin-resistance; linkage

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1. Introduction

A large body of evidence indicates that inflammatory processes play a pivotal role in the initiation and progression of atherosclerotic lesions. On this basis, circulating markers of inflammation have been intensively investigated for their role as predictors of coronary artery disease [1]. C-reactive Protein (CRP) – an acute phase reactant produced by the liver in response to inflammatory cytokines – is the most studied of these markers. Although inflammatory processes are complex, single measures of the circulating levels of this molecule have been consistently shown to predict cardiovascular risk in prospective studies [2]. Elevated levels of serum CRP have also been associated with insulin resistance and type 2 diabetes – two conditions that predispose to coronary artery disease [3,4].

Environmental factors, such as infectious agents and nutrients, have been postulated to contribute to chronic inflammation and CRP levels [1,5]. However, recent evidence indicates that genetic factors also play a significant role, accounting for up to 40% of CRP variance [6-9]. Studies aimed at identifying the genetic variants that are involved in such modulation are still in their infancy. Several reports have described an association between single nucleotide polymorphisms in the CRP gene and circulating CRP levels [10-14]. Other studies have implicated candidate genes involved in both inflammation and lipid metabolism [15-19]. However, the complexity of the pathways controlling inflammation and the fact that these effects are relatively small compared to the magnitude of the heritability of CRP levels suggest that additional genes are involved. Some of these genes may have modulating effects independent of the exposure to proinflammatory stimuli, others may be specific to a particular proinflammatory environment such as diabetes.

Here we present the results of a 10 cM genome screen for linkage to circulating levels of CRP in a set of extended families including members with and without type 2 diabetes. Our findings point to chromosome 5p15 as a genomic region harboring a major genetic modulator of this trait. Another locus is present on 3p21, with an effect on CRP levels that appears to be specific to the diabetic state.

2. Methods

2.1. Families with type 2 diabetes

Thirty-eight extended families with type 2 diabetes were included in this study. These families belong to the Joslin Study on Genetics of Type 2 Diabetes – a collection of 104 extended families in which type 2 diabetes segregates as an autosomal dominant disorder. The ascertainment of these families and the recruitment procedures have been previously described [20]. The screening criteria were: 1) a proband and at least one sibling with type 2 diabetes diagnosed between ages 10 and 59 years; 2) three or more generations affected by diabetes; and 3) unilineal transmission of diabetes. Diabetes in a proband was considered non-insulin dependent if hyperglycemia was managed without insulin for at least two years after diagnosis. An additional selection criterion was the availability of a large number of family members (with and without diabetes) willing to participate in the study. The 38 families included in the present study were those in the collection that had an average age at diagnosis between 35 and 59 years and had stored plasma available for the measurement of CRP and IL6. These families are a subset of the 63 families that were analyzed by Krolewski et al. for linkage to albuminuria [20]. Of the 38 families, 37 were Caucasian and one was African-American.

2.2. Study subject examination and laboratory methods

The study protocol and informed consent procedures were approved by the Committee on Human Studies of the Joslin Diabetes Center. Fasting blood was drawn for DNA extraction,

blood glucose determination and other biochemical measurements. Diabetic subjects who were treated only by diet, and all non-diabetic participants, had an additional blood sample drawn 2h after an oral challenge with 75g of glucose. Diabetes was diagnosed if any of the following criteria were met: 1) treatment with insulin or oral agents and the presence of diabetic hyperglycemia was confirmed by the study examination; 2) OGTT blood glucose values meeting World Health Organization (WHO) criteria (fasting ≥ 140 mg/dl or 2h ≥ 200 mg/dl); or 3) HgbA1c $\geq 7.0\%$ (normal values $< 6.1\%$) in individuals who declined the OGTT or were not fasting when examined. Individuals who did not meet any of these criteria but were not normoglycemic according to WHO criteria were classified as having impaired fasting glucose or impaired glucose tolerance (n= 34). Women who were normoglycemic at interview but had a history of documented diabetes during pregnancy were considered as having gestational diabetes (n=9). Both of these groups were considered to have type 2 DM in this analysis. Plasma C-reactive protein (CRP) levels were determined on fasting samples by a high sensitivity ELISA assay (ICN Pharmaceuticals). All assays were performed in duplicates by investigators blinded to the clinical or genetic characteristics of study subjects. Intra- and inter-assay coefficients of variations were $< 10\%$. ELISA results were calibrated to the widely used N High Sensitivity CRP assay from Dade Behring (Deerfield, IL).

2.3. DNA Extraction and Genotyping

Genomic DNA was prepared from peripheral blood by phenol-chloroform extraction. A genome scan was performed by the NHLBI Mammalian Genotyping Service (MGS) at the Marshfield Medical Research Foundation by means of PCR and automated fragment analysis [21]. Each individual was genotyped for 383 autosomal microsatellite markers with an average heterozygosity of 0.75 (Marshfield Set 12), resulting in a mean distance between markers of 9.1 cM over 22 autosomes. Markers on sex chromosomes were not analyzed. Information on the screening set and genotyping protocols is available at the Marshfield website (<http://research.marshfieldclinic.org/genetics>). The proportion of markers successfully genotyped for each individual ranged from 53 to 100%, with 75% of the subjects having information on more than 97% of the markers. The overall error rate, as measured on replicate CEPH samples, was 0.7%.

Genotypes from the genome screen were analyzed by means of the program PREST [22] to check the accuracy of relationships between individuals within families, and adjustments of the family structures were made accordingly. The program PEDCHECK [23] was then used to detect genotyping errors and inconsistencies. If the mistyping was not resolved by further review, the genotypes were set to be unknown. Genotypes from 494 individuals were used in the reported analyses. Since allele frequency estimates obtained using samples containing related individuals can be significantly biased unless pedigree structure is taken into account [24], maximum likelihood techniques that account for pedigree structure were used to estimate allelic frequencies, using the program SOLAR.

2.4. Data Analysis

Univariate analyses were performed to characterize the distribution of each variable used in the analysis. A skewness between -1.0 and $+1.0$ and a kurtosis between -0.5 and $+1.0$ were considered as indicators of normality. The distribution of CRP values was not normal (skewness=1.6, kurtosis=2.4), but the values transformed to the natural logarithm approximated a normal distribution (skewness= -0.5 , kurtosis= -0.2). Characteristics of diabetic and non-diabetic subjects groups were compared by t-test or Wilcoxon Rank Sum depending on the normality of the respective variable's distribution. Univariate associations with diabetes status and variables of interest were adjusted for possible family effects in a mixed model with the variable 'family' treated as a random effect using the PROC MIXED procedure in SAS (Cary, NC).

Heritability of logCRP levels and their correlation with BMI due to genetic (ρ_G) and environmental (ρ_E) factors were estimated by pedigree-based variance component analysis as implemented in the computer program SOLAR (version 2.1.4, Southwest Foundation for Medical Research (San Antonio, TX, USA). Heritability (h^2) is defined as the ratio of the variance of the trait accounted for by genes (σ_G^2) divided by the total phenotypic variance (σ_T^2) minus the variance due to covariates (σ_C^2). Analyses were initially conducted in the overall group of relative pairs with age, sex, and diabetes status as covariates. Since BMI was shown to have a strong environmental correlation with CRP, subsequent analyses were performed including BMI among the covariates. To examine further the effect of diabetes, analyses were also performed separately in relative pairs with diabetes (defined as DM-DM pairs) and without diabetes (defined as NDM-NDM pairs).

Pedigree-based variance components linkage analysis was used to detect and localize quantitative trait loci (QTLs) controlling the variation of CRP levels. This powerful linkage analysis technique [25,26] uses information from all possible biological relationships simultaneously and represents an extension of a method developed by Amos [27] that specifies the expected genetic covariances between relatives as a function of the identity-by-descent (IBD) relationships at a marker locus. Linkage analysis of logCRP was first performed in all relative pairs and then in the DM-DM subset to investigate the interaction between diabetes and the QTLs for this measure. This analysis was performed by making CRP values unknown in all NDM family members. The analyses in all pairs were adjusted for age, sex, BMI, and diabetes status; the analysis of the DM-DM subset was adjusted for age, sex, and BMI. The hypothesis of “no linkage” was tested by comparing the likelihood of a model in which h^2 was estimated but h^2_q (heritability due to the QTL) was constrained to 0 with a model in which both h^2 and h^2_q were estimated. LOD scores were obtained by converting the difference in $-2 \log$ likelihood values into values of $\log_{10}$. Empirical p-values were calculated on the basis of the LOD-score distribution under the hypothesis of no linkage obtained from 10,000 or 50,000 replicates. In addition, we calculated genome-wide p values for the two strongest linkage findings (i.e., $\text{LOD} \geq 2.0$) in our data using the method of Feingold et al. [28], which takes into account the finite marker density in the genetic map used for the multipoint linkage analysis of a given phenotype and the mean recombination rate for the pedigreed population studied.

3. Results

Thirty-eight families from the Joslin Study on the Genetics of Diabetes were included in this study. Genotypes from 494 family members were available for the analysis. On average, 4.7 diabetic and 8.3 non-diabetic individuals were included in each family. Salient characteristics of study subjects are shown in Table 1. The mean age at diagnosis of diabetes was 49 ± 13 yrs. Individuals with diabetes were older (59 ± 14 vs. 43 ± 17 years) and had higher BMI (30.5 ± 6.4 vs. 27.5 ± 6.8) at examination than non-diabetic subjects. They also had higher CRP levels than non-diabetic family members ($p=0.0027$). At the time of examination, 38% of diabetic subjects used insulin to treat their disease, 39% used oral agents, and 23% used a modified diet or no treatment.

The distribution of different relative pairs according to genetic relationship and diabetes status is summarized in Table 2. The 38 extended families included a total of 2547 relative pairs, of which 356 were concordant for diabetes (DM-DM pairs), 1059 were concordant for the absence of diabetes (NDM-NDM pairs), and 1159 were discordant for diabetes (DM-NDM pairs).

In an initial variance component analysis including age, sex, and diabetes status as covariates, the heritability of CRP in all pairs was 0.27 ± 0.08 ($p<0.0001$). Since CRP levels showed a significant environmental, but not genetic correlation with BMI ($\rho_E=0.43 \pm 0.08$, $p<0.0001$, $\rho_G=0.015 \pm 0.25$, $p=0.95$), the analysis was repeated after including this variable among the

covariates. In this model, familial factors accounted for almost 40% of the variability CRP levels not explained by BMI or other covariates ($h^2 = 0.39 \pm 0.10$, $p < 0.0001$). After stratification by diabetes status, the heritability was 0.42 ± 0.18 ($p = 0.003$) among DM-DM pairs and 0.34 ± 0.14 ($p = 0.0015$) among NDM-NDM pairs.

A genome wide scan was performed to identify potential locations of genes modulating CRP levels. Based on the heritability results, linkage analysis was conducted using a variance component model that included age, sex, diabetes status, and BMI as covariates. Genome-wide LOD score plots for the linkage analysis of all pairs are shown in Figure 1. The highest multipoint LOD score (LOD=3.41) was on chromosome 5p15 at 14 cM from p-ter on the Marshfield map (Table 3). Two other locations supported linkage – with LOD scores equal or greater than 1.2. One site was on 5q35 at 161 cM from p-ter (LOD=1.35) and the other on 17p11 at 22 cM (LOD=1.33).

To determine whether diabetes modified the effect of the loci identified in the analysis of all pairs, linkage with CRP levels was also evaluated separately in the subset of relative pairs concordant for diabetes (DM-DM relative pairs). As discussed in detail by Krolewski et al. [20], comparisons of support for linkage from the analyses with all pairs and with DM-DM pairs provide information on the specificity of the effects of genetic loci in relation to diabetes. The evidence supporting linkage on chromosome 5p15 was present in DM-DM pairs (LOD=1.38) but was lower than that observed in all pairs (LOD=3.41), indicating that NDM-NDM and/or NDM-DM pairs also contributed to the linkage signal (Table 3). This suggests that this locus has a modulating effect on CRP levels that is independent of the presence of diabetes. The same pattern was observed for the locus on 5q35, whereas all the linkage at 17p11 came from NDM-NDM and/or NDM-DM pairs since the LOD score was 0 in DM-DM pairs. The analysis of DM-DM pairs also revealed a new linkage peak at 3p21 (70 cM). The maximum LOD score reached at this location was 2.17 in the DM-DM pairs as compared to a value of 0.47 in all pairs, suggesting the presence of a genetic effect specific to diabetes (Table 3). A smaller peak (LOD=1.39) specific to diabetes was also observed on chromosome 1 at 9 cM.

Figure 2 shows the LOD score plots at the two loci with the strongest evidence of linkage with CRP levels (5p15 and 3p21). The genome-wide p values corresponding to these two strongest linkage findings (i.e., D5S817, 5p15, LOD = 3.41 and D3S2409, 3p21, LOD = 2.17) were 0.013 and 0.270, respectively. The locations of the linkage peaks are very similar in all relative pairs as well as in DM-DM relative pairs. Along most of the region on 5p, the support for linkage with logCRP was much stronger in all relative pairs than in the subset that included only DM-DM relative pairs. This contrasts with chromosome 3p, where the linkage support was much stronger in DM-DM pairs than in the group of all relative pairs, despite the fact that the latter group had much larger number of relative pairs. The LOD-1 support interval on 5p spans a genetic region of 18 cM, corresponding to 9 Mb on the latest genome assembly. The LOD-1 support on 3p spans a genetic region of 23 cM, corresponding to 28 Mb on the physical map.

4. Discussion

A rapidly growing body of evidence suggests that inflammatory traits have a substantial genetic component [6-9]. To identify genetic determinants of this phenotype, we performed a 10 cM genome screen for linkage with serum CRP – a well-established marker of inflammation – in a panel of large, predominantly Caucasian families including members with and without type 2 diabetes. We found the strongest evidence of linkage to occur on chromosome 5p and 3p, with weaker signals being present on chromosomes 5q and 17p. Comparison of the patterns of linkage in DM-DM and all relative pairs indicate that the gene on 5p controls variation in

CRP levels independently of the presence of diabetes, whereas the effect of the 3p locus appears to be specific to the diabetic state.

The present study confirms the significant role of genetic factors in modulating CRP levels. Our estimate of CRP heritability – 0.39 among all relative pairs – is within the range of the estimates reported by other studies [6-9]. Estimates were similar when relative pairs were stratified into DM-DM and NDM-NDM pairs, suggesting that familial clustering of diabetes does not contribute to the significant heritability of CRP levels. Familial clustering of BMI also does not seem to account for these findings, since CRP levels were not genetically correlated to BMI levels and CRP heritability was estimated using a model that included BMI among the covariates. These results are in agreement with a recent study of monozygotic twins showing that the observed association between adiposity and CRP levels is independent of genetic influences [29].

The strongest peak in our scan was on 5p15. The LOD score at this location reached a value of 3.4, corresponding to a significant ($p=0.013$) genome-wide p value. The LOD score was lower ($LOD=1.38$) in the subset of pairs concordant for diabetes, consistent with the notion that non-diabetic family members also contributed to the linkage signal. Such linkage pattern suggests that the putative gene at this locus is involved in the constitutive modulation of CRP levels or in the inflammatory response to exposures unrelated to diabetes. CRP is produced by the liver under the control of IL-6, IL-1, TNF α and other inflammatory cytokines through pathways involving NF- κ B, C/EBPs and other transcription factors [30]. None of the 18 RefSeq genes placed in the 9 Mb of the LOD-1 support interval seems to be an obvious component of these pathways (Table 4). However, several other transcripts of unknown function have been assigned to this interval and may contribute to the modulation of CRP levels though as yet unidentified mechanisms.

The second largest peak was on 3p21. In contrast with the linkage evidence at 5p15, this peak was almost exclusively present in DM-DM pairs. Considering that the number of these pairs was much lower than the number of all relative pairs, it is remarkable that the LOD score at this location reached a value of 2.2, even if this was not significant (genome-wide $p=0.27$). The fact that the linkage peak was unique to DM-DM pairs suggests that the putative gene at this location may modulate inflammatory pathways that are especially active in the presence of type 2 diabetes. Individuals with type 2 diabetes are characterized by increased levels of inflammatory markers [3,4,31]. The mechanisms underlying this association are not completely clear but seem to be related to the insulin-resistant state typical of type 2 diabetes [32]. Chronic, low-grade inflammation is increasingly recognized as one of the components of the insulin resistance syndrome, and molecular studies have demonstrated a significant overlap between inflammation and insulin-resistance pathways, especially with regard to the regulation of NF- κ B activation [33]. In this respect, it is interesting to note that the genes located in the 3p LOD-1 interval include IL17RB – an interleukin 17E receptor that has been shown to activate NF- κ B upon binding of its ligand [34]. The interval also includes a cluster of chemokine receptors, which might also act as modulators of inflammatory pathways related to insulin-resistance and type 2 diabetes.

Previous studies of the genetic regulation of CRP levels have mainly focused on candidate genes. Several reports have been published showing a significant association between serum CRP levels and a variety of polymorphisms in the gene coding for this molecule [10-13]. For some of these sequence variants, especially those in the promoter, an effect on gene expression has been documented *in vitro*. Other studies have reported associations with the APOE Cys130Arg, which distinguishes APOE4 from the other isoforms, the TNF α –308 promoter polymorphism, and with variants in the IL-1 β and IL-6 genes [15-18]. None of the regions hosting these genes showed significant or suggestive linkage in our genome screen. The

maximum LOD score was 0.49 in the region of the CRP gene (1q23), 0.027 in that of APOE (19q13), 0.67 in that of IL-1 β (2q13), 0.36 in that of TNF α (6p21), and 0.72 in that of IL-6 (7p15). Whether these small peaks are just noise or correspond instead to the genetic effects described above is difficult to say. What is clear, however, is that the contribution of these genes to CRP variance is minor in our population compared with the effect of the genes at 5p15 and 3p21.

The results of two genome screens for linkage to inflammatory traits have been recently published [8,35]. One of these studies, involving 1054 subjects from 304 families from the Framingham Heart study, identified linkage with monocyte chemoattractant protein-1 (MCP-1) levels on chromosome 1q but failed to find significant or even suggestive evidence of linkage with CRP levels at any genomic location, including 5p15. One factor that might account for these negative results is that subjects in the Framingham study were on average 10 years older than our combined diabetic and non-diabetic family members (59 vs. 48 years). If genetic influences on CRP levels diminish with age, the putative gene on 5p15 might have been more difficult to detect in that study. Another factor to consider is that the Framingham families included fewer relative pairs (about 1200) than our families (n= 2547). Furthermore, all the relative pairs in the Framingham families were sib-pairs, whereas more than 70% of the pairs in our families had more distant relations, which provide more information for linkage analysis. Thus, the Framingham study may have had less power than our study to detect linkage of CRP to the 5p locus.

The other study, conducted in 280 non-diabetic sibling pairs from the HERITAGE family study, identified linkage signals at 20q13 (LOD=3.2) and 5p13 (LOD=2.2) [35]. Of note, the marker showing the strongest linkage at 5p13 is only 30 cM away from our peak at 5p15. While no information is provided in the HERITAGE paper about the 1-LOD confidence interval, it is possible that the two peaks correspond to the same genetic effect and that their different location is due to the relative imprecision of linkage mapping.

Some limitations of our study should be acknowledged. One concerns the inclusion of environmental factors in the variance component model. We adjusted our analysis for BMI, since this variable was shown to have a strong environmental correlation with CRP levels. However, we did not have access to data regarding other environmental exposures that can influence CRP levels such as alcohol intake, smoking habits, and estroprogestinic treatment [36]. Failure to account for these factors may have affected the estimates of CRP heritability and may have reduced the power of linkage analysis. However, previous studies have shown this effect to be minor. For instance, in the Framingham study, only minimal differences were observed between CRP heritabilities before and after adjustment for environmental factors [8]. Similarly, adjustment for environmental factors did not result in major changes in the evidence of linkage [8]. Another issue is the generalizability of our findings. The families from the Joslin Study on the Genetics of Diabetes are characterized by a pattern of diabetes transmission consistent with an autosomal-dominant mode of inheritance. This should not affect the generalizability of the results on 5p15, since this locus was detected both in diabetic and non-diabetic pairs. However, whether the findings for 3p21, which were specific to diabetes, also apply to other forms of the disease remains to be determined.

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Figure 1. Results of genome-wide scans for multipoint linkage analysis of logCRP. The chromosomal position (Marshfield Map) is represented on the x-axis, the LOD scores on the y-axis.

Chromosome 5

Chromosome 3

Figure 2. Detailed genome scan plots for logCRP on chromosomes 5 and 3 for all relative pairs (including DM-DM, NDM-NDM and DM-NDM relative pairs) (thick line) and DM-DM relative pairs only (thin line). Chromosomal positions correspond to the Marshfield map.

Table 1

Clinical characteristics of family members according to diabetes status.

Characteristics	Diabetes Mellitus		p-value	Adj p-value [†]
	Present (n=177)	Absent (n=317)		
Men (%)	44.5	45.8	NS	NS
Age at Examination (yrs) (mean ± SD)	59 ± 14	43 ± 17	<0.001	<0.001
Age at Diagnosis of Diabetes (yrs) (mean ± SD)	49 ± 13	-		
BMI (mean ± SD)	30.5 ± 6.4	27.5 ± 5.8	<0.001	<0.001
Hypoglycemic Rx at Examination (%)				
Insulin	38	-	-	-
Oral Agents Only	39	-	-	-
Diet only or No Rx	23	-	-	-
Plasma CRP Percentile Cutoffs (mg/l)				
25 th	1.6	0.9	<0.001	0.0027
50 th	3.7	2.2		
75 th	7.7	4.6		
95 th	15.1	10.6		

[†] Adjusted for possible family effect

Table 2

Distributions of pairs of relatives in the 38 families according to genetic relationship and diabetes status.

Relationship	Pairs of relatives by Diabetes Status			All Pairs
	DM-DM	NDM-NDM	DM-NDM	
Parental (0.500)	30	98	121	249
Sibling (0.500)	140	147	141	428
Half-sibling (0.250)	0	8	8	16
Grandparental (0.250)	2	7	8	17
Avuncular (0.250)	105	204	393	702
Cousin (0.125)	65	410	313	788
Other (0.063)	14	185	148	347
All pairs	356	1059	1159	2547

Table 3
Maximum LOD scores for linkage to plasma CRP levels in all pairs and DM-DM pairs in the 38 Joslin families.

Chromosome	Marker	Position	All pairs			DM-DM pairs		
			LOD	Nominal p value	Empirical p value	LOD	Nominal p value	Empirical p value
5p15	D5S817	14	3.41 ^a	3.7×10^{-5}	1.2×10^{-4}	1.38	0.0058	0.010
5q35	D5S1456	161	1.35	0.0063	0.01	0.37	0.096	0.11
17p11	D17S974	22	1.33	0.0067	0.01	0.00	-	-
3p21	D3S2409	70	0.47	0.07	0.08	2.17 ^b	7.9×10^{-4}	0.0021
1p31	D1S551	9	0.00	-	-	1.32	0.0068	0.011

^a Genome-wide p value = 0.013;
^b genome-wide p value = 0.270

Table 4

RefSeq genes placed in the 1-LOD interval at 5p15.

Gene Symbol	Location (Mb)	Gene Product	Function
<i>AYTL2</i>	1514544-1577076	Acyltransferase like 2	Lipid metabolism
<i>MRPL36</i>	1851500-1852946	Mitochondrial ribosomal protein L36	Protein synthesis within the mitochondrion
<i>NDUFS6</i>	1854509-1869163	NADH dehydrogenase (Fe-S protein) 6	Oxidative phosphorylation
<i>IRX4</i>	1930549-1935880	Iroquois homeobox protein 4	Embryogenesis and transcriptional regulation
<i>IRX2</i>	2799880-2804769	Iroquois homeobox protein 2	Embryogenesis and transcriptional regulation
<i>IRX1</i>	3649168-3654516	Iroquois homeobox protein 1	Embryogenesis and transcriptional regulation
<i>ADAMTS16</i>	5193443-5373411	Metalloprotease disintegrin 16	Secreted protein
<i>MED10</i>	6425040-6431639	Mediator of RNA polymerase II transcription	RNA polymerase II transcription
<i>NSUN2</i>	6652355-6686157	NOL1/NOP2/Sun domain family 2 protein	RNA methylation
<i>SRD5A1</i>	6686563-6722673	Steroid-5-alpha-reductase 1	Testosterone metabolism
<i>POLS</i>	6767718-6810161	DNA polymerase sigma	DNA repair
<i>ADCY2</i>	7449345-7883194	Adenylate cyclase 2	Signal transduction
<i>FASTKD3</i>	7912273-7922115	FAST kinase domains 3	Protein kinase, control of apoptosis
<i>MTRR</i>	7922275-7954233	Methionine synthase reductase isoform 2	Protein synthesis and one carbon metabolism
<i>SEMA5A</i>	9091859-9599158	Semaphorin 5A	Neural development
<i>TAS2R1</i>	9682109-9683463	Taste receptor T2R1	Bitter taste signaling
<i>CCT5</i>	10303282-10319500	Chaperonin containing TCP1, subunit 5	Protein folding
<i>MARCH6</i>	10406828-10488491	Membrane-associated ring finger 6	Protein ubiquitination