Modulation of ADAR mRNA expression in patients with congenital heart defects

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Abstract

Adenosine (A) to inosine (I) RNA editing is a hydrolytic deamination reaction catalyzed by the adenosine deaminase (ADAR) enzyme acting on double-stranded RNA. This posttranscriptional process diversifies a plethora of transcripts, including coding and noncoding RNAs. Interestingly, few studies have been carried out to determine the role of RNA editing in vascular disease. The aim of this study was to determine the potential role of ADARs in congenital heart disease. Strong downregulation of ADAR2 and increase in ADAR1 expression was observed in blood samples from congenital heart disease (CHD) patients. The decrease in expression of ADAR2 was in line with its downregulation in ventricular tissues of dilated cardiomyopathy patients. To further decipher the plausible regulatory pathway of ADAR2 with respect to heart physiology, miRNA profiling of ADAR2 was performed on tissues from ADAR2-/- mouse hearts. Downregulation of miRNAs (miR-29b, miR-405, and miR-19) associated with cardiomyopathy and cardiac fibrosis was observed. Moreover, the upregulation of miR-29b targets COL1A2 and IGF1, indicated that ADAR2 might be involved in cardiac myopathy. The ADAR2 target vascular development associated protein-coding gene filamin B (FLNB) was selected. The editing levels of FLNB were dramatically reduced in ADAR2-/- mice; however, no observable changes in FLNB expression were noted in ADAR2-/- mice compared to wild-type mice. This study proposes that sufficient ADAR2 enzyme activity might play a vital role in preventing cardiovascular defects.

Introduction

Congenital heart disease (CHD) is defined as a structural or functional heart defect. It belongs to a heterogeneous group of diseases and can be classified anatomically, clinically, epidemiologically and developmentally [1–3]. The most common conditions associated with CHD among hospitalized patients are ventricular septal defect (VSD), Tetralogy of Fallot (TOF), patent ductus arteriosus (PDA), transposition of the great arteries (TGA), atrial septal defect (ASD) and atrioventricular septal defect (AVSD) [4]. A recent study indicated that, due to
cardiac anomalies, 11% of Pakistani newborns die within the first postnatal month [5]. Genetic conditions and environmental factors, such as maternal diabetes and rubella, have been identified as factors leading to CHD defects [6].

The most common type of RNA editing in humans replacement of adenosine (A) with inosine (I) [7]. ADARs perform this complex hydrolytic posttranscriptional deamination reaction. The ADAR family consists of three members, ADAR1, ADAR2 and ADAR3. ADAR1 and ADAR2 play active roles in the deamination of adenosine, while ADAR3 is nonfunctional [8]. Several studies have shown that the extent of RNA editing not only varies between individuals but also has high tissue specificity [9–11]. Approximately 2.5 million sites in the human transcriptome have been edited; a large majority of them are in double-stranded Alu elements, and the sites are mainly in introns and untranslated regions (UTRs) [8]. However, the functional consequences of a majority of the RNA editing events remain elusive.

RNA editing is known to modulate splicing, mRNA coding potential, and transcript stability and even to alter microRNA processing and targeting [7, 9, 10]. Initially majority of the RNA editing targets have been reported in the neuronal receptors such as AMPA and glutamate receptors [11, 12]. A recent study focusing on RNA editing events in six different tissues identified an average of 79,976 editing sites within heart tissue [8]. This implies that RNA editing might play a role in other tissues apart from the nervous system. Apart from protein coding targets different non-coding RNAs like microRNAs (miRNAs) are also edited by ADARs [13, 14]. ADARs modulate the processing and expression of miRNA by either editing [10, 14, 15] or interacting with dicer [16]. They also alter target specificity of miRNAs by editing the seed sequence [10]. miRNAs regulate diverse cellular physiology [17] and developmental processes [18]. In the cardiovascular system, miRNAs regulate diverse processes, such as cardiac remodeling, cardiac hypertrophy and fibrosis [19]. A significant decrease in ADAR2 RNA was observed in cyanotic congenital heart disease (CHD while expression of ADAR1,did not show a significant difference [20]. Another study demonstrated an increase in ADAR1 mediated RNA editing of the CTSS transcript is increased in hypoxic or proinflammatory conditions as well as in patients with clinical or subclinical vascular damage [21]. CTSS plays a role in vascular inflammatory processes [21]. Moreover, decrease in ADAR2 mediated editing of another actin-binding protein, filamin A (FLNA), have been linked to cardiovascular disease and reduced systolic output [22]. Thus, these studies demonstrate that ADARs might be contributing towards heart disease.

The current study determined the RNA levels of ADAR1 and ADAR2 in congenital heart disease patients. The relative gene expression of a crucial angiogenesis-related transcription factor, FOXP1, was found to be downregulated in CHD cases. A dramatic decrease in ADAR2 mRNA levels and an opposing upregulation of ADAR1 were also found. To further explore the role of ADAR2 in heart physiology, ADAR2 knockout mouse heart tissue was used. Although previously documented, no strong anomalies in heart physiology were observed [23].

Materials and methods

Collection of samples

This study was initiated after receiving approval from the ethics committees of both COMSATS Institute of Information Technology (CIIT) (No./Bio/ERB/15/75) and the collaborating hospital, the Rawalpindi Institute of Cardiology (RIC). Patient echocardiography reports were consulted to confirm the presence of congenital heart defects. Blood samples from 35 patients with different defects from the Rawalpindi Institute of Cardiology (RIC) were collected before surgery and stored on ice during transportation. The samples were segregated on the basis of
age (3 months–16 years) and sex. Thirteen control samples were collected from healthy individuals using the same parameters. Interviews were conducted using specified questionnaires that gathered information on age, sex, medication and family history. The details of these persons are provided in S1 and S2 Tables in accordance with the Ethical Review Board (ERB) approval.

**cDNA synthesis**

Five milliliters of whole blood was collected from each patient and stored in ethylenediaminetetraacetic acid (EDTA) test tubes. To avoid RNA degradation, the blood was kept at 4°C for up to 24 hours between sample collection and RNA extraction. RNA was isolated from peripheral blood mononuclear cells with TRIzol Reagent (Invitrogen, Germany) according to the manufacturer’s instructions. The optical density of the RNA was measured immediately following extraction. RNA samples showing 260/280 ratios below 1.8 or above 2.0 were not used in further analyses. Complementary DNA (cDNA) was synthesized from one microgram of RNA using a RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA). A negative control, termed minus reverse transcriptase (−RT), that lacked reverse transcriptase was prepared for each of the samples.

**Real-time PCR**

The relative mRNA expression of genes was examined using quantitative PCR with gene-specific primer sets (IDT, USA and Macrogen, South Korea); the TUB1 gene was used as an internal control. 5X HOT FIREPol EvaGreen qPCR Mix Plus master mix (ROX) (Solis BioDyne, Tartu, Estonia) was used for qPCR. The sequences of the genes (ADAR1 p110, ADAR1 p150, ADAR2, FOXP1, FLNB, COL1A2, and IGF1) were obtained from Ensemble, and primers were synthesized with the Integrated DNA Technologies Primer Quest Tool. The primers are listed in S1 Table.

**Statistical analysis**

Statistical analyses were performed with GraphPad Prism 7.0b. For expression data, the target gene (ADAR1 p110, ADAR1 p150, ADAR2, FOXP1, FLNB, COL1A2, and IGF1) Ct values were normalized to the control gene (TUB1) Ct value. Statistical significance was determined using the Mann-Whitney U test, and \( P < 0.05 \) was considered to indicate significance.

**Phenotype of ADAR2-/- mice**

Adar2\(^{-/-}\) mice were a kind gift from Peter Seeburg. These transgenic mice were bred on an SV129 background. As ADAR2 deficiency leads to early postnatal lethality, the mice were rescued with a pre-edited Gria2 receptor (Gria2R/R) [24, 25]. The mice were bred at the Vienna BioCenter facility animal house. Gria2R/R; ADAR2\(^{+/+}\) mice were intercrossed. The resulting sibling female offspring of genotype Gria2R/R;Adar2\(^{-/-}\) and Gria2R/R;ADAR2\(^{+/+}\) were euthanized at the age of postnatal day 6 (P6) by cervical dislocation. The whole hearts were dissected and subsequently used for RNA preparation from three biological replicates [24, 26]. These mice displayed phenotypic features described previously [23].

**RNA extraction and microRNA cloning**

RNA was extracted from the whole hearts of euthanized postnatal day 6 (P6) female mice (Adar2\(^{-/-}\) Gria R/R and Adar2\(^{+/+}\), Gria R/R siblings) using TriFast (Peqlab, Erlangen,
Generation of libraries for small RNA next-generation sequencing (NGS) was performed according to a method described previously [15].

Quantification of the complete library was performed using a Bioanalyzer and a qPCR NGS Library Quantification Kit (both from Agilent Technologies). Cluster generation and sequencing were carried out using an Illumina HiSeq 2000 system. After single-end sequencing at a read length of 50 base pairs, the adaptor sequences were removed using Cutadapt (http://code.google.com/p/cutadapt/). At least 200,000 reads per sample were generated and mapped to the genome using Bowtie [27]. The differential expression of microRNAs was analyzed using DESeq [28]. Only microRNAs with a P-value smaller than 0.1 were considered.

**Sequencing and clipping of reads**
The completed libraries were quantified with an Agilent Bioanalyzer dsDNA 1000 Assay Kit and an Agilent qPCR NGS Library Quantification Kit. Cluster generation and sequencing were carried out using an Illumina Genome Analyzer IIx System according to the manufacturer’s guidelines. Illumina sequencing was performed at the Vienna BioCenter Core Facility Next Generation Sequencing (VBCF NGS) Unit (csf.ac.at). After sequencing at a read length of 36 base pairs, the adaptor sequences were removed using Cutadapt [29]. The RNA sequencing data are available in the GEO database (GSE122397).

**Mapping to mature microRNA sequences**
Mapping of the clipped reads to mature microRNA sequences was performed as described previously. Mapping was performed using the NextGenMap tool, restricting the mapped reads to have at least 90% identity (# differences/alignment length) [30]. Two microRNA target genes, COL1A2 and insulin-like growth factor-1 (IGF1), were amplified using the primers listed in S4 Table.

**Results**

**ADAR2 expression is reduced in CHD patients**
RNA was extracted from blood samples taken from 35 patients diagnosed with congenital heart disease and from 13 otherwise healthy individuals, which were used as controls. Most of the patients had VSD. Initially, ADARs were thought to play an important role in the nervous system, as most of the editing targets, such as glutamate and AMPA receptors, were found in the brain [10, 11, 31, 32]. Recent studies indicate that ADARs might contribute towards cardiovascular disease [21, 22]. GTEX differential expression analysis of different tissues showed higher expression of the ADAR1 p110 in the nervous system, whereas ADAR1 p150 RNA level was higher in the vascular system (Fig 1A & 1B). ADAR1 p110 was strongly expressed in the brain cerebral hemispheres, followed by the spleen and tibial artery. In the Fig 1, the whiskers represent the maximum and minimum values. The median is represented as a black line in the box. GTEX gene expression analysis of different tissues showed higher expression of ADAR2 and FOXP1 vascular system compared to nervous system tissues (Fig 1C & 1D). As a control for CHD, we selected a member of the forkhead box family of transcription factors, FOXP1. FOXP1 plays a critical role in mouse and human heart development, and high expression has been observed during cardiomyocyte proliferation [33]. qPCR analysis showed a significant decline in ADAR2 expression in CHD patients compared to controls (Fig 1E). However, the expression of both ADAR1 isoforms (p110 and p150) was significantly increased in CHD patients. This elevated ADAR1 expression is consistent with recent findings demonstrating that ADAR1 expression is elevated in patients undergoing carotid endarterectomy [21].
three genes, FOXP1 and ADAR2 were downregulated in patients (Fig 1E), indicating their expression modulation with regard to heart disease.

**Heart defect-specific function**

The differential expression of ADARs in the vascular tissues has further intrigued us to determine their expression in heart tissues. The expression analysis was compared between the atrium and ventricular tissues of the heart. GTex expression analysis of heart tissue showed higher levels of ADAR1 p110 compared to p150 in the atrium. However, ADAR1 p150 showed similar expression in both atrial and ventricular tissue (Fig 2A & 2B). In addition, the expression of ADAR2 and FOXP1 was also high in the atrium compared to the ventricle (Fig 2C &
2D). The high atrial specific expression of ADARs was further correlated with the expression pattern of the three selected genes with the heart defect. The strongest upregulation was observed of both ADAR1 isoforms (p150 & p110) isoforms in ASD, followed by VSD. However, the results also showed an approximately 3-fold increase in the expression of these isoforms in patients with TOF and AVSD compared to controls (Fig 2E & 2F). In contrast, the ADAR2 mRNA level was decreased in AVSD, TOF and VSD patients (Fig 2G). The FOXP1 mRNA levels were decreased significantly in TOF patient samples compared to control samples as it is an important transcriptional regulator during cardiovascular development (Fig 2H). Thus, the high expression of both the ADAR1 isoforms in ASD implies that they might...
have a critical function in the atrium. The strong downregulation of ADAR2 indicated that it might play a role in atrium and ventricle development.

The marked decrease in ADAR2 expression in CHD patients was further investigated in ADAR2−/− mouse hearts. ADAR2 mediated editing occurs both in coding and non-coding genes[34] Non-coding RNAs such as miRNAs play a significant role in heart development [35, 36]. The small RNA sequencing of ADAR2−/− mouse heart samples was performed in biological triplicates. No upregulated miRNAs were observed in the absence of ADAR2 editing activity. However, a consistent ~2-fold decrease in miR-29b levels was consistently observed at the P6 stage (GSE122397) in ADAR2−/− mouse hearts compared to wild-type mouse hearts. miR-29b inhibits cardiac fibrosis induced by angiotensin II through the TGF-β/Smad3 pathway[36]. The deregulation of miR-29b can induce cardiac fibrosis [36]. Other miRNAs like miR-451b, miR-451a, and miR-19b (Table 1) showed ~ 1.5-fold downregulation. Apart from these, various members of the let-7 family also showed downregulation of approximately 1- to 1.5-fold in ADAR2 knockout mouse hearts (Table 1). Aberrant expression of the let-7 family has been linked to a variety of cardiovascular diseases, including fibrosis, hypertrophy, dilated cardiomyopathy (DM), myocardial infarction (MI), atherosclerosis and hypertension [37]. This reduction in the regulation of ADAR2 microRNAs points to the existence of a potential regulatory mechanism mediated by ADAR2 in cardiac development and physiology.

Table 1 shows the 1- to 1.9-fold-downregulated miRNAs obtained by RNA sequencing analysis of the noncoding RNA dataset GSE122397.

miR-29 family functions as downregulators of genes involved in cardiac fibrosis and extra cellular matrix production [19, 39]. The impact of the downregulation of miR-29b was determined on its target genes. Two genes Col1A2 and IGF1 were selected based on their role in cardiac fibrosis and hypertrophy. The upregulation of Col1A2 as a result of the miR-29b downregulation has been observed in the myocardial infarcted region [40]. Deficiency of IGF1 alleviates hypertrophic markers and mitigates cardiac hypertrophic remodelling [41]. qPCR analysis of two target genes, Col1A2 and IGF1, showed that these genes exhibited slight upregulation in knockout mouse hearts. This mild increase in Col1A2 and IGF1 can be due to the observed 2 fold decrease in miR-29b expression (Fig 3).

### Filamins and cardiac defects

Actin-binding proteins such as FLNA and FLNB play important roles in the development of the vascular system [42]. ADAR2-mediated FLNA and FLNB RNA editing occurs at the same

| No. | miRNA   | Log2 fold change | P value  | Reference |
|-----|---------|------------------|----------|-----------|
| 1   | miR-29b | -1.97            | 0.001599 | [34]      |
| 2   | miR-451b | -1.51            | 0.0005   | [37]      |
| 3   | miR-451a | -1.51            | 0.0005   | [37]      |
| 4   | miR-19b1 | -1.48            | 0.015    | [34]      |
| 5   | let7c-2  | -1.47            | 0.0017   | [13]      |
| 6   | let7c-1  | -1.39            | 0.003    | [13]      |
| 7   | let7-1   | -1.23            | 0.01     | [13]      |
| 8   | let7-b   | -1.23            | 0.01     | [13]      |
| 9   | miR-382  | -1.21            | 0.04     |          |
| 10  | miR-26a  | -1.19            | 0.03     |          |
| 11  | miR-378  | -1.19            | 0.011    | [14]      |
| 12  | miR-378a | -1.14            | 0.019    | [14]      |
| 13  | miR-130a | -1.09            | 0.024    | [38]      |

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position, leading to changes in glutamine (Q) to arginine (R) at the protein level [43]. Impaired FLNA editing has been linked to arterial remodeling, thickening of the left ventricular wall, and increased phosphorylation of the myosin light chain[22]. FLNB showed high expression in vascular tissues [44]. High RNA editing of FLNB has been observed in the cardiovascular system (62% in the heart and 66% in the aorta) and musculoskeletal system. [44]. However, in ADAR2−/− mouse hearts compared to wild-type mouse hearts FLNB editing was reduced by 24% (Fig 4). We propose that the elevated expression of FLNB and the decline of editing in ADAR2−/− mouse heart tissues might be associated with the cardiovascular disease similar to FLNA. Since the editing of FLNB was correlated with FLNB expression in the mouse cerebral cortex [44] therefore, the mRNA level of FLNB in ADAR2−/− hearts was also determined. No significant difference was observed in FLNB mRNA levels between ADAR2−/− mouse heart tissues and wild-type mouse heart tissues (Fig 4). Thus, FLNB editing might have a heart-specific function independent of its expression.

**ADAR1 expression in ADAR2 KO mouse hearts**

ADAR1 and ADAR2 both edit double stranded RNA. The elevated expression of ADAR1 in the absence of ADAR2 raised the possibility of a underlying compensatory mechanism for editing the target genes. Therefore, the expression of ADAR1 in ADAR2 KO mice was determined to rule out this possibility. ADAR1 mRNA levels remained unchanged in the absence of
Therefore, we can conclude that the observed increase in ADAR1 was solely because of the CHD defect.

**Discussion**

Adenosine deamination by ADARs is a posttranscriptional event that can diversify transcripts at both the sequence and structural levels. Deregulation of editing has been associated with a number of diseases [7, 20, 34]. The tissue-specific gene expression implies that ADAR1 p150, ADAR2 and FOXP1 might play critical roles in the vascular system. In this study, we observed a strong downregulation of ADAR2 and FOXP1 at the mRNA level in PBMCs of congenital heart defect patients. The observed differences at the mRNA level might be due to differential
expression patterns in atrium versus ventricular heart tissues. RNA sequencing analysis of left ventricular myocardium from 13 DCM individuals compared to 10 controls showed significant declines of ~1.5-fold and ~1.3-fold for ADAR2 and FOXP1, respectively [45] (GSE55296). However, no significant difference was seen at the ADAR1 mRNA level [45] (GSE55296). In addition, no significant deregulation was observed for the three candidate genes in ischemic cardiomyopathy cases [45] (GSE55296). This implies that the decrease in ADAR2 and FOXP1 is associated with dilated cardiomyopathy. However, ADAR1 mRNA levels were found to be significantly upregulated in dilated aortas (n = 35) compared to nondilated aortas (n = 8) [21]. A similar pattern of ADAR1 overexpression was observed in human atherosclerotic plaques [21]. In this study, a significant increase in ADAR1 p150 mRNA levels was observed in the ASD and VSD patient samples signifying the upregulation of ADAR1 in response to CHD.

Two candidate genes FOXP1 and ADAR2 showed a significant decline in CHD. FOXP1, which acts as a transcription factor, has a DNA-binding domain that binds to the consensus sequence 5’-TRTTKRY-3’ located in the promoters and enhancers of many genes [46]. FOXP1 is associated with cardiovascular development as the FOXP1-null embryos usually die at embryonic day E14.5 with defects in ventricular septation, myocardial maturation and outflow tract septation [47]. Moreover, FOXP1 maintains cardiac homeostasis by counteracting the hypertrophy-associated effects of NFAT3 [48]. Thus, FOXP1 serves as a regulator for maintaining cardiomyocyte size and proliferation [48]. Thus, the observed deregulation of FOXP1 in PBMCs and DCM tissues might be indicating disturbed cardiac homeostasis. The other candidate gene ADAR2, editing activity has been reported previously in the brain AMPA receptor, leading to seizures and, consequently, death [49]. However, a recent study has documented high ADAR2 activity in the vascular system compared to the nervous system [22]. ADAR2 edits a plethora of coding and non-coding transcripts. ADAR2 modulates the expression and processing of non-coding RNAs such as miRNAs [10, 14, 50]. miRNA profiling of ADAR2 knockout mouse heart tissues showed ~1.5- to 2-fold downregulation of miR-29b, miR-451, miR-19 and members of the let-7 family (Table 1). Most of these downregulated miRNAs like miR-29b, miR-19 and let-7 family are associated with cardiac fibrosis, hypertrophy and cardiomyocyte proliferation [51–53]. This miRNA deregulation in the absence of ADAR2 points towards its involvement in the regulation of the heart development and physiology. Since ADAR2 downregulation was associated with DCM we selected two cardiac hypertrophy and fibrosis associated genes (COL1A2 and IGF1) that are potential targets of miR-29b. COL1A2 functions in pathways associated with extracellular matrix receptor interactions and focal adhesion [54]. High amounts of collagen may lead to cardiac fibrosis [54] whereas increased expression of IGF1 have been reported previously in hypertrophic cardiomyopathy [55]. Increases in the expression of COL1A2 and IGF1 were observed on the corresponding decrease of miR-29b in the absence of ADAR2. This slight increase in COL1A2 and IGF1 in ADAR2-/- mouse heart tissues indicated that ADAR2 is not the core factor related to cardiomyopathy. However, it might be one of the factors involved in this pathway.

Among the protein coding targets of ADAR, filamins have gained importance as they are involved in cardiovascular development [42]. Filamins are actin-binding proteins comprising three members: filamins A, B and C. Filamins not only bind to actin via their N-terminal domains but also serve as docking sites for different cytoplasmic proteins, membrane receptors and integrins [56]. Filamin A and filamin B are exclusively edited in a highly interactive region of the Q/R site by ADAR2 [43]. ADAR2-mediated RNA editing of FLNA is 100-fold higher in vascular tissue than in nervous tissues [22]. Mice with impaired FLNA editing show increased vascular contraction, cardiac remodeling and reduced systolic output [22]. However, no changes in steady-state levels or stability of FLNA was observed in the absence of FLNA editing signifying the importance of editing independent of FLNA expression [22]. The same editing
site and the high editing activity of FLNB in the heart (62.3%) and skeletal muscle (68.5%) made it a suitable candidate for this study [44]. Moreover, FLNB deficiency in mice is associated with vascular defects. FLNB also plays a critical role in endothelial cell migration and angiogenesis [56]. In this study, the 24% decrease in FLNB editing was also accompanied with no change in FLNB at RNA level in ADAR2-KO mouse heart tissues.

These findings also imply that ADAR2 might have a cardioprotective function as it modulates the expression of miRNAs and their corresponding targets. Further studies are also needed to elucidate the role of edited FLNB in the heart. We propose that editing of FLNB might be altering its interaction with different docking proteins. Therefore, we can conclude that ADAR2 might not be the critical factor for cardiac development. However, the deregulation of miRNAs and FLNB editing might contribute to hypertrophic cardiomyopathy. Future studies, may help to decipher the link between cardiac defects and ADAR2 activity along with the impact of ADAR2 mediated editing on its target genes.

Supporting information

S1 Table. CHD patient data. Age and gender distribution of the congenital heart defect patients along with their medication status and family history. (DOCX)

S2 Table. Normal individual data. Age and gender distribution of the normal individuals. (DOCX)

S3 Table. Primer sets used for the qPCR analysis of gene expression. The sequences of the primers used in this study are given. (DOCX)

S4 Table. Primer sets used for the real-time PCR analysis of miR-29b target gene expression in the ADAR2-KO mouse heart tissues. (DOCX)

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References

1. Hobbs CA, Cleves MA, Melnyk S, Zhao W, James SJ. Congenital heart defects and abnormal maternal biomarkers of methionine and homocysteine metabolism. The American journal of clinical nutrition. 2005; 81(1):147–53. https://doi.org/10.1093/ajcn/81.1.147 PMID: 15640474

2. Botto LD, Muline J, Erickson JD. Do multivitamin or folic acid supplements reduce the risk for congenital heart defects? Evidence and gaps. American Journal of Medical Genetics Part A. 2003; 121(2):95–101.

3. Verkleij-Hagoort AC, Verlinde M, Ursem NT, Lindemans J, Helbing WA, Ottenkamp J, et al. Maternal hyperhomocysteinemia is a risk factor for congenital heart disease. BJOG: An International Journal of Obstetrics & Gynaecology. 2006; 113(12):1412–8.

4. Al-Hamash SM. Pattern of congenital heart disease: a hospitalbased study. Al Kindy College Medical Journal. 2006; 3:44–8.

5. Pathan IH, Bangash SK, Khawaja AM. SPECTRUM OF HEART DEFECTS IN CHILDREN PRESENTING FOR PEDIATRIC CARDIAC SURGERY. Pakistan Heart Journal. 2016; 49(1).

6. Botto LD, Lynberg MC, Erickson JD. Congenital heart defects, maternal febrile illness, and multivitamin use: a population-based study. Epidemiology. 2001; 12(5):485–90. PMID: 11505164

7. Tariq A, Jantsch MF. Transcript diversification in the nervous system: A to I RNA-editing in CNS function and disease development. Frontiers in neuroscience. 2012; 6:99. https://doi.org/10.3389/fnins.2012.00099 PMID: 22787438

8. Picardi E, Manzari C, Mastropasqua F, Aiello I, D’Erchia AM, Pesole G. Profiling RNA editing in human tissues: towards the inosinome Atlas. Scientific reports. 2015; 5:14941. https://doi.org/10.1038/srep14941 PMID: 26492020

9. Wang IX, So E, Devlin JL, Zhao Y, Wu M, Cheung VG. ADAR regulates RNA editing, transcript stability, and gene expression. Cell reports. 2013; 5(3):849–60. https://doi.org/10.1016/j.celrep.2013.10.002 PMID: 24183664

10. Nishikura K. A-to-I editing of coding and non-coding RNAs by ADARs. Nature reviews Molecular cell biology. 2016; 17(2):83–96. https://doi.org/10.1038/nr.2015.4 PMID: 26648264

11. Tariq A, Jantsch MF. Transcript diversification in the nervous system: A to I RNA editing in CNS function and disease development. Frontiers in neuroscience. 2012; 6.

12. Huntley MA, Lou M, Goldstein LD, Lawrence M, Dijkgraaf GJ, Kaminker JS, et al. Complex regulation of ADAR-mediated RNA-editing across tissues. BMC genomics. 2016; 17(1):61.

13. Chawla G, Sokol NS. ADAR mediates differential expression of polycistronic microRNAs. Nucleic acids research. 2014; 42(8):5245–55. https://doi.org/10.1093/nar/gku145 PMID: 24561617

14. Vesely C, Tauber S, Sedlazeck FJ, Tajaddod M, Haeseler Av, Jantsch MF. ADAR2 induces reproducible changes in sequence and abundance of mature microRNAs in the mouse brain. Nucleic acids research. 2014; 42(19):12155–68. https://doi.org/10.1093/nar/gku844 PMID: 25260591

15. Vesely C, Tauber S, Sedlazeck FJ, von Haeseler A, Jantsch MF. Adenosine deaminases that act on RNA induce reproducible changes in abundance and sequence of embryonic miRNAs. Genome research. 2012; 22(8):1468–76. https://doi.org/10.1101/gr.133025.111 PMID: 22310477

16. Ota H, Sakurai M, Gupta R, Valente L, Wulff B-E, Ariyoshi K, et al. ADAR 1 forms a complex with Dicer to promote microRNA processing and RNA-induced gene silencing. Cell. 2013; 153(3):575–89. https://doi.org/10.1016/j.cell.2013.03.024 PMID: 23622424

17. Mendell JT. MicroRNAs: critical regulators of development, cellular physiology and malignancy. Cell cycle. 2005; 4(9):1179–84. https://doi.org/10.4161/cc.4.9.2032 PMID: 16096373

18. Johnson CD, Esquela-Kerscher A, Stefani G, Byrom M, Kelner K, Ovcharenko D, et al. The let-7 microRNA represses cell proliferation pathways in human cells. Cancer research. 2007; 67(16):7713–22. https://doi.org/10.1158/0008-5472.CAN-07-1083 PMID: 17699775

19. Sassi Y, Avramopolous P, Ramanujam D, Grüter L, Werfel S, Giossele S, et al. Cardiac myocyte miR-29 promotes pathological remodeling of the heart by activating Wnt signaling. Nature Communications. 2017; 8(1):1614. https://doi.org/10.1038/s41467-017-01737-4 PMID: 29158499

20. Borik S, Simon AJ, Nevo-Caspi Y, Mishali D, Amargillo N, Rechavi G, et al. Increased RNA editing in children with cyanotic congenital heart disease. Intensive care medicine. 2011; 37(10):1664. https://doi.org/10.1007/s00134-011-2296-z PMID: 21720910

21. Stellos K, Gatsiou A, Stamatakopoulos K, Matic LP, John D, Lunella FF, et al. Adenosine-to-inosine RNA editing controls cathepsin S expression in atherosclerosis by enabling HuR-mediated post-transcriptional regulation. Nature Medicine. 2016.
22. Jain M, Mann TD, Stulić M, Rao SP, Kirsch A, Pullirsch D, et al. RNA editing of Filamin A pre-mRNA regulates vascular contraction and diastolic blood pressure. The EMBO journal. 2018; 37(19):e94813. https://doi.org/10.15252/embj.201694813 PMID: 30087110

23. Horsch M, Seeburg PH, Adler T, Aguilar-Pimentel JA, Becker L, Calzada-Wack J, et al. Requirement of the RNA-editing enzyme ADAR2 for normal physiology in mice. Journal of Biological Chemistry. 2011; 286(1):1864–22. https://doi.org/10.1074/jbc.M110.200881 PMID: 21467037

24. Brusa R, Zimmermann F, Koh D-S, Feldmeyer D, Gass P, Seeburg PH, et al., editors. Early-onset epilepsy and postnatal lethality associated with an editing-deficient GluR-B allele in mice. Cold Spring Harbor Symp Quant Biol; 1995: JSTOR.

25. Higuchi M, Maas S, Single FN, Hartner J, Rozov A, Burnashev N, et al. Point mutation in an AMPA receptor gene rescues lethality in mice deficient in the RNA-editing enzyme ADAR2. Nature. 2000; 406(6791):78–81. https://doi.org/10.1038/35017558 PMID: 10894545

26. Hartner JC, Schmittwolf C, Kispert A, Müller AM, Higuchi M, Seeburg PH. Liver disintegration in the mouse embryo caused by deficiency in the RNA-editing enzyme ADAR1. Journal of Biological Chemistry. 2004; 279(6):4894–902. https://doi.org/10.1074/jbc.M311347200 PMID: 14615479

27. Langmead B, Trapnell C, Pop M, Salzberg SL. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome biology. 2009; 10(3):R25. https://doi.org/10.1186/gb-2009-10-3-r25 PMID: 19261174

28. Anders S, Huber W. Differential expression analysis for sequence count data. Genome Biol. 2010; 11(10):R106. https://doi.org/10.1186/gb-2010-11-10-r106 PMID: 20979621

29. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMNet journal. 2011; 17(1):pp. 10–2.

30. Sedlazeck FJ, Rescheneder P, Von Haeseler A. NextGenMap: fast and accurate read mapping in highly polymorphic genomes. Bioinformatics. 2013; 29(21):2790–1. https://doi.org/10.1093/bioinformatics/btt468 PMID: 23975764

31. Kawahara Y, Ito K, Sun H, Ito M, Kanazawa I, Kwas S. Regulation of glutamate receptor RNA editing and ADAR mRNA expression in developing human normal and Down’s syndrome brains. Developmental brain research. 2004; 148(1):151–5. PMID: 14757529

32. Seeburg PH, Hartner J. Regulation of ion channel/neurotransmitter receptor function by RNA editing. Current opinion in neurobiology. 2003; 13(3):279–83. PMID: 12850211

33. Chang SW, Mislankar M, Misra C, Huang N, DaJusta DG, Harrison SM, et al. Genetic abnormalities in FOXP1 are associated with congenital heart defects. Human mutation. 2013; 34(9):1226–30. https://doi.org/10.1002/humu.22366 PMID: 23766104

34. Tomasselli S, Galeano F, Alon S, Raho S, Galardi S, Polito VA, et al. Modulation of microRNA editing, expression and processing by ADAR2 deaminase in glioblastoma. Genomic biology. 2015; 16(1):5.

35. Liu N, Olson EN. MicroRNA regulatory networks in cardiovascular development. Developmental cell. 2010; 18(4):510–25. https://doi.org/10.1016/j.devcel.2010.03.010 PMID: 20412767

36. Liu N, Bezprozvannaya S, Williams AH, Qi X, Richardson JA, Bassel-Duby R, et al. microRNA-133a regulates cardiomyocyte proliferation and suppresses smooth muscle gene expression in the heart. Genes & development. 2008; 22(23):3242–54.

37. Liu D, Liu C, Wang X, Ingvarsson S, Chen H. Universal RNA editing in a human liver at the fetal stage. 2012.

38. Ekdahl Y, Farahani HS, Behm M, Lagergren J, Öhman M. A-to-I editing of microRNAs in the mammalian brain increases during development. Genome research. 2012; 22(8):1477–87. https://doi.org/10.1101/gr.131912.111 PMID: 22645261

39. Caravia XM, Fanjul V, Oliver E, Roiz-Valle D, Morán-Álvarez A, Desdín-Micó G, et al. The microRNA-29/PGC1α regulatory axis is critical for metabolic control of cardiac function. PLoS biology. 2018; 16(10):e2006247. https://doi.org/10.1371/journal.pbio.2006247 PMID: 30346946

40. Van Rooij E, Sutherland LB, Thatcher JE, DiMaio JM, Naseem RH, Marshall WS, et al. Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. Proceedings of the National Academy of Sciences. 2008; 105(35):13027–32.

41. Hua Y, Zhang Y, Ren J. IGF-1 deficiency resists cardiac hypertrophy and myocardial contractile dysfunction: role of microRNA-1 and microRNA-133a. Journal of cellular and molecular medicine. 2012; 16(1):83–95. https://doi.org/10.1111/j.1582-4934.2011.01307.x PMID: 21418519

42. Zhou X, Borén J, Akyürek LM. Filamins in cardiovascular development. Trends in cardiovascular medicine. 2007; 17(7):222–9. https://doi.org/10.1016/j.tcm.2007.08.001 PMID: 17936203

43. Stulić M, Jantsch MF. Spatio-temporal profiling of Filamin A RNA-editing reveals ADAR preferences and high editing levels outside neuronal tissues. RNA biology. 2013; 10(10):1611–7. https://doi.org/10.4161/rna.26216 PMID: 24025392
44. Czermak P, Amman F, Jantsch MF, Cimatti L. Organ-wide profiling in mouse reveals high editing levels of Filamin B mRNA in the musculoskeletal system. RNA biology. 2018;1–9.

45. Molina-Navarro MM, Triviño JC, Martínez-Dolz L, Lago F, González-Juanatey JR, Portolés M, et al. Functional networks of nucleocytoplasmic transport-related genes differentiate ischemic and dilated cardiomyopathies. A new therapeutic opportunity. PloS one. 2014; 9(8):e104709. https://doi.org/10.1371/journal.pone.0104709 PMID: 25137373

46. Costa RH, Kalinichenko VV, Lim L. Transcription factors in mouse lung development and function. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2001; 280(5):L823–L83. https://doi.org/10.1152/ajplung.2001.280.5.L823 PMID: 11290504

47. Wang B, Weidenfeld J, Lu MM, Maika S, Kuziel WA, Morrisey EE, et al. Foxp1 regulates cardiac outflow tract, endocardial cushion morphogenesis and myocyte proliferation and maturation. Development. 2004; 131(18):4477–87. PMID: 15342473

48. Bai S, Kerppola TK. Opposing roles of FoxP1 and Nfat3 in transcriptional control of cardiomyocyte hypertrophy. Molecular and cellular biology. 2011:MCB. 00925–10.

49. Dillman AA, Hauser DN, Gibbs JR, Nalls MA, McCoy MK, Rudenko IN, et al. mRNA expression, splicing and editing in the embryonic and adult mouse cerebral cortex. Nature neuroscience. 2013; 16(4):499. https://doi.org/10.1038/nn.3332 PMID: 23416452

50. Paul D, Sinha AN, Ray A, Lal M, Nayak S, Sharma A, et al. A-to-I editing in human miRNAs is enriched in seed sequence, influenced by sequence contexts and significantly hypoedited in glioblastoma multiforme. Scientific Reports. 2017; 7.

51. Faccini J, Ruidavets J-B, Cordelier P, Martinis F, Maoret J-J, Bongard V, et al. Circulating miR-155, miR-145 and let-7c as diagnostic biomarkers of the coronary artery disease. Scientific Reports. 2017; 7.

52. Zhang Y, Huang X-R, Wei L-H, Chung AC, Yu C-M, Lan H-Y. miR-29b as a therapeutic agent for angiotensin II-induced cardiac fibrosis by targeting TGF-β/Smad3 signaling. Molecular Therapy. 2014; 22(5):974–85. https://doi.org/10.1038/mt.2014.25 PMID: 24569834

53. Qin D-N, Qian L, Hu D-L, Yu Z-B, Han S-P, Zhu C, et al. Effects of miR-19b overexpression on proliferation, differentiation, apoptosis and Wnt/β-catenin signaling pathway in P19 cell model of cardiac differentiation in vitro. Cell biochemistry and biophysics. 2013; 66(3):709–22. https://doi.org/10.1007/s12013-013-9516-9 PMID: 23443808

54. Zhao J, Lv T, Quan J, Zhao W, Song J, Li Z, et al. Identification of target genes in cardiomyopathy with fibrosis and cardiac remodeling. Journal of biomedical science. 2018; 25(1):63. https://doi.org/10.1186/s12929-018-0459-8 PMID: 30115125

55. Cambroner F, Marín F, Roldán V, Hernández-Romero D, Valdés M, Lip GY. Biomarkers of pathophysiology in hypertrophic cardiomyopathy: implications for clinical management and prognosis. European heart journal. 2009; 30(2):139–51. https://doi.org/10.1093/eurheartj/ehn538 PMID: 19136482

56. del Valle-Pérez B, Martínez VG, Lacasa-Salavert C, Figueras A, Shapiro SS, Takafuta T, et al. Filamin B plays a key role in vascular endothelial growth factor-induced endothelial cell motility through its interaction with Rac-1 and Vav-2. Journal of Biological Chemistry. 2010; 285(14):10748–60. https://doi.org/10.1074/jbc.M109.062984 PMID: 20113058