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Signature MicroRNA expression patterns identified in humans with 22q11.2 deletion/DiGeorge syndrome

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Abstract Patients with 22q11.2 deletion syndrome have heterogeneous clinical presentations including immunodeficiency, cardiac anomalies, and hypocalcemia. The syndrome arises from hemizygous deletions of up to 3 Mb on chromosome 22q11.2, a region that contains 60 genes and 4 microRNAs. MicroRNAs are important post-transcriptional regulators of gene expression, with mutations in several microRNAs causal to specific human diseases. We characterized the microRNA expression patterns in the peripheral blood of patients with 22q11.2 deletion syndrome (n=31) compared to normal controls (n=22). Eighteen microRNAs had a statistically significant differential expression ($p<0.05$), with miR-185 expressed at 0.4× normal levels. The 22q11.2 deletion syndrome cohort exhibited microRNA expression hyper-variability and group dysregulation. Selected microRNAs distinguished patients with cardiac anomalies, hypocalcemia, and/or low circulating T cell counts. In summary, microRNA profiling of chromosome 22q11.2 deletion syndrome/DiGeorge patients revealed a signature microRNA expression pattern distinct from normal controls with clinical relevance.

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1. Introduction

Chromosome 22q11.2 deletion syndrome is one of the most common human genetic deletion disorders with a frequency of 1 in every 4000 births [1]. The syndrome develops following aberrant interchromosomal exchanges involving 8 large, paralogous low copy repeats of DNA (LCRs A-H) that span chromosome 22q11.2 [2–4]. These LCRs, long nucleotide stretches with extremely high sequence-homology, mispair during meiosis, which causes hemizygous chromosomal deletions of variable lengths. Most patients (90%) have a 3 million base pair (Mb) deletion between LCR-A and D (~60 genes), while 5–8% have a 1.5 Mb loss spanning LCR A and B (~30 genes) [5]. There are infrequent deletions between LCR D and E and/or the more distal LCRs [6,7]. The deletions contribute to variable clinical presentations including immunodeficiency, cardiac anomalies, hypocalcemia, feeding difficulties, facial dysmorphology, skeletal and renal anomalies, developmental and speech delay, learning disabilities, and/or schizophrenia [1,8,9]. The T cell immunodeficiency results from inadequate development of the thymus [10]. Up to 60% of patients with confirmed deletions on 22q11.2 have some degree of T cell lymphopenia. The peripheral T cells in these patients can undergo an unusual homeostatic expansion, resulting in an age-dependent T helper cell 2 (Th_2) bias [9,11]. The cardiovascular defects primarily stem from the haploinsufficiency of the T-box1 transcription factor (Tbx1), which is required for formation of the pharyngeal apparatus during mammalian embryogenesis [12–16]. The 4th pharyngeal arch and the 3rd pharyngeal pouch give rise to the aortic arteries, the thymus and parathyroid organs, respectively. Point mutations in Tbx1 have been reported in several patients, and these are causal to the cardiac anomalies [15]. Yet, the thymic hypoplasia is only seen in a subset of such patients, suggesting that additional genetic components contribute to the thymic defects and ensuing T cell lymphopenia [17].

DiGeorge Syndrome Critical Region 8 (DGCR8), another gene deleted on chromosome 22q11.2, is known to be a pri-microRNA-binding protein required for microRNA (miR) biogenesis [18]. MiRs are a family of small, non-coding RNAs (18-22 nucleotides) that modulate gene expression by targeting specific messenger RNAs for degradation, translational repression, or both [19,20]. MiRs affect a wide range of biological responses including proliferation, differentiation, apoptosis, and/or stress responses [21]. In 22q11.2-deletion mouse models, a deficiency of DGCR8 causes a 20–70% reduction in a subset of miRs in the brain [22,23]. These miR changes contribute to a cognitive impairment, with altered short-term plasticity in the prefrontal cortex [23].

How the hemizygous deletions of 60 genes and 4 miRs encoded on chromosome 22q11.2 influence the complex clinical symptoms presented by 22q11.2 deletion syndrome patients is not well understood. We profiled the miRs in the peripheral blood of 31 patients with 22q11.2 deletion syndrome along with 22 normal controls. Statistically significant differences in the expression of 18 miRs were identified. MiR-185 was expressed at 0.4× normal levels, consistent with its hemizygous deletion on chromosome 22q11.2. This was the only miR of the 4 encoded within the locus detected in the peripheral blood. Mosaic cluster analyses revealed groups of miRs with both positive and negative correlation coefficients that were dramatically altered and/or absent in the patients [24–26]. Putative targets of these clusters were linked to various biological responses. The changes in particular groups of miRs matched selected clinical conditions including low numbers of circulating T cells, hypocalcemia, and cardiac anomalies. MiR differences were not related to changes in the cell populations of the peripheral blood, as expression profiling with 177 genes that define selected cell-based modules revealed similar patterns between the patient cohort and normal groups. These findings indicate that miR profiling of 22q11.2 deletion syndrome patients can uncover a clinically informative miR pattern significantly distinct from normal individuals.

2. Materials and methods

2.1. Patient cohort

Our study was a prospective and retrospective analysis of clinical and laboratory data of a total of thirty-one patients with 22q11.2 deletion syndrome. For this study, DiGeorge syndrome was defined as patients with low CD3 counts (<10% percentile for age and <1500 cells/μl), hypocalcemia and congenital heart disease. All patients were referred to the outpatient Immunology Clinic and/or admitted to Children's Medical Center in Dallas, TX between May 2009 and April 2011. The Institutional Review Board at UT Southwestern Medical Center approved this study (IRB # 072010-003). Informed consent was obtained from all patients and control subjects (or parents, when necessary).

2.2. Patient characteristics

A total of 31 subjects, 64% females (20/31), mean age 5.34 ± 4.81 years, participated in the study (Supplemental Tables 1–3). Ethnic distributions were as follows: 48% Hispanic, 45% Caucasian, and 6% African-American. All 31 patients had fluorescent in-situ hybridization (FISH) evidence for the 22q11 deletion. One patient had documented autoimmunity (thrombocytopenia, MRNAP060), 1 had low IgG (MRNAP040) as

Abbreviations: ASD, Atrial septal defects; Btk, Bruton's tyrosine kinase; Camk4, Calcium/calmodulin-dependent kinase 4; Cy3, Cyanine 3; DGCR8 (dgcr8), DiGeorge Syndrome Critical Region 8; DGS, DiGeorge syndrome; FISH, Fluorescent *in situ* hybridization; IRB, Institutional Review Board; Itk, Interleukin-2 inducible T cell kinase; KEGG, Kyoto Encyclopedia of genes and genomes; LCR, Low copy repeats; MiR, microRNA; Mzb1, Marginal zone B- and B1-cell specific protein 1 (pERp1); NF-ATc3, Nuclear factor of activated T cells, cytoplasmic, calcineurin 3; PIDs, Primary immunodeficiency diseases; SERCA2, sarcoplasmic/endoplasmic reticulum calcium ATPase 2; TA, Truncus arteriosus; Tbx1, T-box transcription factor; TOF, Tetralogy of Fallot; Th2, T helper 2 subset; VCF, Velo-cardio-facial syndrome; VSD, ventricular septal defects.