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Human CXADR Gene – Coxsackie Virus Interaction Analysis to Identify Mutations and SNPs: An Exploratory In–Silico Approach

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Abstract:

Coxsackie viruses belong to the enterovirus family which are usually isolated from the stomach and intestinal regions of the human body. The research we have undertook here is an exploration of the human *CXADR* gene using an *in–silico* approach to demonstrate the power and effectiveness of various online analytical tools. In this attempt to recognize the various mutations that occur in the *CXADR* gene, we have chosen 11 SNPs that have deleterious clinical significance. The junction expression profile reveals, the expression of *CXADR* gene in various tissues. In addition, gene expression profiles of various tissues have enabled us to predict the various diseases that might be caused due to the mutations with varying levels of confidence. The *CXADR* gene plays a very vital role in the interaction of the Coxsackie virus at the molecular level, as evidenced by their part played in causing a disease named Viral Myocarditis.

Keywords: *CXADR* gene, SNPs, Exploration, Coxsackie virus, clinical significance.

Introduction:

Coxsackie viruses are viral infectious agents that belong to the enterovirus family and these viruses are usually extracted from the digestive system of the human body. These viruses can cause a number of infections and have great clinical significance. These viruses have first been identified in the town of Coxsackie in New York, USA in 1947 [1]. Using molecular and cytological studies, their genetic characteristics have been well documented, ever since their discovery. Several strains of this virus such as CVA16, CVA6, CVA10 and CVA9 have been well demonstrate their role in causing disease [2]. Coxsackie virus is known to cause hand, foot and mouth disease (HFMD) among children below five years and generally absent

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in adult and in some cases fatality have also been reported if left untreated. This phenomenon is usually considered rare from type A strains, a key pathogen which tend to infect the skin and mucous membrane thereby causing the syndrome like HFMD while fatal cases have been reported by Wang et al in their research [2,3].

In India, several cases of viral HFMD and other associated diseases caused by the entero-viruses strains have been reported since 1993. Most of the cases have been observed in the states of Tamil Nadu, Kerala, Karnataka, Bihar, Maharashtra and Uttar Pradesh [20, 22]. Latest observations show that small scale epidemics of HFMD have occurred in the cities of Bangalore between 2013 and 2015 [20], while cases have also been observed recently in the states of Kerala, Bihar, and Uttar Pradesh. This makes it more imminent and essential to further analyze the characteristics of this virus to reach a viable solution.

On analyzing the fundamental biological properties and molecular characteristics it was understood that the Coxsackie virus infects humans with the *CXADR* gene. [4]. **Coxsackievirus and adenovirus receptor (CAR)** is a protein that in humans is encoded by the *CXADR* gene. The protein encoded by this gene is a type I membrane receptor for group B coxsackie viruses and subgroup C adenoviruses. This gene is located at 21q21.1 and has 10 exon. It was conserved among other organisms such as mice, rat, rhesus monkey, cow, dog, zebra fish, chicken, and frog [4].

This study was aimed to focus on the fundamental properties and the mutational relationships of *CXADR* gene. Even though, several *in vitro* studies have been done in regards to the attributes of this virus, an *in–silico* study can help us better document the genomic and drug interacting mechanisms of this virus.

Materials and Methods:

Retrieval of *CXAR* gene dataset:

Complete datasets of the *CXADR* gene were retrieved from the Online Mendelian Inheritance of Man (OMIM) [22] database and National Centre for Biotechnology Information (NCBI-GenBank). The single nucleotide polymorphisms (SNPs) were retrieved from NCBI and UNIPROT Databases [8, 9, 10].

Interpretation of similar genes of *CXADR*:

STRING is a database consisting of known and predicted protein-protein interaction data. It shows the relationship between the loaded gene and other genes. The relationship mentioned in STRING are derived on the basis of Genomic context predictions, Lab experiments, Co-Expression, Automated Text mining, and previous knowledge in databases [6]. The result obtained from the STRING represents the linkage between *CXADR* and other correlational genes. The obtained genes are the predicted functional partners, among these genes AMICA1 gene possess the high score with 0.975 followed by NCOA1 with 0.867, LNX1 with 0.828, TJP1 with 0.721, MPDZ with 0.718, PVRL1 with 0.698, CD46 with 0.686, NR1I3 with 0.684, CD55 with 0.678, NR1I2 with 0.672. The scores are obtained on the basis of

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homology, text mining, databases, experiments, co-expression, co-occurrence, gene fusion neighbourhood.

Prediction of number of variations in *CXADR* and unmasking mistrust SNPs on *CXADR*:

Exome variant server provided by the National Heart, Lung and Blood Institute (NHLBI) was used to determine the number of mutations possible with reference to the European American (EA) and African American population (AA) variants. The suspected SNPs for CXADR gene and allele frequency were evaluated and identified through this database. The SIFT algorithm and the Polyphen-2 algorithm are used to forecast the damaging effects of nonsynonymous variant and also the possibly damages tend to cause by the variants of genes. Consequently, the PROVEAN algorithm is used to predict whether an amino acid substitution or indel has any large scale impact over the biological functions of a protein [11, 12, 13]. The Non synonymous SNPs are 11 and those are chosen for the process.

KEGG Analysis of *CXADR*:

The Kyoto Encyclopaedia of Genes and Genomes (KEGG) is a database resource for understanding high-level functions and utilities of the biological system, such as the cell, the organism and the ecosystem, from molecular level information. Here we used the KEGG database to map the pathway of Viral Myocarditis [7].

Prediction of *CXADR* gene-related diseases and expression profile in Humans:

The Ensembl Genome Browser and UCSC Browser were used to elucidate gene expression, junction expression and exon expression of the *CXADR* gene [17]. DISEASES database was used to predict gene-related diseases [14]. Further results are compared with Uniprot and Bioxpress. The Bioxpress was used to investigate the Gene/miRNA expression relationship with cancer [15, 16]. All the results were confirmed with each other and GTEx portal.

RESULTS:

Prediction of *CXADR* related Genes:

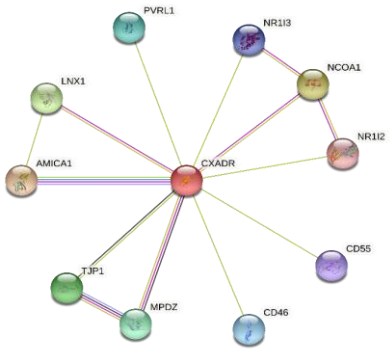

Fig 1: Relationship of *CXADR* with closely related genes.

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Prediction of suspected SNPs in *CXADR*:

The suspected SNPs of *CXADR* gene are mentioned along with the results of SIFT, POLYPHEN-V2, PROVEAN tools respectively (Table 1)

Table 1: Suspected SNPs of *CXADR* gene and pathogenicity predictions

rs Ids	Alleles	GVS Functions	SIFT Prediction (Class: Score)	Polyphen Prediction (Class: Score)	Provean Prediction
rs374465561	C>A	Missense	Deleterious(0.022)	Probably damaging(0.999)	Neutral
rs370613074	A>G	Missense	Deleterious(0.011)	Probably damaging(1.00)	Neutral
rs372788152	G>A	Missense	Deleterious(0.031)	Probably damaging(1.00)	Neutral
rs437470	A>G	Missense	Deleterious(0.026)	Benign(0.0)	Neutral
rs145951623	G>A	Missense	Deleterious(0.00)	Benign(0.007)	Neutral
rs370031613	G>A	Missense	Deleterious(0.015)	Probably Damaging(1.00)	Neutral
rs138470268	C>T	Missense	Deleterious(0.043)	Possibly Damaging(0.955)	Neutral
rs376434359	G>A	Missense	Deleterious(0.00)	Possibly Damaging(0.907)	Neutral
rs143764073	G>A	Missense	Deleterious(0).035	Benign(0.06)	Neutral

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rs142651712	**C>T**	**Missense**	**Deleterious(0.00)**	**Probably Damaging(1.00)**	**Neutral**
rs149825627	**G>C**	**Missense**	**Deleterious(0.00)**	**Probably Damaging(0.999)**	**Neutral**

KEGG Pathway Analysis:

The KEGG analysis is a collection of databases dealing with genomes, biological pathways, diseases, drugs, and chemical substances. The analysis shows about the viral myocarditis (fig.2). Myocarditis is a cardiac disease associated with inflammation and injury of the myocardium. It results from various etiologies, both non-infectious and infectious, but coxsackievirus B3 (CVB3) is still considered the dominant etiological agent. Myocarditis may be caused by direct cytopathic effects of virus, a pathologic immune response to a persistent viral infection, or autoimmune responses triggered by the viral infection. The virus enters the myocyte through internalization of the coxsackie-adenoviral receptor (*CAR*) and its coreceptor, decay-accelerating factor (*DAF*). Viral proteases cleave various proteins in the host cell. One example is viral protease 2A, which cleaves eukaryote initiation factor 4G (eIF4G) and the dystrophin protein, resulting in a complete shutdown of cap-dependent RNA translation and cytoskeletal destruction in infected cardio myocytes, respectively.

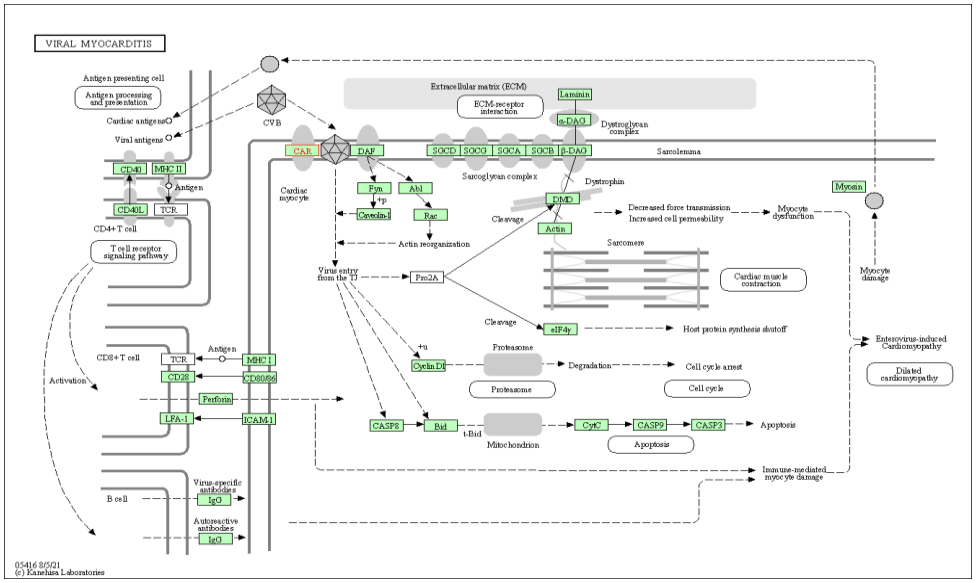

Fig 2: The KEGG Pathway of myocarditis system

Analysing Gene related diseases and expression in Normal tissues and cancer tissues:

We have used the Bioxpress database to analyse the Cancer expressed tissues. The Bioxpress database for differential expression in cancer where RNA-seq and miRNA-seq derived read counts have been analysed for differential expression. The Bioxpress has shown that in

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CXADR Liver cancer (p-Value:0.977, log2FC:-0), Urinary bladder cancer (p-Value:0.059, log2FC: 0.99), Head and neck cancer (p-Value:0.891, log2FC:-0.03), colon adenocarcinoma (p-Value: 0.085, log2FC:-0.27), lung squamous cell carcinoma ((p-Value:0, log2FC:0.79), prostate cancer ((p-Value:0.001, log2FC:0.34) are over expressed and Papillary renal cell carcinoma (p-Value:0.57, log2FC:0.15), stomach cancer (p-Value: 0.344, log2FC: 0.31), breast cancer (p-Value: 0.114, log2FC:-0.27), Thyroid cancer (p-Value: 0.008, log2FC: -0.34), Chromophobe adenocarcinoma (p-Value: 0.204 , log2FC: -0.41), kidney cancer (p-Value: 0, log2FC: -0.9),Oesophageal cancer (p-Value: 0.682, log2FC: -0.2) are under expressed.

We have predicted the Gene related Diseases of *CXADR* and found that it was mostly expressed in skin, prostate, oesophagus, bladder and minor salivary gland (Figs: 3,5)

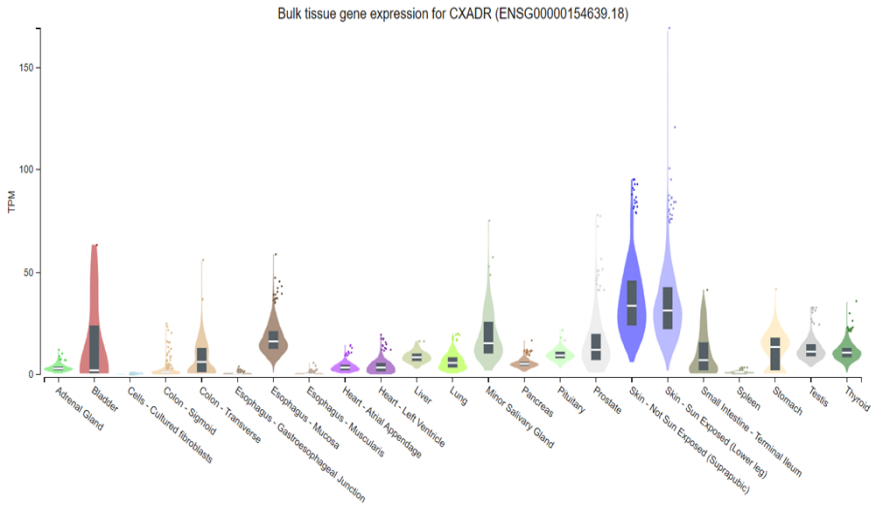

Fig 3: Expression of *CXADR* gene among various tissues

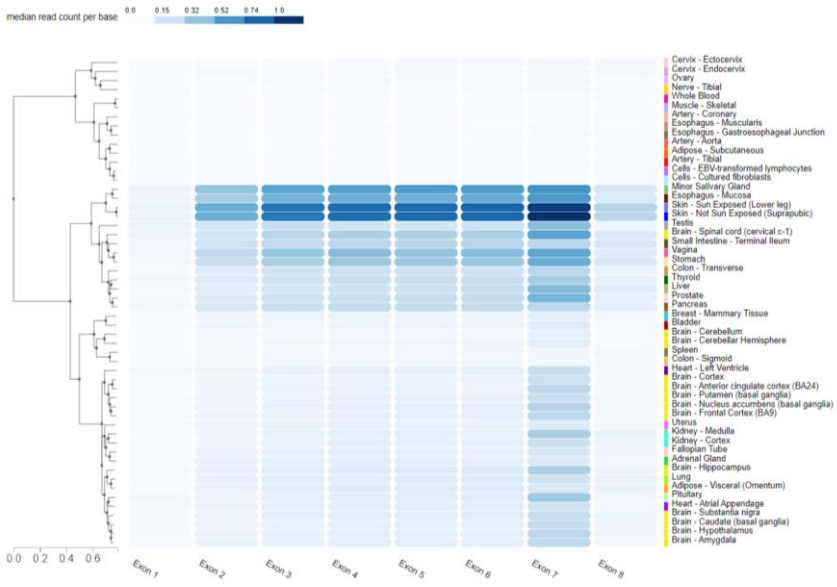

Figure 4: The exon expression of *CXADR* gene.

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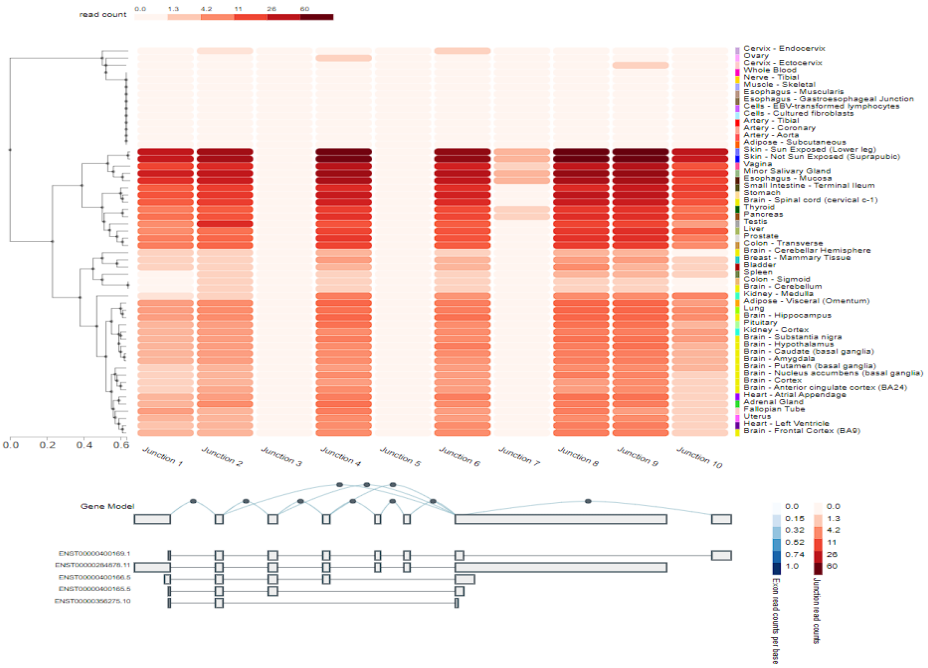

Fig 5: The junction expression of *CXADR* gene

And eventually, with these observations, we have analysed the gene related diseases (Table 2).

Table 2: Predictions of *CXADR* gene with other gene related diseases.

Name	Z-score	Confidence
Myocarditis	**5.1**	★★★☆☆
Cancer	**4.2**	★★★☆☆
Dilated cardiomyopathy	**3.9**	★★☆☆☆
Gastrointestinal system disease	**3.6**	★★☆☆☆
Eye disease	**3.4**	★★☆☆☆
Meningitis	**3.4**	★★☆☆☆
Autosomal dominant hypocalcemia	**3.1**	★★☆☆☆
Brain disease	**3.1**	★★☆☆☆
Lung disease	**3.0**	★★☆☆☆

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DOID:0081062	**2.9**	★★☆☆☆
Connective tissue disease	**2.9**	★★☆☆☆
Carbohydrate metabolic disorder	**2.9**	★★☆☆☆
Poliomyelitis	**2.9**	★★☆☆☆
Breast disease	**2.9**	★★☆☆☆
Pancreatitis	**2.8**	★★☆☆☆
Hematopoietic system disease	**2.8**	★★☆☆☆
Hand, foot and mouth disease	**2.8**	★★☆☆☆
Prostate disease	**2.7**	★★☆☆☆
Herpes simplex	**2.5**	★★☆☆☆
Coronary artery disease	**2.5**	★★☆☆☆

Discussion:

The *CXADR* gene is responsible for the binding of the coxsackie virus to the human tissues and can cause HFMD [3]. The gene expression profiles shows that most gene expression occurs in the Skin cells exposed to sun. Alongside this data, this gene is also expressed in tissues like cardiac tissues, muscle cells, endothelial and epithelial cells. A molecular bioinformatic approach has been undertook to understand the pathogenic properties of this gene.

In developing nations like India, the HFMD and other related diseases caused by this family of viruses, represent a huge problem due to the high population density, and as a result, increased virus transmission rates. This was appropriately evident during the COVID pandemic, since India recorded the highest number of cases as well as deaths, across the globe. Even though the pandemic has mellowed down, it has taught an important lesson about infectious diseases. With the population expected to reach nearly 200 million by the beginning of 2050, the chances of future pandemic events seem all the more possible. These factors are an important reason for why these works are important. Hence, in order to combat these imminent future threats, we need to improve upon our capability to understand viral diseases and produce novel drug compounds and products.

Exomes are the collection of all coding sequences in an organism. By understanding the particular region in an exome where the gene is present, we can become aware of its gene expression patterns in various tissues of the body.

Using KEGG, we have mapped out the gene expression pathway of the *CXADR* gene, the path by which viral myocarditis takes place.

This research venture has the capability to give rise to new ideas about this gene, and this data can be used to further progress in giving rise to new drug compounds that can act against this virus. In addition to this data, supplemental research efforts can also be undertaken, to understand its epidemic causing abilities and to take effective measures to ensure safe drug administration procedures, that can narrow down the infection rates in highly transmissible regions.

Conclusion:

We have found 11 SNPs in the *CXADR* gene which have been observed to be missense, non – synonymous and deleterious variants in comparison with the wild type gene. The gene expression data has illustrated in the expression patterns in various tissues of the human body. The exon expression data shows the organs or tissues where the specified gene is highly expressed. The junction expression data has demonstrated the consistent expression of the *CXADR* gene in the Oesophagus, Minor Salivary gland, skin and testes. With the help of this data, we were able to find or predict what diseases can occur on a higher probability, in correspondence to the different types of mutations that might occur in the *CXADR* gene.

The research we have carried out here has showed the varying effects of the *CXADR* gene under several possible mutations (SNPs) and these outputs can serve as further steps to devise a new drug compound to inhibit the coxsackie virus. Gene expression profiles, their relationships with other similar genes, protein variant effects and gene pathway analysis are important in terms of their bioinformatic research approach. In addition to this, machine learning approaches can also be undertaken to speed up the process of variant identification. Several questions still need to be asked and they can be addressed in further research. This research effort is a venture to contribute to the scientific community, to address meaningful questions in regards to drug discovery and vaccine designing for this rather, persisting viral agent.

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