Role of discs large homolog 5

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Abstract
In 2004, an association of genetic variation in the discs large homolog 5 (DLG5) gene with inflammatory bowel disease (IBD) was described in two large European study samples. The initial report of DLG5 as a novel IBD susceptibility gene sparked a multitude of studies investigating its effect on CD and IBD, respectively, leading to controversial findings and ongoing discussions concerning the validity of the initial association finding and its role in the aetiology of Crohn disease. This review aims to summarize the current state of knowledge and to place the reported findings in the context of current concepts of complex diseases. This includes aspects of statistical power, phenotype differences and genetic heterogeneity between different populations as well as gene-gene and gene-environment interactions.

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Key words: DLG5; Association; Gender; Genetic heterogeneity; Inflammatory bowel disease; Crohn disease

INTRODUCTION
In 2004, we described replicated association of genetic variation in the discs large homolog 5 (DLG5) gene with IBD and CD in two large European study samples consisting of German and UK patients[1]. The DLG5 gene product is a member of the group of membrane-associated guanylate kinases (MAGUKs) and as such contains 4 PDZ domains, one SH3 domain followed by one guanylate kinase (GUK) domain. Additionally, this MAGUK contains an N-terminal domain of unknown function, DUF622. All of these domains are assumed to be involved in protein-protein interactions, supporting the notion of DLG5 as a multifunctional adapter and scaffold protein. DLG5 has been reported to be involved in maintaining cell shape and polarity[2] and to be located at sites of cell-cell contact[3]. The DLG5 gene is located in a region of strong linkage disequilibrium which is independent of the surrounding genes due to substantial recombination. Analysis of the extended DLG5 haplotype identified 4 common haplotypes, with haplotype A tagged by 8 htSNPs of equivalent genetic information content. While haplotype A was significantly undertransmitted in IBD and CD, haplotype D is significantly overtransmitted in both IBD ($\chi^2 = 8.08, P = 0.004$) and CD ($\chi^2 = 4.15, P = 0.04$). The only tagging SNP for haplotype D that allows discrimination from the underlying frequent haplotype C is the single nucleotide polymorphism 113G→A (reference SNP ID: rs1248696). The polymorphism results in the amino acid substitution R30Q in the DUF622 domain of DLG5. Comparisons with homolog proteins indicate that the R30Q variant may disturb binding to a Rab GTPase and thus is likely to have functional implications[4]. In addition, we described 5 additional rare, so-called 'private' mutations that are also likely to have functional implications but are running at a very low frequency in the general population and are thus unlikely to considerably contribute to the observed effect[5].

The initial report of DLG5 as a novel IBD susceptibility gene sparked a multitude of studies investigating its effect on CD and IBD, respectively, unfortunately leading to discrepant findings and ongoing discussions concerning the validity of the initial association finding.

ROLE OF DLG5 IN JAPANESE PATIENTS WITH CROHN DISEASE
Shortly after the initial report, DLG5 was analysed for its association to CD in Japanese patients[6]. The CD associated variant R30Q in individuals of Caucasian decent was absent in the Asian population. However, another variant in the DLG5 gene, rs3758462, located in intron 15 of the DLG5 gene, was significantly associated with CD ($P = 0.023$) in this Japanese population with an evaluated odds ratio (OR) of 1.39 confirming that DLG5 is a susceptibility gene for CD with a moderate effect size. More important, the study by Yamazaki et al highlights the fact that ethnically divergent populations, although they share the same phenotype, need not necessarily share the same predisposing variants. One reason is that ethnically
divergent populations do not necessarily share the same risk genes as highlighted by the example of CARD15 variants in ethnically divergent populations. While certain mutations in the CARD15 gene, namely SNP8 (R702W), 12 (G908R) and 13 (1007insC), are strongly associated to CD in populations of Caucasian descent, the same mutations are completely absent in Asian populations. In fact, the CARD15 gene is not even associated to the disease in Asian populations\(^8,9\), thus the finding that a variant in DLG5 is - although modestly - associated with CD in a Japanese population implicates DLG5 as a susceptibility gene that confers risk in populations of divergent ethnic origin. However, further studies involving additional cohorts of Asian or African origin will need to be studied to conclusively address the role of DLG5 in ethnically divergent populations.

**DLG5 AND HETEROGENEITY OF RISK VARIANTS IN POPULATIONS OF CAUCASIAN DESCENT**

Daly et al\(^7\) investigated the contribution of DLG5 in three study cohorts consisting of patients from Quebec, Italy and UK. This study failed to replicate the reported undertransmission of haplotype A and suggested that the original observation could conceivably have been partially a statistical fluctuation and partially a result of LD with the observed R30Q association on haplotype D. However, they found evidence for an association of the R30Q variant with an estimated OR for R30Q of approximately 1.25, supporting the fact that DLG5 is a moderate susceptibility gene in populations of Caucasian decent and weaker than in the initial report\(^10\). This finding implicates that if the true association was as modest as observed by Daly et al several thousand cases and controls would be required to have a > 90% power to replicate this effect with confidence (\(P < 0.01\)), an effort that can only be managed by a consortium effort as none of the individual investigators has a sufficiently powered study population at their disposal. Of note, Daly and colleagues\(^7\) found evidence of replication of the DLG5 association in only two of three of their sample collections, with the study sample from the UK failing to replicate the DLG5 association. This observation pointed towards the possibility of either phenotypic differences between the populations studied or genetic heterogeneity within populations of Western European descent. The latter explanation was further supported by a more recent study which failed to replicate this association\(^11\) in a Scottish population. In fact, in their study, the 113A allele was less common in IBD and CD patients than in healthy controls (11.4% in IBD vs 13.2% in healthy controls). The authors concluded that the observed discrepancies in allele frequencies might be explained by genetic heterogeneity within European populations. Such genetic heterogeneity has been observed even for the well established CARD15 gene within Europe with Northern European populations exhibiting the lowest frequencies and Southern European populations exhibiting the highest frequencies of the variant alleles\(^9\). Accordingly, divergent association results for the R30Q variant in the DLG5 gene may be indicative of the observed genetic heterogeneity in European populations. This finding is further corroborated by a recent study by Medici et al\(^10\) who observed extreme heterogeneity for both CARD15 and DLG5 variants between a German and a Norwegian study sample, with a lower population attributable risk percentage for CARD15 and DLG5 variants in the Norwegian cohort than observed for other European cohorts. These results are consistent with an emerging pattern of a low frequency of e.g. CARD15 variants in Northern countries where the prevalence of IBD is greatest.

**ROLE OF GENDER**

Environmental factors as well as gender may contribute to the complex basis of CD. While gender-specific gene effects are well documented in other complex diseases, i.e. cardiovascular diseases, gender has previously not been investigated in this context in inflammatory bowel disease. Interestingly, a follow-up study of the original genomewide linkage study in German and UK IBD families\(^13\) examined the data set for gender specific linkage\(^14\) and identified the chromosomal region 10 q, harbouring DLG5, as a male specific CD susceptibility locus. In a recent study we investigated whether the association of the DLG5 variant R30Q to CD shows the same gender specificity as the underlying linkage signal. Using multivariate logistic regression analyses in a combined sample consisting of individuals from Germany, Italy and Quebec that were part of the studies reported by us\(^13\) and Daly et al\(^7\), we examined whether gender is a relevant factor to the association between CD and the R30Q variant in DLG5. Remarkably, our analyses revealed that the R30Q variant was associated with CD in men (OR = 2.49, \(P < 0.001\)) but not women (OR = 1.01, \(P = 0.979\)), thus establishing the DLG5 R30Q variant as a gender-specific susceptibility factor for CD\(^15\). This gender dependence may in part explain the divergent association studies relating to DLG5 and IBD outlined in the paragraphs above, as gender composition of the cohorts at study may have been different, and gender was not included as an independent variable in previous analyses. Hopefully, reanalysis of the previous study data involving gender will help to further clarify the role of DLG5 in the aetiology of IBD.

Of note, similar to the observation made by Tenesa
Table 1 Summary of genetic studies that analyzed DLG5 variants for their association to IBD or CD

| Study                      | Summary of results                                                                                                                                                                                                 | Country of sample collection | Number of participants | Detailed results                                                                                                                                                                                                 | Further sample info: gender/age |
|----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------|------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|
| Stoll et al 2004[1]        | First finding of association between DLG5 variants and IBD/CD Positive association between R30Q (haplotype D tagging SNP) and IBD/CD Negative association between haplotype A and IBD/CD Positive association between P1371Q (rare haplotype) and IBD/CD Interaction between R30Q variant and CARD15 risk alleles | Germany, United Kingdom     | 457 IBD trios (302 CD) | TDT (T:U) single SNPs R30Q
R30Q
IBD: 92/64, P = 0.025; CD: 60/42, P = 0.075
Haplotype A (DLG5_e26)
IBD: 162/225, P = 0.001; CD: 108/151, P = 0.008
P1371Q
IBD: 40/24, P = 0.046; CD: 24/17, NS                                      | Controls age and sex matched |
| Daly et al 2005[1]         | Replication of positive association Canada, between R30Q and CD in two of three Italy independent study samples No replication of negative association between haplotype A and CD P1371Q variant not polymorphic | Northern Europe              | 249 CD patients, 207 controls | Allele frequencies (cases versus controls)
R30Q
CD: 13.2 %, controls: 9%, OR = 1.62, P = 0.001
Haplotype A
CD: 32.4 %, controls: 36.1%, OR=0.74, P = 0.01
P1371Q
CD: 4.2 %, controls: 2.5%, OR=1.51, P = 0.01 | Allele frequencies (cases versus controls) |
| Newman et al 2006[1]       | Non replication of positive association Canada between R30Q variant and IBD/CD Association between haplotype A and IBD/CD, however positive association in this study instead of negative Replication of positive association between P1371Q and IBD/CD | United Kingdom               | 402 CD patients, 179 UC patients, 537 controls | Allele frequencies (cases versus controls)
R30Q
Controls: 8.7 %; IBD: 8.2 %, NS; CD: 7.8 %, NS
Haplotype A
Controls: 30.8 %; IBD: 36.1 %, P = 0.01;
CD: 36.6 %, P = 0.01
P1371Q
Controls: 6.1 %; IBD: 9.6 %, P = 0.01;
CD: 10.3 %, P = 0.01
Frequencies for both variants did not differ between males and females in the cases and controls | Mean age CD patients: 20.4 yr Controls: 31 yr |
| Török et al 2005[2]       | Non replication of positive association Southern between R30Q and IBD/CD Non replication of negative association between haplotype A and IBD/CD Non replication of positive association between P1371Q and IBD/CD Non replication of interaction between R30Q variant and CARD15 risk alleles | Germany                      | 625 CD patients, 363 UC patients, 1012 controls | Allele frequencies (cases versus controls)
R30Q
Controls: 10.8%; IBD: 10.1%, NS; CD: 9.8%, NS
Haplotype A
Controls: 35.2%; IBD: 34.4%, NS;
CD: 34.6%, NS
P1371Q
Controls: 4.4%; IBD: 4.5%, NS; CD: 3.9%, NS | Controls age and sex matched |
| Noble et al 2006[3]        | Non replication of positive association Scotland between R30Q and IBD/CD Non replication of negative association between haplotype A and IBD/CD Non replication of interaction between R30Q variant and CARD15 risk alleles | Scotland                     | 374 CD patients, 305 UC patients, 294 controls | Allele frequencies (cases versus controls)
R30Q
Controls: 13.2%; IBD: 11.4%, P = NS;
CD: 11.4 %, NS
Haplotype A
Controls: 31.5%; IBD: 35 %, P = 0.17;
CD: 36.9 %, P = 0.078
P1371Q
Controls: 4.4%; IBD: 4.5%, NS; CD: 3.9%, NS | Numbers Male/female (median age) CD patients: 181/193 (28 yr) Controls: 143/151 (39 yr) |
| Vermeire et al 2005[4]     | Non replication of positive association Belgium between R30Q and IBD/CD Non replication of negative association between haplotype A and IBD/CD Replication of interaction between R30Q variant and CARD15 risk alleles | Belgium                      | 373 IBD trios (80 % CD) | TDT (T:U) single SNPs R30Q
IBD: 51.79, P = 0.01; CD: 42.71, P = 0.01
Haplotype A (DLG5_e26)
IBD: 161/149, NS; CD: 134/123, NS                                      | Numbers Male/female (mean age) IBD patients: 162/211 (37 yr) Controls: 139/166 (41 yr) |
|                          |                                                                        |                              | 472 CD patients, 120 UC patients, 305 controls | Allele frequencies (cases versus controls)
R30Q
Controls: 10.8%; IBD: 11.8%, NS; CD: 11.5%, NS
Haplotype A
Controls: 33.8%; IBD: 33.9 %, NS; CD: 35.2 %, NS | IBD patients: 256/352 (45 yr) Controls: 139/166 (41 yr) |
et al.\(^{12}\), the observed difference for men and women is result of divergent allele frequencies in the healthy controls and not in the CD patients. The allele frequencies for the Q allele were almost identical among men and women affected with CD (CD males: 10.1%, CD females 10.9%) while there was a significant difference in healthy individuals (males controls: 5.2%, female controls: 11.3%). These data suggest gender-specific genetic heterogeneity or, more likely, the presence of transmission ratio distortion (TRD) of the Q allele among healthy controls. This hypothesis was further supported by two independent population-based study samples, where similar observations were made\(^{13}\). Interestingly, one of the two study samples consisted of a study sample of newborns (Q allele: 7.1% in males vs 11% in females, \(P = 0.036\)) indicating that the observed gender-specific disparity of Q allele frequencies is in fact consequence of prenatal processes, and likely of gender-dependent transmission distortion. However, other mechanisms or a combination of all possible causes for TRD, i.e. biased meiotic segregation, gametic selection and viability selection are conceivable and the true underlying mechanisms are subject to further research. The question remains why the observed gender-dependent disparity in Q allele frequencies is offset in male CD patients. In our opinion, the increase in Q alleles in affected individuals could be either consequence of a yet unknown, disease related mechanism or result of an accumulation of the risk associated Q allele in affected men but not women. However, further studies will need to be conducted to conclusively address this question.

**ROLE OF GENE-GENE INTERACTION**

Prior to the identification of DLG5 as a susceptibility gene for IBD and CD, respectively, two CD risk genes were identified, CARD15 and the IBDS risk haplotype that since then have been unequivocally replicated several times\(^{16-23}\). As IBD is a polygenic, multifactorial disease, the potential gene-gene interaction between the known susceptibility variants in CARD15, IBDS and DLG5 needs to be investigated to estimate the overall genetic risk. However, most of the studies on IBD genetics are hampered by the lack of statistical power to address gene-gene interactions due to small sample size, rendering such studies a challenging endeavour. This is particularly true for DLG5, as DLG5 is a susceptibility gene that only confers a modest risk\(^{7}\). The non replication of negative association\(^{24}\) of the Q allele frequencies is offset in male CD patients. In our study sample (40 yr), R30Q gender specificity was analyzed without considering the genotype status of the affected child. Interestingly, in CD trios, we observed a significant difference in transmissions of R30Q to the affected child. Interestingly, in CD trios, we observed a significant difference in transmissions of R30Q to the affected child. Interestingly, in CD trios, we observed a significant difference in transmissions of R30Q to the affected child. Interestingly, in CD trios, we observed a significant difference in transmissions of R30Q to the affected child. Interestingly, in CD trios, we observed a significant difference in transmissions of R30Q to the affected child. Interestingly, in CD trios, we observed a significant difference in transmissions of R30Q to the affected child. Interestingly, in CD trios, we observed a significant difference in transmissions of R30Q to the affected child. 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