

# Evaluation the Polymorphism of IFITM3 Gene and its Role in Covid 19 Infection Outcome and Disease Severity in Babylon Province

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## Abstract

By preventing viral membrane hemifusion between the host and viral cell cytoplasm, the interferon-induced transmembrane protein 3 (IFITM3) contributes significantly to the adaptive and innate immune response. Incidence and severity of influenza or other viral infections have been linked to single nucleotide polymorphisms (SNPs) in the gene IFITM3. Our goal was to examine how SNPs in the gene IFITM3 affected SARS-CoV-2 infection. The study's sampling took place on Covid-19, a subject patient in Babylon Governorate, between October 24, 2021 and November 1, 2022.

There were Four single-nucleotide polymorphisms (SNPs) were genotyped in the 305 base-pair PCR results of the IFITM3 center advertiser locale around record start site (rs6598045), (rs7478728), (rs71452596), (rs7479267). Every one of these SNPs were in Hardy-Weinberg harmony (P.0.05) both in the Covid -19 and control gatherings. was found among three SNPs, rs7478728, rs71452596 and rs7479267.

**Keywords:** SARS-CoV-2; infection; IFITM3; polymorphism

## 1. Introduction

The interferon-induced transmembrane (IFITM) proteins are essential for the immune system's innate and adaptive defence against viruses. On chromosome 11p15.5, the human IFITM locus contains five genes, including IFITM3. The IFITM3 protein is mostly expressed on endosomes and lysosomes and is an IFN-stimulated gene (ISG). It stops hemifusion of a wide range of enveloped viruses, such as influenza A, Ebola, Marburg, or SARS-CoV, between the viral membrane and the host cellular membrane [1]. IFITM2 and IFITM3 enhance cellular entry of human CoV-OC43 [2]. Similar to this, production of an IFITM3 variation with improved plasma membrane localization vs endosomal localization increases infections by virus-like particles carrying SARS-CoV or MERS-CoV glycoprotein (pseudovirus) [3]. Single nucleotide polymorphisms (SNPs) in the gene IFITM3 have been linked to increased infection susceptibility and illness severity, thus according previous research [4]. IFITM2 and IFITM3 polymorphisms have been hypothesised to enhance their plasma membrane localization [5].

## 2. Methodology

Using the FAVORGEN kit, DNA was extracted and purified. Several extraction and purification solvents were kept at 28 °C until needed. Other substances were utilized in the extraction and purification of DNA in addition to the solution already present in a number of extraction solvents.

Primers Used in this research in sequence (5' → 3')  
IFITM3 F: TTCATGGTGTCCAGCGAAGA and rs6598045 R:

TGTGGAGACCCCAACACAG was is designed for this research the size 305 bp the Tm=59 GC% = 50-60.

The primers were prepared by adding distilled water free of nuclease in the different volume according to the manufacturing company instructions to obtain a solution of base stock with a concentration of 100 Pico mole / μl, mixed by vortex, then centrifuged for 10sec at 4000 rpm. Then 10 μl of each primer was taken and putt in the micro centrifuge tube with 90 μl of nuclease free distilled water to prepare the working solution.

Volumes of chemical materials uses in PCR assay Master Mix (5 μl) DNA(1-2 μl)Forward Primer(1 μl)Reveres Primer(1 μl)Deionizer D. W(18 μl) and the conditions of PCR reaction for three primers used for polymorphism of human genes were Pre denaturation(95°C) for 5 min (1 cycle) (Denaturation (95°C) for (30 sec) Annealing (55°C) for (30 sec) Extension (72°C) for (40 sec) all these for (30 cycles)) Final extension (72°C) for (5 min) (1 cycle) Cooling (4°C) for (∞) in (1 cycle). Agar gel electrophoresis was used to find the amplified PCR products.

## 3. Results and Discussion

### 1. Quality DNA extracted from blood of Covid 19 patients

The profile gel-electrophoresis 50 blood samples of Covid 19 patients (Figure1) was shown all samples gave huge bright bands of DNA.

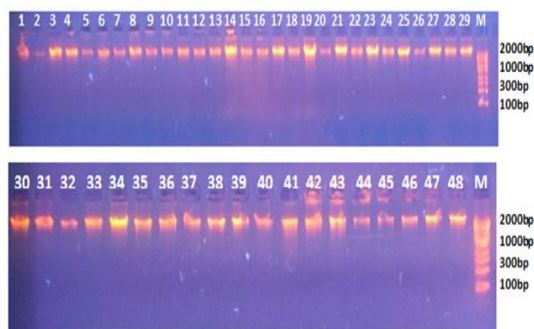


Figure 1: Gel-electrophoresis illustration quality and quantity of DNA extracted from Covid-19 patients group, 1-48 patient samples, M=molecular marker 100bp for each step.

2-The profile gel-electrophoresis 20 blood samples of control group (Figure 2) show all samples gave huge bright bands of DNA except sample 3 shown faint band.



Figure 2: Gel-electrophoresis illustration quality and quantity of DNA extracted from healthy group, 1-10 control samples, M=molecular marker 100bp for each step.

### 3-Localization of IFITM gene

The IFITM gene in humans is located in chromosome 11 at position NC\_000011.10 (319676-320860), previous literatures referred to the role of IFITM1, have been an important restriction factors in viral infections. the Figure illustrated the location of IFITM gene on Chr:11.

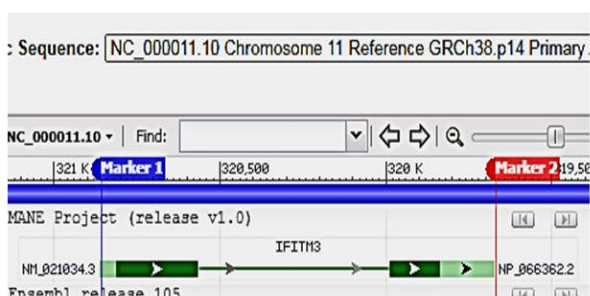


Figure 3: The location and boundaries of IFITM gene, Marker 1 and 2 delimited this gene on Chr. 11.

### 4-Targeted region in IFITM3 gene and SNPs validity:

(Figure 4) represents the boundaries primer pair F3 sites (320809 & 321113) on the region of IFITM3 gene at Chr:11 on reference strain NO. NC\_000011.10. The length of the gene segment was 310bp marked by the Forward and revers primers at sites on 320809 and 321113 respectively.



Figure (4): The targeted region of partial sequence of IFITM3 gene amplified by primer pair F1&F2 covering the many SNPs.

### 5. Amplification of targeted of partial sequence of IFITM3 gene

Amplification of targeted of for Covid-19 cases: show success the primer pair efficiency to amplification region 320809-321113 as target DNA region of IFITM3 gene included many SNPs, the amplification region with flanking primers 305bp for patient group (Figure 5) and for healthy group (figure 6)

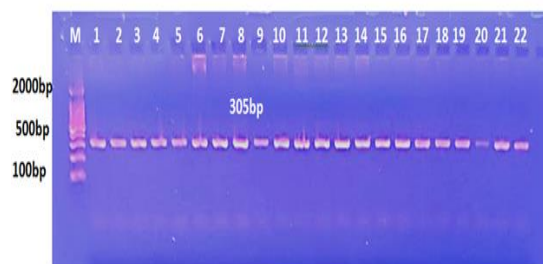


Figure (5): Gel electrophoresis of amplicon span from 320809-321113 as target DNA region of IFITM3 gene amplification region with primer flanking regions, 1-22 patient Covid patients PCR products 305bp, M1= molecular marker 100bp for each step.

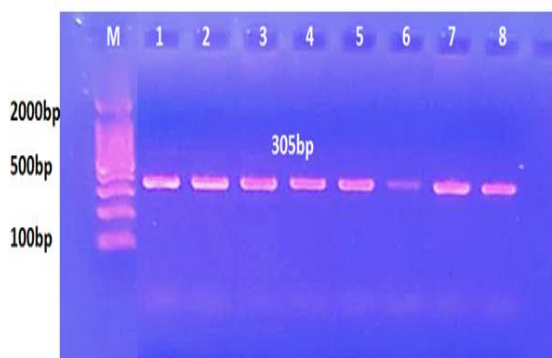


Figure (6): Gel electrophoresis of target DNA region for IFITM3 gene amplification region with flanking region, 1-8 control group, PCR products 305bp, M=molecular marker 100bp for each step.

Four single-nucleotide polymorphisms (SNPs) were genotyped in the 305 base-pair PCR findings of the IFITM3 centre advertiser locality surrounding record start site, according to genotyping and heredity study. (rs6598045), (rs7478728), (rs71452596), (rs7479267). Every one of these SNPs were in Hardy-Weinberg harmony (P.0.05) both in the Covid -19 and control gatherings. was found among three SNPs, rs7478728, rs71452596 and rs7479267.

The results of multiple alignment of partial sequence of gene IFITM3 shown 4 mutants located on sites 155 G>A, 158 G>T, 161 G>A and 168 A>G. these mutants shown high frequent ranged this indicated to be SNPs. After detected validity of these mutants, the results shown that mutant on site 155 was rs7479267 G>A; on site 158 was rs71452590 G>T, on site 161 was rs7478728 G>A and on 168 was rs6598045 A>G.

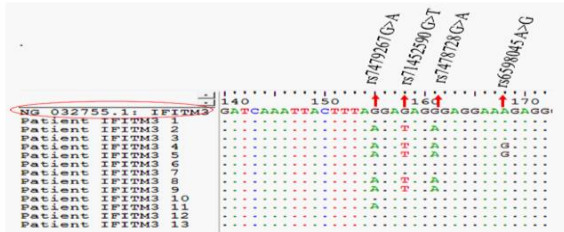
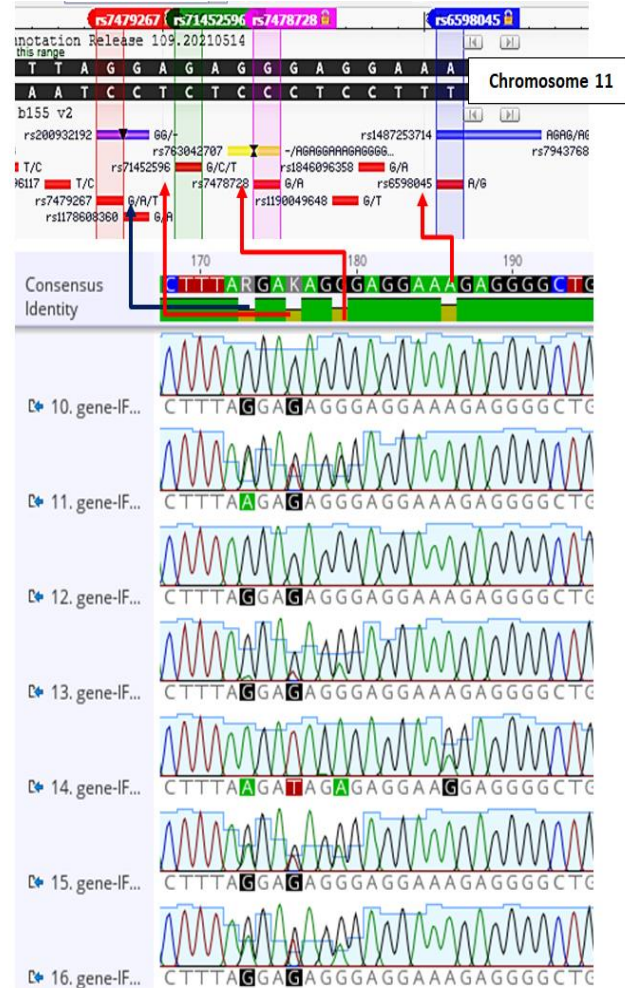


Figure (7): Multiple alignment of partial sequence of Targeted region of Chromosome 11-IFITM3 gene, shown SNPs on sites: on 155 was rs7479267 G>A; on site 158 was rs71452590 G>T, on site 161 was rs7478728 G>A and on 168 was rs6598045 A>G based on BioEdit software.

The properties of the BioEdit software was illustration only homozygous SNPs but neglected the heterozygous SNPs.

The properties of genius software more advance software was illustration all types of SNPs: homozygous wild type allele, homozygous mutant allele and heterozygous allele of Covid-19 cases and control. The results of targeted region 320809 & 321113 shown four SNPs : rs4479267, rs71452596, rs7478728 and rs6598045.



Figure(8 )(continue):The multiple alignment of chromatograms of targeted region 52185415-52186396 shown three SNPs : rs4479267, rs71452596, rs7478728 and rs6598045. 1-16 Covid-19 patient disease cases.

The Alignment of control cases performed by Genious prime software figure 12.

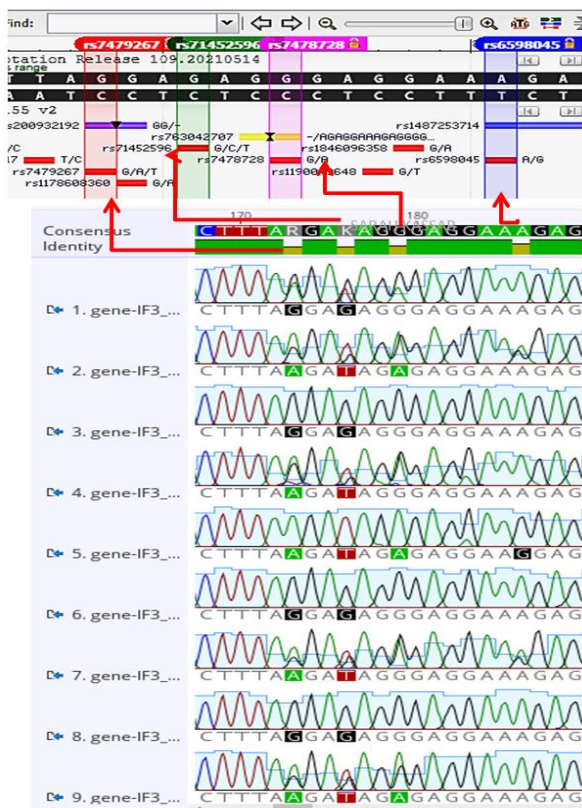


Figure (8): The multiple alignment of chromatograms of targeted region 52185415-52186396 shown three SNPs : rs4479267, rs71452596, rs7478728 and rs6598045. 1-16 Covid-19 patient disease cases.

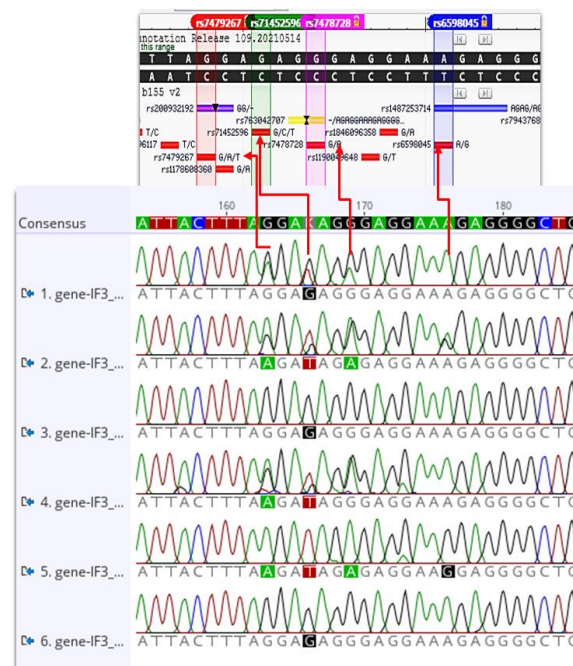


Figure (9): The multiple alignment of chromatograms of targeted region 52185415-52186396 shown three SNPs : rs4479267, rs71452596, rs7478728 and rs6598045. 1-6 healthy group.

The results of multiple alignment based on genius software Figures . The rs4479267 shown high frequent compared with two SNPs: rs71452596, rs7478728, this result paved away to considers these SNPs to be correlated risk with Covid -19 disease.

### 6. Determination the allele risk among SNPs valid in this study

Due to the results of statistical analysis based on Chi-Square test and genotype distribution, percentage and allele frequency, and evaluated the Odd ratio values (OR) for each genotype of SNPs under interest, the results were clearest in tables (1-4).

**Table 1: Genotypes of IFITM3 gene SNP (rs7479267G>A and polymorphisms.**

IFITM3 gene SNP (rs7479267 G>A>T; polymorphisms					
SNPs	Genotype	Patients N=50	Control N=50	OR(95%CI)	P-value
rs7479267 G>A>T	GG	16(32%)	25(50%)	0.45(0.199-1.02)	0.5
	GA	12(24%)	16(32%)	0.4(0.19-1.17)	0.1
	AA	22(44%)	8(16%)	*4.02(1.5-10.3)	0.03
Allele Frequency	G	44(44%)	66(67.3%)	0.35(0.2-0.63)	0.0004
	A	60(56%)	32(32.4%)	2.81(1.58-4.9)*	

\*High value of OR explained that Allele A was risk allele.

**Table 2: Genotypes of IFITM3 gene SNP (rs71452596G>A and polymorphisms.**

IFITM3 gene SNP (rs71452596 G>C>T; polymorphisms					
SNPs	Genotype	Patients N=50	Control N=50	OR(95%CI)	P-value
rs71452596 G>C>T	GG	15(30%)	17(34%)	0.83(0.36-1.9)	0.6
	GT	16(32%)	8(16%)	2.5(0.9-6.5)	0.06
	TT	19(38%)	25(50%)	0.61(0.27-1.36)*	0.22
Allele Frequency	G	46(46%)	42(42%)	1.38(0.7-2.4)	0.22
	T	53(54%)	67(58%)	0.7(0.4-1.2)	

\* T allele not considered risk allele based on the low value of OR.

**Table 3 : Genotypes of IFITM3 gene SNP (rs7478728G>A and polymorphisms.**

IFITM3 gene SNP (rs7478728G>A; polymorphisms					
SNPs	Genotype	Patients N=50	Control N=50	OR(95%CI)	P-value
rs7478728G>A	GG	15(30%)	17(34%)	0.83(0.35-1.9)	0.6
	GA	22(44%)	17(34%)	1.43(0.63-3.2)	0.3
	AA	13(26%)	16(32%)	0.7(0.3-1.8)*	0.5
Allele Frequency	G	52(52%)	51(51%)	1.04(0.59-1.8)	0.8
	A	48(48%)	49(49%)	0.75(0.3-1.8)	

\* A allele not considered allele risk based on low value of OR.

**Table 4: Genotypes of IFITM3 gene SNP (rs65598045 A>G and polymorphisms.**

IFITM3 gene SNP (rs65598045 A>G; polymorphisms					
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SNPs	Genotype	Patients N=60	Control N=60	OR(95%CI)	P-value
rs65598045 A>G	AA	37(74%)	34(68%)	1.34(0.56-3.2)	0.5
	AG	9(18.4%)	8(16%)	1.15(0.41-3.3)	0.7
	GG	4(8%)	8(16%)	0.45(0.12-1.63)*	0.22
Allele Frequency	A	74(83%)	76(76%)	0.95(0.5-1.58)	0.8
	G	51(17%)	50(24%)	1.05(0.63-1.7)	

\*G allele not considered as risk allele based on low value of OR.

The genotyping results of Allele AA, AG and GG of SNP rs65598045 A>G, shown that A allele shown high percentage in patient and healthy groups, with high Odd Ratio (OR=1.34(0.56-3.2) more than mutant allele G with low Odd Ratio (OR=0.45(0.5-1.63).these results indicated that wild allele A defiance against disease. These results were conflict with the results of table (1)

The Allele Frequency Calculator shown high percentage of A wild allele in both patients and control groups 83% and 76% receptacle, while the mutant allele G shown low percentage 17% and 24% receptively

The A allele frequency percentage was high in is used to reflect the genetic diversity of a population species. It is also referred to as allele frequency. It is a measure of relative frequency of allele on a genetic locus in a population. The frequency is expressed in terms of percentage. Hardy-Weinberg Equation can be used to find the frequency of allele.

The results of detection the main genotypes in Covid 19 patients presented with higher frequencies of AA genotype in rs65598045 and TT genotype in rs65598045 of IFITM3 gene than healthy controls (both P<0.001).

As seen in Figures 10,11,12, for IFITM3 gene SNPs polymorphism the presence of TT genotype of rs7478728G>A and AA genotype in rs7478728G>A of IFITM3 was fundamentally higher in control bunch than Covid 19 patients . Moreover, the strategic relapse model showed a diminished danger for Covid 19 improvement among people with AA genotype (OR 1.43(0.63-3.2), and transporters of An allele (AG+GG) (changed OR = 1.05).

### 7. Genotypes illustration based on phylogeny tree:

The phylogeny tree constructed based on sequence data of IFITM3 gene was shown five main genotypes: genotype 1 include cases 3 and 6; genotype 2 include 15, 20, 25; genotype 3:31, 32; genotype 4:10, 14; genotype 5:11, 19, 21, 24. Figure(10).

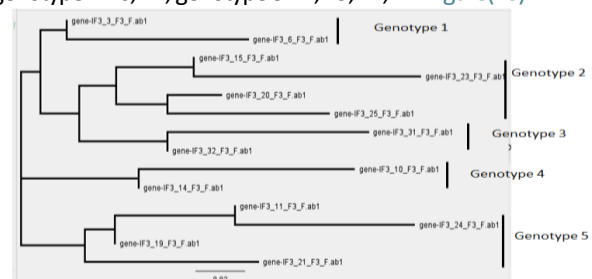


Figure (10): Genotyping closeness among patients constructed based on sequences of IFITM 3 gene performed by phylogeny tree.

In few years ago, interferon-instigated transmembrane (IFITM) proteins have been distinguished as significant antiviral variables. Newly, IFITM3 is found in the early and late endosomes, individually, and is intense inhibitors of infections that rely upon endosomal pathways for

disease.

Concentrates in vitro, show that exhaustion of *IFITM 3*, while overexpression of *IFITM 3* proteins hinders viral replication [6].

Sanger approval of a subset of variations for the following stage of the undertaking, I chose to approve four SNPs inside the locale: Chr11:320,988-321,138 by Sanger sequencing. This locale is situated at the 5' finish of the *IFITM3* quality and it is for the most part inadequately addressed by sequencing information.

The genotypes for three SNPs rs7479267, rs71452596, rs7478728 were mistakenly called as heterozygous in the 1000 Genomes Project and the Illumina designated sequencing dataset. Sanger it is truth be told homozygous to succession affirmed that these SNPs at those positions. Final finding: we obtained the local hospitals in Babylon province case statistical indicators based on Odd Ratio of COVID-19 and genetic information on the *IFITM3* gene. We performed genotypes and allele frequency analysis between the case of COVID-19 and allele frequencies of the polymorphisms of the *IFITM3* genes in patients group and compared with healthy one. We identified a weak to strong effects between the case group of COVID-19 and the allele frequency of the rs6598045 SNP *IFITM3* gene. To the best of our knowledge, this report is the first to describe relationship between COVID-19 and many SNPs: rs6598045 SNP of the *IFITM3* gene at the population-level.

Because *IFITM3* plays a pivotal role in IFN- $\gamma$  signaling and successful immunity against *M. tuberculosis* requires strong IFN- $\gamma$  [7].

Interferon-instigated layer protein that hinders the section of infections into the host cell cytoplasm by forestalling viral combination with cholesterol exhausted endosomes is encoded by the *IFITM3* quality [8]. It has an ability to inactivate new encompassed infections, which bud out of the tainted cell. It has been demonstrated to be dynamic against different infections, including flu A infection, SARS Covid (SARS-CoV), Ebola infection (EBOV), Dengue infection (DENV), human immunodeficiency infection type 1 (HIV-1), and so on. [9]. Pathways through which *IFITM3* capacities are: Innate Immune System what's more, Interferon gamma flagging. Studies have shown that the initial 21 amino acids of the N-end of *IFITM3* quality are expected for constriction of vesicular stomatitis infection replication, also, that shortened *IFITM3* protein neglects to confine the replication of different kinds of flu infection, as well as HIV-1 [10, 11]. According to [12, 13] showed that even full-length *IFITM3* confines passage and replication of H1N1. Polymorphisms of this quality have been concentrated in a few diseases. The polymorphism rs6598045 c.-188T > C (4.85% in our partner) prompts a contrast in the limiting limit of the record factor causing a distinction in the record proficiency of the *IFITM3* quality, which was accounted for to show a solid relationship with flu H1N1 2009 pandemic infection contamination [7]. Another practical polymorphism rs3888188, showed that fringe blood mononuclear cells conveying GG genotype had decreased *IFITM3* mRNA level contrasted with those with TT or GT genotype, which inclines toward aspiratory tuberculosis

in Iranian and Han Chinese populaces [7].

Past examinations anticipated that rs12252 C allele could create an other joined record that encodes a deviant shortened protein 121 of *IFITM3*, which lessens the cell obstruction to flu infections by obstructing beginning phase of viral replication [4].

Polymorphism has been seen in Chinese populace with pandemic flu (H1N1 09pdm), occasional flu (H3N2 what's more, flu B), and avian flu (H7N9) [14]. In our partner, 11.65% of the people conveyed this allele. Occasional flu medical clinic affirmations were related with rs7948108, which was seen with a low (0.97%) rate in our companion.

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