The Level of Genome's Epigenetic Variability in Hashimoto's Disease

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Summary of master's thesis.
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Epigenetic changes refer to stable, heritable, and reversible modifications (6) Epigenetic processes (particularly DNA methylation) together with environmental and genetic factors (including genome instability and synthetic processes variability) are key to understanding the pathogenesis of many diseases including autoimmune thyroiditis (Hashimoto's disease) (2,3) Disturbances in DNA methylation may be implicated in Hashimoto's disease (1). Epigenetic changes including DNA global methylation are tissue-specific for most diseases, involves pathologic tissues and cells. Changes in DNA methylation in some cases expresses in homocysteine level variability (8). Homocysteine level varies in different ethnic groups (7).

Hashimoto's thyroiditis is prevalent disease all around the world including Georgia with increasing frequency predominantly among women. During disease antibodies against thyroid gland are produced by immune system resulting in thyroid tissue lymphocytic impregnation, which cause inflammation – thyroiditis (4,5).

Research relevance: genetic studies in Hashimoto's thyroiditis are first made in Georgia.

Research purpose was to investigate the level of genome's epigenetic variability in Hashimoto's thyroiditis patients – middle-age (25-45) Georgian females.

Research objectives:

Determination of global DNA methylation and homocysteine level;

Determination of level of chromosome mutations (aberrations, aneuploidy, polyploidy, fragile sites);

Determination of frequency of acrocentric chromosome associations and evaluation of nucleolus-forming sites activity in Hashimoto's thyroiditis patients in Georgia.

On the first stage to specify diagnosis following studies were made: anti-TPO, anti-TG antibodies, TSH, fT4, fT3, coagulation and lipid profile, also some other parameters level.

Study material: Vein blood serum and lymphocyte cells culture from Hashimoto's thyroiditis patients.

Research methods: DNA extraction method; Immunoferment assay (ELISA); Lymphocytes cultivation method; Mutations registration method; Acrocentric chromosomes nucleolus-forming sites Ag-banding method.

Based on gotten clinical analysis results and ultrasonography patients were exactly diagnosed Hashimoto's thyroiditis. They were taken vein blood - research material for serum and lymphocyte culture extraction for next genetic investigation.

Next step involved determination of levels of DNA global methylation and homocysteine via immunoferment assay, ELISA). First step of DNA global methylation detection included DNA extraction from lymphocyte culture using special DNA-extraction kit.

The analysis of DNA global methylation results showed significantly low level in all Hashimoto's thyroiditis patients, averaged 5,45% compared with healthy control group – 13,92%. This result

corresponds to earlier studies for the list of other autoimmune diseases where DNA global hypomethylation is presented. In addition, DNA global methylation in Hashimoto's thyroiditis has never been detected before.

Homocysteine concentration was normal in all patients, but reached norm upper limit in some cases (patients – 4.8-7.8 8μmol/l; norm - 4.5-7.9 μmol/l).

Next step of research included determination of chromosome structure (aberrations, ESC, fragile sites) and quantity (aneuploidy, polyploidy) disorders in Hashimoto's thyroiditis patients. We have found high frequency of aberrant (patient–6,9±1,1; control–1,7±0.3) and polyploidy (patient–1,3±0,49; control–0,1±0.01) cells in Hashimoto's thyroiditis patients. There was no significant difference in case of aneuploidy that also corresponds with normal ESC chromosomes amount.

Fragile sites frequency and location was also identified. We found significant increased number of fragile sites containing cells (%) in Hashimoto's thyroiditis patients (71,5±1,98%) over against healthy controls (40,24±0,32%). The number of fragile sites in per cell was also increased. We have also identified specificity fragile sites location at chromosome groups – increased at E, F and G chromosome groups.

Ag-positive chromosomes amount and acrocentric chromosomes associative activity were studied to identify synthetic processes intensity. We have found significantly increased level of associations containing metaphases (patient–80,24±2,28; control–49,2±2.2), increased associations in per cell (patient–2.07±0.05; control–1.39±0.03) and also increased chromatids amount in per cell, on account of D group chromosomes activation. The number of chromosomes containing active nucleolus organizer regions (both 1- and 2-point) was also significantly increased (total: patient–15.3±0,11; control–7,62±1.16).

In lymphocyte culture cells of Hashimoto's thyroiditis patients, we can conclude:

- Significantly decreased DNA global methylation level, this corresponds to earlier studies for the list of other autoimmune diseases where DNA global hypomethylation is presented;
- Increased chromosomes aberrations and polyploidy frequency;
- Increased fragile sites frequency, specifically at E, F and G chromosome groups.
- Increased associations containing metaphases frequency, chromatid associations and 1- and 2-point Ag-positive chromosomes in per cell, which indicates synthetic processes high intensity.

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