Evaluation of *MLH1* I219V Polymorphism in Unrelated South American Individuals Suspected of Having Lynch Syndrome

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Abstract. *Background: Some single-nucleotide polymorphisms are associated with higher risk of colorectal cancer development and are suggested to explain part of the genetic contribution to Lynch syndrome. Aim: To evaluate the mutL homolog 1 (MLH1) I219V polymorphism in 124 unrelated South American individuals suspected of having Lynch syndrome, based on frequency, association with pathogenic MLH1 and mutS homolog 2 (MSH2) mutation and clinical features. Materials and Methods: DNA was obtained from peripheral blood and polymerase chain reaction (PCR) was performed, followed by direct sequencing. Results: The Val allelic of the I219V polymorphism was found in 51.61% (64/124) of the individuals, with an allelic frequency of 0.3. MLH1 or MHS2 pathogenic mutations were found in 32.81% (21/64) and in 23.33% (14/60) of Val-carriers and non-carriers, respectively. Conclusion: The Val-carrying genotype was frequent in the studied population; however, it does not appear to exert any modifier effect on MLH1 or MSH2 pathogenic mutations and the development of colorectal cancer.*

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Lynch syndrome (LS) is the most common form of familial colorectal cancer (CRC) and is associated with germline mutations in the DNA mismatch repair (MMR) genes, i.e. mutL homolog 1 (*MLH1*) on 3p21.3, mutS homolog 2 (*MSH2*) on 2p22-p21, mutS homolog 6 (*MSH6*) on 2p16.3 and post-meiotic segregation increased 2 (*PMS2*) on 7p22.2. Mutations in the above mentioned genes have been reported to account for more than 85% of LS kindreds fulfilling the Amsterdam criteria and exhibiting microsatellite instability (MSI) (1). Recently, the term LS has been restricted to those families with germline mutations in one of the MMR genes. Familial aggregation of CRC with no evidence of MMR deficiency was shown to be clinically and molecularly distinct from classical LS tumors and, therefore, is designated as familial colorectal cancer type X (FCCTX). Given the above distinction, the term hereditary non-polyposis colorectal cancer (HNPCC), which was formerly used to refer to families clinically diagnosed with colorectal cancer that might or might not have mismatch repair deficiency, is replaced by one of the more informative names: LS and FCCTX (2).

To date, over 513 different DNA alterations have been reported in HNPCC, in which the majority are represented by single-nucleotide substitutions, deletions or insertions (3). In total, 308 different pathogenic mutations have been identified in *MLH1*, along with 45 polymorphisms (3-5). The characterization of the functional effects of MMR mutations is essential, not only for effective diagnosis of cancer predisposition, but also for selecting the appropriate screening and treatment (6).

The common definition of single-nucleotide polymorphisms (SNPs) requires that the relative frequency of the least frequent allele is greater than 0.01, except for two polymorphisms in *MLH1*: a TTC deletion in the 3'untranslated region and a CA repeat polymorphism assigned to locus D3S1611, whose allelic frequencies range from 0.4% to 50% (7).

One of the most common polymorphisms related to LS is I219V located on the *MLH1* gene. The I219V polymorphism lies in *exon 8* and is conserved throughout evolution, which can be seen as a sign of its importance for the good functioning of the protein. HNPCC databases, *in silico* programs and various functional studies described it as a benign polymorphism. Recently, a functional study based on a transient transfection of *hMLH1* complementary DNA carrying the variant into a human embryonic kidney fibroblast cell line lacking *hMLH1* expression, demonstrated *in vivo* that the I219V SNP does not affect the MMR capacity (8).

Several studies have shown a high frequency of the I1219 polymorphic variant in patients with LS, however, none have tested whether this variant exerts a modifier effect on pathogenic mutations. Therefore, the aim of our study was to evaluate the frequency of I219V polymorphism in 124 individuals and its association with *MLH1*/*MSH2* pathogenic mutations and clinical characteristics, as part of a larger South American Collaborative Study evaluating MMR gene mutations in unrelated individuals with suspected LS (9).

Materials and Methods

Participating centers. One hundred and twenty-four unrelated individuals enrolled in this study were referred from the following South American hospitals and medical centers: 105 from the Department of Pelvic Surgery, A.C. Camargo Hospital, Sao Paulo, Brazil; 6 from the Colorectal Surgery Service, Uruguayan Collaborative Group, Montevideo, Uruguay; and 13 from the Department of Colorectal Surgery, Italian Hospital, Buenos Aires, Argentina. As part of a South American Collaborative Study, all cases were studied after signed informed consent was obtained.

Samples and DNA extraction. Genomic DNA from 124 patients fulfilling the Amsterdam I, II or Bethesda guidelines (10-13) was directly extracted from 3 ml of whole-blood using the Puregene Genomic DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA), according to the manufacturer's instructions.

Table I. *Allelic and genotypic frequency of the mutL homolog 1 (MLH1) I219V polymorphism in South American individuals suspected of having Lynch Syndrome*.

Polymorphism	Genotype	N+/N total	Genotypic frequency	Allelic frequency
I219V c.655A->G	Ile/Ile	60/124	0.48	0.7
	Ile/Val	54/124	0.44	
	Val/Val	10/124	0.08	0.3

Ile/Il, homozygosis for wild allele; Ile/Val, heterozygosis; Va/Va, homozygosis for polymorphic allele; N+, positive cases for each genotype; N total, total number of individuals evaluated.

PCR conditions. The *MLH1* [GenBank: NM.000249] and *MSH2* [GenBank: NM.000251.1] genes were divided into 20 fragments covering all 19 and 16 exons, respectively, and substantial parts of intronic regions. The PCR reactions contained 0.2 μM of each primer, 1.25 U AmpliTaq Gold (Applied Biosystems, SP, Brazil), 10 μM of each dNTP, 3 mM $MgCl_2$ and 25 ng DNA in a final reaction volume of 25 μl. The following cycling conditions were used: initial denaturation at 95˚C for 9 min, followed by 35 cycles of denaturation at 95˚C for 45 s, annealing steps at 56-60˚C for 45 s and extension at 72˚C for 1 min, followed by 72˚C for 7 min.

DNA sequencing and analysis. PCR products were purified with exonuclease I and alkaline phosphatase (EXO-SAP IT-USB/GE, SP, Brazil) and sequenced in both directions. Sequencing reactions were separated on an ABI Prism 3130 instrument (Applied Biosystems, SP, Brazil). All sequences were analyzed by the CLC software (CLCbio, Aarhus, Denmark).

Mutation nomenclature. Mutation nomenclature followed the Human Genome Variation Society (HGVS) guidelines (www.hgvs.org/ mutnomen/). All identified mutations were compared with those previously reported in databases for *MLH1* and *MSH2* genes, maintained by the MMR Gene Unclassified Variants Database (www.mmruv.info), the Mismatch Repair Genes Variant Database (http://www.med.mun.ca/mmrvariants/) and the International Society for Gastrointestinal Hereditary Tumors (http://www.insight-group.org).

Statistical analysis. Statistical analyses were carried out to evaluate if the mutL homolog 1 (*MLH1*) I219V polymorphism has implications on age of tumor onset in patients harboring or not pathogenic mutations in *MLH1*/*MSH2* genes. The comparison was performed by Wilcoxon and Kruskal-Wallis tests (with significance set at $p<0.05$), using the statistical software package IBM SPSS Statistics 20 (SPSS, Chicago, IL, USA).

Results

Genetic analyses of I219V polymorphism in individuals with suspected LS. The *MLH1* I219V polymorphism frequencies are shown in Table I. Val-carriers were found in 64 (51.6%) out of 124 unrelated individuals with

Table II. *MutL homolog 1 (MLH1) I219V polymorphism and MLH1 or mutS homolog 2 (MSH2) pathogenic mutations in South American individuals suspected of having Lynch Syndrome.*

I219V plymorphism	Total	*MLH* and/or *MSH2* pathogenic mutation	*MLH1* mutation	*MSH2* mutation
Yes	64/124 (51.61%)	21/64 (32.81%)	12/21 (57.1%)*	9/21 (42.9%)*
No	60/124 (48.39%)	14/60 (23.33%)	6/14 (42.9%)	8/14 (57.1%)

*One individual harbored pathogenic mutations in both genes.

suspected LS, of whom 49 were from Brazil, two from Uruguay and 13 from Argentina. Of them, 54 (44%) and 10 (8%) were *Ile/Val* heterozygotes and *Val/Val* homozygotes, respectively. The allelic frequency of *Ile* and *Val* was 0.7 and 0.3, respectively.

Correlation of I219V polymorphism with MLH1 and/or MSH2 pathogenic mutations. The characterization of germline mutations of *MLH1* and *MSH2* in unrelated South American individuals with suspected LS were provided by the South American Collaborative Study (9). Twenty-one out of 64 (32.81%) of Val-carriers harbored pathogenic mutations in *MLH1* and/or *MHS2*, and one case presented pathogenic mutations in both genes. In non-carriers (60/124), *MLH1* and/or *MHS2* pathogenic mutations were found in 23.33% (14/60) (Table II).

Clinical characteristics of I219V carriers associated with the presence of MLH1 and/or MSH2 pathogenic mutations. The Val polymorphism was found in 51.61% (64/124) of cases, out of which 51.5% (33/64) fulfilled the Bethesda guideline and 48.5% (31/64) the Amsterdam I or II criteria. The median age of diagnosis was 28-62 and 29-71 years in Val-carriers with and without *MLH1/MSH2* pathogenic mutation (Table 3), respectively (Table III). Clinical criteria, type and number of tumors in these cases are presented in Table III.

In order to evaluate if this polymorphism exerts any influence on the age of tumor onset, we analyzed the mean age of carriers and non-carriers of the I219V polymorphism according to their mutation status (*MLH1* and/or *MSH2* mutation). However, no statistically significant association was found between these groups (Table IV).

Table III. *Clinical features of 64 suspected of Lynch syndrome, carriers of MutL homolog 1 (MLH1) I219V polymorphism, according the status of MLH1/MSH2 pathogenic mutations.*

Clinical characteristics of I219V cases		
Characteristics	Carriers of pathogenic mutations	Non-carriers of pathogenic mutations
Age, years	28-62	29-71
Clinical criteria		
Amsterdam	15 (71.43%)	16 (37.21%)
Bethesda	6 (28.57%)	27 (62.79%)
Type of cancer		
Colonic	12	18
Adenoma	1	4
Rectal	0	9
Stomach	1	0
Endometrial	1	1
Two or more tumors in the same patient		
Colon/stomach	1	0
Colon/adenoma	1	0
Colon/hepatobiliary system	1	0
Colon/endometrial	1	0
Colon/sebaceous glands	1	1
Colon/breast	0	1
Colon/ small bowel	0	2

Table IV. *Mean age (years) at diagnosis of carriers of MLH1/MSH2 pathogenic mutations and mutL homolog 1 (MLH1) I219V genotype.*

	I219V	Non I219V
MLH1 mutation carriers	40.2 (n=13)	41.8 (n=7)
MSH2 mutation carriers	44 (n=7)	40.1 (n=8)
Non-carriers	44.9 (n=44)	41.7 (n=45)

Discussion

SNPs may play a small role in CRC risk. It is also possible that a combination of several variants in different genes might work synergistically to increase the risk of CRC (8). In this series, we investigated the I219V polymorphism in order to analyze its frequency, its correlation with *MLH1/MSH2* pathogenic mutations and its influence in hereditary CRC risk (based on clinical characteristics).

Trojan *et al*. (14) postulated that the majority of *MLH1* missense variants associated with LS, lead to structural changes within the amino- or carboxy-terminal regions containing the ATPase site and the domain of PMS2 interaction, respectively. However, according to Hudler *et al*. (15), the missense polymorphism located in the *exon 8*

leads to a substitution of conservative hydrophobic amino acid, namely isoleucine, by valine, which could affect the speed and fidelity of protein synthesis, due to the lower abundance of the tRNA or due to changes in the secondary structure of mRNA.

First studies regarding the I219V polymorphism reported an incidence of 31-80% in different populations (16-18). The allelic frequencies found in this series for *Ile* and *Val* were 0.7 and 0.3, respectively. Similar results were reported for German LS probands (0.69 and 0.31), Swedish families with LS (0.64 and 0.34) and Italian families with suspected LS (0.33 for *Val*) (16-19). This similarity might be explained by the African influence exerted through the slave population for three centuries, as well as the Native Indian and colonizing European populations in Brazil (20-22). It is known that ethnicity can impact molecular pathways of various types of human cancer and as a result, different clinical and pathological features can be found (23).

Regarding the association of the I219V polymorphism and *MLH1*/*MSH2* pathogenic mutations, the results of this series showed no significant differences between Val-carriers with *MLH1* and/or *MSH2* pathogenic mutation (32.81%) and non-carriers with *MLH1* and/or *MSH2* pathogenic mutations (23.33%). We can deduce that the presence of I219V polymorphism is not linked to pathogenic mutation in *MLH1* or *MSH2* genes in LS.

Regarding clinical characteristics, 51.5% (33/64) of Val-carriers fulfilled the Bethesda criteria, whereas the *MLH1*/*MSH2* pathogenic mutation was observed in 48.4% (15/31) of those fulfilling the Amsterdam criteria, confirming that the strict Amsterdam criteria are much better for selecting individuals suspected of having LS (9). However, no statistical difference was observed related to clinical criteria and tumor onset in carriers and non-carriers of Val in the I219V polymorphism.

Conclusion

To our knowledge, this is the first study regarding the evaluation of I219V according to *MLH1* and/or *MSH2* pathogenic mutations and clinical characteristics in unrelated South American LS individuals suspected of having LS. The Val-carrying variant was very frequent in this series and showed no association with *MLH1*/*MSH2* pathogenic mutations, clinical criteria (Amsterdam or Bethesda), or age of tumor onset. According to the computational prediction methods, it is suggested that this polymorphism does not have a pathogenic consequence.

Conflicts of Interest

No Author has any conflict of interest with regard to this study.

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