Identification of essential regulatory elements responsible for the explicit expression of IL-28Rα and their effect on critical SNPs using *in-Silico* methods

Hashaam Akhtar[1, 3*], Gibrail Islam[2], Syed Umer Jan[4], Ayesha Nawaz[6], Samar Akhtar[5], Rune Hartmann[3] and Hajra Sadia[1]

[1]Atta-Ur-Rahman School of Applied Biosciences, National University of Sciences and Technology, Islamabad, Pakistan
[2]Ghulam Ishaq Khan Institute of Engineering Sciences and Technology, Topi, Khyber Pakhtunkhwa, Pakistan
[3]Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark
[4]Faculty of Pharmacy, University of Balochistan, Quetta, Pakistan
[5]Riphah Institute of Pharmaceutical Sciences, Riphah International University, Islamabad, Pakistan
[6]Research Center of Modelling and Simulation, National University of Sciences and Technology, Islamabad, Pakistan

Abstract: IL-28Rα and IL10Rβ collectively construct a fully functional hetero-dimeric receptor for type III interferons (IFNs). IL-28Rα is the private chain for type III IFNs since their involvement in any other pathway has not been reported yet and they are highly expressed in response to certain viral attack or cancers. IL-28Rα is specific in their expression pattern and it expresses within few cell types only. The regulatory mechanisms governing the expression of IL-28Rα at the molecular level are not completely known yet and need to be scrutinized at primary levels. In the present study, various *in-silico* techniques were applied and it was observed that AP1-2, STAT 1-6, P-53, LyF-1 (lymphoid transcription factor), c-Jun, PU.1, CREB (cAMP response element-binding), PLAG (pleotropic adenoma gene), MYOD (myoblast determination protein 1), NOFL and KLFS as transcription factors that are selected with preference. Interestingly AP-2, c-Jun, LyF-1, STAT, NF-Y and P53 have also been reported in literature recently as some of the key regulatory elements as well. Based on the fact that interlinking between different interferon stimulation genes (ISGs) is also not very clear and induction of one type of interferon can affect the efficacy of the other, we found that IFN-λ4 induction can increase the expression of IL-28Rα, similar to IFN-λ3 but contrary to type I IFNs, which has either no effect on the expression of IL-28Rα or can down regulate its expression at higher concentrations (data not published).

Keywords: Interferon Lambda, Interferon lambda receptor (IFNλRα), SNP, Transcription factors, ISG's.

INTRODUCTION

Interferon (IFN) based responses are triggered naturally during viral infections and mediate innate immune responses like the production of type 1 interferon (IFN-α and –β) and a series of other pro-inflammatory cytokines (De Maeyer and De Maeyer-Guignard, 1998; Lefevre *et al*., 1998; Samuel, 2001). In 2003, a class of novel antiviral cytokines IFN-λ1, IFN-λ2, and IFN-λ3 and now IFN λ4 (recent addition) were classified as type III IFNs that evolved independently of type I IFNs (Prokunina-Olsson *et al*., 2013). IFN-λ expression has been shown to depend on the same triggers, i.e. viral infections, Toll like receptor (TLR) ligands and signal transduction pathways (Uze and Monneron, 2007; Ank *et al*., 2008). Regulatory mechanisms of type I IFNs are well elucidated, whereas the expression pattern of all four IFN-λ's are not well understood to date. IFN-λ4 has raised many questions on the characterization of these antiviral proteins (Onoguchi *et al*., 2007). Upstream of *IFNL3* (*IL28B*) on chromosome 19q13.13, a dinucleotide variant ss469415590 (TT or ΔG), is in high linkage disequilibrium with and has been established as potent genetic marker that is strongly associated with HCV clearance (Halfon *et al*., 2011; Lin *et al*., 2011). ss469415590 [ΔG] is a frame shift variant that creates a novel gene, designated as *IFNL4*, encoding the IFN-λ4 protein, which is moderately similar to IFN-λ3 (Prokunina-Olsson *et al*., 2013). Although the reason for this is mainly unclear the disruption of the IFNL4 gene is beneficial for humans in response to HCV infection (Prokunina-Olsson *et al*., 2013; *Hashaam et al., 2013*).

However IFN-λ's are distinct from the other two types of IFNs (i.e. Type I & Type II), as type III subset of IFNs basically differ in their gene locations, they share their own signaling receptor among their own group, they signal through a heterodimer receptor complex like type I interferons but their own private chain known as Interferon lambda receptor 1 (CRF2/12, IFNLR, IL-28R1) is shared with a β chain of IL-10R (Kotenko *et al*., 2003; Sheppard *et al*., 2003).

IL-28Rα (also known as IFN-λRα), a foremost member of class II cytokine receptors, is primarily present on the epithelial cells, B cells, certain macrophages and hepatocytes. An interesting fact has recently been put forth, showing the expression of IL-28Rα on monocyte derived macrophages however; they are neither present on

**Corresponding author:* e-mail: hashaamakhtar@gmail.com

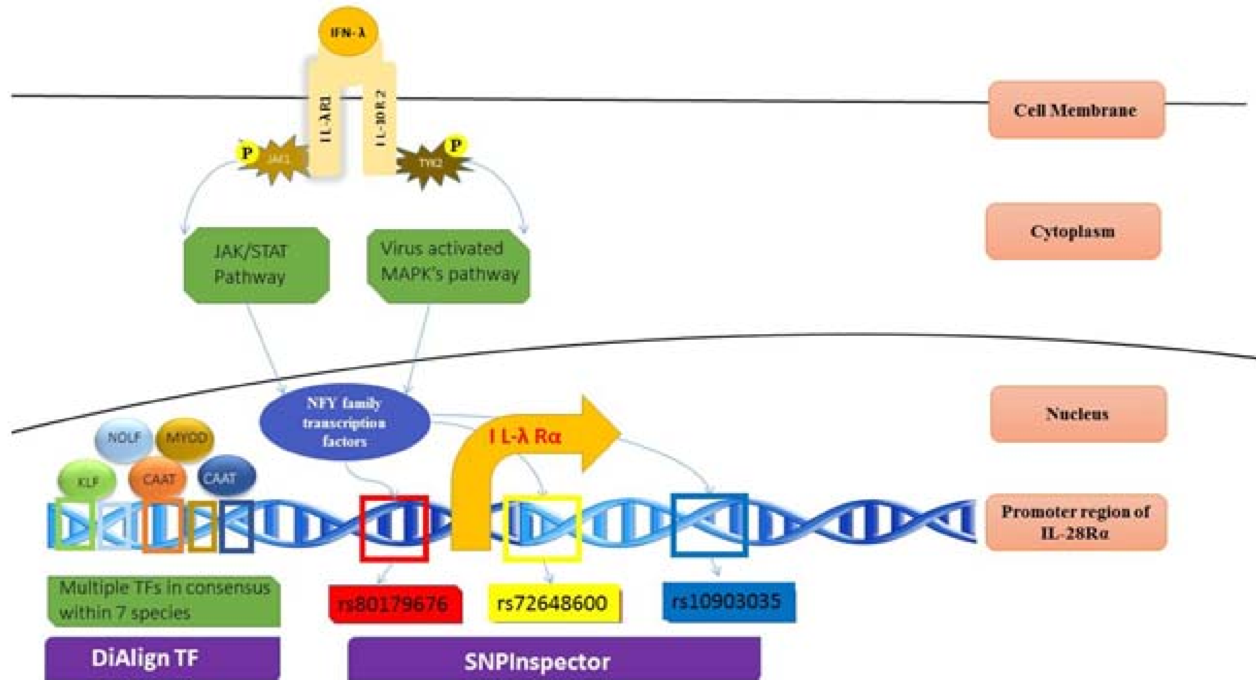

Fig. 1: Key elements describing the expression of IL-28Rα, the receptor protein gets expressed with the combined effect of a series of transcription regulatory elements as highlighted by our study via various computational tools. NFY-A as per supported by our results from SNPInspector are; family of TFs shown to play significant role in the expression (reported as various SNPs in a series of literature as well). Alongside are the results from DiAlign TF suggesting the binding sites for a series of differentially expressed TFs that are in consensus to 7 of the notable vertebrate species.

the precursor monocytes nor on monocytes driven dendritic cells (Liu *et al*., 2011a). It is for this reason that IFN-λ plays a role in inducing TLRs induced cytokine production in monocyte derived macrophages only (Liu *et al*., 2011b). In addition to this, the epigenetic reprogramming/silencing has also been shown to play a role in the selective expression of IL-28Rα (Ding *et al*., 2014).

Selective expression of IL28Rα/IL10Rβ has made type III IFN's more specific or a targeted drug and clinical trials are being conducted by zymogenetics using these biomarkers (ZymoGenetics, 2010). Whereas type I IFN receptors are relatively more expressed on most cell types, including white blood cells, which result in abnormalities like anemia, neutropenia, and thrombocytopenia, after or during combined therapy using pegylated (or standard) interferon and ribavirin in chronic hepatitis C treatment (Ong and Younossi, 2004).

To further elucidate this difference, it is important to study the development and differentiation process of both these cells. The haematopoietic stem cells (HSC) in the bone marrow give rise to lymphoid and myeloid lineages of immune cells (Stec *et al*., 2013). The monocytes are produced from the myeloid lineage -which later gives rise to macrophages as well as dendritic cells (Stec *et al*., 2013; Galibert *et al*., 2001; Gaudernack and Bjercke, 1985; Van Voorhis *et al*., 1983).

In viral infections, innate immune responses are initiated when either viruses or their genetic material is recognized by cellular pattern recognition receptors such as TLRs or RIG-1/ MDA-5 leading to the activation of several transcription factor systems and their receptors' gene expression (Onomoto *et al*., 2012; Saito and Gale, 2008; Takeuchi and Akira, 2008).

IL-28Rα is largely restricted to cells of epithelial origin (Donnelly and Kotenko, 2010). We hereby propose a model for IL-28Rα gene regulation, where we show that the expression of IL-28Rα depends on specific transcription regulating factors that activate the gene expression independently via promoter elements. Factors regulating the post transcriptional regulation of this IL-28Rα are also covered in this study.

Type III IFN genes have great influence in regulation at transcriptional levels (Ong and Younossi, 2004). Both type I and type III IFNs are induced through transcriptional mechanisms involving the transcription factors, IFN regulatory factors (IRFs) and nuclear factor (NF) kB (Iversen and Paludan, 2010). According to a study, the inhibition of the NF-kB pathway in DCs and in

animals strongly inhibits the induction of IFN-λ expression but only has a minor effect on the expression of type I IFN's (Iversen and Paludan, 2010).

There are gaps in the studies related to the factors regulating the expression as well as the induction of IL-28Rα, therefore the current study is aimed to identify those responsible key regulatory factors involved in the expression of IL-28Rα receptor and in addition to this we explore the single nucleotide polymorphism reported on those transcription factor binding sites to increase the understanding about the effects of transcription factors in expression as well as treatment outcomes of interferon therapies.

In the start of 2014, Ding *et al*. found relationship between the expression of IL-28Rα and a transcription factor called NF-YA, which functions in collaboration with E2F family TFs, CBFA2 and RUNX1: also predicted in our research (Ding *et al*., 2014). PNRC2 (Proline-rich Nuclear Receptor co-activator 2) is a novel co-activator for multiple nuclear receptors and it is widely expressed in lungs, spleen, ovary, thymus, and colon. Two transcription factors; NFY (nuclear factor Y) and E2F1have been shown to have effect on the expression pattern of other co-activators of various receptors. These transcription factors work when DNMT and histone deacetylases (HDAC) are blocked naturally in IL-28Rα expressing cell lines or artificially by inhibitors like 5azadC or MS-275 in non- IL-28Rα expressing cell lines.

MATERIAL AND METHODS

Sequence data

The sequence data used during the searches was Homo sapiens interferon, lambda receptor 1 (IFNLR1 also called CRF2/12; IFNLR; IL-28R1; IL28RA; LICR2), transcript variant 1, mRNA. The NCBI reference or accession ID for the transcript used is NM_170743.3. The reference sequence is 4563bp long and the CDS region ranges between 28-1590 nucleotides. We applied various computational tools in our present study to predict transcription factors (TF) and transcription factor binding sites (TFBS) involved in the selective expression of IFNλRα. Some of the noticeable softwares are discussed below with some basic introduction.

1. Gene-regulation\BIOBASE

(http://www.biobase international.com/product/transcription-factor-binding-sites)

We initially started with using 'Transfac professional' software, provided by Biobase to predict the TFs involved in the expression of IL-28Rα, as Transfac provides the most comprehensive assortment of TFs, majority of which are usually experimentally proven through chromatin immunoprecipitation (ChIP) or many labs trust on its authenticity in carrying on their further experiments. The options in prediction of TFBS given by TRANSFAC® are as follows

a. Match

It is prediction software, which uses library of positional weight matrices (PWMs) from TRANSFAC® Public 6.0 to predict TFBS in DNA sequences for potential TFBS. The output file contains list of matched matrices, for each factor there is a list of supported literature information about the transcription factor and its preferred binding sequence (Kel *et al*., 2003). The MATCH option uses the Match algorithm, in combination with a selected profile containing a list of matrices and their assigned cut-offs to search for individual transcription factor binding sites that meet the specified cut-offs.

b. F-match

Statistically over-represented TFBS are compared with control sets in this program and it assumes the binominal distribution of TFBS frequency. F-Match uses the Match algorithm with the same library of positional weight matrices from TRANSFAC®6.0.

Both the MATCH and F-match softwares was used using the default parameters set as p-value threshold as 0.01, and the cut off was set so as to reduce false positives.

c. AliBaba2.1

AliBaba2 was also used to predict the transcription factor binding sites using TRANSFAC® Public for the prediction in our DNA sequence.

2. Genomatix Software Suite

Genomatix Software GmbH is a German company with many biological and computational types of software (http://www.genomatix.de) and four of them, which we used in the present study are:

a. Mat Inspector

It was used for finding physical TF binding sites (TFBS), this tool uses the library of matrix descriptions for TFBS to identify matches in DNA sequences. The filters and the quality of selection are quite reliable as compared to other softwares. Its use is in practice since 2005 and has been cited in many publications (Quandt *et al*., 1995; Cartharius *et al*., 2005).

Key features of Mat Inspector include various vital and helpful outputs like TFBS grouping as matrix families, graphical representation of results, promoter finding and the overlapping involved, lastly the TFBS predictions and ChIP seq data.

b. DiAlign TF

It displays TFBS with help of Mat Inspector with in a multiple alignment. It shows its results in colour boxes

with the most realistic ones to be on top and is very user friendly. It performs pair wise alignment and displays the diagonal similarity as output. The output is generated as common TF matches located in the aligned regions common to 7 (70%) of sequences.

c. SNPInspector

It was used to identify TF sites affected by SNPs. SNPInspector analyses the potential effects of a single nucleotide polymorphism (SNP) associated with your sequence. For each SNP allele the transcription factor binding sites either deleted or generated by the nucleotide exchange are determined. The analysis is based on MatInspector and Genomatix' library of matrix descriptions for transcription factor binding sites.

d. Overrepresented transcription factor binding sites or modules

Searches for all transcription factor binding sites (TFBS) within the input regions and generates statistics on TFBSs and TFBS pairs (modules) together with over-representation values and Z-scores compared against genomic and promoter background. Limit: max. 20000 regions with at most 10000 bp each. Database version ElDorado 12-2013 for our sequence file input was used. The data was compared against the genomic background of Homosapiens, NCBI build 37. The matrix library version of the software used was Matrix Library 9.2.

3. Qiagen

Qiagen is a web based tool available which uses SA Bio sciences' proprietary database (http://www.sabiosciences.com/online_order.php) and combines text mining application (uses information from published data and other public resources) and data from the UCSC Genome Browser (developed and maintained by the Genome Bioinformatics Group) to compile a list of predicted binding sites for over 200 human transcription factors for every gene in the human genome.

4. TESS

This program is pretty easy to use and established the confidence in results through various statistical score values. This program converts CDS input into reads (selected) Partial Weight Matrices (PWMs) from a file and predicts binding sites on DNA sequences read from another file. Hits that have a good enough score are reported as statistical scores (Schug, 2008).

RESULTS

With the application of various software tools we were able to short list few dependable hits of TFs, which included many of those, which are already part of JAK-STAT pathway or many of those TFs, which are identified in nearly all essentials pathways involved in the differentiation of cells. Literature review has also helped us in showing some nearest hits like AP-2, c-JUN, STAT-1 or LyF-1 (Yang *et al*., 2010). tables 4 and 5 show the summary of all the predicted TFs as per reported by using various computational tools and many of them are reported with significant confidence as being similar to ones predicted before in literature.

1. Gene-Regulation\BIOBASE

a. Match(gene-regulation.com)

A portion of results from MATCH (gene-regulation.com) having various transcription factor binding sites with their sequences, positions, core matches and the matrix matches in IL-28Rα gene of Homo sapiens was selected. Matrix match scores are describing the score of the complete matrix match (more important values, ranges between 0-1) and the core similarity is the score of the highest conserved positions of a matrix match (Kel *et al*., 2003). Both thresholds have to be reached for a matrix match and selected summary of results is listed in table 4. c-Rel, ElK-1, c-Ets-1(p54), STATx, AP-1, NF-kappaB, NKx2-5 and v-Myb are reported with matrix similarity values of 0.987, 0.990, 0.993, 1.0, 0.976,1.0, 1.0, 0.971 respectively as the TFs with greater confidence (Complete result is attached as supplementary data).

b. F-MATCH

Table 1: Summary table from the results obtained using F-Match searches showing important Transcription factors as reported. These results were obtained from analyzing our sequence of length: 4563 having total number of sequences: 1 & Total number of sites: 267 Number of sequences with sites: 1, Frequency of sites: 0.05851 and Average number of sites per sequence: 267.00 against the background human genome version: human/hg38

STAT1	STAT3	ELF4	BR-C Z2	ZIC3	Mbp1p	HNF-I alpha	FPM315	PLAG	T3Rbeta	YLR278C
GABP alpha	ER81	ATF3	ISL2	COE1	ETS1	C-Rel	Tcf1DEC 2	ELF1	Sin3A	STATB1
Zic2	STAT5B	Arnt	Zic3	P50 (REL-P65)	NF-KappaB (P50)	Dde box	c-Myb	Zic1	CTCF	CPBP
TF3C -beta	Stb5p	Myogenin /NF-1	STRE	Eve	FOXN1	RXR-alpha	CHES1L	FOXN4	Ybr239c	Prrx2

c. AliBaba2.1

Table 2: Some of the notable transcription factors in IL-28Rα gene in Homo sapiens selected from a set of 164 segments (complete in supplementary data) as potential binding sites reported by AliBaba2.1.

Sp1	NF-1	MyoD	YY1	Myf-3	NF-1
GATA1	NFkappaB	C/EBP beta	REB1	REVErbA alpha	RSRC4
GBF1/2	sox2	ICSBP	EBP-1	NF-EM5	ISGF-3

2. Genomatix software suite
a. MatInspector

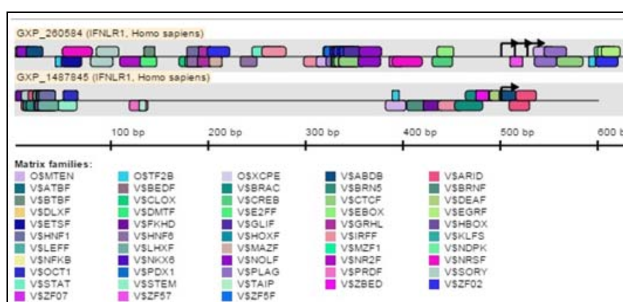

Fig. 2: Graphical representation from the results obtained using Mat Inspector from Genomatix software suite of various transcription factor-binding sites in multiple sequences of IL-28Rα gene in Homo sapiens. By examining the detailed Mat Inspector result file some TFs (complete supplementary data) were found to be the preferred Matrix family with p-values lesser than 0.5 out of the 127 obtained matrix families as shown in summary table 4 at the end.

b. DiAlign TF

Fig. 2: Results from MatInspector shows various transcription factor binding sites in IL-28Rα gene of various species, which have an influence in the evolution of this receptor. It has compared the gene in Homo sapiens, rhesus monkey, chimpanzee, mouse, rat, rabbit, horse, cow, pig, dog (vertebrates). KLFS, NOLF, CAAT, MYOD, PLAG as common TFs located in the aligned regions in consensus within 7 organisms (70%) of their sequences.

c. SNPInspector: Identify TF sites affected by SNPs

Significant results from SNPInspector displaying SNPs located in coding exons, which influence the protein sequence were selected. The variant calls and reported factor family NFY are duly supported by literature as well (table 5). These results were obtained using NM_170743 (1 sequence, 4563 bp) found on chromosome 1 of Homo sapiens, NCBI build 37, ElDorado 08-2011 Extracted region: NC_000001 between 24472207 and 24522206 (50000 bp) as analysis parameters.

d. Overrepresented transcription factor binding sites or modules

Genomatix Overrepresented TF families tool displays the binding sites were distributed based on the Z-score that shows the distance of our sequence from the population mean in units of population standard deviation. Values of promoter association that shows how many TF Families known to occur more than twice as often in promoters as in genomic sequence were also taken into consideration for choosing the results. The results reveal CTCF, GLIF, PLAG, NOLF, NFKB, AP2F, WHNF, SP1F, ZFHX, ZF02, NGRE, NRSF, NRF1, SAL2, PRDM, MAZF, STAF, HESF, KLFS, ZF07, MTEN, CP2F, ESRR, BEDF, HIFF, MYBL, XBBF, TF2D, ZF01, PURA, TELO, INSM, HAML, ZF5F, CSEN, OAZF, MYOD, HICF, NACA, RXRF, SNAI, CDEF as over represented TF families with Z-score (number of standard deviations an observation or datum is above the mean) values greater than 2.5 are listed in table 5.

3. Qiagen

Qiagen online tool provided results in graphical form; which displays the most relevant transcription factors up to 10 in 20kb upstream and 10kb downstream of gene IL28Ra as per reported by Qiagen. Results from Qiagen shows various transcription factor binding sites in IL-28Rα gene of Homo sapiens, where STAT 1, 3 and 5 have various TFBS, in addition to this Rel A ,c-Fos, c-Jun, Lyf-1 NF-KappaB and others with significant sequence complementarity for binding at various transcription factor binding sites.

4. TESS

Table 3: Selected results from TESS software shows various transcription factor binding sites in IL-28Rα gene in Homo sapiens like Sp1, MIG1, FSADR1, LGC, LVC as over-represented transcription factors with significant statistical values passing the threshold filters (log likelihood, core similarity and p-values) providing confidence in our results.

Sp1	LBP1	MIG1	T-Ag	FACB	TEF	CAC	TEF	Lvc
FACB	GR	NFY	UCRF-L	GCF	ER-alpha	ADR1	ADR1	

Table 4: Summary table of the results obtained using Gene regulation Biobase TRANSFAC suite, reported here are TF's chosen based on supporting statistical threshold values.

Gene Regulation Biobase TRANSFAC®		
a) Match	b) F-Match	c) AliBaba 2.1
COMP1	STATx	Sp1
c-Rel	USF	YY1
CHOP-C/EBPalpha	AML-1a	Myf-3
Elk-1	SRY	NF-kappaB1
c-Ets-1(p54)	ZID	AP-2alphaA
STATx	CBF-A	C/EBPalpha
Pax-4	c-Rel	MyoD
AP-1	NF-kappa B	
c-Rel	Nkx-2	
ZID	Lyf-1	
NF-kappaB	AML-1a	
Nkx2-5		
Pax-4		
v-Myb		
NF-1		

DISCUSSION

Interferon Lambda receptor is a heterodimeric receptor comprising of two subunits IL-28Rα (α-subunit; encoded on chromosome 1) and IL-10Rβ (β-subunit; encoded on chromosome 21 (Jimenez-Sousa *et al*., 2014; Miknis *et al*., 2010; Mihm *et al*., 2014; Mihm *et al*., 2004). When the ligand binds with the IFN-λR it results in the dimerization of the receptor which further leads to the activation of Janus kinase (JAK1) & tyrosine kinase 2 (Tyk 2), phosphorylation of STAT1 & STAT2 whereby Interferon Regulatory Factor 9 (IRF9) combines to form the IFN-stimulated gene factor-3 (IRF-3) transcription factor complex, which induces the expression of multiple interferon stimulatory genes (ISGs). The beta part of the heterodimeric receptor (IL-10Rβ) has a broad expression pattern, whereas expression of IL-28Rα is tissue specific and is restricted to limited type of cells only (Josephson *et al*., 2001). The regulation of the expression of IL-28Rα at its molecular level is not fully known yet and needs to be scrutinized at primary levels. To date, no complete model for the IL-28Rα -IL-10RB complex expression exists (Egli *et al*., 2014). Action of type III IFNs is mediated through a heterodimeric receptor (IL28Rα and IL10Rβ),

Table 5: Summary table of the results obtained using Genomatix software suite reported here are TF's chosen based on supporting statistical threshold values.

Genomatix Software Suite											
a. MatInspector											
MTEN	TF2B	TF2D	XCPE	AARF	ABDB	AHRR	AIRE	AP1F	AP2F	ZF03	SORY
ARID	ATBF	BCDF	BCL6	BEDF	BRAC	BRN5	BRNF	BTBF	CART	ZF07	SPZ1
CDEF	CDXF	CEBP	CHOP	CHRE	CIZF	CREB	CTCF	DEAF	DLXF	ZF08	SRFF
DMRT	DMTF	E2FF	EBOX	EGRF	ESRR	FKHD	FXRE	GFI1	GLIF	ZF13	STAF
GRHL	HAML	HDBP	HDBP	HDGF	HESF	HICF	HMTB	HNF1	HNF6	ZF35	STAT
HNFP	HOMF	HOXC	HOXH	HZIP	IKRS	INSM	IRFF	KLFS	LTSM	ZF57	STEM
MAZF	MEF2	MOKF	MYOD	MYT1	MZF1	NDPK	NF1F	NFKB	NKX1	ZF5F	TAIP
NKX6	NOLF	NRF1	NRSF	OSRF	P53F	PARF	PAX3	PAX5	PAX9	ZFHX	WHNF
PAXH	PDX1	PLAG	PRDF	PURA	RORA	RXRF	SATB	SF1F	SMAD	ZBED	YY1F

a. DiAlign TF	**b. SNPInspector**
KLFS	rs80179676 (NFY.01)
NOLF	rs10903032 (NRF.01)
CAAT,	rs10903034 (NYMC.01)
MYOD	rs10903035 (NFY.03)
PLAG	rs11249002 (TCFAP2B.01)
TSS	rs11249006 (MYBL.01)
	rs72648600 (NFY.03)

c. C. Overrepresented TFBS									
CTCF	GLIF	PLAG	NOLF	NFKB	AP2F	WHNF	SP1F	ZFHX	ZF02
NGRE	NRSF	NRF1	SAL2	PRDM	MAZF	STAF	HESF	KLFS	ZF07
MTEN	CP2F	ESRR	BEDF	HIFF	MYBL	XBBF	TF2D	ZF01	PURA
TELO	INSM	HAML	ZF5F	CSEN	OAZF	MYOD	HICF	NACA	

which upon activation leads to the downstream activation of series of transcription factors that drive the ISGs expression. So it is a matter of immense importance to explore the regulation of the receptors so as to define better drugs targeting the expression of receptors responsible for activation as well as for inactivation resulting in the inhibition of various pathways. Moreover there is a need to develop an interlinking among different ISGs, which is really obscure under current scenario. Induction of one type of interferon can affect the efficacy of the other, with reference to its expression levels or therapeutic effects, therefore a gap is still to be filled with findings answering this correlated expression patterns, synergistic and antagonistic effects of this class of cytokines. We in this *in-silico* study have shown the prediction of transcription factors involved in regulating the expression of IL-28Rα, we have furthermore answered the reason behind the resistance of IFN-α therapy and few crucial single nucleotide polymorphism (SNP) site that is one of the most important transcription factor binding site (TFBS) and treatment outcome of IFN-α therapy is somehow interconnected with the expression pattern of IL-28Rα. Important TFBS have been identified within the promoter region of the IL-28Rα gene (Maher *et al*., 2008).

Resistance in IFN-α therapy has long been a matter of concern, though many considerable points have been raised by scientists and studies have proven their relevance with many human and viral factors like genotype, age, viral load, co-infections, insulin resistance, obesity and IL-28 polymorphism but still we are unable to eradicate this issue in HCV treatment (Akhtar *et al*., 2013). Scientists have developed diagnostic tests to predict the outcomes of IFN-α therapy but all of them are ethnic based and many of them are unable to answer the mystery of its dependency on single nucleotide polymorphism (Knapp *et al*., 2011). IL28B gene polymorphism is a reliable baseline predictor of response to IFN-α -based antiviral therapies in chronic hepatitis C (Holmes *et al*., 2011; Imran *et al*., 2015; Ionita-Radu *et al*., 2011; Mangia *et al*., 2010; Susser *et al*., 2014). The expression pattern of all four IFN-λ's are not well understood to date as IFN-λ4 has raised many questions on the beneficial expression of these antiviral proteins recently. Due to confounding role of this IFN-λ4, there has been a lot of research going around exploring the pathways involved and their behavior of activations and deactivations.

Softwares like MatInspector and TESS were used in this study, where MatInspector utilizes a large library of matrix descriptions for transcription factor binding sites to locate matches in DNA sequences and TESS implements a Bayesian clustering algorithm for spatial population genetic analyses. It can perform both individual geographical assignment and admixture analysis. It is designed for seeking genetic discontinuities in continuous populations and estimating spatially varying individual admixture proportions (Cartharius *et al*., 2005). Our results highlighted few responsible TFs, where we employed. DiAlign TF it displays TFBS with help of Mat Inspector with in a multiple alignment. It shows its results in color boxes with the most realistic ones to be on top and is very user friendly.

We employed Genomatrix software, which is an integrated solution for comprehensive visualization and interpretation for gene regulation. It has various tools like SNPInspector which is a genomatrix genome annotation which is used to shortlist the most prevalent SNPs. Multiple SNPs found in IL-28Rα were studied for their further importance (rs10903032, rs10903034, rs10903035, rs11249002 and rs11249006) and their role as TFBS were also premeditated in this contest. SNP rs10903035 (GA or GG) is within the 3′ untranslated region of the IL-28Rα mRNA sequence and was an independent risk factor for IFN-α treatment failure against HCV (Egli *et al*., 2014; Wang *et al*., 2014). In the previous study, we showed that inducing IFN-α or IFN-λ on monocyte differentiated to macrophages had effected the expression of IL-28Rα, whereas the higher concentration of IFN-α can also down regulate the expression of IL-28Rα even in hepatocytes (data under process of publication). Out of many SNPs found, we narrowed our search to those, which were important in the binding of various important transcription factors or involves (creates or vanishes) the TFBS. Out of the considered sites, we selected those TFBS, which were involved in the transcription of IL-28Rα through binding of mainly NFY TF-family.

We emphasized on four dbSNPs i.e. rs10903035, rs80179676, rs72648600 and rs6698365, where rs10903035 had its significance in literature previously and was linked with the outcomes of HCV infections in Chinese population (Cui *et al*., 2011). Results of our study have disclosed the reason behind consequences of rs10903035 as a crucial polymorphism; the AA genotype had a significant increased risk of persistent HCV infection. G → A mutation makes a new site for the transcription factor NFY, which has been proven to play an important role in the expression of IL-28Rα, as the TFBS of NFYA is easily accessible in IL-28Rα expressive cells as compared to the non-expressive cells and HDAC mediated closed chromatin conformation are the main silencing mechanism of IL-28Rα expression in IFN-λ unresponsive cells (Ding *et al*., 2014).

Over expression of IL-28Rα could create redundancy and can be the reason behind resistance of IFN-α therapy in non-responsive hepatitis patients. In addition to this, the AA genotype of rs10903035 has been found to be associated with the insulin resistance in HIV\HCV co-

infected patients, as it seems to have effects in the glucose homeostasis (Jimenez-Sousa *et al*., 2014).

Duong *et al*., has shown in their article that the IFN-λ3 genotype was associated with the expression level of IL-28Rα but not with differential expression of IFN-λ and confiding our results, the missing link between the IFN-λ3 genotypes and the non-responder phenotypes of IFN-α could be the transcription factor binding sequences and the polymorphism involved in them (Duong *et al*., 2014). Expression of IL-28Rα is tissue specific and the epigenetic reprogramming of the IL-28Rα gene is dependent on the binding of TFs at the particular sites. There is a possibility that the IFN-λ3 genotype may also contain some SNP(s) that effect the expression of IL-28Rα as we have seen rs10903035 as the binding site for NFY-A.

CONCLUSION

The current study reveals that the single nucleotide polymorphism in IL-28Rα is crucial in its expression and can create or remove the transcription factor binding sites, which can ultimately affect the efficacy of IFN-α in obtaining sustained virologic response (SVR). It can therefore be strongly implied that individualization of IFN-α - therapies based on a combination of IL28B/ IFN-λRα polymorphisms may help to optimize virologic outcomes and save economic resources. It has the potential for predicting the treatment outcomes of HCV infections. Meta-analysis of IL-28Rα genes with reference to their prognostic value elucidates that it's expression is altered in cancer prognosis, viral attacks and IFN therapies. Keeping an eye on the expression pattern and the TFBS available before the start of any therapy may contribute imperatively for better and efficient disease prognosis in future.

ACKNOWLEDGMENT

We are thankful to Assoc. Prof. Rune Hartmann, (Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark) for their assistance in developing the baseline for the science performed in the current article and to Dr. Hamaad Ahmed (Yusra Institute of Pharmaceutical sciences) for critical reading of the manuscript and for discussion. We are also grateful of National University of Sciences and Technology (NUST) and Higher education commission (HEC), Pakistan for supporting us in conducting this research.

REFERENCES

Akhtar H, Akhtar S, Raheel U, Faheem M, Arshad M, Yameen M and Zaidi NS (2013). Interferons as immune regulators: A rivalry between HCV and interferons. *J. Clin. & Cellu. Immunol.,* **04**: 136-143.

Akhtar H, Akhtar S, Jan SU, Khan A, Zaidi NS and Qadri I (2013). Over expression of a synthetic gene encoding interferon lambda using relative synonymous Codon usage bias in Escherichia coli. *Pak. J. Pharm. Sci.,* **26**(6):1181-1188.

Ank N, Iversen Mb, Bartholdy C, Staeheli P, Hartmann R, Jensen Ub, Dagnaes-Hansen F, Thomsen Ar, Chen Z, Haugen H, Klucher K and Paludan Sr (2008). An important role for type III interferon (IFN-lambda/IL-28) in TLR-induced antiviral activity. *J. Immunol.,* **180**(4): 2474-2485.

Cartharius K, Frech K, Grote K, Klocke B, Haltmeier M, Klingenhoff A, Frisch M, Bayerlein M and Werner T (2005). MatInspector and beyond: Promoter analysis based on transcription factor binding sites. *Bioinformatics,* **21**(13): 2933-2942.

Cui Q, Zhang Yx, Su J, Chen X, Ding K, Lei N, Liu Y, Li J, Zhang Y and Yu Rb (2011). Genetic variation in IL28RA is associated with the outcomes of HCV infection in a high-risk Chinese population. *Infect. Genet. Evol*., **11**(7): 1682-1689.

De Maeyer E and De Maeyer-Guignard J (1998). Type I interferons. *Int. Rev. Immunol.,* **17**(1-4): 53-73.

Ding S, Khoury-Hanold W, Iwasaki A and Robek Md (2014). Epigenetic reprogramming of the type III interferon response potentiates antiviral activity and suppresses tumor growth. *PLoS Biol.,* **12**(1): e1001758.

Donnelly Rp and Kotenko Sv (2010). Interferon-lambda: A new addition to an old family. *J. Interferon Cytokine Res.,* **30**(8): 555-564.

Duong Fh, Trincucci G, Boldanova T, Calabrese D, Campana B, Krol I, Durand SC, Heydmann L, Zeisel MB, Baumert TF and Heim MH (2014). IFN-lambda receptor 1 expression is induced in chronic hepatitis C and correlates with the IFN-lambda3 genotype and with nonresponsiveness to IFN-alpha therapies. *J. Exp. Med.,* **211**(5): 857-868.

Egli A, Santer DM, O'shea D, Tyrrell DL and Houghton M (2014). The impact of the interferon-lambda family on the innate and adaptive immune response to viral infections. *Emerg. Microbes Infect*, **3**(7): e51.

Galibert L, Maliszewski CR and Vandenabeele S (2001). Plasmacytoid monocytes/T cells: A dendritic cell lineage? *Semin. Immunol.,* **13**(5): 283-289.

Gaudernack G and Bjercke S (1985). Dendritic cells and monocytes as accessory cells in T-cell responses in man. I. Phenotypic analysis of dendritic cells and monocytes. *Scand. J. Immunol*., **21**(5): 493-500.

Halfon P, Bourliere M, Ouzan D, Maor Y, Renou C, Wartelle C, Penaranda G, Tran A, Botta D, Oules V, Castellani P, Portal I, Argiro L and Dessein A (2011). A single IL28B genotype SNP rs12979860 determination predicts treatment response in patients with chronic hepatitis C Genotype 1 virus. *Eur. J. Gastroenterol Hepatol,* **23**(10): 931-935.

Holmes JA, Sievert W and Thompson AJ (2011). IL28B polymorphism and genetic biomarkers of viral

clearance in hepatitis C virus infection. *Biomark. Med.,* **5**(4): 461-478.

Imran M, Manzoor S, Azam S and Resham S (2015). Genetic variant of IL28B rs12979860, as predictive marker of interferon-based therapy in Pakistani population. *APMIS*., **123**(4): 342-349.

Ionita-Radu F, Rascanu A and Cheiab B (2011). IL28B polymorphism - predictive factor of HCV infected genotype 1 individuals to treatment response and management of therapy. *Rom. J. Intern. Med*., **49**(2): 99-104.

Iversen MB and Paludan SR (2010). Mechanisms of type III interferon expression. *J. Interferon Cytokine Res*., **30**(8): 573-578.

Jimenez-Sousa MA, Berenguer J, Fernandez-Rodriguez A, Micheloud D, Guzman-Fulgencio M, Miralles P, Pineda-Tenor D, Garcia-Alvarez M, Lopez JC, Aldamiz-Echevarria T, Carrero A and Resino S (2014). IL28RA polymorphism (rs10903035) is associated with insulin resistance in HIV/HCV-coinfected patients. *J. Viral Hepat*., **21**(3): 189-197.

Josephson K, Logsdon NJ and Walter Mr (2001). Crystal structure of the IL-10/IL-10R1 complex reveals a shared receptor binding site. *Immunity*, **15**(1): 35-46.

Kel AE, Gossling E, Reuter I, Cheremushkin E, Kel-Margoulis OV and Wingender E (2003). MATCH: A tool for searching transcription factor binding sites in DNA sequences. *Nucleic Acids Res*., **31**(13): 3576-3579.

Knapp S, Warshow U, Ho KM, Hegazy D, Little AM, Fowell A, Alexander G, Thursz M, Cramp M and Khakoo Si (2011). A polymorphism in IL28B distinguishes exposed, uninfected individuals from spontaneous resolvers of HCV infection. *Gastroenterology*, **141**(1): 320-325.

Kotenko SV, Gallagher G, Baurin VV, Lewis-Antes A, Shen M, Shah NK, Langer JA, Sheikh F, Dickensheets H and Donnelly RP (2003). IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. *Nat. Immunol*., **4**(1): 69-77.

Lefevre F, Guillomot M, D'andrea S, Battegay S and La Bonnardiere C (1998). Interferon-delta: The first member of a novel type I interferon family. *Biochimie*. **80**(8-9): 779-788.

Lin CY, Chen JY, Lin TN, Jeng WJ, Huang CH, Huang CW, Chang SW and Sheen IS (2011). IL28B SNP rs12979860 is a critical predictor for on-treatment and sustained virologic response in patients with hepatitis C virus genotype-1 infection. *PLoS One*., **6**(3): e18322.

Liu BS, Janssen Hl and Boonstra A (2011a). IL-29 and IFN alpha differ in their ability to modulate IL-12 production by TLR-activated human macrophages and exhibit differential regulation of the IFN gamma receptor expression. *Blood*, **117**(8): 2385-2395.

Liu X, Zhan Z, Li D, Xu L, Ma F, Zhang P, Yao H and Cao X (2011b). Intracellular MHC class II molecules promote TLR-triggered innate immune responses by maintaining activation of the kinase Btk. *Nat. Immunol*., **12**(5): 416-424.

Maher SG, Sheikh F, Scarzello AJ, Romero-Weaver AL, Baker DP, Donnelly RP and Gamero AM (2008). IFN alpha and IFN lambda differ in their antiproliferative effects and duration of JAK/STAT signaling activity. *Cancer Biol. Ther*., **7**(7): 1109-1115.

Mangia A, Thompson AJ, Santoro R, Piazzolla V, Tillmann HL, Patel K, Shianna KV, Mottola L, Petruzzellis D, Bacca D, Carretta V, Minerva N, Goldstein DB and Mchutchison JG (2010). An IL28B polymorphism determines treatment response of hepatitis C virus genotype 2 or 3 patients who do not achieve a rapid virologic response. *Gastroenterology*, **139**(3): 821-827.

Mihm S, Frese M, Meier V, Wietzke-Braun P, Scharf Jg, Bartenschlager R and Ramadori G (2004). Interferon type I gene expression in chronic hepatitis C. *Lab. Invest*, **84**(9): 1148-1159.

Mihm S, Spengler U, Amanzada A and Ramadori G (2014). Does the interferon-lambda rather than the interferon-alpha pathway determine the outcome of hepatitis C virus infection? *Hepatology*, **60**(4): 1437-1439.

Miknis ZJ, Magracheva E, Li W, Zdanov A, Kotenko SV and Wlodawer A (2010). Crystal structure of human interferon-lambda1 in complex with its high-affinity receptor interferon-lambdaR1. *J. Mol. Biol.,* **404**(4): 650-664.

Ong JP and Younossi ZM (2004). Managing the hematologic side effects of antiviral therapy for chronic hepatitis C: Anemia, neutropenia and thrombocytopenia. *Cleve. Clin. J. Med.,* **71**(Suppl 3): S17-21.

Onoguchi K, Yoneyama M, Takemura A, Akira S, Taniguchi T, Namiki H and Fujita T (2007). Viral infections activate types I and III interferon genes through a common mechanism. *J. Biol. Chem*., **282**(10): 7576-7581.

Onomoto K, Jogi M, Yoo JS, Narita R, Morimoto S, Takemura A, Sambhara S, Kawaguchi A, Osari S, Nagata K, Matsumiya T, Namiki H, Yoneyama M and Fujita T (2012). Critical role of an antiviral stress granule containing RIG-I and PKR in viral detection and innate immunity. *PLoS One.,* **7**(8): e43031.

Prokunina-Olsson L, Muchmore B, Tang W, Pfeiffer RM, Park H, Dickensheets H, Hergott D, Porter-Gill P, Mumy A, Kohaar I, Chen S, Brand N, Tarway M, Liu L, Sheikh F, Astemborski J, Bonkovsky HL, Edlin BR, Howell CD, Morgan TR, Thomas DL, Rehermann B, Donnelly RP And O'brien TR (2013). A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. *Nat. Genet*, **45**(2): 164-171.

Quandt K, Frech K, Karas H, Wingender E and Werner T (1995). MatInd and MatInspector: New fast and versatile tools for detection of consensus matches in

nucleotide sequence data. *Nucleic Acids Res.,* **23**(23): 4878-4884.

Saito T and Gale M JR (2008). Differential recognition of double-stranded RNA by RIG-I-like receptors in antiviral immunity. *J. Exp. Med.,* **205**(7): 1523-1527.

Samuel CE (2001). Antiviral actions of interferons. *Clin. Microbiol. Rev*., **14**(4): 778-809.

Schug J (2008). Using TESS to predict transcription factor binding sites in DNA sequence. *Curr. Protoc. Bioinformatics*. Chapter 2: Unit (2): 6.

Sheppard P, Kindsvogel W, Xu W, Henderson K, Schlutsmeyer S, Whitmore TE, Kuestner R, Garrigues U, Birks C, Roraback J, Ostrander C, Dong D, Shin J, Presnell S, Fox B, Haldeman B, Cooper E, Taft D, Gilbert T, Grant FJ, Tackett M, Krivan W, Mcknight G, Clegg C, Foster D and Klucher KM (2003). IL-28, IL-29 and their class II cytokine receptor IL-28R. *Nat. Immunol*., **4**(1): 63-68.

Stec M, Baran J, Szatanek R, Mytar B, Lenart M, Czupryna A, Szczepanik A, Siedlar M and Zembala M (2013). Properties of monocytes generated from haematopoietic CD34 (+) stem cells from bone marrow of colon cancer patients. *Cancer Immunol. Immunother*., **62**(4): 705-713.

Susser S, Herrmann E, Lange C, Hamdi N, Muller T, Berg T, Perner D, Zeuzem S and Sarrazin C (2014). Predictive value of interferon-lambda gene polymorphisms for treatment response in chronic hepatitis C. *PLoS One*., **9**(11): e112592.

Takeuchi O and Akira S (2008). MDA5/RIG-I and virus recognition. *Curr. Opin. Immunol*., **20**(1): 17-22.

Uze G and Monneron D (2007). IL-28 and IL-29: newcomers to the interferon family. *Biochimie*., **89**(6-7): 729-734.

Van Voorhis WC, Valinsky J, Hoffman E, Luban J, Hair LS and Steinman RM (1983). Relative efficacy of human monocytes and dendritic cells as accessory cells for T cell replication. *J. Exp. Med*., **158**(1): 174-191.

Wang Y, Li J, Wang X, Ye L, Zhou Y, Thomas RM and Ho W (2014). Hepatitis C virus impairs TLR3 signaling and inhibits IFN-lambda 1 expression in human hepatoma cell line. *Innate Immun*., **20**(1): 3-11.

Yang L, Luo Y, Wei J and He S (2010). Integrative genomic analyses on IL28RA, the common receptor of interferon-lambda1, -lambda 2 and -lambda 3. *Int. J. Mol. Med*., **25**(5): 807-812.

Zymogenetics (2010). Zymogenetics and Bristol-Myers Squibb to present PEG-interferon lambda phase 2a interim clinical trial results at AASLD 2010. *Interferon Lambda*.