Graves' disease (GD) is an autoimmune disorder and type of hyperthyroidism. Etiopathology and clinical view of GD are compound and multifactorial, while genetic ones occur in the first place, but also environmental and endogenous ones contribute to it. Graves orbitopathy (GO) is the main extrathyroidal sign of GD and is a syndrome secondary to autoimmune processes in orbital soft tissue. It is present in about 30% of all GD patients and occurs at various disease's stages. As regards to its risk factors, smoking seems to be of great importance. As for its treatment, intravenous glucocorticoids (iv GCS) play a vital role.

In literature, there are reports focusing on genetic susceptibility to GD with a greatest influence of major histocompatibility complex system and its genes (human leukocyte antigen system, HLA) resulting in "autoactivity". However, due to the multifactorial disease character and the existence of a variety of other causative factors, also capable of interfering with each other in terms of their effects – epigenetics could serve here as an linking issue for i.e. environmental agents. It seems to influence GD occurrence, as well as worsen its course and possibly response treatment too.

As relevant literature makes clear, the primary epigenetic changes entail DNA methylation (DNAm), and a few studies relating this to GD suggest incidences of aberrant DNAm pattern (lymphocytes) in peripheral blood cells different genome regions. Interestingly, both antithyroid drugs (ATD) and radioiodine therapy have been shown to cause DNAm restoration in newly-diagnosed GD patients with global hypomethylation at baseline. Moreover, genome regions presenting altered methylation pattern occur in cytokines'; adhesive particles and second-signal agents (important autoimmune factors) genes' loci, in GO itself as well.

It seems fully justified to study potential DNAm pattern alterations in both GD and GO, though. It could ensure greater insight into disease pathogenesis, course and clinical implications. The aim of this prospective study is to characterize genome-wide DNAm pattern in peripheral whole-blood of patients with GD and GO following ivGCS therapy.

Patients will be recruited at the Department of Internal Medicine and Endocrinology, University Clinical Center, Medical University of Warsaw – European Group on Graves' Orbitopathy (EUGOGO) Unit. The individuals' written consent on participation will be obtained successively. Patients will be recruited and then assigned to either Group 1 and 2 (GR1-2), in line with the picture of GD and/or GO: either active, moderate-to-severe (GR1) or mild, inactive/no GO (GR2). The third one will consist solely of healthy subjects (GR3). A total of three consecutive whole blood samples will be collected from each GD patient (assigned to GR1-2), one at diagnosis (S1), while one during ATD treatment until euthyroidism achievement (time $\leq$12 months; S2) and finally (S3) one either post-complete treatment with iv GCS protocol (12 pulses according to EUGOGO) or in the course of follow-up (GR2). There will be one blood sample collected from healthy control individuals (GR3), who will be subject to matching for gender and age ratio, and be non-sufferers of either autoimmune or thyroid disorders.

In essence, the project's experiments will include the following steps focused on mDNA assessment: blood sampling (both patients and controls), material's storage (samples'freezing), DNA isolation and library preparation (methyl-rich fragments), and also reduced representation bisulfite sequencing (RRBS), all performed at the Department of Medical Genetics of the Medical University of Warsaw. Post to mentioned procedures, there will be bioinformatic and biostatistical analyses.

The DNAm pattern will be compared among different GD patients and with healthy individuals, as well as there will be DNAm association with the clinical view and response to treatment studied.