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TOPIC HIGHLIGHT

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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.

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

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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[1]. 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[1].

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[4]. 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[5,6]; 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[1]. 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[8] 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. Interestingly, a recent study involving Greek patients with Crohn disease[11] showed that the R30Q variant was completely absent in their study population, further demonstrating the presence of extreme heterogeneity in *DLG5* variants between Northern and Southern European populations.

A recent meta analysis by Tenesa *et al*[12] combining the data from the studies reported by Stoll *et al*, Daly *et al* and Noble *et al* found substantial heterogeneity among the studies that reached statistical significance using the Cochran's Q statistic ($P = 0.0004$), and when including a fixed effect model, the R30Q variant was associated with an OR of 1.19 ($P = 0.035$)[12]. When further exploring the source of heterogeneity it became obvious that the frequencies among IBD samples tended to be more similar than between controls, an intriguing observation that will be further detailed in the following paragraphs.

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 genome-wide 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[1] 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
Association findings	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	457 IBD trios (302 CD)	*TDT (T:U) single SNPs* 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	
			Germany, United Kingdom	1 Replication: 485 IBD trios (271 CD)	*TDT (T:U) single SNPs* R30Q IBD: 90:73, $P = 0.09$; CD: 58:43, $P = 0.065$ Haplotype A (DLG5_e26) IBD: 165:214, $P = 0.006$	
			Northern Europe	2 Replication: 538 CD patients, 548 controls	*Allele frequencies (cases versus controls)* R30Q CD: 13.2 %, controls: 9%, OR = 1.62, $P = 0.001$ HaplotypeA CD: 32.4 %, controls: 36.1%, OR=0.74, $P = 0.01$ P1371Q CD: 4.2 %, controls: 2.5%, OR=1.51, $P = 0.01$	Controls age and sex matched
	Daly *et al* 2005[7]	Replication of positive association between R30Q and CD in two of three independent study samples No replication of negative association between haplotype A and CD P1371Q variant not polymorphic	Canada, Italy	249 CD patients, 207 controls	*Allele frequencies (cases versus controls)* R30Q CD: 11%, controls: 5.9%, $\chi^2 = 7.8$, $P = 0.003$ Haplotype A CD: 36.5%, controls: 40.1 %, $\chi^2 = 1.4$, $P = 0.12$ P1371Q Not polymorphic	
			United Kingdom	353 CD patients, 336 UC patients, 493 controls	*Allele frequencies (cases versus controls)* R30Q CD: 9.3%, controls: 9.7%, NS Haplotype A CD: 34%, controls: 35.7%, NS	
			Canada, United Kingdom	306 IBD trios (88 % CD)	*TDT (observed:expected transmissions)* R30Q IBD: 76:65.1, $P = 0.018$ Haplotype A IBD: 304: 307.8, NS	
	Newman *et al* 2006[25]	Non replication of positive association 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	Canada	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
Non replication findings	Török *et al* 2005[26]	Non replication of positive association 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	Southern 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[8]	Non replication of positive association 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 %, $P = 0.078$	Numbers Male/female (median age) CD patients: 181/193 (28 yr) Controls: 143/151 (39 yr)
	Vermeire *et al* 2005[24]	Non replication of positive association 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)
				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)

R30Q not polymorphic	Yamazaki *et al* 2004[4]	R30Q polymorphism not polymorphic in Japanese patients Non replication of negative association between haplotype A and CD Non replication of positive association between P1371Q and CD Other DLG5 variant risk associated instead	Japan	484 CD patients, 345 controls	*Allele frequencies (cases versus controls)* R30Q Polymorphism absent in 48 Japanese patients tested Haplotype A CD: 24.6 %, controls: 22%, NS P1371Q CD: 16.5%, controls: 19.2%, NS DLG5 SNP rs3758462 CD: 18.5 %, controls: 22.1%, $P = 0.068$ ($P = 0.023$, additive inheritance model for rare variant)	
	Gazouli *et al* 2005[11]	R30Q polymorphism absent in Greek study sample	Greece	120 CD patients, 85 UC patients, 100 controls	R30Q Polymorphism absent	
R30Q gender specificity	Friedrichs *et al* 2006[15]	Gender specific analysis of R30Q polymorphism in German, Italian and Canadian study samples with positive association findings[1,7] R30Q confers susceptibility to CD in males but not females Observation of possible transmission ratio distortion (TRD) in general population TRD confirmed by two independent population based samples, one of which was a birth cohort	Germany, Italy, Canada	613 CD patients, 749 controls	*Allele frequencies (cases versus controls)* R30Q Q allele frequencies male CD patients: 10.1%, male controls: 5.2% $OR_{males} = 2.5$, 95% confidence interval = 1.5-4.1, $P < 0.001$ female CD patients: 10.9%, female controls: 11.3% $OR_{females} = 1$, NS TRD Q allele frequencies newborn study sample males: 7.1%, females: 11%	Numbers Male/female (mean age) CD patients: 235/375 (36 yr) Controls: 403/346 (40 yr)

TDT: Transmission disequilibrium test; T:U: Transmitted alleles versus untransmitted alleles (transmissions from heterozygous parents to affected offspring); OR : Odds ratio; NS: Not significant.

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[15]. 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 *IBD5* 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, IBD5* 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 DLG5 R30Q risk variant is comparably infrequent (5%-10%) and subject to genetic heterogeneity[12]. Despite the before mentioned restrictions in the investigation of gene-gene interactions, we examined potential locus-locus interactions with variants of *CARD15* in our initial study describing genetic variation in DLG5[1]. Interactions between *DLG5* and *CARD15* were examined by stratifying trios in two groups on the basis of the *CARD15* genotype of the affected child. Interestingly, in CD trios, we observed a significant difference in transmissions of R30Q between the $CARD15^{risk}$ and $CARD15^{nonrisk}$ group with an increased association of the risk allele in the $CARD15^{risk}$ group, while the association was no longer seen in the $CARD15^{nonrisk}$ group. This suggested a potential interaction of the DLG5 R30Q variant with $CARD15^{risk}$ variants. Recently, Vermeire *et al*[24] reported that *DLG5* was not associated in a large Flemish IBD cohort when *DLG5* was analyzed without considering the genotype status at *CARD15*[24] variants. However, after stratification for $CARD15^{risk}$ variants in the CD patients, the R30Q frequency turned out to differ significantly in the $CARD15^{risk}$ group (Q frequency 12.2%) compared to the $CARD15^{nonrisk}$ group (Q frequency 8.7%; $P = 0.033$), providing further evidence for a potential interaction between $CARD15^{risk}$ variants and DLG5 R30Q. However, further studies involving large study samples will need to be conducted, although due to the observed genetic heterogeneity in *DLG5* variation even within Europe, a consortium effort will be difficult to conduct, and the results are likely difficult to interpret. (Table 1).

In summary, genetic variation in *DLG5* and its role in IBD is a prime example for the difficulties encountered in the dissection of the genetic basis of complex disease. Similar to many examples from other complex diseases, the primary association signal could not be unequivocally replicated in other independent study populations. However, with more and more studies emerging and the growing awareness about the complexity of multifactorial diseases in general, the picture appears to clear off - although there are still a lot of loose ends to tie. The example of *DLG5* shows for the first time that gender needs to be considered as an independent factor contributing to the genetic risk for IBD, a fact that has not been considered previously as the prevalence of IBD is almost equal among genders.

In order to further elucidate the contribution of present and emerging susceptibility genes to the aetiology and clinical presentation of IBD, there is the need to have well-characterized and homogeneous patient populations as well as appropriate controls, preferentially with standardized phenotypes to allow for comparisons between independent study cohorts. In addition, investigators need to be aware of the extreme heterogeneity in IBD disease variants among European populations and between ethnically divergent populations. Finally, the investigation of gene-gene and gene-environment interaction and its influence on disease-onset and progression will gain more importance. Due to the availability of whole genome genotyping arrays, more susceptibility variants will become available in the near future, all of which require further validation in the context of existing IBD susceptibility genes and environmental factors.

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