

Lack of inherited mutations of PTPRD in familial melanoma and melanoma-astrocytoma syndrome

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Dear Sir,

The CDKN2A gene on chromosome 9p21.3 is the major known high-risk melanoma susceptibility gene. However, despite the fact that more than half of familial melanoma has been linked to chromosome 9p, only about 20–40% of melanoma-prone families have mutations in CDKN2A (De Snoo and Hayward, 2005; Goldstein et al., 2006). Several groups have found recurrent regions of loss of heterozygosity (LOH) and deletion on chromosome 9p in both sporadic and familial melanoma that do not encompass the CDKN2A locus (Figure 1A; Puig et al., 1995; Parris et al., 1999; Pollock et al., 2001). Therefore, an additional unidentified tumor suppressor gene(s) on chromosome 9p may also be responsible for inherited susceptibility to melanoma.

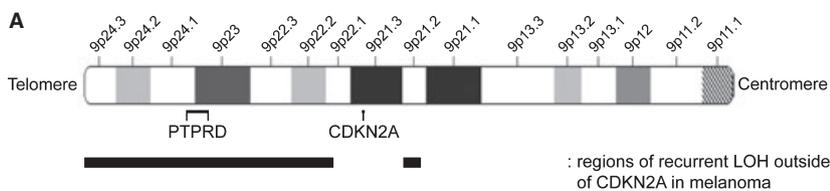
The PTPRD gene at chromosome 9p23–24.1 encodes one of 21 known human receptor-type protein tyrosine phosphatases, a family of proteins which are increasingly thought to be important in cancer development and progression (for reviews see Tonks, 2006; Ostman et al., 2006). PTPRD was first theorized to be a tumor suppressor by Urushibara et al. (1998) who described a selective reduction in PTPRD expression in hepatomas. This theory was supported by the discovery of genomic deletions of PTPRD in several human cancer cell lines by Cox et al. (2005). Subsequent studies have reported homozygous deletions of PTPRD in a broad spectrum of

human tumor types including lung adenocarcinoma (Nagayama et al., 2007; Sato et al., 2005; Weir et al., 2007; Zhao et al., 2005), pancreatic carcinoma (Calhoun et al., 2006), melanoma (Stark and Hayward, 2007), neuroblastoma (Stallings et al., 2006), glioblastoma multiforme (Solomon et al., 2008), and cutaneous squamous cell carcinoma (Purdie et al., 2007).

Somatic mutations of PTPRD in human cancer were first discovered by Sjoblom et al. (2006) who identified three missense substitutions in a panel of 35 colorectal cancers. Two additional studies have since described mutation of PTPRD in lung adenocarcinoma (Ding et al., 2008; Weir et al., 2007). Most recently, somatic mutations of PTPRD were discovered in glioblastoma multiforme (GBM) and melanoma (Solomon et al., 2008). While, PTPRD was mutated in only a modest fraction of lung cancers (6%) and GBMs (6%), the 12% mutation frequency in melanoma makes PTPRD among the most commonly mutated genes in sporadic melanoma reported to date, which include B-Raf (~60%), p53 (0–25%), N-Ras (10–15%), PTEN (~10%), CDKN2A (0–5%), and PIK3CA (<1%) (Curtin et al., 2005; Fecher et al., 2007). Functional experiments have now definitively established that PTPRD has a growth suppressive role in human cancer cells and that tumor-derived mutations compromise the tumor suppressive function of PTPRD (Solomon et al., 2008).

Intriguingly, this study by Solomon et al. (2008) also reported an inherited mutation of PTPRD in a GBM patient with a history of multiple primary tumors. This inherited mutation was accompanied by somatic loss of the wild-type allele in the tumor, suggesting that inherited mutations of this emerging tumor suppressor gene might result in a predisposition syndrome for GBM and other tumor types. Interestingly, melanoma-prone families often have a high rate of gliomas and other neural system tumors, a syndrome that has been termed 'melanoma-astrocytoma syndrome' (Azizi et al., 1995; Kaufman et al., 1993). Based on frequent somatic alterations in sporadic melanoma, its location on chromosome 9p, and the presence of an inherited mutation in a GBM patient with a history of multiple primary malignancies, we thus considered PTPRD an attractive candidate as a cancer susceptibility gene in familial melanoma and melanoma-astrocytoma syndrome.

To test this hypothesis, we sequenced the PTPRD gene in constitutional DNA from probands of twelve melanoma-prone families lacking mutations of CDKN2A.



B

Family	Proband age at diagnosis	No. melanoma patients in family	Brain cancer in family	9p21-haplotype sharing
M	37	13	N	Y
B0	67	3	Y	ND
BD	28	5	Y	Y
AM	58	5	Y	N
A6	47	3	Y	Y
W	51	5	N	Y
E1	42	5	N	Y
A2	38	4	Y	Y*
E2	46	3	N	Y
E3	39	3	N	Y
Z	31	7	N	N
B1	46	3	N	Y

C

		PTPRD	CDKN2A	References
Sporadic melanoma	Deletions	6/76 tumors (8%)	63/126 tumors (50%)	Stark and Hayward, 2007; Curtin et al., 2005
	Mutations	7/57 tumors (12%)	Rare	Solomon et al., 2008; Fecher et al., 2007
Familial melanoma	Deletions	0/12 probands (0%)	4/358 families (1%)	Present study; Goldstein et al., 2006
	Mutations	0/12 probands (0%)	178/466 families (38%)	Present study; Goldstein et al., 2006

Figure 1. (A) Schematic of chromosome 9p showing the location of the CDKN2A gene at 9p21.3 (21.958–21.984 Mb) and the PTPRD gene at 9p23–24.1 (8.304–10.603 Mb). Recurrent regions of LOH in a panel of 37 melanomas (Pollock et al., 2001) are depicted with black boxes. (B) Details of the 12 familial melanoma probands assessed in this study. *, three of the four patients in family A2 have 9p21-haplotype sharing. ND, not determined. (C) Frequency of PTPRD and CDKN2A alterations in sporadic melanoma tumors (top) and kindreds with familial melanoma (bottom).

Five of these families had multiple melanoma patients plus at least one brain cancer patient, six families showed putative linkage to chromosome 9p or a haplotype consistent with linkage in the CDKN2A region, and the twelfth family had seven melanoma patients (see Figure 1B for details on these 12 probands). The 35 coding exons and flanking intronic sequence of PTPRD were PCR amplified from genomic DNA isolated from whole blood using conditions and primer pairs described by Sjoblom et al. (2006). PCR products were purified using the Exo/SAP method followed by a Sephadex spin column. Sequencing reactions were performed using Big Dye v3.1 (Applied Biosystems, Foster City, CA, USA) and M13-Fwd primer, and were run on an Applied Biosystems 3730XL capillary sequencer. Sequence traces were analyzed using Mutation Surveyor (Softgenetics, State College, PA, USA). Traces with putative mutations were re-amplified and re-sequenced.

Though several common SNPs were identified, no mutations of PTPRD were found (Figure 1C). Although the presence of common somatic mutations and homozygous deletions has clearly implicated inactivation of PTPRD in the pathogenesis of sporadic melanomas, these data provide no evidence for PTPRD as the long sought 'other' chromosome 9p familial melanoma susceptibility gene.

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