Table 4: Association studies of common DNA variants of the *NRIP1* gene in relation to human endometriosis.

NRIP1 polymorphism (change in codon)	Genotypes	Patients (n = 59)	Unselected Controls (n = 94)	Super Controls (n = 47)	All Controls (n = 141)	Statistical Analysis*
Gly75Gly (ggg to gga)	aa	15	19	9	28	P = 0.34 (Heterozygous test)
	ag	27	46	27	73	
	gg	17	28	11	39	
Arg448Gly (cga to gga)	cc	44	83	40	123	P = 0.027 (Allele positivity test)
	cg	14	10	7	17	
	gg	1	1	0	1	
Ser803Leu (tcg to ttg)	cc	56	85	46	130	P = 0.59 (Armitage's trend test)
	ct	3	9	1	10	
	tt	0	0	0	0	

*Compares patients versus merged controls. Best p value employing tests for genetic association according to Sasieni (1997). Ile441Val and His221Arg are not analyzed due to small or null sample size in endometriosis samples.

more than 150 chromosomes. The rest of the SNPs are the result of the bioinformatic alignment of different cDNA and genomic clones. The allele frequencies here presented for *Gly75Gly* (dbSNP rs2229741) and *Arg448Gly* (dbSNP rs2229742) polymorphisms are very similar to those included in dbSNP (data not shown).

Overall, our results are preliminary providingt suggestive, but not definitive, evidence of *NRIP1* gene involvement in human endometriosis. We think that conclusive proofs of involvement will be achieved throughout re-analyses of this study in independent cohorts of patients and controls, rather than performing functional analyses of the missense mutations observed. The detection of functionality of DNA variants involved in complex traits such endometriosis, is near to be impossible using conventional technologies because the effect from single genetic variant/mutation is expected to be very small and it is only the joint effect of several susceptibility genes that leads to the disease [25]. In this sense, we are currently recruiting a higher number of patients and controls to perform a proper re-analysis of our results.

Regarding *Arg448Gly* polymorphism, we propose that the variant could act as a low penetrance allele related to human endometriosis. The molecular mechanism of this mutation is not well understood, although its location and degree of conservation provide some interesting clues. In fact, Arg448 residue of RIP140 protein is completely conserved among humans, rats, mice, gallus and xenopus (Fig. 1b). Moreover, the non-conservative substitution detected (*Arg448Gly*) might affect the Carboxyl terminal binding protein (CTBP) interacting motif of RIP140 protein that is located close to this amino acid residue (Fig. 1b).

On the basis of genotype analysis in affected women, we propose that *Arg448Gly* mutation could act in concert with other genetic variants within *NRIP1* or other loci. In this way, we found a single woman affected by endometriosis simultaneously carrying *Val1079Phe* mutation and *Arg448Gly* polymorphism both in a heterozygous state. *Val1079Phe* also arises in an inter-specific conserved residue. Moreover, this mutation is located close to (and may disrupt) the retinoid acid receptor interacting motif "LTKTNPILYYMLQK" of RIP140 protein (Fig. 1b). Supporting its involvement in the disease, we have not identified the *Val1079Phe* mutation in 282-control chromosomes. Intriguingly, retinoid acid receptors alpha, gamma 2 and its regulator cnot7 have been involved in male sterility [24,26,27]. Moreover, the presence of multiple specific functional rare variants in affected patients have been recently proposed and evaluated [28]. This hypothesis is an alternative to explain the genetic component of complex traits in front of the widely accepted common disease common variant hypothesis [29].

Finally, we identified a single patient carrying a unique genotype combination comprising *His221Arg* rare variant and, again, *Arg448Gly* polymorphism. The absence of inter-specific amino acidic conservation and the inexistence of known functional domains close to *His221Arg* variant do not support the functionality of *His221Arg* allele. However, *His221Arg* only appears combined with *Arg448Gly* in a woman affected by endometriosis. This combination never appears in 141 unrelated controls.