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# Gene expression profile in fibroblasts of Huntington's disease patients and controls

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ABSTRACT

Huntington's disease is an inherited disorder caused by expanded stretch of consecutive trinucleotides (cytosine–adenosine–guanine, CAG) within the first exon of the huntingtin (*HTT*) gene on chromosome 4 (p16.3). The mutated huntingtin (mHTT) gains toxic function, probably through mechanisms that involve aberrant interactions in several pathways, causing cytotoxicity. Pathophysiology of disease involves several tissues; indeed it has been shown that there is a broad toxic effect of mHTT in the peripheral tissue of patients with HD, not only in the central nervous system. In this study we compared gene expression profiles (GEP) of HD fibroblasts and matched controls using microarray technology. We used RT-PCR to test the consistency of the microarray data and we found four genes up-regulated in HD patients with respect to control individuals. The genes appear to be involved in different pathways that have been shown to be perturbed even in HD models and patients. Although our study is preliminary and has to be extended to a larger cohort of HD patients and controls, nevertheless it shows that gene expression profiles seem to be altered in the fibroblasts of HD patients. Validation of the differential expressions at the protein level is required to ascertain if this cell type can be considered a suitable model for the identification of HD biomarkers.

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## 1. Introduction

Huntington's disease (HD, OMIM #143100) is a rare and late onset hereditary neurodegenerative disorder characterized by progressive symptoms and prevalence, in the Caucasian population, of about 1/10,000.

The disease is clinically characterized by motor symptoms, cognitive impairment and psychiatric disturbances, which generally appear between 30 and 50 years, but a broad variability in the age of onset has been also described. The course of the pathology is progressive with life expectancy of about 15–20 years after the onset.

In juvenile HD (JHD) symptoms appear before the age of 20 years and the course is more rapidly progressive; in a recent study it has been shown that the mean proportion of JDH cases is less than 5%, but frequency varies in different populations [1].

HD is inherited in autosomal dominant manner and it presents full penetrance, with the offspring of an individual with a mutant allele having a 50% chance of inheriting the disease-causing allele.

The clinical characteristics of the disease are known since 1872, but the gene was mapped only in 1983, on 4p16.3 [2] and was isolated in 1993 [3]. *HTT* gene encodes for a 350kDA ubiquitously expressed protein called huntingtin (HTT). The causative mutation is an anomalous expansion of a tract of CAG trinucleotide repeats within the coding sequence of the gene, leading to an abnormally expanded polyglutamine tract in huntingtin. There is a strong inverse relationship between the age of onset of HD and the number of CAG repeats: longer repeats are correlated with an earlier age of onset [4,5]. Normal individuals have less than 36 repeats, commonly 15–25. Alleles of 40 CAG and above are fully penetrant and cause Huntington's disease, while individuals with 27-35 repeats do not have the disease but those alleles are potentially unstable during reproduction [6,7]. Indeed, alleles of 36–39 (CAG) are incompletely penetrant and can be found in affected individuals as well as in individuals who do not show clinical symptoms so those alleles confer an increasing risk of developing Huntington disease [8].

Like other diseases caused by CAG repeats, HD exhibits anticipation, an earlier disease onset from generation to generation [9]. The repeat instability is more likely observed during spermatogenesis than ovogenesis [10].

As for many neurodegenerative disorders, conventional therapeutics function as symptom relief, and have no effects on disease progression.

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Many efforts have been made to:

1) Improve the knowledge of the pathogenic pathway, to target related molecules.
2) Identify peripheral markers that can be useful for biological monitoring of the disorder and to test the effectiveness of emerging therapies.

The biological function of the HTT protein is not fully understood and it would be useful to dispose of *in vitro* tools for the evaluation in depth of its role in neuronal cells since the genetic mutation in *HTT* gene defines the pathology, but differential gene expression could be useful to indicate the activation of abnormal processes.

In neurons affected by neurodegenerative disease significant variations in morphology, physiology and function have been observed that are related to discrepancies in gene expression profiles of affected cells [11]. Moreover it has been shown that there is a broad toxic effect of mHTT in the peripheral tissues of patients with HD [12]. Hence, the study of gene expression profiles could provide information about the onset and the progression of the disease.

Microarray technology is a valuable tool for quantifying the trascriptome in a unique experiment and it allows to identify differences in gene expression by comparing pathological cells and normal ones. Several gene expression profiling (GEP) studies have been performed in post-mortem HD brain, lymphocytes from HD patients and on transgenic mouse models and cellular models of HD (reviewed by Cha [13]).

The latest studies of gene expression in HD have been performed by Dalrympe et al. [14], Anderson et al. [15], Chang et al. [16], and Krzyszton-Russian et al. [17] in blood cells from HD patients, whereas Pouladi et al. [18] identified a reduced IGF-1 expression in skin-derived fibroblasts from HD patients. Furthermore, del Hoyo et al. [19] showed a decreased activity of catalase in skin fibroblast cultures from HD patients, Mazzola and Sirover [20] reported a decrease of the GAPDH glycolytic activity in fibroblasts from HD patients and Seo et al. [21] showed the altered activity of the ubiquitin proteasome system (UPS) in the same cell type.

We choose to analyze fibroblast's gene expression because mHTT is expressed ubiquitously, therefore molecular changes detected in fibroblasts may reflect peripheral processes promoted by mHTT. The study of cultured fibroblasts has some drawbacks: gene expression profiles are influenced by culture conditions, age of cultures and growth phase. However fibroblasts can be easily obtained and cultured, allowing the extraction of high quality RNA in a simple and reliable way; this is not necessary true for post-mortem brain tissue because RNA has a fragile nature and it is degradable. Last but not least, fibroblasts have the same embryonic origin as neurons.

In this study we compared the gene expression profile of HD fibroblasts and healthy ones to identify differentially expressed genes, which may be used as biomarkers for the disease.

To the best of our knowledge, there is not any published study that had solely investigated the gene expression profile in dermal fibroblasts from HD patients.

## 2. Materials and methods

### 2.1. Cell lines

Dermal fibroblast cell lines from six HD individuals and five normal controls were collected for this study.

HD fibroblasts have been collected from punch biopsies of patients of the Neurology Unit of University of Brescia, Italy. Punch biopsies have been obtained even from healthy volunteers of the same unit.

Subjects with inflammatory or infective conditions were excluded.

All patients and volunteers gave their written informed consent.

Besides, we used GM04476 and GM04799 fibroblast HD cell lines obtained from the Coriell Cell Repositories (CRC), Camden, NJ, and two additional cell lines as controls deriving from CRC, GM04735 and GM04781. The skin biopsy samples were placed in phosphate buffer saline (PBS) solution prior to being cut into small pieces and transferred into a second dish containing RPMI with 20% fetal bovine serum (FBS), 100 mg/mL streptomycin, 100 U/mL penicillin and incubated at 37 °C in a humidified chamber with 5% $CO_2$. All fibroblast cells were cultured in Eagle's minimum essential (MEM) (Euroclone Life Sciences) supplemented with 10% FBS (HyClone), 100 mg/mL streptomycin, and 100 U/mL penicillin and incubated at 37 °C in a humidified chamber with 5% $CO_2$. Cells were harvested once they reached confluence by treating with trypsin (0.05% trypsin with 0.25% EDTA, Invitrogen). RNA samples were extracted when all cell cultures were at passages 8–10.

### 2.2. RNA isolation

Total RNA was isolated from cells using TRIzol (Invitrogen, Life Technologies), followed by RNeasy Minin Kit (QIAgen, Venlo, The Netherlands) and eluted in 30 μL RNase free water, according to the manufacturer's protocol. The RNA was quantified using the NanoDrop ND-1000 spectrophotomer (Thermo Scientific, Wilmington, DE) and the quality of the total RNA was determined electrophoretically by the RNA Nano Assay Chip (RNA 6000 Nano Kit) on a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA). We normally obtain a total RNA with a 260/280-absorbance ratio of two roughly.

### 2.3. Microarray hybridization

RNA was prepared according to manufacturer's one cycle target labeling procedure (Affymetrix, Inc, Santa Clara, CA), according to the standard protocol described in the Affymetrix GeneChip Expression Analysis Manual.

Quickly, cDNA was generated using GeneChip® Expression-3′ Amplification One-Cycle cDNA Synthesis Kit with a starting amount of 2 μg of high-quality total RNA. The labeled cRNA obtained after GeneChip® Expression 3′-IVT Labeling Kit was cleaned, quantified and after fragmentation 15 μg of cRNA was hybridized on Affimetrix GeneChip® Human Genome U133 Plus 2.0 (HG-U 133 plus 2.0). This array is a single GeneChip composed of more than 54,000 probe sets representing 39,000 well-substantiated human gene transcripts.

The GeneChip was scanned and data extracted using GeneChip scanner 3000 7G (Affimetrix, Santa Clara, CA).

### 2.4. Data processing and statistical analysis

Raw data (CEL files) were background corrected, normalized and summarized into probe set expression values using the robust multichip average (RMA) algorithm within Bioconductor v2.7 using OneChannelGUI package on R 2.11.0 environment [22].

Normalized data were filtered by applying an interquartile (IQR) filter (IQR < 25% of mean total IQR) to remove the non-significant probe sets (i.e., not expressed and those not changing) [23]. To assess differential expression, an empirical Bayes method [24] was used to moderate the standard error of the estimated log-fold changes together with a false discovery rate (FDR) correction of the *P*-value [25].

The list of differentially expressed genes was selected considering a $P < 0.01$ together with an absolute fold-change threshold of 2.

All microarray data are MIAME compliant and the raw data have been deposited in the MIAME compliant GEO database (accession number GSE45516).

Subsequently, the dataset of differentially expressed genes was submitted to the GeneOntology (GO) database, via OntoExpress software [26] in order to identify the most representative categories, such as cellular compartment and biological processes within a more meaningful biological frame.

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