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A microRNA cluster (let-7c, miRNA-99a, miRNA-125b, miRNA-155 and miRNA-802) encoded at chr21q21.1-chr21q21.3 and the phenotypic diversity of Down's syndrome (DS; trisomy 21)

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Down's syndrome (DS) is the most common genetic cause of intellectual disability and cognitive deficit attributable to a naturally-occurring abnormality of gene dosage. DS is caused by a triplication of all or part of human chromosome 21 (chr21) and currently there are no effective treatments for this incapacitating disorder of neurodevelopment. First described by the English physician John Langdon Down in 1862, propelled by the invention of karyotype analytical techniques in the early 1950s and the discovery in 1959 by the French geneticist Jerome Lejune that DS resulted from an extra copy of chr21, DS was the first neurological disorder linking a chromosome dosage imbalance to a defect in intellectual development with ensuing cognitive disruption. Especially over the last 60 years, it has been repeatedly demonstrated that DS is not an easily defined disease entity but rather possesses a remarkably *wide variability* in the '*phenotypic spectrum'* associated with this trisomic disorder. This commentary describes the presence of a 5 member cluster of chr21-encoded microRNAs (miRNAs) that includes let-7c, miRNA-99a, miRNA-125b, miRNA-155 and miRNA-802 located on the long arm of human chr21, spanning the chr21q21.1-chr21q21.3 region and flanking the beta amyloid precursor (βAPP) gene, and reviews the potential contribution of these 5 miRNAs to the remarkably diverse DS phenotype.

42 amino acid amyloid-beta (Aβ42) peptide | Alzheimer's disease (AD) | beta amyloid precursor protein (βAPP) | Down's Syndrome | microRNA (miRNA) | small non-coding RNAs (sncRNAs) | systemic inflammation

Introduction - the DS disease spectrum

Diseases often associated with DS, DS progression and aging in the DS patient are from a remarkably broad spectrum of human disorders, many of which associate with neurodevelopment, brain aging, inflammatory neurodegeneration and/or carcinogenesis [1-5]. These include atherosclerosis, altered lipogenesis, Alzheimer's disease (AD), age-related macular degeneration (AMD), amyotrophic lateral sclerosis (ALS; Lou Gehrig's disease), autoimmune disease (such as chronic systemic inflammation and lupus erythematosus), astrogliosis, various cancers, including prominently, glioma, glioblastoma, lymphoma, prostate cancer and leukemia (DS children have a ~10- to 20-fold higher risk for developing acute lymphoblastic and myeloid leukemia compared with non-DS children), celiac disease, congenital heart disease, diabetes, digestive disorders, endocrine abnormalities, hearing loss, Hirschsprung disease, hypothyroidism, hypotonia, immunological deficits and infections (such as a 62-fold higher rate of pneumonia), mental health and emotional problems that include anxiety, depression, aggression, autism, psychosis, social withdrawal and attention deficit hyperactivity disorder (ADHD), manic depression, motor disorders, sleep disorders, status epilepticus (epilepsy and seizures), Usher syndrome, visual problems (such as cataracts, near-sightedness, "crossed" eyes, rapid involuntary eye movements) and various physical anomalies such as early aging that occur at elevated frequencies. All are part of the remarkably wide-ranging Down syndrome '*phenotypic spectrum*' [5-51] (**Figure 1**). Interestingly, the majority of DS patients suffer from 2 or more of these maladies simultaneously, thus DS presents itself as a highly complex and multifaceted human genetic syndrome often consisting of both neurological and non-neurological components.

microRNAs in DS

The question arises of how could the relatively small number of identified protein genes (~225) encoded on chr21, the smallest human chromosome, impact so many diseases? This may not be a question of chr21-encoded genes *per se*, but may, in part, be explained by the five chr21-encoded microRNAs (miRNAs). These chr21 encoded miRNAs include let-7c, miRNA-99a, miRNA-125b, miRNA-155 and miRNA-802, clustered on the long arm of human chr21, and spanning the chr21q21.1-chr21q21.3 region (**Figure 1**). Recent research has shown **(i)** that because of the extra copy of chr21 in DS, there is also an extra dosage of each of these 5 microRNAs in DS tissues; **(ii)** that these 5 microRNAs are easily detected in control brains and significantly increased in abundance in both AD and DS tissues; **(iii)** that at least 3 of these miRNAs including miRNA-99a, miRNA-125b and miRNA-155 are under NF-kB transcriptional control and are inducible from outside of the cell; and **(iv)** are involved in the down-regulation of important inflammation signaling and innate-immune regulatory genes including the down-regulation of the innate-immune system suppressor glycoprotein complement factor H (CFH) [32,52-55].

miRNAs and neurological disease

Since the original discovery of miRNAs in *Caenorhabditis elegans* in 1993 and the first study of alterations of miRNAs in neurodegenerative disease in *Homo sapiens* (in the AD hippocampus) in 2007, the scope and importance of miRNA-based regulation of gene expression have become part of a much larger regulatory role for RNA not previously anticipated or appreciated [55-61]. miRNAs are an intriguing family of small (18-24 nucleotide) non-coding RNAs (sncRNAs) widely distributed amongst all species so far studied in both the plant and

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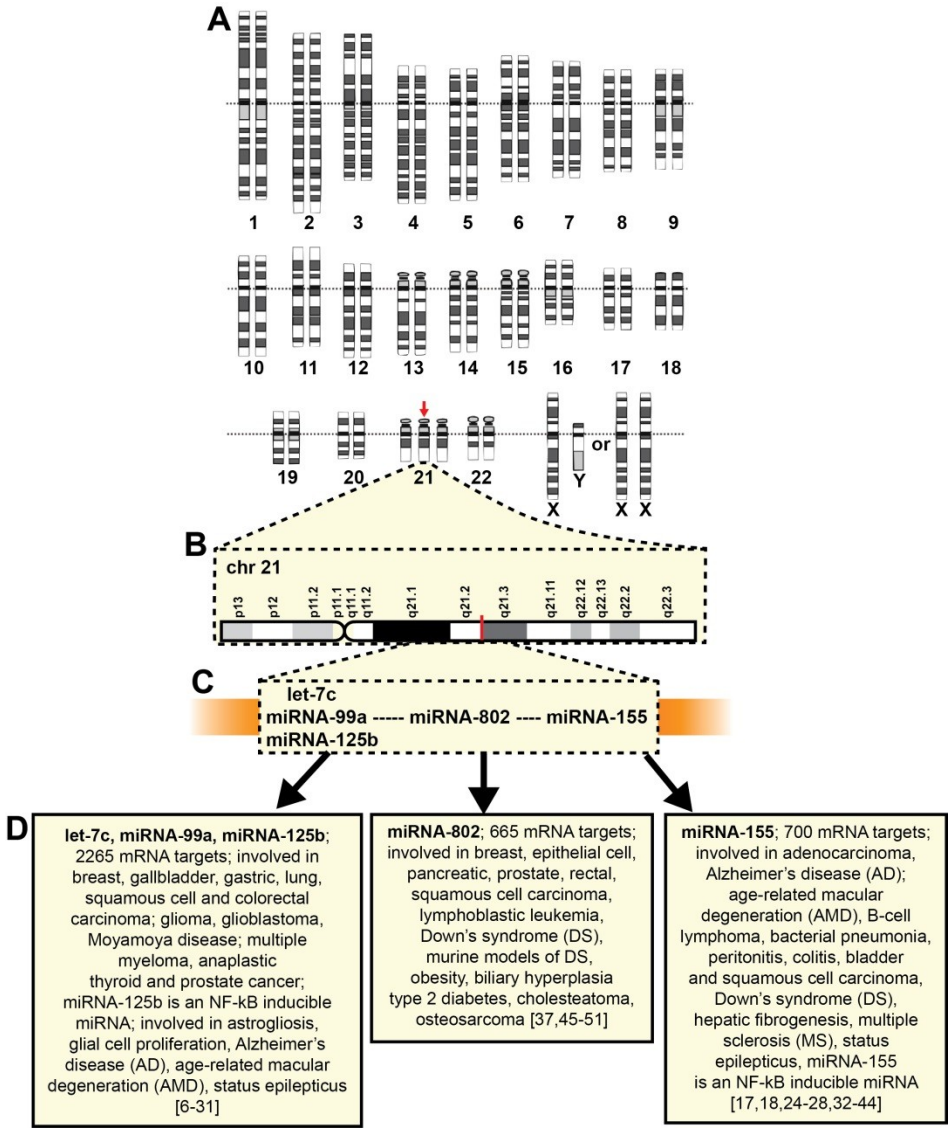

Figure 1. In Down's syndrome (DS; trisomy 21) the activities of five microRNA (miRNA) genes, encoding let-7c, miRNA-99a, miRNA-125b, miRNA-155 and miRNA-802 and clustered on chr21 (chr21q21.1-chr21q21.3) may explain the remarkably wide diversity of the DS phenotype: (A) highly schematicized DS (trisomy 21) karyotype illustrating a typical triplication of chromosome 21 (chr21; vertical red arrow); chromosomes are drawn to the approximate scale; (B) chr21, the smallest human chromosome consists of 48 million base pairs (Mbp) and represents ~1.5% of total cellular DNA; compared to other chromosomes chr21 contains a relatively low number of identified genes (~225); for example 545 genes are encoded on the 49 Mbp chromosome 22 (chr22) [52-54]; the extra copy of chr21 in DS provides an additional gene dosage of all of the miRNA genes encoded on chr 21 [52,54; this communication]; (C) the chr21 region chr21q21.1 to chr21q21.3 encodes just 5 miRNAs, let-7c, miRNA-99a and miRNA-125b at chr21q21.1; miRNA-802 at chr21q21.12 and miRNA-155 at chr21q21.3; (compared to the ~46 miRNAs encoded on chr22); interestingly, the gene encoding βAPP just flanks miRNA-155 at chr21q21.3 [53,54,67,72]; (D) (leftmost panel) diseases associated with let-7c, miRNA-99a and miRNA-125b at chr21q21.1 [6-31]; (middle panel) diseases associated with miRNA-802 [37,45-51]; (rightmost panel) diseases associated with miRNA-155 [17,18,24-28,32-44]; together let-7c, miRNA-99a, miRNA-125b, miRNA-155 miRNA-802 have potential to target the mRNA 3'-UTRs of approximately 3630 protein-coding genes whose dysregulation may help explain the exceedingly high complexity of the DS phenotype [6-54]; see text for additional information.

animal kingdoms. Their major mode of microRNA action is to bind, using base-pair complementarity, to the 3′-untranslated region (3′-UTR) of their target messenger RNAs (mRNAs), and in doing so, decrease the expression of that particular target mRNA. Hence miRNAs act as negative post-transcriptional regulators of gene expression. In the brain and retina where transcription rates are very high, this miRNA-mRNA regulatory system for gene expression may be a particularly active and important genetic process [15,32]. Total human miRNAs currently number about 2650, although there are only about 35-40 abundant miRNAs in the brain and retina [8,15,41]. In neurological diseases, both RNA sequencing and ribosome profiling have shown that up-regulated miRNAs act predominantly to decrease their target mRNA levels, and this along with miRNA-mediated destabilization of mRNAs is the main reason for the observed reductions in gene expression, as are characteristic in both AD and DS brain tissues [57-63]. It is further intriguing that a single miRNA species is capable of the

regulation of multiple target mRNAs encoded from genes dispersed throughout all somatic chromosomes, indicating that miRNAs may regulate multiple signaling pathways and participate in numerous physiological and pathological processes both in health and disease. To date, human diseases most often linked to alterations in the abundance of let-7c, miRNA-99a, miRNA-125b, miRNA-155 or miRNA-802 are neurological disorders that include DS, AD, multiple sclerosis (MS), status epilepticus and astrogliosis, and cancers of many different types **(Figure 1D).** In cancers of the CNS, miRNAs may have either tumor suppressive or oncogenic role in carcinogenesis. The use of multiple miRNA bioinformatics algorithms indicates that over 3630 genes have potential to be regulated by the 5 chr21 miRNAs that include let-7c, miRNA-99a, miRNA-125b, miRNA-155 and miRNA-802, 3 of which are inducible from outside of the cell (see **Figure 1**) [16,23,31,44,72].

Beta amyloid precursor protein (βAPP) is also encoded at chr21q21.3 near the chr21 miRNA cluster

The linkage of the chr21 gene dosage disparity in DS with the significant variability in the DS phenotype has been an elusive goal in the study of trisomy 21 genetics, epigenetics and epidemiology [1,52,65,72]. In DS, there is variability in the biophysical nature and extent of the chr21 triplication as well as in the age of DS onset and the time-course of the disease progression and intensity. The severity and degree of impairment in the DS patient fluctuates significantly [1-4,64-66]. In fact evidence associating a specific chr21 chromosomal domain or chr21-encoded gene to a particular DS phenotype has so far been relatively limited in chr21 genetic linkage studies [52,64-66]. Of interest is that neurological diseases such as those associated with aging, AD and DS are in part characterized by the progressive deposition and aggregation of 40 and 42 amino acid amyloid beta (Aβ) peptides derived from a larger ~770 amino acid beta amyloid precursor protein (βAPP) encoded as a single copy gene on chromosome 21 [52,67]. Interestingly, **(i)** miRNA-155 is encoded immediately upstream of the βAPP gene (encoded at chr21q21.3; the other 4 miRNAs are up to several megabases distant); **(ii)** the accumulation of Aβ peptides is characteristic of aging DS patients and may in part be a consequence of the extra gene dosage of chr21 in DS; and **(iii)** all DS patients exhibit AD-type neuropathological change as they age. Both AD and DS neuropathology include the progressive deposition of Aβ42 peptides into insoluble and pro-inflammatory senile plaque deposits, and these neurotoxic peptides further support the appearance of elevated pro-inflammatory biomarkers in the aging CNS [5,64-67].

Summary

As our understanding of the epidemiology, molecular-genetics and neurobiology of DS continue to advance, DS genetics, epigenetics, DNA bioinformatics, sequence analysis and other metrics have recognized that an increasing number of both neurological and non-neurological diseases are linked to the presence of a complex DS phenotype. This communication provides a plausible explanation of why so many human diseases are associated with DS and the extra copy of chromosome 21, which may be linked to extra dosage of chr21-encoded miRNAs. Here we provide 6 new observations hitherto unrecognized or undocumented in the trisomy research field involving the molecular-genetics of chr21 triplication in DS: **(i)** for the first time we point out that the 5 microRNAs encoded on the extra copy of chr21 in Downs syndrome have potential to regulate the expression of over 3630 human genes located on all somatic chromosomes (see **Figure 1** and text); **(ii)** these findings provide an example of what was classically considered as a neurological-developmental-dementing disorder is also a potential contributor to the development of disease in other major organ systems including the bladder, blood, circulatory system, CNS, heart, gastrointestinal (GI) tract, lung, neuro-vasculature, prostate, and thyroid, including a strong predisposition to many forms of cancer in these tissues; **(iii)** that chr21 via miRNA expression may be an important '*regulatory chromosome'* in the expression of all other somatic chromosomes in both health and disease; **(iv)** that further study and analysis of these chr21-encoded miRNAs, their mRNA interactions and induction of pathogenic biological pathways provides a greatly expanded list of potential therapeutic targets which would ultimately define the basis for more effective treatments in the clinical management of not only DS but other diseases associated with development of chr21-linked neuropathology; **(v)** that the significance, if any, of a 5-member miRNA cluster (3 of which are inducible and under NF-kB control) flanking the βAPP gene remains to be established, and (**vi**) that the activity of miRNA-155, encoded immediately upstream of the βAPP gene, may have some ancillary control of some aspect of βAPP gene expression and perhaps the generation of amyloid peptides. In fact the miRNAs let-7c, miRNA-99a, miRNA-125b, miRNA-155 and/or miRNA-802 may have some regulatory role on βAPP gene activation and/or the secretases, cleavage enzymes or related molecules which in part control βAPP expression and hence amyloidogenesis. Examining miRNA expression and miRNA targeting of mRNA networks in DS patients may identify factors linked to the development of DS-associated disease and may lead to potentially new therapeutic strategies including anti-microRNA approaches to treat this tragic neurological disorder, and the many human diseases associated with the DS phenotype [1,15,32,65,71]. For example, employing anti-miRNA-based therapeutic strategies directed toward a single or a few chr21 specific miRNAs: **(i)** could be of therapeutic use in the restoration of essential and homeostatic mRNA and gene expression patterns in DS patients; and/or **(ii)** may ultimately provide more effective treatments in the clinical management of serious and often fatal human diseases that accompany a highly complex and multidimensional DS phenotype.

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