The **NF1** gene: a frequent mutational target in sporadic pheochromocytomas and beyond

**Jenny Welander¹, Peter Söderkvist¹ and Oliver Gimm¹,²**

¹Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University, SE-58185 Linköping, Sweden
²Department of Surgery, County Council of Östergötland, SE-58185 Linköping, Sweden

**Abstract**

Patients suffering from the neurofibromatosis type 1 syndrome, which is caused by germline mutations in the **NF1** gene, have a tiny but not negligible risk of developing pheochromocytomas. It is, therefore, of interest that the **NF1** gene has recently been revealed to carry somatic, inactivating mutations in a total of 35 (21.7%) of 161 sporadic pheochromocytomas in two independent tumor series. A majority of the tumors in both studies displayed loss of heterozygosity at the **NF1** locus and a low **NF1** mRNA expression. In view of previous findings that many sporadic pheochromocytomas cluster with neurofibromatosis type 1 syndrome-associated pheochromocytomas instead of forming clusters of their own, **NF1** inactivation appears to be an important step in the pathogenesis of a large number of sporadic pheochromocytomas. A literature and public mutation database review has revealed that pheochromocytomas are among those human neoplasms in which somatic **NF1** alterations are most frequent.

**Key Words**

- **NF1**
- pheochromocytomas
- molecular genetics
- somatic mutations
- sporadic tumors

The neurofibromatosis type 1 syndrome, caused by germline mutations in the **NF1** gene, is a multisystem tumor predisposition disorder associated with neurologic, cutaneous, and orthopedic manifestations. Only a small fraction, about 1% (range 0.1–5.7%), of patients with neurofibromatosis type 1 develop pheochromocytomas (Walther et al. 1999). Hereditary pheochromocytomas can also be observed in von Hippel–Lindau disease (10–26%) and multiple endocrine neoplasia type 2 (about 50%), as well as in patients with mutations in the succinate dehydrogenase (**SDH**) genes (having a wide range depending on the gene affected) and a few additional more recently discovered susceptibility genes (reviewed in Welander et al. (2011)). Until recently, somatic mutations in any of the genes involved in hereditary pheochromocytoma have been thought to be rare in the more common sporadic form of the tumor, as has repeatedly been reported for the **RET**, **VHL**, and **SDHx** genes (Burnichon et al. 2011). While ~15% of all the pheochromocytomas or paragangliomas are thought to be associated with germline **SDHx** mutations (Gill et al. 2010, Welander et al. 2011), no single **SDHx** gene is affected in more than 10% of the cases at the most. Genome-wide expression studies have revealed that pheochromocytomas and paragangliomas cluster into two distinct groups based on their transcription profile: **VHL**- and **SDHx**-related tumors display a similar gene expression profile associated with hypoxia and angiogenesis, whereas **RET**- and **NF1**-related tumors express genes linked to an activation of kinase signaling pathways (Eisenhofer et al. 2011).
Interestingly, sporadic pheochromocytomas cluster into either of the two distinct groups instead of forming clusters of their own.

Two independent studies (Burnichon et al. 2012a, Welander et al. 2012) have recently revealed that the NF1 gene is the most frequent target of somatic, truncating mutations in sporadic pheochromocytomas known to date. This suggests one cause for the observation that subgroups of sporadic pheochromocytomas share a common transcriptional profile with hereditary NF1/RET-related tumors. The study carried out by Burnichon et al. (2012a, b) reported mutations in 25 of the 61 (41%) of investigated tumors. Here, the authors pre-selected pheochromocytomas by genome-wide expression cluster analysis and carried out NF1 mutation analysis on a subset of those displaying a NF1/RET-like gene expression pattern, giving a mutation frequency in their entire cohort of 21.8%. This is in agreement with the study carried out by Welander et al. (2012), where 10 of the 42 (23.8%) unselected sporadic pheochromocytomas exhibited somatic NF1 mutations. Thus, current data suggest that roughly one-fifth to one-fourth of the sporadic pheochromocytomas harbor somatic NF1 mutations. In agreement with the classic tumor suppressor gene model, a majority of the tumors in both studies displayed loss of heterozygosity (LOH) at the NF1 locus and a low NF1 mRNA expression. In addition, a previous study has reported LOH at the NF1 locus in a substantial proportion of sporadic pheochromocytomas (Sandgren et al. 2010), while another early study has reported a lack of neurofibromin protein expression in one of the four sporadic pheochromocytomas (Gutmann et al. 1995). It is noteworthy that high plasma levels of catecholamines emerged as common features in affected patients of the NF1-mutated pheochromocytomas (Welander et al. 2012). However, the biochemical data were non-centralized and incomplete in this study, limiting the significance of this finding. Still, the observation of high catecholamine levels in patients with neurofibromatosis type 1 and pheochromocytomas and the finding that PNMT, the enzyme responsible for the conversion of norepinephrine to epinephrine, is normally expressed in NF1 and RET tumors in contrast to the other hereditary pheochromocytomas would support this finding (Eisenhofer et al. 2011a, b, Burnichon et al. 2012b).

![Figure 1](http://erc.endocrinology-journals.org)  
**Figure 1**  
Frequency of somatic NF1 gene alterations in different sporadic human neoplasms. MPNST, malignant peripheral nerve sheath tumor; AML, acute myelogenous leukemia.
Table 1  NF1 mutation distribution in human neoplasms according to the COSMIC public mutation database (http://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=NFI#dist) as of May 8th 2013

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\(^a\)Site indeterminate.
Inactivation of neurofibromin leads to the activation of the RAS/RAF/MEK/ERK pathway (Ballester et al. 1990, Martin et al. 1990). The importance of this signaling pathway in sporadic pheochromocytoma development has been very recently confirmed when somatic H-RAS mutations were reported in these tumors (Crona et al. 2013), and studies of additional factors in the pathway may thus be warranted.

Somatic NF1 gene mutations have in recent years also been detected in other forms of sporadic neoplasms, including glioblastomas (The Cancer Genome Atlas Research Network 2008), malignant peripheral nerve sheath tumors (MPNSTs; Bottilo et al. 2009), acute myelogenous leukemia (Parkin et al. 2010), neuroblastomas (Holzel et al. 2010), lung adenocarcinomas (Ding et al. 2008), and ovarian carcinomas (The Cancer Genome Atlas Research Network 2011), indicating that the NF1 gene may represent a significant mutational target in both neural- and non-neural-derived sporadic tumors (Fig. 1). The public mutation database COSMIC (http://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=NF1#dist) has revealed that NF1 is a target with a varying mutation frequency in a large number of different human neoplasms (Table 1). Apart from MPNSTs and the large group of soft-tissue tumors (including blood vessels, fat, and fibrous tissue, as well as smooth and striated muscle), sporadic pheochromocytomas appear to be the most frequent target. Interestingly, LOH at the NF1 locus, not associated with the mutation, appears in a proportion of all the neoplasms studied (Fig. 1). Concerning pheochromocytomas, NF1 mutations are sometimes observed in samples without LOH (Burnichon et al. 2012a). This opens up a discussion of other potential factors affecting NF1 expression (hypermethylation has been excluded in some but not all positions in the promoter (Welander et al. 2012)) or possibly haploinsufficient behavior.

Many, but not all, of the neoplasms with somatic NF1 mutations have also been reported in patients with neurofibromatosis type 1 syndrome. Thus, the role of NF1 as a tumor suppressor in other malignancies not associated with neurofibromatosis type 1 syndrome warrants further studies.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the commentary reported.

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References


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