Table 2: Anchor and Sensor probes sequences employed for coding Single Nucleotide Polymorphism (cSNP) analysis using Fluorescence Resonance Energy Transfer (FRET) technology.

Mutation	PROBE	SEQUENCE 5' → 3
Gly75Gly	75-ANCHOR	AGTAATGGTCCAGTTCTCAATACAC – F
	75-SENSOR	Cy5 – TACATATCAGGGGTCTGGC – Ph
His221Arg	221-ANCHOR	Cy5 – AGTGGAACAAAGGTCATGAGTGAAC – Ph
	221-SENSOR	TCTCCTCATCATGTTGGACA – F
Ile441Val	448-ANCHOR	TATTCCAACTGTGTTCCCATAGACT – F
Arg448Gly	448-SENSOR	Cy5 – GTCTTGCAAACACCGAACTG – Ph
Ser803Leu	803-ANCHOR	GCGCACCTGCCTTACCAGTGTCCCGA – F
	803-SENSOR	Cy5-GACTTTAAATCGGAGCCTGTT – Ph
Val1079Phe	1079-ANCHOR	Cy5 – CGAGAAACACAAGACAAGGACATTT – Ph
	1079-SENSOR	GGAGGCAATTCTGTTACCAG – F

Nomenclature: F: Fluoresceine, Ph: Phosphate.

finished 80,600 bp of DNA. Using our methodology, we identified six single nucleotide DNA variants within the coding sequence of the *NRIP1* gene in various unrelated somatic DNA samples. Two of these variants have been previously identified and they are included in the Single Nucleotide Polymorphism Database (dbSNP) at the National Centre for Biotechnology Information (NCBI) http://www.ncbi.nlm.nih.gov/ (Table 3). Five of these mutations alter the amino acid coding sequence of RIP140 protein generating missense mutations (Fig. 1 and Table 3). Although all mutations were detected in somatic DNA, direct molecular analyses of the corresponding blood samples of mutated tissue also contain the same DNA change. This last result implies that the genetic variants identified are germ-line and, consequently, somatic mutations at *NRIP1* locus are not commonly involved in the pathogenesis of human endometriosis.

To evaluate the polygenic role of *NRIP1* gene variants in human endometriosis, we decided to preliminary explore the allelic frequencies and genotypes of these mutations in women affected by endometriosis and unselected controls. To conduct genetic association studies, we developed real-time PCR detection protocols using FRET probes for each DNA mutation identified at *NRIP1* locus. Using these techniques, we genotyped the mutations in 200 unrelated women (59 endometriosis patients, 94 unselected controls and 47 super-control women). Overall, 400 different chromosomes have been scored for each DNA variant (Table 4).

By analyzing the allelic frequencies of DNA variants detected in Spanish population, we classified these variants as common polymorphisms if observed in >1% of chromosomes in controls (*Gly75Gly*, *His221Arg*, *Ile441Val*, *Arg448Gly* and *Ser803Leu*) or rare variants if observed with a frequency <1% of chromosomes in controls (Table 3). In contrast, *Val1079Phe* allele appears only in a single patient in heterozygous state and none of 141 controls. This data could suggest its involvement in the disease. Reinforcing this hypothesis, Val1079Phe is located close to high-conserved domain of the carboxylic end of RIP140 protein that interacts with retinoic acid nuclear receptor (Fig. 1).

Direct inspection of genotypes in patients revealed three genotype patterns within the *NRIP1* gene that appear to be over-represented in women affected by endometriosis ($p = 0.006$, Fisher exact test). The patterns consist of a combination of *Arg448Gly* together with *His221Arg* or *Val1079Phe* variants. We identified three unrelated women affected by endometriosis carrying double heterozygotes (*His221Arg*/*Arg448Gly* and *Val1079Phe*/*Arg448Gly*) or homozygote (*Arg448Gly*/*Arg448Gly*) genotypes for these alleles, respectively. The homozygote (*Arg448Gly*/*Arg448Gly*) genotype pattern appeared only in 1 of 94 unselected controls ($p = 0.016$ Fisher exact test) and none of 47 super-control women ($p = 0.023$, Fisher Exact test), whereas double heterozygotes genotypes did not appear in any control individual. These results suggest that specific combinations of amino acid changes at *NRIP1* locus could be related to endometriosis etiology with a 99.4% of reliability, although given the scarce sample size the presence of polygenes within *NRIP1* locus must be proven with a larger and independent re-analysis.

Finally, given the preliminary results, we conducted an small case-control study analyzing all common variants detected within the *NRIP1* locus (*Gy75Gly*, *Arg448Gly* and *Ser803Leu*). Table 4 shows the results for those test that