Spatial and Temporal Expression of High Mobility Group Nucleosome-Binding (HMGN) Genes in Brain Areas Associated With Cognition in Individuals With Down Syndrome

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Research Article

Keywords: Down syndrome, Gene expression, Epigenetics, Human brain, HMGN, Neuroinformatics

Posted Date: August 11th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-784407/v1>

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Version of Record: A version of this preprint was published at Genes on December 17th, 2021. See the published version at <https://doi.org/10.3390/genes12122000>.

Abstract

Background: DNA methylation and histone posttranslational modifications are epigenetics processes which contribute to neurophenotype of Down Syndrome (DS). Previous reports present strong evidence that nonhistone High mobility group N proteins (HMGN) are epigenetic regulators. They play important functions in several process to maintain the brain homeostasis. We aimed to analyze the differential expression of five human HMGN genes along some brain structures and age ranks from DS postmortem brain samples.

Methodology: We performed a computational analysis of human HMGN expression from a DNA microarray experiment data (GEO database ID GSE59630). Using the transformed log2 data, we analyzed the differential expression of five HMGN genes in several brain areas associated with cognition in patients with DS. Moreover, using information from several genome databases, we explore the coexpression and protein interactions with the histones of nucleosome core particle and linker H1 histone.

Results: We registered that HMGN1 and HMGN5 were significantly overexpressed in hippocampus and areas of prefrontal cortex including DFC, OFC and VFC of DS patients. Age ranks comparisons between euploid control and DS individuals showed that HMGN2 and HMGN4 were overexpressed in the DS brain of 16 to 22 weeks of gestation. From BioGRID database we registered high interaction scores of HMGN2 and HMGN4 with Hist1H1A and Hist1H2BA, Hist2AG, and Hist1H3A respectively.

Conclusions: Overall our results give strong evidence to propose that DS would be an epigenetics-based aneuploidy. Remodeling the brain chromatin by HMGN1 and HMGN5 would essential pathway in the modification of brain homeostasis in DS.

Introduction

The continuous chromatin modification and the binding of tissue specific transcription factors to their specific targets in chromatin, maintain the epigenetic landscape necessary to regulate the cell-type specific transcription [1] [2]. However, additional chromatin modifiers, including the H1 linker histones [2] and the high mobility group N (HMGN) proteins, can remodel the chromatin organization and transcription regulation, playing important functions in several process to maintain the general homeostasis [3]. Down syndrome (DS), is a chromosomal aneuploidy caused by a total or partial triplication of chromosome 21, but in rare cases it can be associated with a process of chromosome translocation [4]. In persons with DS the gene dose imbalance by triplication of genes on HSA21 is mostly associated with a wide spectrum of pathologies that include neurological and systemic diseases [4]. The incidence of trisomy 21 is influenced by maternal age and differs in population [5] [6]. In developed countries, the average life span for DS population is 55 years [7]. Although DS is the result of the increased copy number of a single 21 chromosome, the regulation of gene expression is affected at a genome-wide level [8-11].

There is an increasing line of evidence to propose that beyond of HSA21 trisomy, DS is an epigenetics-based syndrome [12]. For instance, a previous study with fetal skin fibroblasts from a set of monozygotic twins, revealed regions that were predominantly hypermethylated in DS in genes involved in embryonic organ morphogenesis. Reprogramming of the DS fibroblasts to induced pluripotent stem cells (iPSCs), showed that these regions were maintained in the pluripotent state and correlated with differential gene expression and increased expression of the DNA methyltransferases DNMT3B and DNMT3L [13]. Thus, the genome-wide differences seen in DS tissues are correlated with epigenetic modifications would be responsible, in part, for the establishment and/or maintenance of differential expression of genes in and outside of HSA21 in DS

HMGN proteins are a nonhistone protein family that include 5 members encoded by 5 specific genes with a similar intron-exon organization that are localized along human genome in different chromosome loci (Table 1) [13-18]. Previous reports show that HMGN proteins are the only nuclear proteins known to specifically recognize the generic structural features of the 147 base pair nucleosome core particles. In vitro analyses showed that at low ionic strength nucleosome core particles can bind to HMGN proteins with high affinity [19-21]. The interaction of HMGN proteins with nucleosomes is dynamic and the proteins compete with the linker histone H1 chromatin binding sites [22-24].

Since the role of high mobility group N proteins (HMGN) as important molecules that reshape the organization of chromatin and transcription levels, we perform a bioinformatic simulation analysis of its spatial and temporal expression in several areas of the brain with Down's Syndrome. To perform these analyses, we obtained log2 data from a free access microarray experiments previously consigned in the GEO DataSets set of NCBI (<https://www.ncbi.nlm.nih.gov/>). To explore the differential brain expression of HMGN genes, we calculate the Z-ratio from DS brain postmortem samples associated with cognitive processes in those brain areas previously described by Olmos-Serrano et al, 2016 [25]. Our results gave strong evidence to support the hypothesis of the crucial role of non-histones HMGN-1 and HMGN-5 proteins as important spatial and temporal remodelers that would change, by epigenetic process, the brain proteostasis in patients with Down syndrome.

Methodology

Data Mining

Raw gene expression data of Down syndrome samples and normal samples were downloaded from the Gene Expression Omnibus (GEO) (<http://www.ncbi.nlm.nih.gov/geo/>) of the National Center for Biotechnology Information (NCBI). For the analyses performed in the present study, we selected the human genes encoding for the five high mobility group N (HMGN) proteins previously consigned in the Gene Entrez of the NCBI database (<https://www.ncbi.nlm.nih.gov/gene>), (table 1). Moreover, for all calculations we used the log2 transformed expression values of free a access DNA microarray experiment whose registration code in the GEO database is GSE59630

(<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE59630>), previously deposited by Olmos-Serrano et al. 2016. [25].

According with information consigned in the GEO database, the selected microarray experiment included gene expression data of more than 17,000 probes from 58 post-mortem brain samples of DS patients (25 from females and 33 from males) and 58 euploid samples as normal controls (25 from females and 33 from males), that were classified by gender, age and also by some brain areas including the hippocampus (HIP), cerebellar cortex (CBC), dorsolateral prefrontal cortex (DFC), orbital prefrontal cortex (OFC), ventrolateral prefrontal cortex (VFC), medial prefrontal cortex (MFC), primary somatosensory cortex (S1C), inferior parietal cortex (IPC), primary visual cortex (V1C), superior temporal cortex (STC), Inferior temporal cortex (ITC). Nevertheless, for the present study we decided to analyze, not only the brain as a whole but also OFC, MFC, HIP and CBC brain regions which are highly associated to neurophenotype of Down syndrome.

Data preprocessing

The robust multiarray analysis (RMA) algorithm [30] in Affymetrix Power Tools (APT; <http://www.affymetrix.com/>) was applied to perform background correction and standardization for all raw data, aiming to filter false-positive data. The applied criterion was as follows: at least half the samples had PLIER signal intensity values greater than 100. [27].

Quantification of the differential HMGN genes expression

Raw intensity log2 data of each experiment were used for the calculation of Z-score [28]. Z-scores of the protein coding genes analyzed, were calculated according to the equation (1):

$$Z - score = \frac{(\text{Log intensity of } G - \text{mean log intensity } G \dots Gn)}{\text{Standard Deviation log } G \dots Gn}$$

Equation 1. Z-score formula

All Z-score values were normalized on a linear scale $-3.0 \leq 0 \leq +3.0$ (two-tailed P value <0.001). From Z-score data we calculated the mean values per gene and per structure in brain samples of DS and euploid controls. This data was used to calculate the Z-ratio (Equation 2) with is a measure to estimate differential gene expression; genes with values over 1.96 are considered over-expressed [28].

$$Z - ratio = \frac{[(Z - score_{G_{1ave}})DS - (Z - score_{G_{1ave}})Con]}{SD \text{ of } Z - score \text{ differences}_{G_{1\dots Gn}}}$$

Equation 2. Z-ratio formula

Gene-dosage imbalanced quantification

To find out the gene dosage imbalance of that HSA21 genes that coexpressed with the five HMGN genes along the cerebral structures of DS brain samples, first we calculate the M values according to the equation 2.1, and then we used the M value to calculate the ratio of the dosage imbalance R (DS Control ratio) as shown in Equations 2.1 and 2.2 [29].

Equation 2.1 M-value formula

$$M = [\text{Mean Log}_2(\text{DS}) - \text{Mean Log}_2(\text{Control})]$$

Equation 2.2. R (DS/Control ratio) formula

$$R = 2^M$$

R values ranging from 0.80 to 1.30 were considered as normal balanced (two copies of gene); on contrary, if R values ranged $1.4 \leq 1.5 \leq 1.7$, genes are dosage-imbalanced by triplication (three copies per gene), but if R ratio is greater than 1.8 genes are amplified (more than three copies).

Construction of HMGN genes network using GeneMania

To build the gene interaction networks of HMGN genes, we applied the free access platform GeneMANIA (<http://www.genemania.org>) a real-time multiple association network actively developed at the University of Toronto, in the Donnelly Centre for Cellular and Biomolecular Research that use a massive set of functional association data. [30]. All calculation carried out in the present study were processed using the updated 2018 version

Protein-Protein interaction analysis

To computational simulate the interaction between each HMGN with several histones of the core particle and H1, we obtain the data from BioGRID (Database of Protein, Chemical, and Genetic Interactions) a free access database (<https://thebiogrid.org/>) [31]. BioGRID is an interaction repository with data compiled through comprehensive curation efforts. The current index is version 3.5 and permits to perform searches of 69,922 publications for 1,706,694 protein and genetic interactions from humans. All data are freely provided via their search index and available for download in standardized formats. The different searches performed in the present study, were from data updated by January of 2019. [31]

Statistical analysis

For comparing mean values of Z-ratio of DS brain, we performed multivariate statistical analyses among the different brain cortex structures between DS patients and euploid controls. The Z test/Two-tailed was used to calculate differences in HMGN differential expression. The p-values were calculated using the web tool P-value from Z-score Calculator (<https://www.socscistatistics.com/pvalues/normaldistribution.aspx>). In all cases we use an alpha of 0.05 to test the significance of H_0 . To calculate the statistical differences in the mean log2 values of DS and

Controls for gender, age, hippocampus, cerebellar and brain cortex structures, we apply the t-test for two paired samples/Two-tailed test with an alfa of 0.05.

Principal Component Analysis (PCA), and a Hierarchical Cluster (Heat-map) were employed as a computational procedure for the classification of multiclass gene expression in brain structures between DS and control samples per sex and age. All analyses were run in SPSS program version 25.0 (<https://spss.softonic.com/>) and Cytoscape 3.6 (https://cytoscape.org/release_notes_3_6_0.html).

Results

Expression of HMGN genes in brain areas from individuals with DS

In general, we observed the expression of five HMGN genes was variable along all structures under analysis. Moreover, we recorded significant differences in their overexpression values depending of brain area under analysis. In this sense, the genes encoding for HMGN1 and HMGN5 were overexpressed not only in HIP, CBC and V1C but in some areas of prefrontal cortex including DFC, OFC and VFC (values of Z-ratio>1.96); in contrast with not significant over expression of HMGN2, HMGN3 genes. Only in ITC HMGN5 gene registered significant Z-ratio (Z-ratio=2.0) (Table 2).

Since the HMGN1 gene is localized at 21q22.2 band, we calculated the level of dose imbalance in those brain structures including in the present study. Our results showed that HMGN1 was dosage imbalanced, in OFC, VFC and CBC by triplication ($R > 1.4$), but in HIP, DFC and ITC was dysbalanced by amplification ($R > 1.8$).

Age dependent expression of HMGN genes in the brain of individuals with DS

HMGN4 (Z-ratio=4.72) and HMGN2 (Z-ratio=2.13), were significant overexpressed in prenatal samples of DS brain (16 to 22 weeks of gestation) in comparison with other age ranks (table 3). The data of Z-ratio for HMGN3 showed significant overexpression values in the brain of DS during the first year (0-12 months), childhood (2 to 10 years), 12 to 22 years old samples, and adulthood (32 to 42 years old) but not in brain samples of prenatal brain samples (16 to 22 weeks of gestation; Z-ratio=1.08) (table 3). In contrast, the Z-ratio values for HMGN1 and HMGN5 along the different age ranks were non-significant, except for HMGN1 that was overexpressed in rank of 12 to 22 years old (Z-ratio=2.0) (Table 3)

Protein-Protein Interaction Network and GO categories

The Protein-Protein Interaction (PPI) network made with the five HMGN genes accounted for a total of 73 nodes, two connected components, one multi-edge node pair, an average number of neighbors of 2.374, and a heterogeneity of 2.012 (Figure 1). The node with the highest number of interactions was HMGN1 with 30, followed closely by HMGN2 with 28. GO categories of biological processes most relevant of the network included indispensable epigenetic processes for chromatin activation or inactivation such as histone deacetylation (P-value $2.46E-08$) and histone H3-K4 methylation (P-value $1.88E-07$). -

HMGN proteins interaction with Histones of nucleosome core and linker H1

From data of several experimental methods reported in BioGRID data base and consigned in table 4, we observed differential high interaction scores of HMGN proteins with histones of the nucleosome core HIST1H2AG, HIST1H2BA, HIST1H3A and HIST1H4A (table 3). Only HMGN2 has a significant high score of interaction with the linker histone HIST1H1A and the histones of nucleosome core HIST1H3A and HIST1H2AG. HMGN1 and HMGN5 showed significant interaction scores only with HIST1H4A. Finally, HMGN3 interacts with the HIST1H4A

Discussion

Previously, some studies presented strong evidence that in DS individuals the genome-wide epigenomic alterations occur not only in chromosome 21 but in some other chromosomes [32-34]. these include changes in gene expression, RNA content, and epigenetics histone modifications, nucleosome spacing, and DNA methylation process which are dependent of health status and age [35]. Specifically, there are strong evidences that HMGN proteins play a role in epigenetic regulation of gene expression and play important functions in several biological process to maintain the normal homeostasis and altered gene expression in disease [36-37]. Thus, we aimed to analyze from a bioinformatics approach, the gene expression of human HMGN genes in different human brain structures and age ranks comparing DS brain samples and euploid controls

Most of the literature reports about the expression of HMGNs in the brain as whole and also in neuronal derived cells, come from experiments carried out in mice [38]. In this sense its interpretation could be cautiously extended to humans and also to the brain of Down syndrome. In this scenario, our results are the first to analyze in a representative sample of euploid individuals and Down syndrome patients the differential expression of the five human HMGN in several area of the brain which are involved in learning and memory and also its age ranks variation. Our bioinformatics approach permitted to obtain strong statistical evidence of the differential regulation of HMGNs in the disruption of the normal brain homeostasis in some areas associated with the Down syndrome neurophenotype.

HMGN1 is in a region of human chromosome 21 and it is frequently found triplicated in DS samples. Our results not only confirmed the previous reports but extended the data to the brain of individuals with DS. HMGN1 gene was dose dysbalanced by triplication in the whole brain, and brain cortex, and dysbalanced by amplification in the hippocampus [39, 40]. The hippocampus is a brain structure that plays a major role in neural plasticity and cognition [39], which is known to be dysregulated in individuals with DS. Our results showed that methyl CpG-binding protein 2 (MeCP2) is underexpressed in several structures of the brain of DS, which can be linked to the dysregulation of the gene HMGN1, given that this latter gene can affect the expression of MeCP2 by changing the chromatin structure and histone modifications in the MeCP2 promoter [40].

According to the PPI network the nodes with the highest number of interactions were HMGN1 and HMGN2. Moreover, the GO categories showed a global implication of these genes in chromatin

remodeling processes such as acetylation, methylation of histones, and dendritic spine morphogenesis. The dysregulation of gene expression recorded in these genes would most certainly affect the interactions with others, and would possibly leading to the epigenomic changes found in individuals with DS.

Some analysis suggests that HMGNs could differentially modulate not only the global gene transcription in some brain structures but also in other tissues [41, 42]. Therefore, the contribution of HMGN1 and HMGN5 to the transcriptional dysregulation of DS neurophenotype need to be studied separately in specific developmental scenarios. In mice HMGN1 is a negative regulator of the brain expression of Mecp2 (methyl CpG-binding protein 2), that promotes HMGN1 overexpression associated with some effects not only in general behavioral activities but in anxiety and social deficits too [43, 44]. On the other hand, HMGN5, is thought to reduce the compactation of the chromatin fiber nucleosomes, thereby enhancing transcription from chromatin templates, nevertheless, it has not been related to DS.

Excluding the gestational period (12-16WG), only HMGN3 had a statistically significant expression across different age-ranks. It contrasts with the expression of HMGN2 and HMGN4 which had significant differential expression in the gestational period but not in the rest of the age-ranks we evaluated. In this sense, our results support some data found in literature that HMGN3 control part some epigenetic mechanisms during the neuronal development [43]. HMGN2 expression has been widely associated with embryogenesis [45]; anti-sense manipulation of HMGN2 gene leads to early embryonic abnormalities [46, 47] Our results suggest that HMGN2 regulates active and bivalent genes by promoting an epigenetic landscape of active histone modifications at promoters and enhancers. The conclusion is that HMGN2 stabilize the epigenetic landscape necessary for maintaining the pluripotent identity of pluripotent stem cell [47].

Overall, our results give strong evidence to propose that DS would be an epigenetics-based aneuploidy and the spatial and temporal differential expression of HMGNs would play important roles in the modification of brain homeostasis in DS. We report the differential interaction of HMGN family proteins with histones of the nucleosome core HIST1H2AG, HIST1H4A, HIST1H2AG, HIST1H3A, HIST2H2AB, HIST1H4A and also with the linker HIST1H1A. In this scenario, we propose that HMGN proteins play an important role in the topological process of remodeling the chromatin in several brain areas of Down's syndrome that are associated with memory and learning process. Global effect of those epigenetics deregulation would be the alteration of the brain homeostasis that potential conditioned the brain in Down's syndrome epigenetics mode.

Declarations

We certified that have no commercial associations (e.g., consultancies, stock ownership, equity interest, patent/licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted article, except as disclosed on a separate attachment. All funding sources supporting the work and all institutional or corporate affiliations of the authors are acknowledged in a footnote in the Work

Funding: No funding was required for this work

Conflicts of interest/Competing interests: None of the authors presents a conflict of interest.

Ethics approval: DNA microarray experiment whose registration code in the GEO database is GSE59630 (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE59630>).

Consent to participate: DNA microarray experiment whose registration code in the GEO database is GSE59630 (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE59630>).

Consent for publication: DNA microarray experiment whose registration code in the GEO database is GSE59630 (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE59630>).

Availability of data and material: DNA microarray experiment whose registration code in the GEO database is GSE59630 (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE59630>).

Code availability: None

Acknowledgements and Authors' contributions

Acknowledgements: To the Universidad del Valle and the Molecular Biology and Patogenesis research group

AR-O, FG-V and YM-P searched data from a DNA microarray experiment in SD and found microarray experiment with ID GSE59630 previously deposited in the GEO DataSet of NCBI database. AR-O and YM-P using the transformed log2 data, analyzed the differential expression of five HMGN genes in several brain areas associated with cognition in patients with DS. AR-O, YM-P and JCM explored the coexpression and protein interactions with the histones of nucleosome core particle and linker H1 histone. All authors worked on the results and conclusions. Similarly, they read and approved the final manuscript.

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Tables

Table 1. Description of gene data of five encoding human high mobility binding nucleosome group proteins (HMGN). [Source of NCBI <https://www.ncbi.nlm.nih.gov/gene/>].

Gene	ID*	Locus	Gene name
HMGN1	3150	21q22.2	High mobility group nucleosome binding domain 1
HMGN2	3151	1p36.11	High mobility group nucleosome binding domain 2
HMGN3	9324	6q14.1	High mobility group nucleosome binding domain 3
HMGN4	10473	6p22.2	High mobility group nucleosome binding domain 4
HMGN5	79366	Xq21.1	High mobility group nucleosome binding domain 5

(*). According with the classification of the National Center for Biotechnology Information (NCBI).

Table 2. Mean Z-ratio values of expression for the five human High Mobility Group Nucleosome Binding Human (HMGN) genes in several brain structures of individuals with Down syndrome.

Gene	Gene ID	Brain	HIP	CBC	DFC	OFC	V1C	VFC	ITC
HMGN1	3150	2.81	3.00	2.35	2.63	2.07	3.00	1.86	2.45
HMGN2	3151	0.06	0.23	0.13	0.54	0.05	-0.62	0.68	0.99
HMGN3	9324	2.48	1.29	1.60	1.78	1.57	2.82	2.31	1.86
HMGN4	10473	0.37	0.85	0.77	0.27	0.84	0.41	0.19	-0.27
HMGN5	79366	2.65	2.18	1.60	2.49	1.13	1.49	2.53	2.88

Z-ratio value >1.96 means significant gene overexpression in the brain of Down syndrome patients (Z test alpha 0.05. Two tails) (*). Gene ID and data source were obtained from the information consigned in NCBI GeoDataset of a microarray experiment with registration code of GSE59630. (HIP). Hippocampus; (CBC). Cerebellar brain cortex; (DFC). Dorsolateral prefrontal cortex; (OFC), Orbital prefrontal cortex; (V1C). Primary visual cortex; (VFC). Ventrolateral prefrontal cortex and (ITC). Inferior temporal cortex.

Table 3. Mean values of Z-ratio for the five HMGN genes expressed in different age ranks of the brain of human DS individuals.

Gene	16-22 WG	0-12 M	2 -10 Y	12-22 Y	30-39 Y	40-42 Y
HMGN1	0.66	1.45	1.94	1.97	2.18	1.55
HMGN2	2.13	0.22	0.54	0.54	0.31	0.49
HMGN3	1.08	3.61	2.71	2.55	2.59	2.94
HMGN4	4.71	1.12	1.51	1.65	1.84	1.67
HMGN5	0.16	1.89	0.78	1.15	0.76	0.82

(WG). Weeks of Gestation. (M). Months. (Y). Years. Data source were previously consigned in the NCBI GeoDataset of a DNA microarray experiment under the registration code of GSE59630.

Table 4. BioGRID data of the five human HMGN interactions with the nucleosome core histones H2BA, H2AG, H2AB, H3A and H4A and with the linker histone H1A.

Interactor	Interaction	Experimental Evidence	Throughput	Score*
HMGN1	HIST1H4A	Affinity Capture-MS (§)	High	> 0.75
	HIST1H2BA	Affinity Capture-MS	High	0.99
	HIST1H3A	Affinity Capture-MS	High	0.90
HMGN2	HIST1H2AG	Affinity Capture-MS	High	0.77
	HIST1H1A	Proximity Label-MS (§§)	High	> 0.75
HMGN3	HIST1H4A	Affinity Capture-MS	High	> 0.75
	HIST1H2AG	Affinity Capture-MS	High	0.92
HMGN4	HIST1H3A	Proximity Label-MS	High	> 0.75
	HIST2H2AB	Affinity Capture-MS	High	0.88
HMGN5	HIST1H4A	Affinity Capture-MS	High	> 0.75

* The cut-off threshold is > 0.75. Data from BioGRID (<https://thebiogrid.org/>)

(§). *Affinity Capture-MS* interaction is inferred when a bait protein is affinity captured from cell extracts by either polyclonal antibody or epitope tag and the associated interaction partner is identified by mass spectrometric methods.

(§§). *Proximity Label-MS* interaction is inferred when a bait-enzyme fusion protein selectively modifies a vicinal protein with a diffusible reactive product, followed by affinity capture of the modified protein and identification by mass spectrometric methods, such as the BioID system.

Figures

Figure 1

Protein to protein interaction network between the five human HMGNs and several cellular human genes. (A). Topological structure of the network using Cytoscape program. (B). The statistically significant cellular genes that interact with some of one HMGN. Data were obtained from GeneMania database.