IL23R Haplotypes Provide A Large Population Attributable Risk for Crohn's Disease

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Abstract

The IL-23 pathway plays a pivotal role in the development of chronic mucosal inflammation seen in the inflammatory bowel diseases. Multiple studies have now established the contribution of the interleukin 23 receptor gene (IL23R) to Crohn's Disease (CD) risk in general and of the IL23R R381Q variant in particular. The aim of this work was to estimate the total contribution of this gene to CD risk test using a haplotype approach.

Methods—763 CD subjects and 254 controls were genotyped for single nucleotide polymorphisms in the IL23R gene using Illumina and ABI methods. Haplotypes were assigned using PHASEv2 and tested for association with CD by chi-square and permutation.

Results—Haplotypes with both increased and decreased risk for CD were observed in 2 of the 4 observed blocks (Block 2 H1: 55.4% control, 64% CD, p=0.019; H2: 64.5% control, 54.4% CD, p=0.006; Block 3 H1: 55.8% control, 64.4% CD, p=0.013; H2: 47.0% control, 36.6% CD, p=0.001). The population attributable risk for these haplotypes was substantially larger than that estimated for the IL23R R381Q variant (Block 2 H1 and block 3 H1 ~20%, compared with ~4% for Block 3 H6, containing the variant).

Discussion—These observations suggest that IL23R makes a substantial contribution to Crohn's disease susceptibility, larger than that estimated from the population frequency of the R381Q variant. These observations also support the expectation that finding "hits" from genome wide association studies will be but an important chapter in the story of unraveling the genetic contribution to Crohn's disease, rather than the final chapter that brings clarity to all the plot twists of a complicated story.

The IL-23 pathway plays a pivotal role in the development of chronic mucosal inflammation seen in the inflammatory bowel diseases (McGovern and Powrie 2007). Furthermore, the contribution of the interleukin 23 receptor gene (IL23R) to Crohn's Disease (CD) risk has now been established by a genome wide association study (GWAS) (Duerr et al. 2006), confirmed in a pediatric cohort (Dubinsky et al. 2007), and confirmed the large multi-disease study from the UK (2007). The association of IL23R variants with CD has also been observed in populations ascertained from Scotland (Van Limbergen et al. 2007), New Zealand (Roberts et al. 2007), Continental Europe (Buning et al. 2003; Libioulle et al. 2007; Weersma et al. 2007), North America (Baldassano et al. 2007), Brazil (Baptista et al. 2008), and Israel (Leshinsky-Silver et al. 2007), but not Japan (Yamazaki et al. 2007). In general, most of the confirmation studies have focused on the "protective" effect of the single-nucleotide polymorphism (SNP), rs11209026, a coding SNP that alters Arginine 381 to Glutamine

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(R381Q). In the original report, the Glutamine allele was present in ~7% of controls and 2-3% of CD cases (Duerr et al. 2006).

When NOD2 (CARD15) was identified as the first gene for CD, the initial findings suggested that three mutations within the gene were associated with disease susceptibility (Hugot et al. 2001; Ogura et al. 2001). However, the initial observations also suggested that the CD association with these three SNPs did not account for all of the linkage signal with CD at the IBD1 locus (Hampe et al. 2002). A portion of the linkage signal, at least, could be explained by other mutations within NOD2 that were not identified in the early NOD2 association studies (Lesage et al. 2002). This initial experience with NOD2 suggests that, once a susceptibility gene has been identified for a complex genetic trait, additional association studies with other variants are necessary in order to evaluate the role of a particular gene in disease etiopathogenesis more fully.

One strategy to test for the role of other variation in a putative susceptibility gene is to examine the association of major haplotypes within the disease under study (Crawford and Nickerson 2005). The rationale for this approach is that, by testing the major haplotypes in a population, the genetic variation at a high frequency in a population may be tested prior to knowing all of the individual variants that may be present. A further advantage of the haplotype approach is that the identification of haplotypes associated with disease susceptibility identify those patients that should be given priority in a deep sequencing project aimed at uncovering variation.

The aim of this study was to estimate the full contribution of IL23R variation to CD by examining the association of IL23R haplotypes with CD in our Caucasian population from Los Angeles. We report that haplotypic variation in IL23R accounts for a substantially larger proportion of the population attributable risk for CD than would be observed by genotyping only the IL23R R381Q variant.

MATERIALS AND METHODS

Subjects

Recruitment of subjects at the Cedars-Sinai Medical Center Inflammatory Bowel Disease center was conducted under the approval of the Cedars-Sinai Medical Center Institutional Review Board. Disease phenotype was assigned using a combination of standard endoscopic, histological, and radiographic features (Mow et al. 2004).

Selection of SNPs

SNPs were selected using "Tagger" (de Bakker 2004) and data from the International HapMap Project (2003; Barrett et al. 2005; Frazer et al. 2007). SNPs that "tagged" major Caucasian haplotypes and that were compatible with Illumina technology were genotyped in the initial phases of this study. Since we were interested in major genetic effects rather than rare alleles, the goal of "tagging" was to find a set of tagSNPs in linkage disequilibrium with all SNPs in the HapMap data with a minor allele frequency ≥5%.

Genotyping

DNA was isolated from Epstein Barr virus transformed lymphoblastoid cell lines using proteinase K digestion, organic extraction, and ethanol precipitation (Sambrook et al. 1989). Single nucleotide markers (SNPs) were genotyped following the respective manufacturer's protocols using either: (1) the oligonucleotide ligation assay, Illumina Golden Gate technology (Shen et al. 2005) (Illumina, San Diego, CA) or (2) the 5'-extension reaction, TaqMan MGB technology (Livak 1999; Kutyavin et al. 2000) (Applied Biosystems, Foster City, CA).

Consistency of SNP genotyping between the two methods was checked by genotyping 100 samples with both methods. The dbSNP accession numbers or "rs numbers" for the SNPs tested in this study are listed in Table 1.

Statistical Analyses

Haplotype blocks were determined using Haploview v4 (Barrett et al. 2005) using the confidence interval method (Gabriel et al. 2002). Haplotypes of subjects were inferred from individual genotyping data using PHASE v2 (Stephens et al. 2001). The association of the presence of a haplotype was tested using logistic regression. The impact of multiple testing was assessed by permuting the phenotypes with respect to the genotypes. All p-values reported have been corrected by this permutation test. Since the haplotypes were inferred from individual SNP data, we corrected for uncertainty in the haplotype assignment by including the probability of haplotype assignment as a covariate in the analysis. This correction for uncertainty usually had no impact because most of the assignments had a greater than 90% probability of assignment. However, all p-values shown have also been corrected for the uncertainty in haplotype assignment (Cordell 2006). Population attributable risk was estimated by assuming: (1) the frequency of a particular haplotype in the controls reflected the population frequency of that haplotype, and (2) the odds ratio for the association of a given haplotype reflected the relative risk of that haplotype for Crohns disease (Sugimura et al. 2003). Haplotypes are numbered in the order of frequency in controls seen in both our own controls and in publicly available data.

RESULTS

Single nucleotide polymorphisms (SNPs) in IL23R were genotyped in a CD case-control cohort and used to infer haplotype block and haplotype structure in the Caucasian sample from Los Angeles using Haploview v4. The existence of the blocks and haplotypes studied here was also confirmed using trio data from the International HapMap Project (result not shown). Using the "confidence interval method" (Gabriel et al. 2002), four haplotype blocks were observed plus one region of linkage disequilibrium (LD) defined by an individual SNP, rs1569922, between blocks 1 and 2. The SNPs forming these blocks are given in Figure 1 and Table 1.

Haplotypes in cases and controls were assigned using PHASEv2 and tested for association with Crohn's disease. No association was observed between CD and haplotypes in blocks 1 and 4; but CD was significantly associated with SNP rs1569922 and haplotypes in blocks 2 and 3 (Table 1). A "risk" haplotype, or a haplotype inferring increased CD risk, and a "protective" haplotype, or a haplotype inferring decreased CD risk, was observed in both blocks 2 and 3 (Block 2 H1: 55.4% control, 64% CD, p=0.019; H2: 64.5% control, 54.4% CD, p=0.006; Block 3 H1: 55.8% control, 64.4% CD, p=0.013; H2: 47.0% control, 36.6% CD, p=0.001). Furthermore, within each block, the odds ratio (OR) for CD significantly increased with the number of copies of the risk haplotype and significantly decreased with the number of copies of the protective haplotype (Figure 2a & 2b).

As Figure 2c shows, when the risk haplotypes from blocks 2 and 3 were combined, the OR for CD significantly increased with the number of copies of risk haplotypes from OR=0.77 for 0 risk copies to OR=1.3 for 3 copies, p(trend)=0.0072. Concomitantly, when the protective haplotypes from blocks 2 and 3 were combined, the OR significantly decreased with the number of copies of protective haplotypes from OR=1.3 for 0 protective copies to OR=0.45 for 4 copies, p(trend) <0.0001.

The population attributable risk (PAR) for a genetic risk factor estimates the percentage of CD cases that would not exist if that risk factor were not present in a given population and the prevented fraction estimates the protection due to the presence of a protective factor. While

this type of analysis is widely recognized to be a simplification of the contribution of any single genetic locus to disease susceptibility, it does provide a rough estimate of the relative contribution of the effect size that different loci have on disease susceptibility. The prevented fraction was approximately 4% for the protective IL23R R381Q variant (rs11209026, located in block 3, on H6). A substantially greater reduction in CD risk was observed for the protective haplotypes reported here, a PAR of 22% for block 2 H2 and 16% for block 3 H2 in this sample (Table 1). In addition, a large PAR was observed for the risk haplotypes reported here, approximately 10% for either block 2 H1 or block 3 H1. These values are visually compared in Figure 3.

DISCUSSION

The significant genetic associations and high population attributable risks reported here underscore the importance of IL23R genetic variation to CD susceptibility. While 10 SNPs were associated with CD in the original report of a genome wide association of ileal CD, the greatest association for an individual SNP was observed for rs11209026 (R381Q) (Duerr et al. 2006). For this SNP, the prevented fraction, or the amount of disease that would not be present if the genetic variant did not exist in the Caucasian population, is roughly 4%. In contrast, the IL23R risk and protective haplotypes identified here (Figure 1, Table 1) are at a higher frequency in our sample of the Caucasian CD population and thus substantially raise the population attributable risk and prevented fraction (Figure 3).

The limitations of this study include the fact that the haplotypes were inferred from data for individual SNPs and multiple comparisons were required for haplotype analysis. We have attempted to account for these problems by including the uncertainty in haplotype assignment as a covariate in the logistic regression analysis and by calculating empirical p-values using permutation of the phenotypes with respect to genotypes. Since the probability of correct haplotype assignment was greater than 90% for a large proportion of the subjects, this correction had only a slight effect on the observed significance of the associations reported here.

These data support the concept that the initial observation of an association between a genetic variant and a disease phenotype or quantitative trait should be followed by further genotyping and analysis of the genetic locus under study. Such follow-up should include performing haplotype association studies using haplotype tagging SNPs to "cover" the gene as well as directly sequencing the gene itself in order to identify SNPs that are potentially associated with the phenotype but are rare in the general population and so not included in analysis by "tagSNPs." In this way, the genome wide association studies being performed currently represent the first step, rather than the goal, of the genetic dissection of a complex trait such as CD (Taylor et al. 2007).

While additive to increase CD risk, no interaction between CARD15/NOD2 mutations and IL23R risk haplotypes were observed (analysis not shown). This observation may suggest that CARD15/NOD2 and IL23R variants define two separate pathways to intestinal inflammation in humans. Extensive work with mouse models of intestinal inflammation, developed by "knocking out" many different immune-related genes (Sartor 2006), has demonstrated that there are multiple pathways to intestinal inflammation. If so, then IL23R variation may be useful to distinguish CD subtypes with different underlying pathophysiological mechanisms, and raises the possibility that therapies targeted at IL23 signalling may be more effective in the IL23-related CD subtype(s) rather than in CD as a whole.

Acknowledgements

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Abbreviations

CD
>Crohn's disease

GWAS
>genome wide association study

IBD
>inflammatory bowel disease

IL23
>interleukin 23, alpha subunit p19 (dbGENE id 51561)

IL23R
>interleukin 23 receptor (dbGENE id 149233)

OR
>odds ratio

PAR
>population attributable risk

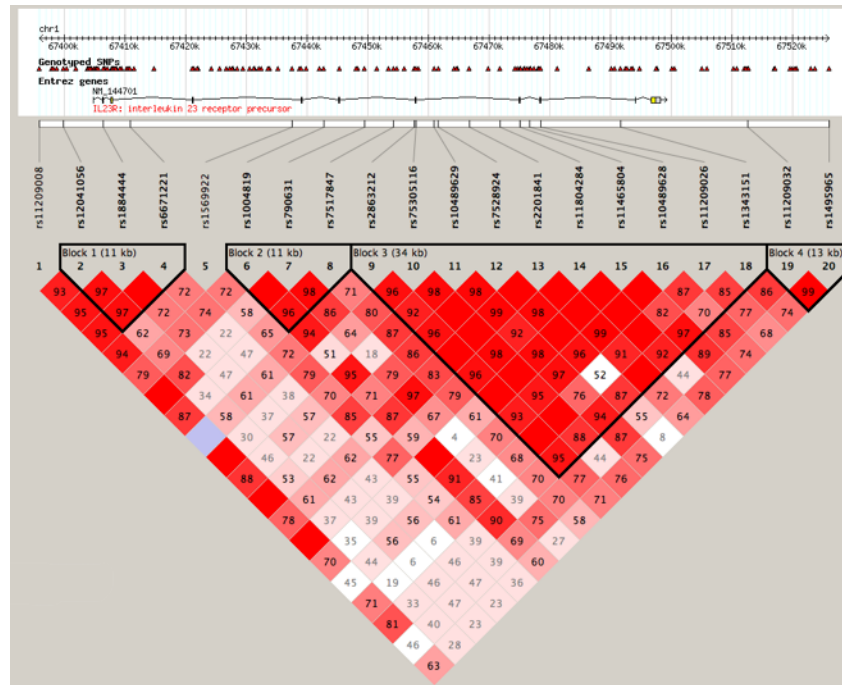**SNP**
>single nucleotide polymorphism

Figure 1. The Four Haplotype Blocks of IL23R

The position of the SNPs genotyped is shown in relationship to the chromosome 1 coordinates (NCBI build 36) and to the IL23R gene. Numbers indicate the extent of D' between two SNPs. The dark lines show the block structure of IL23R as determined using the confidence interval method (Gabriel et al. 2002).
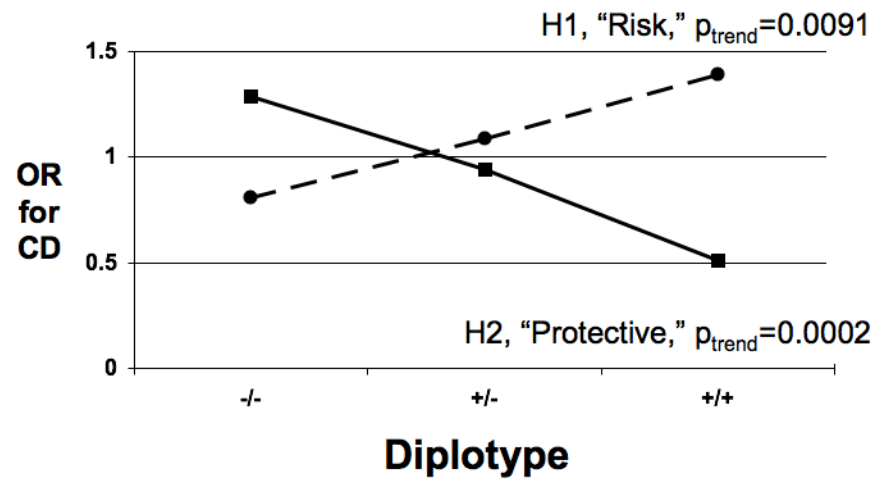
Figure 2. Odds Ratio for CD Increases With Increasing Number of Risk Haplotypes

a. The odds ratio (OR) for CD with increasing number of copies of risk (dotted line) or protective (solid line) haplotypes is shown in the left chart for block 2 and in the right chart for block 3.

b. OR with increasing number of copies of risk (dotted line) or protective (solid line) haplotypes across blocks 2 and 3 combined.

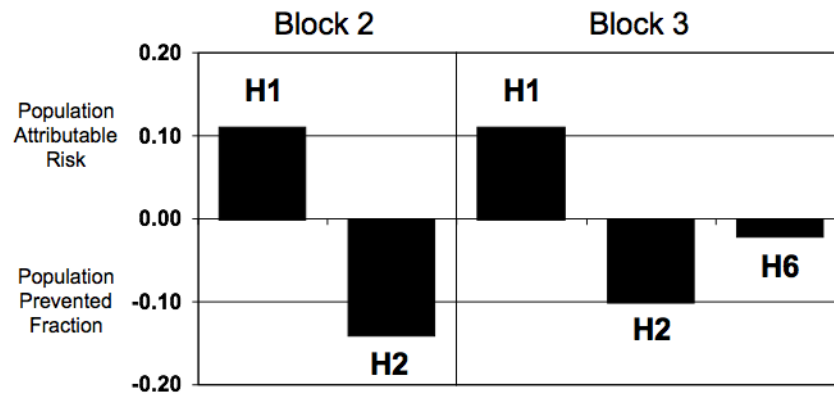**Figure 3. The Effect of the Risk and Protective Haplotypes Is Greater Than The Effect of the IL23R R381Q SNP**

Population attributable risk (positive parts of the bar graph) or prevented fraction (negative parts of the bar graph) was estimated as outlined in Methods. H1 is the risk haplotype in each block and H2 is the protective haplotype in each block as identified in this report. H6 contains the R381Q variant.

Table 1

Association of IL23 Haplotypes with CD

Gene	Haplotype	SNPs (alleles)							Percent Controls with Haplotype Present	Percent CD with Haplotype Present	p empirical*	Odds Ratio	95% Confidence Interval	Estimate of Population Attributable Risk or of Prevented Fraction
IL23R									**n=257**	**n=763**				
1) Block 1		rs12041056	rs1884444	rs6671221										
	H1	G	C	A					42.9%	44.5%				
	H2	A	A	G					43.5%	41.9%				
	H3	G	A	G					13.2%	13.2%				
2) Individual SNP between Block 1 and Block 2		rs1569922												
		C							85.3%	93.0%	0.0001	2.3	1.49,3.65	+ 0.50
3) Block 2		rs1004819	rs790631											
	H1 (risk)	T	A						55.4%	64.0%	0.019	1.43	1.07,1.91	+ 0.19
	H2 (protective)	G	A						64.5%	54.4%	0.006	0.65	0.49,0.88	- 0.22
	H3	G	C						45.4%	49.7%				
4) Block 3		rs7530511	rs7528924	rs2201841	rs10489628	rs11209026	rs1343151							
	H1 (risk)	C	A	C	C	G	C		55.8%	64.4%	0.013	1.43	1.07,1.91	+ 0.19
	H2 (protective)	C	A	T	T	G	T		47.0%	36.6%	0.001	0.65	0.48,0.86	- 0.16
	H3	C	A	T	T	G	C		20.3%	23.0%				
	H4	C	G	T	C	G	C		19.5%	21.2%				
	H5	T	G	T	C	G	C		19.5%	19.8%				
	H6	C	A	T	C	A	T		12.0%	8.4%				
5) Block 4		rs11209032	rs1495965											
	H1	G	A						82.4%	76.9%				
	H2	A	G						57.7%	63.2%				
	H3	G	G						19.2%	18.1%				-.04