The Association of Homozygote T Allele of rs2943641 Polymorphism near of Insulin Receptor Substrate 1 Gene in the Susceptibility to Autism

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Abstract

**Background:** Autism disorder is a neuro-developmental disorder; it is heterogeneous with multiple genes defects that can lead to autism. The incidence has increased from 1980 to 1990, 5/10,000 to 37/10,000 respectively. The increase in the frequency has led to huge studies being carried out in this field. The main causes and the pathway of the disease is as yet unclear. However, several reports have been documented that indicate that CNVs and single genes disorders that are involved in multiple pathways have a role in the development of autism. The main genes that are associated with ASD are involved in mTOR/PI3K pathway. MTOR/PI3K pathway is responsible for the growth rate and pruning of cellular-synapse. Therefore, increase the activity of this pathway due to mutations in the upstream or downstream of the pathway it may cause ASD to develop. The aim of this dissertation is to present a new aspect by indicating the association of Homozygote T Allele of rs2943641 Polymorphism in IRS1 that is involved in the PI3K pathway and increase the susceptibility to ASD. The effect of homozygote T allele of rs2943641 has been previously reported as increasing the expression of IRS1. Increase in the expression leads to an increase in the phosphorylation of PI3K that may hyper-activate the pathway.

**Methods:** An allelic discrimination assay was suggested to determine the most common allelic variation of rs2943641 in autistic patients in Saudi Arabia.

**Results and conclusion:** If the result indicates an association between the T allele of rs2943641 and ASD, a new aspect in the genetic causes for autism will have been added.

**Keywords:** Autism; IRS1-Autism; rs2943641 Polymorphism-Autism; IRS-1; rs2943641; T Allele of rs2943641

Introduction and Background

Introduction

Most patients who have autistic disorders suffer from some form of developmental delay and severe neuro-developmental disorders have an early onset. Frequently shared common clinical features are deficiency in social interaction, language delay and repetitive behaviour. They can also have a lower IQ than normal with some of them suffering from intellectual disability [1]. The phenotype differs from patient to patient with variable severity. In May 2013 the new Diagnostic and Statistical Manual of Mental Disorders, published the fifth edition (DSMV) and together with the International Classification of Diseases, Tenth Revision (ICD-10) the classification of autism is now dependent on age of impairment in social, language and showing an interest in activities. The most common are autistic disorder is ASD, next is Asperger syndrome AS, and the third is pervasive developmental disorder not otherwise specified PDD-nos [2]. Reviewed by Freitag in 2006, an Austrian-US-American Professor of Child Psychiatry in 1943 was the first to recognise a child with mental retardation who was isolated due to the lack of ability to communicate. Eugen Bleuler named this condition autism using Bleulers schizophrenia criteria which was used to classify the isolated schizophrenia. In the same period 1950-1960 Hans Asperger recognised a child with similar autistic feature but without mental retardation. Between 1970-1980 Michael Rutter and Lorna Wing investigated the diagnosis and aetiology of autism [3].

Prevalence

The incidence and the diagnosis of autism has been increasing dramatically raising the concern of parents, physicians and scientists as to the need for more investigations in order to determine if there are any environmental effects that could be causing the elevation in the incidence [4]. In 1980 the frequency was low 5/10,000, however in 1990 in many of the studies carried out in Japan, England, and Sweden to determine the incidence of autism the results show an increase to 37/10,000 [5]. Recently, US studies indicate that in 8 year olds there is one child in 110 with ASD. This study does not indicate a huge increase in prevalence rather that it is an increase in awareness and the ability of the public and scientists to evaluate and find a specific diagnosis. The ratio of boys to girls is 4:1 but, as yet there is no definitive evidence as to the reason for the difference, however interestingly in the severe cases of ASD the ratio becomes 1.8:1 [4].

Clinical feature

The symptoms of autism most commonly occur in infants before they are three years of age. Parents are usually late in recognising the abnormal features of ASD in their child which could have alerted them; however diagnosis of the condition may also take a considerable time. Therefore, the age of diagnosis is much later than the age of onset, as there are no specific signs or symptoms that can be clearly recognised at an early age. Autism can be diagnosed in adolescents or adults as a result of the symptoms occurring at age when involvement in society is required. The main three symptoms for autism are lack of social involvement, communication and repetitive action [6] (Figure 1).

Inheritance

It is not yet known what causes autism therefore; twins and family studies are carried out to investigate if the condition is caused by genetic or environmental factors or both. These studies are carried out...
Autistic patients typically become isolated from people and watch them from a distance, they avoid eye contact when someone speaks to them, usually fail to point to what they want which causes confusion for the parent. Loss of interest or involvement in any entertainment thus making it difficult to form friendships (reviewed by Miles, 2011).

An autistic patient is not interested in computer games or any spontaneous game due to the inability to function spontaneously (reviewed by Miles, 2011). Communication: as previously mentioned autistic patients have language delay and they also start to repeat a particular word or even just part of a word usually they do not know its meaning (Rapin, 1991).

This symptom can be recognised in the first few months. An autistic patient enjoys repeating some stereotypic behaviour and this repetition may go on for hours (Rapin, 1991). However, repetitive action can start to occur in the second years when something disrupts their routine. When subjected to loud sound such as a vacuum cleaner their behaviour becomes stressed and they put their hands over their ears to avoid the sound and start to scream. Also, the type of food that they like can change, preferring some kind of fried food rather than healthy food and this can affect their health (reviewed by Miles, 2011).

Autistic patients have language delay and they also start to repeat a particular word or even just part of a word usually they do not know its meaning (Rapin, 1991). Communication: as previously mentioned autistic patients have language delay and they also start to repeat a particular word or even just part of a word usually they do not know its meaning (Rapin, 1991).


diagnosis of autism. For example, in Saudi Arabia they used MRI as a basic technique of diagnosis autism.

Autism can be diagnosed by neurologists using scan images that can illustrate that the brain pattern. Muller et al. in 2001 shows differentiation between autistic patients and normal controls, autism shows abnormality in the function map. The standard magnetic resonances images detect reduction in the pattern of distinct regional activation-deactivation in autism. This result matches the imprinting in the motor function for autism [9].

**Environmental**

Environmental factors can interact with gene mutations to cause ASD. Exposure to high level of pollution and impairment during gestation such viral infection can also increase the susceptibility of having an autistic child. In addition, testosterone and over activity of the immune system can increase the risk to the foetus of ASD [10].

**The history of genetic diagnosis of ASD**

In 1970 the cause of ASD was widely believed to be a biological effect connected to the appearance of aloof behaviour of the mother. However, in 1980 with the increase of the role of genetics in these phenomena and also with the increase in chromosome abnormality which had been noticed in ASD patients many syndromes have been correlated with ASD [5]. In addition, twins and family study linkage analysis have detected a strong correlation between genetic factors and the etiology of ASD that is lacking specific diagnosis criteria [3]. In the late 1990’s, whole-genome association studies were carried out to identify specific loci leading to ASD, while the genomic technique such as aCGH was used to identify copy number variation CNV and also has highlighted a number of interesting loci. Recently, genetic classification of the causes of ASD show, 5% caused by chromosome abnormality, 20% caused by CNV and 5% due to a single gene disorder. 20 to 25% of the mutated genes in autistic cases were identified and related to ASD, the number identified has been increased due to the use of aCGH. However in 75% to 80% of autistic cases the cause is unknown [4].

There are multiple rare familial mutations and environmental factors that can lead to ASD [10]. As well as heterogeneity, there are biological effects that have been hypothesised as being similar to ASD such as having a defect in synaptic function and abnormality in brain function. As a result, whole genome analysis and pathway mechanism studies must be carried out to correlate phenotype-genotype relations [5].

**Chromosome rearrangement and CNVs**

**Down syndrome:** Down syndrome DS is caused by trisomy of chromosome 21 as a result of meiotic non-disjunction or by Robertsonian translocation. Rasmussen et al., in 2001 has indicated in their experiment that, there is a delay in the diagnosis of ASD in Down syndrome patients when compared with the diagnosis with autism in non-DS patient. In this study they suggested that autism should be considered in DS patient, 7% of DS having ASD. They attempt to diagnose, indicate and fully assess ASD in DS patients as that will facilitate support for them by special education or other supporting elements. They also determined that significant factors lead to the development of ASD in DS patients such as: a history of ASD in a first or second degree relative and early hypothyroidism.
Copy number variations CNVs and Autism: The expected cause of ASD is CNVs, 5-10% in non-syndromic, 10–20% in syndromic patients and schizophrenia 5% (Table 1) [11]. Reviewed by Bauer and Msall in 2011, Array-CGH is the first choice in diagnosing ASD as it has high resolution, and the ability to detect abnormalities of less than 5Mbp even as small as 1M. Shen, et al., in 2010 determined that aCGH is capable of detecting 18.2% of CNVs small deletions and duplications in ASD patients. 7% of the CNVs whereas determined to be abnormal whereas, the remaining 11% of the CNVs were of unknown significance [12]. Therefore, aCGH is a good technique to use in the detection of de novo small deletions or duplications [13] (Table 1).

Reviewed by Miles, 2011 several CNVs that are de novo or inherited events related to ASD. They found duplications in 15q11.2-11.3 in autistic patients which includes the 15q11.2-11.3 region that is documented in OMIM#608636 as being related to autism. They also reported that, 20% of deletions in 16p11.2 are located in the hot spot region that is related to 1% autism with macrocephaly compared to 60% of duplications in the same region 16p11.2 that lead to attention deficit hyperactivity disorder ADHD with microcephaly [4]. 16p11.2 is an area involved in the gene that is important in brain structural development [14]. In addition 7q11.23 duplication, Williams syndrome, has been found to be associated with ASD in 1% of cases [4].

Single gene disorders: A study has been carried out that has focussed on the analysis of a single gene responsible for ASD in familial, de novo or a spontaneous event and illustrate in idiopathic and syndromic ASD the associate loci and candidate genes, however no specific gene has been identified as being responsible for ASD [10]. They analysed five genes that are considered to be the most likely candidates to produce the ASD phenotype (Table 2). The studies show that, syndromic autism is usually caused by a single gene disorder. Recently, SFARIGENE database has a list of the candidate genes associated with ASD [4].

Rett syndrome: Rett syndromes is caused by a mutation in the X-linked gene MECP2 that codes for methyl CpG-binding protein 2, it has been reviewed by Castro et al., in 2013 that, deficiency in MECP2 leads to alteration in the significant intracellulular pathway for example mTOR/P13K signalling pathway that is related to autism. Approximately 25% to 40% of individuals with Rett syndrome are also diagnosed with autism [15].

Genes balance between excitatory inhibitory signals: NRXN1–NLGN3&4–SHANK3 pathway is responsible for maintaining the balance between excitatory and inhibitory therefore; mutation in either of them may cause mental retardation. The association between alteration in the synaptic function and ASD has been observed in 10% to 30% of ASD patients who suffer from epilepsy due to an imbalance in excitatory-inhibitory signal [16]. This association has been proved by the detection of mutations in Neurelins (NLGN) that are postsynaptic cell adhesion molecules. Also, several mutations in SHANK3, NRXN1, CNTNAP2 and CNTNAP3/4, are detected in ASD (Figure 2) [17]. The hypothesis is that the presence of these mutations requires other factors before ASD will be developed and it can also be the cause of other diseases (Table 3).

Genes involved in mTOR/P13K pathways
mTOR/P13K pathway activated by insulin signalling: Phosphoinositide-3kinase mTOR/P13K pathway that is responsible for the growth rate of cellular-synapse, is one of the most important pathways and includes several genes that are associated with syndromic ASD Bourgeron, 2009 (Figure 3). mTOR/P13K pathway connects the extra cellular insulin signalling to activate mTOR. The signalling links to P13K involve insulin receptor substrate IRS. This will activate the P13K/mTOR pathway which is responsible for pruning and the formation of the synapse. Abnormality in the shape and the size of neuron increases the susceptibility for autism. Therefore, mutation in the upstream may lead to an increase in the activity of this pathway that leads to ASD [16].

TSC1/TSC2, and PTEN work in one pathway like a negative control

<table>
<thead>
<tr>
<th>CNV associated with</th>
<th>High resolution array detects</th>
<th>Inherited %</th>
<th>A de novo %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-syndromic</td>
<td>8% CNV</td>
<td>2%</td>
<td>6%</td>
</tr>
<tr>
<td>Syndromic</td>
<td>25% CNV</td>
<td>18%</td>
<td>7%</td>
</tr>
</tbody>
</table>

Table 1: The percentage of the detection rate for autism by high resolution array, and percentage of inherited and de novo event [11].

<table>
<thead>
<tr>
<th>Location</th>
<th>Gene Name</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>7q36.2</td>
<td>EN2 Engrailed homolog 2 gene</td>
<td>Involved in cerebellum development and it has essential role during development in segmentation</td>
</tr>
<tr>
<td>15q11q13</td>
<td>GABR Gamma amino butyric acid receptor genes</td>
<td>Regulate brain cell migration, differentiation, and synapse formation</td>
</tr>
<tr>
<td>16p11.2</td>
<td>OXTR oxytocin receptor genes</td>
<td>Involved in the response to stress and in social skills such as empathy</td>
</tr>
<tr>
<td>7q21-q36</td>
<td>RELN Reelin gene</td>
<td>Involved in neuronal migration in the developing brain</td>
</tr>
<tr>
<td>17q11.1-q12</td>
<td>SLC5A4 Serotonin transporter gene</td>
<td>Could account for phenotypic expression of happiness</td>
</tr>
</tbody>
</table>

Table 2: Five genes associated with autism [10].

<table>
<thead>
<tr>
<th>Name of the locus</th>
<th>Location</th>
<th>Name of the gene</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurexin1</td>
<td>Xp16.3</td>
<td>NRXN1</td>
<td>Involved in cerebellum development and it has essential role during development in segmentation</td>
</tr>
<tr>
<td>Contactin-associated protein-2</td>
<td>CNTNAP2</td>
<td>7q35</td>
<td>mTOR/P13K pathway activated by insulin signalling:</td>
</tr>
<tr>
<td>Like domains 3 SHANK3</td>
<td>22q13</td>
<td>Neuroligins 3&amp;4 NLGN3 3 q13.1 and NLGN4 4 Xp22.3</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Genes regulate the excitatory inhibitory signals [26-32].
The rs2943641 polymorphism of the IRS1

**IRS1 Structure and Function**

Sun et al., in 1991 detected that, insulin receptor substrate 1 IRS1 encoding protein which phosphorylated by insulin receptor kinase is involved in the mTOR/P3K pathway which is in association with ASD. IRS1 is located on the long arm of chromosome 2q36.3 has responsibility for insulin resistance and is associated with type II diabetes "non-insulin-dependent diabetes mellitus" NIDDM.

Interestingly IRS1 is not included in crucial enzymatic activity it and acts as activation for different pathways after phosphorylation with tyrosine by insulin receptor kinase. Normally, IRS1 protein is phosphorylated when it interacts with insulin or insulin like growth factor by insulin receptor tyrosine kinase to regulate the insulin signalling process. IRS1 bind usually interacts with protein included in the SH domain e.g. P13K, p85 and Grb2. Therefore after, the insulin signal has phosphorylated the insulin receptor kinase, IRS1 will then phosphorylate and corroborate with p85 and p110 to activate P3K. IRS1 therefore plays a critical role in the activation of the downstream of P3K pathway [20].

**IRS1 domains structure and function**

Insulin receptor substrate1 IRS1 consists of multiple domains for structure and functional acts. Normally under insulin stimulation 632Y motif in IRS1 binds with insulin receptor kinase to activate IRS1 that activate the P13K pathway. However, in a case of hyperinsulism the increase the insulin signalling for activating the P13K pathway is inhibited by phosphorylation of IRS1 at Ser-636/639 through mTOR1 [21](Table 4).

Tzatsos in 2009 found that, SAIN domain is a critical domain in IRS1 having a role in regulating the phosphorylation of IRS1 at Ser-636/639 by mTOR1 (Figure 4). Raptor plays an important role through protein which has a lipid phosphatase function. PTEN acts as an inhibitor in P13K the the pathway by removing phosphate from the signalling molecule phosphoinositide-3,4,5-triphosphate PIP3. PTEN mutation was reported by Herman et al., in 2007 to be in autistic families. Sequencing the PTEN was carried out in two families that had Cowden syndrome occurring in the parent Cowden syndrome presents where there are mutations in PTEN and they found a nonsense mutation R130X within exon 5 PTEN is present in the essential phosphatase domain of the protein and Y178X in exon 6 in an autistic patient with macrocephaly measuring >2.5 standard deviations SD above the mean. Therefore, sequencing PTEN for autistic patients that have macrocephaly is recommended [18].

Tuberous sclerosis 1 and 2 (TSC1 and TSC2): Tuberous sclerosis is a multisystem autosomal-dominant disorder where the loss of function occurs as a result of mutation in either of TSC1 genes in 9q34 or the TSC2 gene in 16p13.3. The gene product of TSC1 is known as hamartin, and the product of TSC2 is tuberin. Approximately 10% to 30% of cases of tuberous sclerosis are due to mutations in TSC1, 90% to 70% due to mutations in TSC2. Approximately 30%-60% of patients with tuberous sclerosis complex TSC have autism [16].

TSC acts as a negative regulator for the P13K pathway by regulating the upstream of mTOR and downstream of Akt. TSC2 inhibits the activation of S6K and 4E-BP1 by blocking the Akt activity [19]. Chiang et al., in 2013 determined that several missense variations in TSC1 and TSC2 in probands and where inherited increase the susceptibility to autism.

**The rs2943641 polymorphism of the IRS1**
interaction with mTOR1 and mLST8 to form mTOR1 complex that regulates the phosphorylation of IRS1 at Ser636/639 and has been found in non-insulin-dependent diabetes mellitus the activity of Akt is reduced with the increase in phosphorylation of IRS1 at Ser636/639 [22].

The effect of rs2943641 in IRS1

rs2943641 single nucleotide polymorphism SNP located in Inter-genic region 500 kb upstream from the IRS1 in chromosome 2q36 is likely to have an effect on expression. The HGVS named is g.227093745T>C. Meaning that the ancestral nucleotide is a T at position 227093745 in the genomic DNA region has been changed to C. the minor allele is T” (Figure 5) [23].

Explain the rs2943641 hypothesis

Autism is a neuro-developmental disorder with an early onset; autistic patients are unique with due to their repetitive behaviour. The genetic cause is still complicated as we know it is heterogeneous with multiple effects that can lead to ASD. In some case the individual has inherited a genetic defect that when interacting with environmental factors can present a serious disease such as autism. Recently, several studies have been carried out and these revealed essential information for example there are studies that illustrate many pathways that interact with autism. Several syndromes have been also reported are associated with autism as mention before [4]. This research has been carried out to add a new aspect for the genetic causes that can elevate the susceptibility to autism.

rs2943641 polymorphism has been chosen to be the main hypothesis of this study. This polymorphism is located near of IRS1 which is a critical element in the activation the downstream of this in the mTOR/PI3K pathway as a response to the stimulation insulin signalling as mention before [20].

Rung et al., in 2009 has investigated that, the effect of the rs2943641 in transferring insulin signalling that stimulates PI3K pathway. They found that the C allele of rs2943641 has reduced the IRS1 expression that is associated with a decrease in the phosphorylation of PI3K under insulin signalling stimulates. The reason for this is that the C allele reduces the insulin sensitivity leading to insulin resistance. Therefore, this foundation has provided the hypothesis of rs2943641 variation has a role in the IRS1 expression that affects the activity of the PI3K pathway.

This hypothesis is that, this research has determined that specific variation of polymorphisms can affect over-expression of IRS1 gene. This suggested that an increase in the expression of IRS1 can increase the activity of the PI3K pathway that may lead to increase the susceptibility for ASD [16]. Maglio et al., in 2013 determined that homozygote T allele of rs2943641 results in lowering the insulin resistance in other word increase the insulin sensitivity through over-expression of IRS1.

This hypothesis does not suggest that the rs2943641 is the main cause of autism but rs2943641 may one of the genetic factors that triggers the susceptibility for autism through increasing the activity of the PI3K pathway under the insulin signalling. On the other hand, rs2943641 may not increase the susceptibility for autism however, if it may links with another alteration it could then have role in causing autism. No studies have determined that this association therefore, this study aims to determine the association of Homozygote T Allele of rs2943641 Polymorphism near of IRS1 and increase the susceptibility to ASD.

Method “Designing the Experiment”

Aim of the investigation

The aim of investigation is to determine the association of Homozygote T Allele of rs2943641 Polymorphism in IRS1 and increase the susceptibility to ASD.

Facilities

This experiment can be carried out in a laboratory that has polymerase chain reaction PCR availability, and 7700 sequence detection system SDS to measure the florescent intensity after the TaqMan assay proses.
Strategy

Strategy plan has been determined to obtain the desired result. The strategy is clearly explained in Figure 6.

Design the allelic discrimination assay

To design allelic discrimination assay that is able to differentiate between the individual who is homozygote for the T allele of rs2943641 polymorphism from the individual who is homozygote for the C allele and who is heterozygote. TaqMan PCR assay was chosen as the allelic discrimination assay, TaqMan is 5’ nuclease assay used in genotyping studies to distinguish allelic variations of rs2943641. This technique is based on the 5’-3’ exonuclease activity of the Taq polymerase to cleave the labelled probes which are hybridized to the complementary sequence that illustrates fluorescent signals during PCR (Figure 7) [24].

For the rs2943641 SNP TaqMan required two probes one containing the C allele and the other for the T allele. Each probe is labelled with 5’ reported dye that has different colours to distinguish between the C allele probe and the T allele probe, and a 3’ quencher dye that blocks the reported dye from releasing the fluorescence (Figure 7) [24].

In the TaqMan PCR probes are designed to anneal to the specific SNP site sequence. The flanking sequence has been designed using the Ensembl website (Figure 8). The sequence is amplified by specific unlabelled primer sites which are designed by Primer3 left primer ATTCATGGGTCCAAGGATCA and right primer AGCTAGTGGCTACCATGTTGAA the melting temperature TM approximately 59.53°C and the amplicon length is 174 bp (Table 5). The two probes of the TaqMan assay will be designed, one for the C allele labelled with VIC dye as fluorophores and MGB as Quenchers and the other probe for the T allele labelled with FAM dye as fluorophores and MGB as Quenchers the probes then sequencing (Figure 9).

Life technologies are a large company that can provide several brands of laboratory products. This experiment has suggested that the primers and the probes should be ordered from life technology as that will facilitate ordering the single tube custom TaqMan SNP genotyping assays kit. The kit contains two primers and two different probes. TaqMan master mix is ordered separately. Real time PCR instrument will automatically run and analysis the assay. Single tube custom TaqMan SNP genotyping assays kit can run 1,500 reactions [24].

Sample source

Blood sample can be sourced from Prince Salman Central for
**Figure 9:** The design criteria for TaqMan probes and the primers (Life technology).

Disability Research in Saudi Arabia; centre has a blood bank for different types of disability patients that include autistic patients. The blood sample can be provided by the following strategy begin by filling a form explaining to which purpose this sample will be used and if it is for scientific research, if it is ethically acceptable and must follow legal criteria to obtain the approval from the Ministry of Health with this approved the centre will give the sample for free. Normal control samples can be obtained from the blood bank centre in Saudi Arabia which has the blood of the normal children coming from screening tests. These should follow the same criteria. To obtain a good result in these studies 300 samples from autistic patients can be used plus 100 samples from normal individuals used as a control.

**Procedure of the assay**

**DNA extraction:** After collection of the sample, extraction of the DNA from the blood is the critical step in DNA analysis. Therefore, the quality and the accuracy of the result depend on the isolation process. Furthermore, the method used to isolate the DNA depends on the sample type and the storage factor. In this experiment blood samples are collected and stored in a perfect way. DNAzol BD reagent kit from Life technology was ordered to extract 10-20 µg DNA from 500 µL bloods [24].

**Prepare the control samples:** Run the samples using the TaqMan assay and select the first three samples which represent a homozygote of T allele, a heterozygote CT allele and a homozygote of C allele then sequencing them to confirm the result. If the results from sequencing have confirmed the results from the TaqMan assay these three samples can be used as positive controls. If the results are not confirmed that means the polymorphism and the amplification are not in the right location therefore the primers and the probes will require being re-designed. Negative controls also should be used to insure the accuracy of the assay this is done by running all TaqMan assay components with the DNA replaced by water.

**TaqMan assay procedure:** After the DNA was extracted the TaqMan assay was carried out using the negative and positive controls and run in each reaction if it is possible. TaqMan can distinguish between the homozygote C allele, the homozygote T allele and the heterozygote TC allele that will be illustrated by measuring the fluorescence which are emitted on the reported day.

There is useful software which facilitates analysis of the data e.g. Sequence detection systems SDS that records the fluorescent signal for the two reported dyes and are reported by SDS. That will present the data of rs2943641 in a plot or a graph by present homozygote C allele, heterozygote CT allele and homozygote C allele (Figure 10) [24].

**Estimated cost of the experiment:** The estimated cost for the 400 samples can only be roughly calculated due to the possibility that potential problems could lead to re-runs some samples. Therefore, extra cost may need to be added (Table 6) [24].

**Results and Conclusion**

The incidence of autism has increased in the last ten years dramatically; however, the main genetic reason has been unclear until now. The guilty feelings of parents of autistic patient have risen and they want to know where the defect comes from, most of the cases are de novo or spontaneous mutation, however it has been documented that, if the family already has an autistic patient the possibility that the next baby will suffer from ASD is increased as mentioned previously. It is commonly agreed that autism is heterogeneous and multiple gene defects can cause it. Recently, the studies revealed that many CNVs are related to autism. Several genes involving different pathways have been reported as being associated with ASD. Moreover, autistic symptoms have been present in different syndromes. At present there is no cure for ASD due to the causes being as yet unclear, therefore clinical care attempts to develop the quality of the life for autistics by involving them in special courses that trains them how they can develop language ability, behaviour and increase their confidence to become involved in a social environment.

**Table 6:** Estimated cost for the experiment, the 400 samples for free but required to take the approved.
This study tends to concern focusing in new areas to added more information in a new direction. This has been done by investigating the association of Homozygote T Allele of rs2943641 Polymorphism in increasing the susceptibility for autism. The rs2943641 is located near of IRS1 which is involved in the mTOR/P13K pathway which is known to be responsible for the growth rate of cellular-synapse; IRS1 can phosphorylate P13K under the insulin signalling. Increase in phosphorylation can increase the activity of the pathway which has been identified as being associate with autism due to it begin normal. Alteration in the expression of IRS1 can change the activity of mOTR/P13K pathway increasing the expression of IRS1 that may up-regulate the pathway, while a decrease in the expression may down-regulate the pathway. Homozygote T allele for rs2943641 has been reported as affecting an increase in the expression of IRS1 leading to low insulin resistance in other word increase the insulin sensitivity. The aim of this study is to identify the association of the homozygous T allele of rs2943641 polymorphism in autistic patient and comparing them with normal.

Since IRS1 can play an essential role in the activation of the mTOR/P13K pathway through phosphorylated P13K, the polymorphism near of IRS1 role in the susceptibility to autism appear to be sensible? If the result of the experiment shows agreement with the hypothesis i.e. the frequency of the homozygote T allele of rs2943641 is more in autistic patients than the normal control, while the homozygote C allele for the polymorphism is low. This would indicate that, rs2943641 is associated with autism due to the effect of homozygote T allele which has been reported as affecting to insulin resistance with over-expressed IRS1 that in time will increase the phosphorylation of P13K which may cause hyper-activity of the pathway under the insulin signalling stimulate. Of course, the result may show disagreement with hypothesis, the frequency of homozygote T allele of rs2943641 in autistic patients being lower than the normal control, while the frequency of C allele for rs2943641 higher in autistic patient that the normal control. Therefore, rs2943641 will indicate not have a direct association with autism. However, it may alter the function of other causes of autism. The C allele has been documented as being associated with low expression of IRS1 leading to high insulin resistance that may alter another causative for autism.

Stern in 2011 has attempt in her hypothesis to connect and explain the relation between the increase in the incidence of autism and hyperinsulism. Therefore, according to this hypothesis if the result agree with hypothesis further investigation can be carried out by measuring the insulin level in the same samples in autistic and normal control (Figure 11). If the insulin level is indicated as being high in autistics patients who carry the T allele of the polymorphism then that can confirm the hypothesis that hyperinsulinism can increase the insulin signalling that hyper-activate the mTOR/P13K pathway in autistic who are carried the T allele due to they have high insulin sensitivity. On her other hand, the result may show no alteration in the insulin level in autistic patients but unless hyperinsulism hypothesis can be explain be other alternative.

This study was aiming to find a new aspect of polymorphism in IRS1 having a association in the increase in the susceptibility for autism and as IRS1 is in the P13K pathway therefore, this research will also confirm that the pathway has an essential role in autism [32-36].

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4. Life technology. Big company provide several brand for laboratory product (DNAzol BD reagent kit and single tube custom TaqMan SNP genotyping assays kit).


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