SUPPLEMENTAL DIGITAL CONTENT 1: METHODS

Study design:

The study was performed from January 2023 till August 2023. It is an explorative study performing a transcriptome analysis of human biopsies of subcutaneous fatty tissue comparing the expression pattern of lipedema patients with hypertrophied healthy adipose tissue. In order to exclude effects caused by obesity, a subgroup analysis was performed corresponding to a BMI >25 and <34. Further a questionnaire about hormonal substitution, body morphology and co-morbidity was performed. The biopsies were collected by a German specialist hospital of liposuction.

Participants:

Participation in the study was voluntary. The participants were of legal age (>18 years) and gave their informed consent. The participant's data was anonymized. Eligibility criteria were female subjects for whom liposuction is indicated for medical or cosmetic reasons following the medical guidelines. Exclusion criteria were tissue inflammation in the region of interest and infectious diseases. Patients with a confirmed diagnosis of lipedema St. I, II or III were included in the study group, while patients with lipohypertrophy without lipedema symptoms served as control. The diagnosis and staging were assured by preliminary diagnostics of independent doctors and verified by medical specialists of surgery, dermatology and phlebology. The diagnosis and staging were blinded and confirmed via image morphology by a medical specialist of plastic surgery for a third time.

Questionnaire:

A questionnaire about hormonal substitution, body morphology, familial pre-disposition, pain level and co-morbidity was performed by a medical specialist interviewing the patient. The control group consisted of 8 patients. The patients with lipedema were divided stage-dependently into Stage I (n=24); Stage II (n=17) and Stage III (n=13). The questionnaire was anonymized for further analysis.

Sampling and treatment protocol:

Before liposuctioned native samples of subcutaneous fatty tissue of the thigh region were collected after local anesthesia. The skin was incised, and the fatty tissue was sampled via forceps biopsy. Each sample consisted of nearly the same amount of fatty tissue. The tissue samples were incubated with RNA later (Sigma Aldrich) at 4°C following the instruction protocol and sent to Eurofins genomics (Konstanz, Germany) for RNA isolation, cDNA synthesis and NGS-Analysis. The samples had to pass several quality control checkpoints of Eurofins genomics till NGS-Analysis. The control group consisted of 4 patients. The patients with lipedema were stage-dependently divided into Stage I (n=12); Stage II (n=9) and Stage III (n=8). The raw data (fastq files) was downloaded from the servers and further processed as described in the section NGS Analysis.

NGS Analysis

After download of the fastq files, sequences were mapped to the human reference genome (GRCh38) using the aligner hisat2 (Version 2.1.0). After sorting the files for improved data processing using samtools (Version 1.9), featurecount tables were generated using the GRCh38.110 annotation and the program featureCounts (Version 2.0.3). The differential gene expression analysis of NGS data was performed in R using edgeR. Heatmaps were generated with Pyhton (Version 3.11.2) using bioinfokit (Version 2.1.3).

Statistics of clinical data

Data is reported as mean ± SD. The statistical analysis was done with the t-test calculator of GraphPad dotmatics (https://www.graphpad.com/quickcalcs/ttest1.cfm) using an unpaired T-test to determine the significance. Results were marked on their significance level like $p<0.05$ (*); $p< 0.01$ (**); $p< 0.001$ (***).