LETTER

Does germ-line deletion of the *PIP* gene constitute a widespread risk for cancer?

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We wish to draw the attention of cancer geneticists to a particular genetic variant of the Prolactin-Induced Protein (*PIP*) gene that may be an important predisposing factor to cancer because of its high frequency and significant association with cancer, as determined in this study. In an initial copy number variation (CNV) screen for germ-line deletions in 123 Brazilian cancer patients selected as high risk either because of early age of onset (pediatric cancer) or a positive family history (*TP53*-negative Li–Fraumeni and *APC/MUTYH*-negative Familial Adenomatous Polyposis patients), a previously undescribed microdeletion was discovered in four patients. The deletion carried in these patients was apparently identical (Figure 1), with a size of 69kb and similar base-pair position in chromosome region 7q34. All four deletions were validated by qPCR and found to harbor only the *PIP* and *TAS2R39* (taste receptor type 2 member 39) genes. *TAS2R39* is a member of ~30 *TAS2R* bitter taste receptors,¹ several of which are known to exhibit variation in copy number. It is unlikely that *TAS2R39* has a role in tumorigenesis, and

will not be considered further in this submission. We here refer to the deletion encompassing the *PIP* gene as *PIP*-São Paulo, following the convention of adding the place of discovery to the gene name. This deletion was not detected in a control group of 260 non-related individuals from the São Paulo urban area that had attended our genetic service for reasons other than cancer (normal relatives of patients with intellectual disabilities). The difference in deletion frequency between patients and controls was significant at the 0.01 level (Fisher's exact test). Another example of the *PIP*-São Paulo deletion was serendipitously detected in a *TP53*-mutated patient who was not part of these cohorts, but had been investigated by aCGH for presenting with an atypically severe course of cancer that commenced at 4 years of age with the diagnosis of a rhabdomyosarcoma, followed by choroid plexus tumor (7 years of age), liposarcoma and osteochondroma (10 years of age), and finally passed away at 11 years. This patient was not included in the statistics of the patient–control comparison; however, a possible interaction between the *TP53* mutation and *PIP*-São Paulo deletion may have contributed to the severity of the cancer progression.

Following our initial observations, we interrogated the 1000 Genomes Project database and found a comparable deletion to be present in 13 out of 1097 individuals: 9 of European and 4 of Latin-American origin (see Supplementary Table 1). Manual review of the sequence data in this region indicated that 7 out of the 13 individuals had sufficient sequence depth to determine that they shared identical breakpoints. From this information, we designed PCR primers and were able to amplify across the breakpoints in all the five Brazilian deletion cases. Subsequent sequencing of the PCR fragments (see Supplementary Figure 1) demonstrated that all identified *PIP*-São Paulo deletions were identical to the deletions present in the 1000 Genomes Project data set. In a replicate study, we determined by breakpoint PCR the frequency of *PIP* deletions in an independent Brazilian cancer group (219 individuals) that had either presented with more than one primary cancer prior to 60 years of age

Figure 1 *PIP*-São Paulo microdeletion at 7q34, detected by array-CGH in cancer patients. Array-CGH profile of a chromosome region at 7q34 (microarray platform Agilent 180K—Agilent Technologies, Santa Clara, CA, USA), showing heterozygous losses of a similar genomic segment (green bars) (image adapted from the Genomic Workbench software, Agilent Technologies): (a) Patient 1; (b) Patient 2; (c) Patient 3; (d) Patient 4 and (e) Y87 (*TP53*-mutated patient who was not part of the cohorts). In the chromosome 7 ideogram, the red bar marks the microdeletion area, which is shown in detail underneath. The deleted segment (solid green bar) is shown in the context of the genomic region. UCSC genes are also represented (figure adapted from UCSC Genome Bioinformatics, <http://genome.ucsc.edu>, GRCh37).

Table 1 Characteristics of the PIP-São Paulo deletion patients: mutation status, clinical phenotype, type of tumor and age at diagnosis

Patient ID	Cohort	Mutation status	Tumor (age)	Other characteristics
Patient 1	LFS/LFL	Non-mutated	Dermatofibrosarcoma (55)	LFL Eeles 1
Patient 2	LFS/LFL	Non-mutated	Endometrium sarcoma (65)	LFL chompret
Patient 3	Pediatric	—	Pilocytic astrocytoma of the fourth ventricle (9)	Slender build; triangular face; strabismus; bilateral ptosis of eyelids; palpebral fissures slant down; sparse eyebrows; hypoplastic alae nasi; hypoplastic maxilla; right unilateral auricular pit; left unilateral auricular tag and one <i>café-au-lait</i> spot
Patient 4	FAP	Non-mutated	Colorectal cancer (56)	Missense variant not previously reported (c.5365G>C p.Val1789Leu)
Patient 5	Multiple tumors	—	Colorectal cancer (47)	Adenoma (50)
Patient 6	Multiple tumors	—	Rhabdomyosarcoma (13), breast cancer (38)	
Patient 7	Multiple tumors	—	Ewing (16), schwannoma (21)	Dermatofibroma (21)
Patient 8	Multiple tumors	—	Osteosarcoma (13)	fibroadenoma (31), fibroadenoma (35)
Patient 9	Familial melanoma	—	Melanoma (53)	
Patient 10	Familial melanoma	—	Melanoma (46)	
Y87	LFS/LFL ^a	<i>TP53</i> mutation (S241Y TCC>TAC)	Rhabdomyosarcoma (4), choroid plexus tumor (7), liposarcoma (10)	Osteochondroma (10), passed away (11) LFL chompret

Abbreviations: FAP, Familial adenomatous polyposis; LFS/LFL, Li-Fraumeni syndrome/Li-Fraumeni-like.

^aNot included in the analysis.

(166 individuals) or were probands of hereditary melanoma families (53 individuals with no *CDKN2A* or *CDK4* mutations). This identified a further 6 individuals, who had had cancer and carried the *PIP* deletion (2.6%), as opposed to 10 out of 847 individuals (1.2%) from a second control group. As in the initial study, the deletion frequency in the replicate study in cancer patients was higher than that in controls, although this was not significant at the 5% level ($P=0.11$). However, when cancer and control samples from both studies were pooled, statistical significance was attained ($P=0.04$, Fisher's exact test). Importantly, heterogeneity tests demonstrated that no significant differences existed at the 0.05 level between the two control and two patient samples, respectively. We conclude that the relatively frequent germ-line deletion of the *PIP* gene has more than a two-fold increase in frequency in the Brazilian cancer patients compared with the Brazilian controls. The clinical phenotype, mutation status, type of tumor and age of diagnosis of all patients carrying the *PIP*-São Paulo deletion are summarized in Table 1, demonstrating that several types of cancer are involved in this study and that no single type or group of cancers predominates. Presence of the *PIP*-São Paulo deletion was investigated in paraffin blocks from several different primary tumors taken from four of the deleted patients. In all samples, except for one from patient 7, the *PIP*-São Paulo deletion was detected (Supplementary Figure 1); additionally, at least one exon of the *PIP* gene was amplified from each of the tested samples, indicating that one allele was retained, similar to blood. Unfortunately, DNA quality did not allow sequence screening for point mutations or other deletions that may have occurred in the remaining allele. We conclude that if the *PIP*-São Paulo deletion is directly responsible for tumor induction, this has not occurred by induction of homozygosity of the deletion itself in the cases studied.

To date, several publications have reported association between *PIP* expression and tumor progression, particularly for prostate and breast cancers.^{2,3} The gene has been implicated in multiple functions, including apoptosis, cell proliferation and migration,⁴ but mutations have only been studied in tumor cell lines and never

investigated as germ-line mutations. According to the TCGA database (<http://cancergenome.nih.gov/>), *PIP* gains in tumors are more frequent than deletions; however, data in different type of tumors are very heterogeneous and difficult to interpret. We have no specific insights into the mechanisms through which the *PIP*-São Paulo deletion could be oncogenic.

It is evident from the foregoing that the *PIP*-São Paulo deletion is not specific to Brazil. Inspection of the HapMap3 database shows that the haplotype adjacent to the deletion (Table 2) is either absent or extremely rare in African populations. Interestingly, although about ~25% of the 1094 individuals sequenced in data set 1 of the 1000 Genome Project were of African origin, none of the 13 individuals carrying the deletion were African and, except for one Colombian and two Mexican individuals from Los Angeles, CA, USA, all others were of European origin. Ethnic certainty of Brazilian cancer patients and controls is obscured by miscegenation and ethnic diversity; however, they are most likely to be mainly Caucasians, given the relatively high Caucasian composition of São Paulo, and through hospital attendance preferences and other socioeconomic differences. It is probable that *PIP*-São Paulo arose in an European population as a founder mutation that was subsequently exported to the New World.

The crucial question of whether the *PIP*-São Paulo deletion constitutes a widespread cancer risk can only be answered by similar investigations in other countries, which we hope will be stimulated by this report. However, maintenance of a deletion variant associated with cancer at such high frequency is surprising and probably implies that, if the cancer association is confirmed by independent studies, *PIP*-São Paulo appears to have a relatively low cancer penetrance rate so that many carriers either do not manifest cancer or do so at later age, following transmission of the deletion to their offspring, similar to the well-documented Brazilian *TP53* variant R337H4. Whereas R337H has an estimated population frequency of ~0.3% in Southern Brazil and exhibits an ~8% penetrance rate in families with adrenocortical cancer,⁵ *PIP*-São Paulo is practically an order of magnitude more frequent (estimated at ~2% in European

Table 2 SNPs, genomic positions (Hg19) and the common haplotype deduced from the proximal region of the patients with 7q34 deletion

SNP ID	Genomic position (bp)	Haplotype
rs4252461	142616964	G
rs4252400	142627330	G
rs8176049	142637728	T
rs6949788	142638447	T
rs8176025	142642121	G
rs8176009	142645680	C
rs41515149	142648931	T
rs8176001	142649042	A
rs3757853	142650225	C
rs8175963	142657525	C
rs8175950	142661445	T
rs10261905	142679840	A
rs1011672	142697445	C
rs6960104	142727310	C
rs7782579	142747913	C
rs7801166	142747955	A
rs1994497	142816677	A
rs10243198	142821960	G

Abbreviation: SNP, single-nucleotide polymorphism.

populations from the 1000 Genome Project data set) and appears as a very frequent factor associated with increased cancer risk. Further, if the *PIP*-São Paulo deletion frequency is at least two-fold higher in European cancer patients than in controls, as in the São Paulo study, then even allowing for a low penetrance rate, there would be an increased cancer risk for Europeans. In the case of *PIP*-São Paulo, it is the high frequency and not the penetrance that constitutes the main risk parameter. This completely unanticipated aspect deserves a rapid, coordinated response to confirm whether indeed such an increased risk exists, and if so, to establish its magnitude and specificity. Rapid and cost-effective detection of the deletion can be performed using either PCR across the breakpoints, or copy number estimates by RT-PCR, MLPA and so on.

The exact genome location of *PIP*-São Paulo is chr7: 142824847-142893913 (Genome build GRCh37/hg19).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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