Co-occurrence of *MEN1* p.Gly111fs and *AIP* p.Arg16His Variants in Familial *MEN1* Phenotype

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Abstract. *Background/Aim:* Familial multiple endocrine neoplasia type 1 (*MEN1*) is a rare disorder mostly associated with germline *MEN1* mutations. *Materials and Methods:* Genotyping of the *MEN1*, cyclin-dependent kinase inhibitor 1B (*CDKN1B*) and aryl hydrocarbon receptor-interacting protein (*AIP*) genes using Sanger sequencing was carried out in a family with *MEN1* and the resulting germline variants genotyped in an additional 95 ethnically matched controls. *Results:* A missense variant in *AIP* (p.Arg16His) gene and a truncating mutation (p.Gly111fs*8) in *MEN1* gene were both detected in the proband and his father, showing limited co-segregation with phenotype. p.Arg16His *AIP* missense variant was detected in one control. *Conclusion:* There are conflicting data regarding the functional effects of *AIP* p.Arg16His and its role in disease development. We demonstrated the co-occurrence of p.Arg16His *AIP* missense variant in a patient with a bona fide *MEN1* mutation. Our finding of p.Arg16His *AIP* in one of the 95 controls and its co-occurrence with *MEN1* in a patient suggests that it is more likely that this variant is a rare polymorphism, unrelated to *MEN1* pathogenesis.

Multiple endocrine neoplasia type 1 (*MEN1*- OMIM #131100) is characterized by the co-occurrence of tumors in at least two of the following three endocrine tissues: parathyroid, endocrine pancreas and anterior pituitary, following an autosomal dominant inheritance pattern (1). In the majority of familial *MEN1* cases, germline mutations in the *MEN1* gene at 11q13 is detected (2).

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Mutations in the cyclin-dependent kinase inhibitor 1B gene (*CDKN1B*, OMIM #600778) have also been reported in some families with *MEN1* but are far less frequent (3, 4). In some familial *MEN1* cases, no underlying genetic anomaly in either gene has been detected (5). One attractive candidate for harboring germline mutations in familial *MEN1* cases is the aryl hydrocarbon receptor-interacting protein gene (*AIP*, OMIM #605555). This gene is located on the long arm of chromosome 11, a region showing high rates of allelic loss in *MEN1*-associated tumors (6). Previous genotyping of the *AIP* gene in patients with *MEN1*-like phenotype demonstrated that mutations in this gene are extremely rare (5). However, *AIP* frameshift mutation was reportedly detected in a patient with acromegaly and recurrent hyperparathyroidism (7). The putative pathogenicity of sequence variants, especially missense mutations, in genes that underlie hereditary cancer syndromes may be hard to ascertain. These variants may truly be pathogenic, rare polymorphisms or variants of unknown significance. Several techniques can be employed to decipher the pathogenic significance of these variants: functional assays, co-segregation analyses, *in silico* predictive algorithms, and rates in ethnically matched populations (8). Here we evaluated the contribution of germline mutations in the *MEN1*, *CDKN1B*, and *AIP* genes to the *MEN1*-like phenotype in a Brazilian family, where the co-occurrence of sequence variants in the *AIP* and *MEN1* genes are reported.

Materials and Methods

This study was approved by the local Ethics Committee (CAAE-09135912.6.0000.5149), and each participant (proband IV.1; and four family members, III.2, III.3, III.4, III.5) gave their informed consent. Patients were clinically evaluated by history and physical examination and diagnosis was further supported by biochemical testing and imaging analyses. The proband (IV.1, Figure 1A), a 28-year-old male, was referred in 2009 for evaluation of cervical

nodules detected during workup for primary hyperparathyroidism. Cervical ultrasound demonstrated three benign thyroid nodules and two enlarged parathyroid glands (right and left inferior parathyroid glands). At age 25 years, he was diagnosed with gastric carcinoid that clinically manifested as persistent diarrhea. No surgical procedures were advised, and he was prescribed omeprazole with improvement of diarrhea; however, the patient did not return for follow-up. Standard biochemical and imaging tests in 2009 showed hyperparathyroidism (126 ng/ml) associated with hypercalcemia (11.5 mg/dl) and elevated serum gastrin level (180 ng/ml). Basal values of anterior pituitary hormones except prolactin (307 ng/ml) were within normal range. Abdominal computed tomographic scan showed thickening of the gastric antrum and five duodenal and mesenteric fat nodules. Brain magnetic resonance imaging revealed pituitary macroadenoma measuring 1.3×1.2×1.0 cm subsequently treated with cabergoline. The patient underwent pan-parathyroidectomy (with forearm implant of a single gland) and partial thymectomy with subsequent partial gastrectomy and excision of gastric carcinoid tumor. The pathological report showed that all resected parathyroid glands were hyperplastic. Based on the co-occurrence of hyperparathyroidism, macroadenoma of the pituitary and gastric neuroendocrine tumor, a clinical diagnosis of MEN1 was made.

The proband's father (III.4, Figure 1A) died at age 61 years of multiple liver metastases originating from a pancreatic neuroendocrine tumor. According to medical records, the father was also diagnosed with hyperparathyroidism and a pituitary microadenoma with increased human growth hormone level (9.04 ng/ml, reference <0.97 ng/ml) and normal prolactin level 2 years prior to his death. There was no mention in the medical records as to whether the father had clinical features of acromegaly. The proband's 33-year-old phenotypically healthy sister (IV.3) refused DNA testing; their paternal grandfather (I:1) died of thymoma at an unknown age.

Genomic DNA was isolated from peripheral blood leukocytes of the proband as well as his unaffected mother (III.5, Figure 1A) and two paternal aunts (III.2 and III.3, Figure 1A) using the high-salt method of Lahiri and Nurnberger (9). DNA from liver metastasis of the deceased father (III.4) and the carcinoid tumor removed from the proband were isolated using QIAamp DNA FFPE Tissue Kit (Qiagen, Milan, Italy) using the manufacturer's recommended protocol. DNA extracted from blood of 95 ethnically matched elderly (65 years of age or older) controls without personal or family history of benign or malignant tumors was used to access the frequency of the studied variants in the healthy population.

At least 100 ng of DNA were used for each polymerase chain reaction (PCR) to amplify all coding exons of *MEN1*, *CDKN1B*, and *AIP* genes. Primers were either designed according to previously published data (2,10), or using PrimerBlast software, (available at <http://www.ncbi.nlm.nih.gov/tools/primer-blast/>). Gel-verified PCR products were gel purified using QIAquick PCR purification kit (Qiagen, Hilden, Germany) and were bi-directionally sequenced using an ABI BigDye Terminator Cycle Sequencing Kit v3.1 on an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, Foster City, USA). The reference sequences derived from Ensembl (<http://www.ensembl.org>) or mutation analysis were NM_130803.2 for *MEN1*, NM_001302959 for *AIP*, NM_001220778 for *CDKN1A*.

Aiming to obtain a homology model of mutated *AIP* (c.47G>A), a psiBLAST [ProteinDataBank (PDB) <http://blast.ncbi.nlm.nih.gov>] procedure was performed. The crystallographic coordinates were

obtained from a PDB template 2LKN with 100% of identity with the *AIP* translated protein sequence (11). To correlate this point mutation with a possible change in protein behavior, a model using Swiss-Model Platform was built (<https://swissmodel.expasy.org>). The software Pymol (The PyMOL Molecular Graphics System, Version 1.5.0.4; Educational Pymol; Schördinger, LLC, Mannheim, Germany) was used to generate the images.

Results

The proband harbored a germline heterozygous 1bp deletion in exon 2 of the *MEN1* gene, which leads to a frameshift deletion, p.(Gly111Valfs*8) (Figure 1B) in both blood and tumor samples (VSPAVSW followed by stop codon eight positions later). In addition, three known *MEN1* single nucleotide polymorphisms (SNPs) were present: two silent sequence variants p.Asp423 (c.1269C>T; rs2071313) and p.His438 (c.1314C>T; rs540012), and the third SNP was a prevalent non-synonymous coding variant p.Thr546Ala (c.1636G>A; rs2959656). DNA from the proband's unaffected mother and paternal aunts were genotyped for the *MEN1* gene frameshift deletion p.Gly111fs and all displayed wild-type alleles. It was not possible to genotype the patient's and father's tumor DNA for this mutation as the remaining paraffin-embedded tissue DNA was degraded.

Sequencing of *AIP* revealed the heterozygous germline missense variant p.Arg16His (c.47G>A; rs145047094) (Figure 1C) in the proband, and tumor of his MEN1-affected father. The mother and paternal aunts did not harbor this *AIP* variant. No other family members were genotyped for this sequence variant. Mutations in *CDKN1B* gene were not detected in the patient's germline DNA. Since the *AIP* is a tumor-suppressor gene (9), experiments to determine loss of heterozygosity (LOH) of the wild-type allele in the carcinoid of the patient and liver metastases from the father's neuroendocrine tumor were carried out. Neither of the analyzed tumors showed LOH, and both were somatically heterozygous for *AIP* p.Arg16His.

In silico analysis (Figure 1D) of the impact of p.Arg16His on protein structure showed that the lower flexibility and hydrophobicity of the amino acid histidine, compared to arginine (mainly found on α helix) might reduce the stability of the β A strand. This mutation can also modify the N-terminal α 0, a fragment that plays an important role in the stability of *AIP* (12), which is oriented nearly parallel to the first strand β A. This result is in line with the amino acid change prediction tools showing that the change is highly deleterious, as predicted by PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>) with a score of 0.969 (sensitivity=0.77; specificity=0.95).

To assess the population rate of the *AIP* sequence variant, 95 elderly control individuals (see Materials and Methods) were also screened for *AIP* p.Arg16His. This variant was found in heterozygous state in a single 70-year-old control with no personal or family history of cancer or endocrine abnormalities.

Figure 1. A: Pedigree of a Brazilian family affected by multiple endocrine neoplasia type 1 (MEN1). Black filled shapes are patients diagnosed with MEN1 syndrome who were also aryl hydrocarbon receptor-interacting protein (AIP) p.Arg16His mutation carriers. Open shapes represent individuals clinically unaffected by MEN1. Question mark represents an individual with unknown clinical history. The proband is shown by an arrow (IV.1) and was also the only individual tested for MEN1 that was positive for MEN1 p.Gly111fs. Individuals III.2, III.3 and III.5 were tested for MEN1 and AIP mutations and all displayed wild-type alleles. B: Electropherogram of the one base pair deletion found in heterozygosis in MEN1 exon 2. C: Electropherogram of the c.47G>A (p.Arg16His) AIP mutation. D: AIP p.R16H in 3D protein prediction model.

Discussion

In the present study, the p.Gly111fs*8 *MEN1* variant clearly underlies the MEN1-phenotype in this family. This mutation was previously described by Kansain and co-workers in a patient with invasive Hurthle cell thyroid carcinoma (13) and deposited as a somatic mutation in the Catalogue of Somatic Mutations in cancer (<http://cancer.sanger.ac.uk/cosmic>) (14), but there is no report of this mutation in MEN1-positive patients in this

same database, which recently added germline mutations to its repertoire (last updated February 13th, 2018).

Another significant finding emerging from the current study is that of the co-occurrence of p.Arg16His *AIP* missense variant. The p.Arg16His variant was first described by Daly and co-workers, in two members of the same family with familial isolated pituitary adenoma (of 73 families genotyped) (15).

This variant occurs in an evolutionary highly conserved residue suggestive of a possible deleterious effect on

protein function (16) as indeed predicted by established prediction algorithms. To further understand the predicted impact of p.Arg16His on AIP structural integrity, *in silico* analysis using psiBLAST was performed. Even though both arginine and histidine are basic amino acids, the protein-folding predictions suggest that this simple amino acid change is enough to reduce the stability of the β A strand and modify the N-terminal α 0, causing overall AIP instability within an important interaction site. Taken together, these predictions suggest that this missense variant is seemingly pathogenic.

However, despite suggestive data as to the pathogenicity of this AIP variant, it has been regarded as a rare polymorphism (15, 18, 19), in part due to its detection in unaffected controls (16, 18, 20), in agreement with the data reported herein and also the lack of co-segregation of the p.Arg16His variant with disease phenotype in some familial cases (21). Of note, results from a recent study regarding p.Arg16His functional impact on AIP protein demonstrated that this alteration is sufficient to disturb cAMP signaling and growth hormone secretion, suggesting that the p.Arg16His variant alone may not be sufficient to produce a pathological effects but along with other mutations may be associated with the pathological mechanism (22).

Conclusion

The present study demonstrated the co-existence of an AIP missense variant with a most likely pathogenic *MEN1* mutation in the proband, combined with the presence of the variant in one Brazilian control. Taken together these findings are consistent with the assignment of the p.Arg16His AIP variant as a rare polymorphism.

Conflicts of Interest

The Authors declare that they have no conflict of interest regarding this study.

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