Identification of new mutations in patients with head and neck paragangliomas (HNPGL) for better stratification of the clinical course of disease.

Research Project Objectives: Pheochromocytomas (PCC) and paragangliomas (PGL) are rare neuroendocrine tumors originating from adrenomedullary chromaffin cells (PCC), sympathetic ganglia or parasympathetic ganglia in the head and neck (HNPGL). Together they are referred to as PPGL. Those tumors show the highest degree of heritability out of all neoplasms in human, which equals to 40-45%. The main clinical problem related to HNPGL is to make decision either to implement a "wait and see" policy or to undertake the surgery. HNPGL have reach vascularization, which makes surgery extremely difficult. The factors predicting tumor behavior have not been defined yet. There is some knowledge about genetic bases of these neoplasms, but it mostly relates to pheochromocytomas. The better understanding of the nature and prognosis of the disease will be possible thanks to the new mutation which will be defined in the proposed project, and further will be correlated with cancerogenesis and other features of clinical tumor behavior. The correlation of genetic data and clinical course of the disease will constitute the source of the new knowledge and allow to stratify the clinical entity of HNPGL into high and low risk patients.

The scientific aims of the project

1. Identification of the germline mutations in gene panel related with the disease.
2. Detection of somatic gene mutations and rapid detection of coexisting neoplasms in hereditary cases.
3. Defining correlation between genetic factors and the course of the disease (e.g. tumor growth rate, diameter at the time of diagnosis) or its relapses.

Project's research hypotheses: We hypothesis that genetic factors are strongly responsible for HNPGL tumor characteristics such as: hormonal activity, type of vascularization, aggressive rise to the vascular wall and tumor progression.

Description of the study: 80 patients, aged between 18 and 90, treated since 2008 at The Department of Otolaryngology, Head and Neck Surgery at Poznań University of Medical Sciences with head and neck paragangliomas, have been qualified for the study. Patients were categorized according to all for mentioned criteria: localization (carotid body paraganglioma, jugulotympanic paraganglioma, other) aggressiveness of the tumor (e.g. growth rate), surgical difficulties, complications after treatment and recurrences. This enabled the identification of patients with difficult course of the disease. The blood and tumor samples were obtained from all patients for genetic testing and pathology examination. To identify those mutations, next-generation sequencing (NGS) Ion AmpliSeq™ Comprehensive Cancer Panel will be used.

Justification for tackling specific scientific problems: According to common knowledge, the study of germline DNA should be prioritized in head and neck paraganglioma and thorax paraganglioma. There is also a recommendation for genetic testing, somatic as well germline, regardless of the age of diagnosis. Especially since genetic testing is very effective in predicting the incidence of metastatic tumors, the ideal example of which is the *SDHB* gene mutation, which leads to a metastatic disease in 40% or more of cases. Next generation sequencing, in contrast to the Sanger sequencing, enables deeper sequencing which provides more accurate detection of mutations of even single nucleotides. This can provide a better understanding of the mutations underlying the carcinogenesis of HNPGL, e.g. by detecting mosaic mutations.

Most significant expected results: This study will provide better understanding of the genetic landscape of head and neck paragangliomas. The element of novelty at work is finding a new mutation and correlating it with the clinical course. Another value added will be tracing the inheritance of mutations in family trios. This will allow for the positive inheritance of the qualification of a new mutation as a marker. The results of our study may contribute in future to find a set of genetic changes and clinical characteristics, that bind together will answer the main clinical questions, enables the rapid detection of coexisting neoplasms in hereditary syndromes and screening of family members. The new genetic/clinic algorithm may have the significant impact on patients' selection, treatment method and finally, for the medical expeditures and patient quality of life. With gained novel genetic information, acquired in this research, prediction of the disease course, multiple tumors' occurrence, metastases, relapses will be feasible. Adequate treatment: undertaking the surgery or implementation of wait and see policy, sufficient follow-up can be planed. Identification of gene mutations and molecular pathways in HNPGL may bring new knowledge for the whole family of neural-crest derived paraxial autonomic ganglia or sympathoadrenal lineage tumors. The wide context of the research is to provide important insight on the fundamental natural history of PPGL.