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Validation of RS6265 SNP in BDNF in the Involvement of Diabetes Type II

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ABSTRACT

Brain-derived neurotrophic factor (BDNF), which is a member of the neurotrophin family, is widely expressed in the adult mammalian brain and includes growth factors that promote cell survival, differentiation, and cell death. It plays a critical role in the long-term survival, differentiation and outgrowth of neurons during development and maintenance of neuronal systems in adult life .It is also thought that BDNF is one of the key factors in neurotrophin theory which has proposed that the failure of neurogenesis and neuronal plasticity causes various psychiatric diseases like schizophrenia and major depression. They are synthesized as proforms that can be cleaved intracellularly to release mature, secreted ligands. Mature neurotrophins selectively bind to members of the Trk family of receptor tyrosine kinases that promote Trk-mediated differentiation or survival .It is interesting that proneurotrophins are not inactive precursors, since they can be secreted and cleaved extracellularly and serve as high-affinity ligands for NTR, which promotes apoptosis in neurons and oligo dendrocytes. The gene of BDNF is localized to chromosome 11p14.1 and has a single nuclear polymorphism (SNP) at nucleotide position 196/758 which results in amino acid change at codon 66 Valine (Val) ® Methionine (Met) (Val66Met) of the pro BDNF molecule. The SNP is located in a section of BDNF precursor protein that is cleaved away by proteases on the cell surface, rendering the amino acid change absent from mature BDNF Cultured hippocampal neurons transfected with Met-BDNF show reduced depolarization induced secretion and fail to localize BDNF to secretory granules and dendritic process. **Diabetes mellitus**, describes a group of metabolic diseases in which the person has high blood glucose (blood sugar), either because insulin production is inadequate, or because the body's cells do not respond properly to insulin, or both. Patients with high blood sugar will typically experience polyuria (frequent urination), they will become increasingly thirsty (polydipsia) and hungry (polyphagia).

1.1. INTRODUCTION

Diabetes, often referred to by doctors as **diabetes mellitus**, describes a group of metabolic diseases in which the person has high blood glucose (blood sugar), either because insulin production is inadequate, or because the body's cells do not respond properly to insulin, or both. Patients with high blood sugar will typically experience polyuria (frequent urination), they will become increasingly thirsty (polydipsia) and hungry (polyphagia).

Other forms of diabetes mellitus include congenital diabetes, which is due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid

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diabetes induced by high doses of glucocorticoids, and several forms of monogenic diabetes.

Untreated, diabetes can cause many complications. Acute complications include diabetic ketoacidosisand nonketotic hyperosmolar coma. Serious long-term complications include cardiovascular disease,chronic renal failure, and diabetic retinopathy (retinal damage). Adequate treatment of diabetes is thus important, as well as blood pressure control and lifestyle factors such as stopping smoking and maintaining a healthy body weight.

1.2. Types

There are three main types of diabetes mellitus (DM).

1.2.1 Type 1 Diabetes

Type 1 DM results from the body's failure to produce insulin, and currently requires the person to inject insulin or wear an insulin pump. They must also ensure proper blood-glucose levels by carrying out regular blood tests and following a special diet. Some people may refer to this type as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes" or **early-onset diabetes**. Patients with type 1 diabetes must also ensure proper blood-glucose levels by carrying out regular blood tests and following a special diet.

People usually develop type 1 diabetes before their 40th year, often in early adulthood or teenage years. Type 1 diabetes is nowhere near as common as type 2 diabetes. Approximately 10% of all diabetes cases are type 1.Between 2001 and 2009, the prevalence of type 1 diabetes among the under 20s in the USA rose 23%, according to *SEARCH for Diabetes in Youth* data issued by the CDC (Centers for Disease Control and Prevention).

1.2.2. Type 2 Diabetes

Type 2 DM results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency. This form was previously referred to as non-insulin-dependent diabetes mellitus (NIDDM) or "adult-onset diabetes". Approximately 90% of all cases of diabetes worldwide are of this type.

Some people may be able to control their type 2 diabetes symptoms by losing weight, following a healthy diet, doing plenty of exercise, and monitoring their blood glucose levels. However, type 2 diabetes is typically a progressive disease - it gradually gets worse - and the patient will probably end up have to take insulin, usually in tablet form.

Overweight and obese people have a much higher risk of developing type 2 diabetes compared to those with a healthy body weight. People with a lot of visceral fat, also known as central obesity, belly fat, or abdominal obesity, are especially at risk. Being overweight/obese causes the body to release chemicals that can destabilize the body's cardiovascular and metabolic systems.

Being overweight, physically inactive and eating the wrong foods all contribute to our risk of developing type 2 diabetes. Drinking just one can of (non-diet) soda per day can raise our risk of developing type 2 diabetes by 22%, researchers from Imperial College London reported in the journal *Diabetologia*. The scientists believe that the impact of sugary soft drinks on diabetes risk may be a direct one, rather than simply an influence on body weight.

The risk of developing type 2 diabetes is also greater as we get older. Experts are not completely sure why, but say that as we age we tend to put on weight and become less physically active. Those with a close relative who had/had type 2 diabetes, people of Middle Eastern, African, or South Asian descent also have a higher risk of developing the disease.

Men whose testosterone levels are low have been found to have a higher risk of developing type 2 diabetes. Researchers from the University of Edinburgh, Scotland, say that low testosterone levels are linked to insulin resistance.

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Measuring the glucose level in blood

1.2.3. Gestational Diabetes

This type affects females during pregnancy. Some women have very high levels of glucose in their blood, and their bodies are unable to produce enough insulin to transport all of the glucose into their cells, resulting in progressively rising levels of glucose. Diagnosis of gestational diabetes is made during pregnancy.

The majority of gestational diabetes patients can control their diabetes with exercise and diet. 10% to 20% of them will need to take some kind of blood-glucose-controlling medications. Undiagnosed or uncontrolled gestational diabetes can raise the risk of complications during childbirth. The baby may be bigger than he/she should be.

Scientists from the National Institutes of Health and Harvard University found that women whose diets before becoming pregnant were high in animal fat and cholesterol had a higher risk for gestational diabetes, compared to their counterparts whose diets were low in cholesterol and animal fats.

Other forms of diabetes mellitus include congenital diabetes, which is due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids, and several forms of monogenic diabetes.

Untreated, diabetes can cause many complications. Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar coma. Serious long-term complications include cardiovascular disease, chronic renal failure, and diabetic retinopathy (retinal damage). Adequate treatment of diabetes is thus important, as well as blood pressure control and lifestyle factors such as stopping smoking and maintaining a healthy body weight.

1.3. What Is Prediabetes?

The vast majority of patients with type 2 diabetes initially had **prediabetes**. Their blood glucose levels where higher than normal, but not high enough to merit a diabetes diagnosis. The cells in the body are becoming resistant to insulin. Studies have indicated that even at the prediabetes stage, some damage to the circulatory system and the heart may already have occurred.

1.4. Diabetes As A Metabolism Disorder

Diabetes (diabetes mellitus) is classed as a metabolism disorder. Metabolism refers to the way our bodies use digested food for energy and growth. Most of what we eat is broken down into glucose. Glucose is a form of sugar in the blood - it is the principal source of fuel for our bodies.
When our food is digested, the glucose makes its way into our bloodstream. Our cells use the glucose for energy and growth. However, glucose cannot enter our cells without insulin being present - insulin makes it possible for our cells to take in the glucose. Insulin is a hormone that is produced by the pancreas. After eating, the pancreas automatically releases an adequate quantity of insulin to move the glucose present in our blood into the cells, as soon as glucose enters the cells blood-glucose levels drop.

A person with diabetes has a condition in which the quantity of glucose in the blood is too elevated (hyperglycemia). This is because either the body does not produce enough insulin, produces no insulin, or has cells that do not respond properly to the insulin the pancreas produces. This results in too much glucose building up in the blood. This excess blood glucose eventually passes out of the body in urine. So, even though the blood has plenty of glucose, the cells are

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not getting it for their essential energy and growth requirements.

1.5. Why Is It Called Diabetes Mellitus?

Diabetes comes from Greek, and it means a "siphon". Aretus the Cappadocian, a Greek physician during the second century A.D., named the condition *diabainein*. He described patients who were passing too much water (polyuria) - like a siphon. The word became "diabetes" from the English adoption of the Medieval Latin diabetes.

In 1675, Thomas Willis added mellitus to the term, although it is commonly referred to simply as diabetes. *Mel* in Latin means "honey"; the urine and blood of people with diabetes has excess glucose, and glucose is sweet like honey. Diabetes mellitus could literally mean "siphoning off sweet water". In ancient China people observed that ants would be attracted to some people's urine, because it was sweet. The term "Sweet Urine Disease" was coined.

1.5.1. Symptoms

In both types of diabetes, signs and symptoms are more likely to be similar as the blood sugar is high, either due to less or no production of insulin, or **insulin resistance**. In any case, if there is inadequate glucose in the cells, it is identifiable through certain signs and symptoms. These **symptoms** are quickly relieved once the Diabetes is treated and also reduce the chances of developing serious health problems.

1.5.1.1. Symptoms of each type of Diabetes:

(i) Diabetes Type 1:

In type 1, the pancreas stop producing insulin due to autuimmune response or possibly viral attack on pancreas. In absence of insulin, body cells don't get the required glucose for producing ATP (AdenosinTriphosphate) units which results into primary **symptom** in the form of nausea and vomiting. In later stage, which leads to ketoacidosis, the body starts breaking down the **muscle tissue** and fat for producing energy hence, causing fast weight loss. Dehydration is also usually observed due to electrolyte disturbance. In advanced stages, coma and death is witnessed.

(ii) Diabetes Type 2:

- **Increased fatigue** : Due to inefficiency of the cell to metabolize glucose, reserve fat of body is metabolized to gain energy. When fat is broken down in the body, it uses more energy as compared to glucose, hence body goes in **negative calorie effect**, which results in fatigue.
- **Polydipsia** : As the concentration of glucose increases in the blood, brain receives signal for diluting it and, in its counteraction we feel thirsty.
- **Polyuria**: Increase in urine production is due to excess glucose present in body. Body gets rid of the extra sugar in the **blood** by excreting it through urine. This leads to dehydration because along with the sugar, a large amount of water is excreted out of the body.
- **Polyphegia** : The hormone insulin is also responsible for stimulating hunger. In order to cope up with high sugar levels in blood, body produces **insulin** which leads to increased hunger.
- **Weight flactuation** : Factors like loss of water (polyuria), glucosuria , metabolism of body fat and **protein** may lead to weight loss. Few cases may show weight gain due to increased appetite.
- **Blurry vision** : Hyperosmolar hyperglycemia non ketotic syndrome is the condition when body fluid is pulled out of tissues including lenses of the eye, which affects its ability to focus, resulting blurry vision.
- **Irritability** : It is a sign of high blood sugar because of the inefficient glucose supply to the brain and other body organs, which makes us feel tired and uneasy.
- **Infections** : The body gives few signals whenever there is fluctuation in blood sugar (due to suppression of immune system) by frequent skin infections like fungal or bacterial or UTI (urinary tract infection).

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- **Poor wound healing** : High blood sugar resists the flourishing of WBC, (white blood cell) which are responsible for body immune system. When these cells do not function accordingly, wound healing is not at good pace. Secondly, long standing diabetes leads to thickening of **blood vessels** which affect proper circulation of blood in different body parts.

1.5.1.2. Here is a list of the most common diabetes symptoms:

- **Frequent urination**
  Have you been going to the bathroom to urinate more often recently? Do you notice that you spend most of the day going to the toilet? When there is too much glucose (sugar) in your blood you will urinate more often. If your insulin is ineffective, or not there at all, your kidneys cannot filter the glucose back into the blood. The kidneys will take water from your blood in order to dilute the glucose - which in turn fills up your bladder.

- **Disproportionate thirst**
  If you are urinating more than usual, you will need to replace that lost liquid. You will be drinking more than usual. Have you been drinking more than usual lately?

- **Intense hunger**
  As the insulin in your blood is not working properly, or is not there at all, and your cells are not getting their energy, your body may react by trying to find more energy - food. You will become hungry.

- **Weight gain**
  This might be the result of the above symptom (intense hunger).

- **Unusual weight loss**
  This is more common among people with Diabetes Type 1. As your body is not making insulin it will seek out another energy source (the cells aren't getting glucose). Muscle tissue and fat will be broken down for energy. As Type 1 is of a more sudden onset and Type 2 is much more gradual, weight loss is more noticeable with Type 1.

- **Increased fatigue**
  If your insulin is not working properly, or is not there at all, glucose will not be entering your cells and providing them with energy. This will make you feel tired and listless.
- **Irritability**
  Irritability can be due to your lack of energy.
- **Blurred vision**
  This can be caused by tissue being pulled from your eye lenses. This affects your eyes' ability to focus. With proper treatment this can be treated. There are severe cases where blindness or prolonged vision problems can occur.
- **Cuts and bruises don't heal properly or quickly**
  Do you find cuts and bruises take a much longer time than usual to heal? When there is more sugar (glucose) in your body, its ability to heal can be undermined.
- **More skin and/or yeast infections**
  When there is more sugar in your body, its ability to recover from infections is affected. Women with diabetes find it especially difficult to recover from bladder and vaginal infections.
- **Itchy skin**
  A feeling of itchiness on your skin is sometimes a symptom of diabetes.
- **Gums are red and/or swollen - Gums pull away from teeth**
  If your gums are tender, red and/or swollen this could be a sign of diabetes. Your teeth could become loose as the gums pull away from them.
- **Frequent gum disease/infection**
  As well as the previous gum symptoms, you may experience more frequent gum disease and/or gum infections.
- **Sexual dysfunction among men**
  If you are over 50 and experience frequent or constant sexual dysfunction (erectile dysfunction), it could be a symptom of diabetes.

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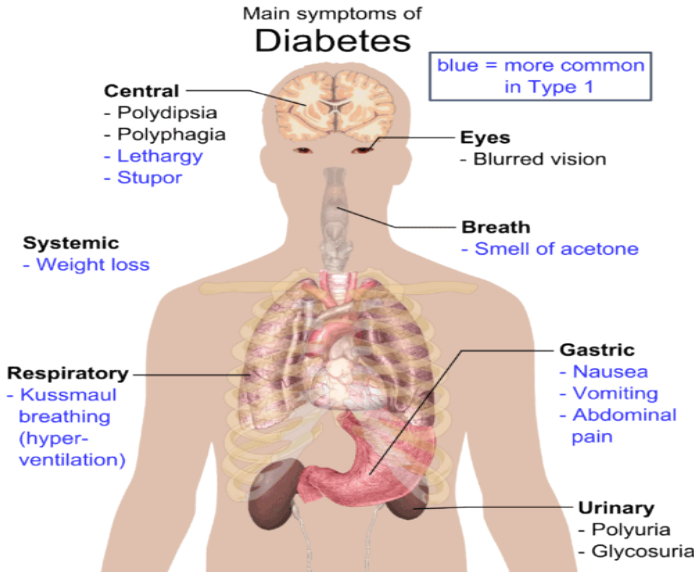
- **Peripheral Neuropathy Numbness or tingling, especially in your feet and hands** If there is too much sugar in your body your nerves could become damaged, as could the tiny blood vessels that feed those nerves. You may experience tingling and/or numbness in your hands and feet.



Symptoms of diabetes mellitus

1.5.2. Causes

Type 2 diabetes is due primarily to lifestyle factors and genetics. A number of lifestyle factors are known to be important to the development of type 2 diabetes, including obesity (defined by a body mass index of greater than thirty), lack of physical activity, poor diet, stress, and urbanization

Dietary factors also influence the risk of developing type 2 diabetes. Consumption of sugar-sweetened drinks in excess is associated with an increased risk. The type of fats in the diet is also important, with saturated fats and trans fatty acids increasing the risk and polyunsaturated and monounsaturated fat decreasing the risk. Eating lots of white rice appears to also play a role in increasing risk A lack of exercise is believed to cause 7% of cases.

The following is a comprehensive list of other causes of diabetes:

Genetic defects of β-cell function
Maturity onset diabetes of the young
Mitochondrial DNA mutations
Genetic defects in insulin processing or insulin action
Defects in pro insulin conversion
Insulin gene mutations
Insulin receptor mutations
Exocrine pancreatic defects
Chronic pancreatitis
Pancreatectomy
Pancreatic neoplasia
Cystic fibrosis
Hemochromatosis
Fibrocalculous pancreatopathy
Endocrinopathies

(i) **_Growth hormone excess_ (acromegaly)**

Cushing syndrome
Hyperthyroidism
Pheochromocytoma
Glucagonoma

(ii) **Infections**

Cytomegalovirus infection
Coxsackievirus B

(iii) **Drugs**

Glucocorticoids
Thyroid hormone
β-adrenergic agonists
Statins

1.5.3. Pathophysiology

Insulin is the principal hormone that regulates uptake of glucose from the blood into most cells (primarily muscle and fat cells, but not central nervous system cells). Therefore, deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus.

Humans are capable of digesting some carbohydrates, in particular those most common in food; starch, and some disaccharides such as sucrose, are converted within a few hours to simpler forms, most notably the monosaccharide glucose, the principal carbohydrate energy source used by the body. The rest are passed on for processing by gut flora largely in the colon. Insulin is released into the blood by beta cells (β-cells), found in the islets of Langerhans in the

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pancreas, in response to rising levels of blood glucose, typically after eating. Insulin is used by about two-thirds of the body's cells to absorb glucose from the blood for use as fuel, for conversion to other needed molecules, or for storage.

Insulin is also the principal control signal for conversion of glucose to glycogen for internal storage in liver and muscle cells. Lowered glucose levels result both in the reduced release of insulin from the β-cells and in the reverse conversion of glycogen to glucose when glucose levels fall. This is mainly controlled by the hormone glucagon, which acts in the opposite manner to insulin. Glucose thus forcibly produced from internal liver cell stores (as glycogen) re-enters the bloodstream; muscle cells lack the necessary export mechanism. Normally, liver cells do this when the level of insulin is low (which normally correlates with low levels of blood glucose).

Higher insulin levels increase some anabolic ("building up") processes, such as cell growth and duplication, protein synthesis, and fat storage. Insulin (or its lack) is the principal signal in converting many of the bidirectional processes of metabolism from a catabolic to an anabolic direction, and *vice versa*. In particular, a low insulin level is the trigger for entering or leaving ketosis (the fat-burning metabolic phase).

If the amount of insulin available is insufficient, if cells respond poorly to the effects of insulin (insulin insensitivity or resistance), or if the insulin itself is defective, then glucose will not have its usual effect, so it will not be absorbed properly by those body cells that require it, nor will it be stored appropriately in the liver and muscles. The net effect is persistent high levels of blood glucose, poor protein synthesis, and other metabolic derangements, such as acidosis.

When the glucose concentration in the blood is raised to about 9-10 m mol/L (except certain conditions, such as pregnancy), beyond its renal threshold(i.e. when glucose level surpasses the transport maximum of glucose reabsorption), reabsorption of glucose in the proximal renal tubuli is incomplete, and part of the glucose remains in the urine (glycosuria). This increases the osmotic pressure of the urine and inhibits reabsorption of water by the kidney, resulting in increased urine production (polyuria) and increased fluid loss. Lost blood volume will be replaced osmotically from water held in body cells and other body compartments, causing dehydration and increased thirst.

1.6. How To Determine Whether You Have Diabetes, Prediabetes or Neither: Diagnosis of diabetes

Doctors can determine whether a patient has a normal metabolism, prediabetes or diabetes in one of three different ways - there are three possible tests:

- **The A1C test (Haemoglobin A1C Test)**
  - at least 6.5% means diabetes
  - between 5.7% and 5.99% means prediabetes
  - less than 5.7% means normal
- **The FPG (fasting plasma glucose) test**
  - at least 126 mg/dl means diabetes
  - between 100 mg/dl and 125.99 mg/dl means prediabetes
  - less than 100 mg/dl means normal

  An abnormal reading following the FPG means the patient has impaired fasting glucose (IFG)
- **The OGTT (oral glucose tolerance test)**
  - at least 200 mg/dl means diabetes
  - between 140 and 199.9 mg/dl means prediabetes
  - less than 140 mg/dl means normal

  An abnormal reading following the OGTT means the patient has impaired glucose tolerance (IGT)
- Diabetes can often be detected by carrying out a urine test, which finds out whether excess glucose is present. This is normally backed up by a blood test, which measures blood glucose levels and can confirm if the cause of your symptoms is diabetes.

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- Diagnosis

Diabetes diagnostic criteria

Condition	2 hour glucose	Fasting glucose	HbA_{1c}
	M mol/l(mg/dl)	M mol/l(mg/dl)	%
Normal	<7.8 (<140)	<6.1 (<110)	<6.0
Impaired fasting glycaemia	<7.8 (<140)	≥ 6.1(≥110) & <7.0(<126)	6.0–6.4
Impaired glucose tolerance	≥7.8 (≥140)	<7.0 (<126)	6.0–6.4
Diabetes mellitus	≥11.1 (≥200)	≥7.0 (≥126)	≥6.5

- Diabetes mellitus is characterized by recurrent or persistent hyperglycemia, and is diagnosed by demonstrating any one of the following:
- Fasting plasma glucose level ≥ 7.0 m mol/l (126 mg/dl)
- Plasma glucose ≥ 11.1 m mol/l (200 mg/dL) two hours after a 75 g oral glucose load as in a glucose tolerance test
- Symptoms of hyperglycemia and casual plasma glucose ≥ 11.1 m mol/l (200 mg/dl)
- Glycated hemoglobin (Hb A1C) ≥ 6.5%.
- A positive result, in the absence of unequivocal hyperglycemia, should be confirmed by a repeat of any of the above methods on a different day. It is preferable to measure a fasting glucose level because of the ease of measurement and the considerable time commitment of formal glucose tolerance testing, which takes two hours to complete and offers no prognostic advantage over the fasting test. According to the current definition, two fasting glucose measurements above 126 mg/dl (7.0 m mol/l) is considered diagnostic for diabetes mellitus.
- People with fasting glucose levels from 110 to 125 mg/dl (6.1 to 6.9 m mol/l) are considered to have impaired fasting glucose. Patients with plasma glucose at or above 140 mg/dL (7.8 m mol/L), but not over 200 mg/dL (11.1 m mol/L), two hours after a 75 g oral glucose load are considered to have impaired glucose tolerance. Of these two pre diabetic states, the latter in particular is a major risk factor for progression to full-blown diabetes mellitus, as well as cardiovascular disease.
- Glycated hemoglobin is better than fasting glucose for determining risks of cardiovascular disease and death from any cause.

Data on the performance of some of these tests in the screening context is limited though it is clear that there is no single ideal test for diabetes screening with high sensitivity and specificity and positive predictive value close to 100 per cent. There are few studies comparing different screening tests in the same population and the populations studied are often very different to the UK population. In comparing tests, specificity and sensitivity from published work is quoted and assessments of positive predictive value (PV+) are made on the basis of a one to two per cent prevalence of undiagnosed diabetes. Based on the available information, the proposed screening tests can be placed in a rough 'order of merit'.

The choice of screening methods should be made on the basis of local circumstances such as the availability of staff, methods of follow up etc. It is not recommended that more than one test be performed on each person.

1. **Two hour post glucose load blood glucose assay** – use of an oral glucose load, usually in the form of a glucose-containing drink, with subsequent measurement of blood glucose is the basis of the oral glucose tolerance test (OGTT) the 'gold standard' for assessment of carbohydrate tolerance. While a full supervised OGTT would represent the best possible screening test for

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diabetes it is not usually practical when large numbers of people are being screened.

An unsupervised glucose load test, in which a person consumes 75g of oral glucose in liquid form and has a single blood glucose assay 120 minutes later (as timed by the individual) gives a reasonable approximation to a formal OGTT and is potentially usable on a larger scale.

Studies have shown a screening laboratory–measured capillary plasma glucose >8.6 mmol/l to have a sensitivity of 90 per cent, specificity of 93 per cent and PV+ of 18 per cent. A result above 11.1 mmol/l is diagnostic of diabetes. However, results between 7.8 mmol/l and 11.1 mmol/l would indicate the need for further testing because of the risk of Impaired Fasting Glucose and Impaired Glucose Tolerance.

2. **Fasting blood glucose** – fasting blood glucose is a remarkably constant parameter on a day-to-day basis in both people without diabetes and those with Type 2 diabetes. In a screening context it is a useful single test which will inevitably miss those people with a carbohydrate intolerance whose hyperglycaemia is only manifest after a carbohydrate load. Sensitivity can be improved by lowering the threshold for a 'positive' test but this is achieved at the expense of a reduced specificity and PV+.
3. **Random blood glucose** – screening with this test is not as sensitive or specific as fasting blood glucose [or a 2 hour OGTT] but may be the most practical test. However its sensitivity and specificity of readings that are slightly raised is not good. Very high results are a good indicator of IFG/IGT, but lower ranges of 6-10 mmol/l may need to be rescreened using a fasting test.
4. **Post prandial/post glucose glycosuria** – testing for glycosuria is most sensitive following ingestion of food, either a specific glucose load or a normal meal. The technique is limited by variations in the renal threshold for glucose, especially the tendency for the threshold to rise with age and by the sensitivity or the glycosuria detection method, usually a commercial glucose-oxidase based dipstick.

These procedures are the recommended screening methods. Method one has the best sensitivity but is the most complex procedure. Method four has the lowest PV+ but is the simplest, while methods two and three lie in the middle in terms of complexity and screening performance. Selection of the best test will depend on local circumstances such as facilities and ease of follow up. Method one, for example may be most appropriate where an individual, perhaps with a family history of diabetes or other risk factor, presents for screening while method three may be the method of choice for screening of a large population (eg a factory or a GP practice population).

Selection of the best test will depend on local circumstances such as facilities and ease of follow up. Method one may, for example, be most appropriate where an individual, perhaps with a family history of diabetes or other risk factor, presents for screening while method three may be the method of choice for screening of a large population (eg a factory or a GP practice population).

Any screening programme will identify people with IFG or IGT. They should be tested, in line with WHO standards, to rule out a diagnosis of diabetes. This population will need to be provided with lifestyle advice, support and information about healthy eating and physical activity and will need to be screened for diabetes on a three yearly basis.

(5) Understanding Test Results

A blood test indicating prediabetes means that insulin resistance has progressed to the point where the beta cells in the pancreas can no longer compensate and a person's blood glucose levels are rising toward type 2 diabetes. The higher the test results, the greater the risk of type 2 diabetes.

- **Test numbers.** For example, people with an A1C below 5.7 percent may still be at risk for

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diabetes if they have a family history of type 2 diabetes or have gained excess weight around the waist. People with an A1C above 6.0 percent should be considered at very high risk of developing diabetes. A level of 6.5 percent or above means a person has diabetes.

- **Follow up.** People whose test results indicate they have prediabetes may be retested in 1 year and should consider making lifestyle changes to reduce their risk of developing type 2 diabetes.
- **Varying results.** Although all these tests can be used to test for prediabetes, in some people one test will indicate a diagnosis of prediabetes or diabetes when another test does not. People with differing test results may be in an early stage of the disease, where blood glucose levels have not risen high enough to show on every test.
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Health care providers repeat laboratory tests to confirm test results. Diabetes develops over time, so even with variations in test results, health care providers can tell when overall blood glucose levels are becoming too high.

1.7. Approaches to Preventing Diabetes

The DPP tested three approaches to preventing diabetes:

- **Making lifestyle changes.** People in the lifestyle change group exercised, usually by walking 5 days a week for about 30 minutes a day, and lowered their intake of fat and calories.
- **Taking the diabetes medication metformin.** Those who took metformin also received information about physical activity and diet.
- **Receiving education about diabetes.** The third group only received information about physical activity and diet and took a placebo—a pill without medication in it.

People in the lifestyle change group showed the best outcomes. However people who took metformin also benefited. The results showed that by losing an average of 15 pounds in the first year of the study, people in the lifestyle change group reduced their risk of developing type 2 diabetes by 58 percent over 3 years.

Lifestyle change was even more effective in those ages 60 and older. People in this group reduced their risk by 71 percent.

People in the metformin group also benefited, reducing their risk by 31 percent. More information about the DPP, funded under NIH clinical trial number NCT00004992.

- **Lasting Results**

The Diabetes Prevention Program Outcomes Study (DPPOS) has shown that the benefits of weight loss and metformin last for at least 10 years. The DPPOS has continued to follow most DPP participants since the DPP ended in 2001. The DPPOS showed that 10 years after enrolling in the DPP

- people in the lifestyle change group reduced their risk for developing diabetes by 34 percent
- those in the lifestyle change group ages 60 or older had even greater benefit, reducing their risk of developing diabetes by 49 percent
- participants in the lifestyle change group also had fewer heart and blood vessel disease risk factors, including lower blood pressure and triglyceride levels, even though they took fewer medications to control their heart disease risk
- those in the metformin group reduced their risk of developing diabetes by 18 percent

Even though controlling weight with lifestyle changes is challenging, it produces long-term health rewards by lowering the risk for type 2 diabetes, lowering blood glucose levels, and reducing other heart disease risk factors.

For more information about the risk of developing diabetes; the DPP, funded under NIH clinical trial number NCT00004992; and the DPPOS, funded under NIH clinical trial number NCT00038727, see the following NDIC publications:

- *Diabetes Prevention Program*

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- *Am I at risk for type 2 diabetes? Taking Steps to Lower Your Risk of Getting Diabetes*

1.8. What steps can help reverse insulin resistance and prediabetes?

By losing weight and being more physically active, people can reverse insulin resistance and prediabetes, thus preventing or delaying type 2 diabetes. People can decrease their risk by

- eating a healthy diet and reaching and maintaining a healthy weight
- increasing physical activity
- not smoking
- taking medication

(i) Eating, Diet, and Nutrition

Adopting healthy eating habits can help people lose a modest amount of weight and reverse insulin resistance. Experts encourage people to slowly adopt healthy eating habits that they can maintain, rather than trying extreme weight-loss solutions. People may need to get help from a dietitian or join a weight-loss program for support.

In general, people should lose weight by choosing healthy foods, controlling portions, eating less fat, and increasing physical activity. People are better able to lose weight and keep it off when they learn how to adapt their favorite foods to a healthy eating plan.

The DASH (Dietary Approaches to Stop Hypertension) eating plan, developed by the NIH, has been shown to be effective in decreasing insulin resistance when combined with weight loss and physical activity. More information on DASH is available atwww.nhlbi.nih.gov/health/health-topics/topics/dash.

The U.S. Dietary Guidelines for Americans also offers healthy eating advice and tools for changing eating habits atwww.choosemyplate.gov.

(ii) Dietary Supplements

Vitamin D studies show a link between people's ability to maintain healthy blood glucose levels and having enough vitamin D in their blood. However, studies to determine the proper vitamin D levels for preventing diabetes are ongoing; no special recommendations have been made about vitamin D levels or supplements for people with prediabetes.

Currently, the Institute of Medicine (IOM), the agency that recommends supplementation levels based on current science, provides the following guidelines for daily vitamin D intake:

- People ages 1 to 70 years may require 600 International Units (IUs).
- People ages 71 and older may require as much as 800 IUs.

The IOM also recommended that no more than 4,000 IUs of vitamin D be taken per day.

To help ensure coordinated and safe care, people should discuss use of complementary and alternative medicine practices, including the use of dietary supplements, with their health care provider.

More information about using dietary supplements to help with diabetes is available from the NDIC atwww.diabetes.niddk.nih.gov/dm/pubs/alternativetherapies/index.aspx.

(iii) Physical Activity

Regular physical activity tackles several risk factors at once. Regular physical activity helps the body use insulin properly.

Regular physical activity also helps a person

- lose weight
- control blood glucose levels
- control blood pressure
- control cholesterol levels

People in the DPP who were physically active for 30 minutes a day, 5 days a week, reduced their risk of type 2 diabetes. Many chose brisk walking as their physical activity.

Most people should aim for at least 30 minutes of exercise most days of the week. For best results, people should do both aerobic activities, which use large muscle groups and make the heart beat faster, and muscle strengthening activities.

Aerobic activities include brisk walking, climbing stairs, swimming, dancing, and other activities that increase the heart rate.

Muscle strengthening activities include lifting weights and doing sit-ups or push-ups.

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People who haven't been physically active recently should talk with their health care provider about which activities are best for them and have a checkup before starting an exercise program.

(iv) Not Smoking

Those who smoke should quit. A health care provider can help people find ways to quit smoking. Studies show that people who get help have a better chance of quitting.

For more information about how to reverse insulin resistance and prediabetes with diet and increased physical activity, see the following National Diabetes Education Program publications at www.yourdiabetesinfo.org:

- *Get Real! You Don't Have to Knock Yourself Out to Prevent Diabetes!*
- *More Than 50 Ways to Prevent Diabetes*
- *Small Steps. Big Rewards. Your Game Plan to Prevent Type 2 Diabetes.*

(v) Medication

The medication metformin is recommended for treatment of some individuals at very high risk of developing type 2 diabetes. In the DPP, metformin was shown to be most effective in preventing or delaying the development of type 2 diabetes in younger, heavier people with prediabetes. In general, metformin is recommend for those who are younger than age 60 and have

- combined IGT and IFG
- A1C above 6 percent
- low HDL cholesterol
- elevated triglycerides
- a parent or sibling with diabetes
- a BMI of at least 35

Metformin also lowers the risk of diabetes in women who have had gestational diabetes. People at high risk should ask their health care provider if they should take metformin to prevent type 2 diabetes.

Several medications have been shown to reduce type 2 diabetes risk to varying degrees, but the only medication recommended by the ADA for type 2 diabetes prevention is metformin. Other medications that have delayed diabetes have side effects or haven't shown long-lasting benefits. No medication, including metformin, is approved by the U.S. Food and Drug Administration to treat insulin resistance or prediabetes or to prevent type 2 diabetes.

(vi) Points to Remember

- Insulin is a hormone that helps cells throughout the body absorb glucose and use it for energy. Insulin resistance is a condition in which the body produces insulin but does not use it effectively.
- Insulin resistance increases the risk of developing type 2 diabetes and prediabetes.
- The major contributors to insulin resistance are excess weight, especially around the waist, and physical inactivity.
- Prediabetes is a condition in which blood glucose or A1C levels—which reflect average blood glucose levels—are higher than normal but not high enough for a diagnosis of diabetes.
- The Diabetes Prevention Program (DPP) study and its follow-up study, the Diabetes Prevention Program Outcomes Study (DPPOS), confirmed that people with prediabetes can often prevent or delay diabetes if they lose a modest amount of weight by cutting fat and calorie intake and increasing physical activity.
- By losing weight and being more physically active, people can reverse insulin resistance and prediabetes, thus preventing or delaying type 2 diabetes.
- People with insulin resistance and prediabetes can decrease their risk for diabetes by eating a healthy diet and reaching and maintaining a healthy weight, increasing physical activity, not smoking, and taking medication.
- The DPP showed the diabetes medication metformin to be most effective in preventing or delaying the development of type 2 diabetes in younger and heavier people with prediabetes and women who have had gestational diabetes.

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1.9. Controlling Diabetes - Treatment Is Effective And Important

All types of diabetes are treatable. Diabetes type 1 lasts a lifetime, there is no known cure. Type 2 usually lasts a lifetime; however, some people have managed, through a lot of exercise, diet and excellent body weight control to get rid of their symptoms without medication.

Research showed that gastric bypass surgery can reverse type 2 diabetes in a high proportion of patients. They added that within three to five years the disease recurs in approximately 21% of them.

Patients with type 1 are treated with regular insulin injections, as well as a special diet and exercise. Patients with Type 2 diabetes are usually treated with tablets, exercise and a special diet, but sometimes insulin injections are also required.
If diabetes is not adequately controlled the patient has a significantly higher risk of developing complications.

1.9.1. Complications linked to badly controlled diabetes:

(i) Eye complications - glaucoma, cataracts, diabetic retinopathy, and some others.
(ii) Foot complications - neuropathy, ulcers, and sometimes gangrene which may require that the foot be amputated
(iii) Skin complications - people with diabetes are more susceptible to skin infections and skin disorders
(iv)) Heart problems - such as ischemic heart disease, when the blood supply to the heart 9vmuscle is diminished
(v) Hypertension - common in people with diabetes, which can raise the risk of kidney disease, eye problems, heart attack and stroke
(vi) Mental health - uncontrolled diabetes raises the risk of suffering from depression, anxiety and some other mental disorders
(vii) Hearing loss - diabetes patients have a higher risk of developing hearing problems
(viii) Gum disease - there is a much higher prevalence of gum disease among diabetes patients
(ix) Gastroparesis - the muscles of the stomach stop working properly
(x) Ketoacidosis - a combination of ketosis and acidosis; accumulation of ketone bodies and acidity in the blood.
(xi) Neuropathy - diabetic neuropathy is a type of nerve damage which can lead to several different problems.
(xii) HHNS (Hyperosmolar Hyperglycemic Nonketotic Syndrome) - blood glucose levels shoot up too high, and there are no ketones present in the blood or urine. It is an emergency condition.
(xiii) Nephropathy - uncontrolled blood pressure can lead to kidney disease
(ivx) PAD (peripheral arterial disease) - symptoms may include pain in the leg, tingling and sometimes problems walking properly
(vx) Stroke - if blood pressure, cholesterol levels, and blood glucose levels are not controlled, the risk of stroke increases significantly
(vxi) Erectile dysfunction - male impotence.
(vxii) Infections - people with badly controlled diabetes are much more susceptible to infections
(vxiii) Healing of wounds - cuts and lesions take much longer to heal

1.9.2. Main Aim of treatment

The main aim of treatment of both types of diabetes is to normalise blood glucose levels to protect against long term damage to the eyes, kidneys, nerves,heart and all the blood vessels. Some experts call diabetes “a blood vessel disease” because preventing narrowing of the blood vessels is key to preventing complications.

Although diabetes cannot be cured, it can be treated successfully. If a high blood sugar (glucose) level is brought down to a normal or near-normal level, your symptoms will ease and you are likely to feel well again. You still have some risk of complications in the long term if your blood glucose level remains even mildly high - even if you have no symptoms in the short term. However, studies have shown that people who have better glucose control have fewer

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complications (such as heart disease or eye problems) compared with those people who have poorer control of their glucose level.

Therefore, the main aims of treatment are:

- To keep your blood glucose level as near normal as possible.
- To reduce any other risk factors that may increase your risk of developing complications. In particular, to lower your blood pressure if it is high, and to keep your blood lipids (cholesterol) low.
- To detect any complications as early as possible. Treatment can prevent or delay some complications from getting worse.

Treatment aim 1 - keeping your blood glucose level at normal levels

1.10. How is the blood sugar (glucose) level monitored?

The blood test that is mainly used to keep a check on your blood glucose level is called the HbA1c test. This test is commonly done every 2-6 months by your doctor or nurse.

The HbA1c test measures a part of the red blood cells. Glucose in the blood attaches to part of the red blood cells. This part can be measured and gives a good indication of your average blood glucose level over the preceding 1-3 months.

Treatment aims to lower your HbA1c to below a target level. Ideally, it is best to maintain HbA1c to less than 48 mmol/mol (6.5%). However, this may not always be possible to achieve and your target level of HbA1c should be agreed between you and your doctor. If your HbA1c is above your target level then you may be advised to step up treatment (for example, to increase a dose of medication) to keep your blood glucose level down.

Some people with diabetes check their actual blood glucose level regularly with a blood glucose monitor. If you are advised to do this then your doctor or nurse will give you instructions on how to do it.

1.10.1. Lifestyle - diet, weight control and physical activity

Lifestyle changes are an essential part of treatment for **all** people with type 2 diabetes, regardless of whether or not they take medication.

You can usually reduce the level of your blood glucose and HbA1c if you:

- **Eat a healthy and balanced diet**. Your practice nurse and/or dietician will give you details on a healthy diet. The diet is the same as recommended for everyone. The idea that you need special foods if you have diabetes is a myth. Diabetic foods still raise blood glucose levels, contain just as much fat and calories and are usually more expensive than non-diabetic foods. Basically, you should aim to eat a diet low in fat, salt and sugar and high in fibre and with plenty of fruit and vegetables.
- **Lose weight if you are overweight**. Getting to a perfect weight is unrealistic for many people. However, losing some weight if you are obese or overweight will help to reduce your blood glucose level (and have other health benefits too).
- **Do some physical activity regularly**. If you are able, a minimum of 30 minutes' brisk walking at least five times a week is advised. Anything more vigorous and more often is even better. For example, swimming, cycling, jogging, dancing. Ideally, you should do an activity that gets you at least mildly out of breath and mildly sweaty. You can spread the activity over the day - for example, two fifteen-minute spells per day of brisk walking, cycling, dancing, etc. Regular physical activity also reduces your risk of having a heart attack or stroke.

Many people with type 2 diabetes can reduce their blood glucose (and HbA1c) to a target level by the above measures. However, if the blood glucose (or HbA1c) level remains too high after a trial of these measures for a few months, then medication is usually advised.

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1.10.2. Medication

There are various medicines that can reduce the blood glucose level. Different ones suit different people. It is fairly common to need a combination of medicines to control your blood glucose level. Some medicines work by helping insulin to work better on the body's cells. Others work by boosting the amount of insulin made by the pancreas. Another type works by slowing down the absorption of glucose from the gut. There is also a type which suppresses a hormone called glucagon, which is released into the bloodstream by the pancreas and stops insulin from working.

Medication is not used *instead* of a healthy diet, weight control and physical activity - you should still do these things *as well* as take medication. See separate leaflet calledTreatments for Type 2 Diabetes for more details.

(i) Related Wellbeing

- Artificially sweetened soft drinks linked to higher risk of type 2 diabetes in middle-aged women
- Walnuts found to reduce risk of type 2 diabetes in women
- Having desk job 'doubles risk' of heart attack

Treatment aim 2 - to reduce other risk factors

You are less likely to develop complications of diabetes if you reduce any other risk factors. These are briefly mentioned below - see separate leaflet called Preventing Cardiovascular Diseases for more details. Although everyone should aim to cut out preventable risk factors, people with diabetes have even more of a reason to do so.

(ii) Keep your blood pressure down

It is very important to have your blood pressure checked regularly. The combination of high blood pressure and diabetes is a particularly high risk factor for complications. Even mildly raised blood pressure should be treated if you have diabetes. Medication, often with two or even three different medicines, may be needed to keep your blood pressure down. See separate leaflet called Diabetes and High Blood Pressure for more details.

(iii) If you smoke - now is the time to stop

Smoking is a high risk factor for complications. You should see your practice nurse or attend a smoking cessation clinic if you have difficulty stopping smoking. If necessary, medication or nicotine replacement therapy (nicotine gum, etc) may help you to stop.

(iv) Other medication

You will usually be advised to take a medicine to lower your cholesterol level. This will help to lower the risk of developing some complications such as heart disease, peripheral vascular disease and stroke.

Treatment aim 3 - to detect and treat any complications promptly

Most GP surgeries and hospitals have special diabetes clinics. Doctors, nurses, dieticians, specialists in foot care (chiropodists), specialists in eye health (optometrists) and other healthcare workers all play a role in giving advice and checking on progress. Regular checks may include:

- Checking levels of blood sugar (glucose), HbA1c, cholesterol and blood pressure.
- Ongoing advice on diet and lifestyle.
- Checking for early signs of complications, for example:
  - Eye checks - to detect problems with the retina (a possible complication of diabetes) which can often be prevented from getting worse. Increased pressure in the eye (glaucoma) is also more common in people with diabetes and can usually be treated.
  - Urine tests - which include testing for protein in the urine, which may indicate early kidney problems.
  - Foot checks - to help to prevent foot ulcers.
  - Other blood tests - these include checks on kidney function and other general tests.

It is important to have regular checks, as some complications, particularly if detected early, can be treated or prevented from getting worse.

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1.11. Genetic Causes Of Diabetes Mellitus Type 2: Candidate Genes

Most cases of diabetes mellitus type 2 involved many genes contributing small amount to the overall condition. As of 2011 more than 36 genes have been found that contribute to the risk of type 2 diabetes. All of these genes together still only account for 10% of the total genetic component of the disease.

Genes associated with developing type 2 diabetes, include *TCF7L2*, *PPARG*, *FTO*, *KCNJ11*,*NOTCH2*,*WFS1*, *IGF2BP2*, *SLC30A8*, *JAZF1*, *HHEX* among others. *KCNJ11* (potassium inwardly rectifying channel, subfamily J, member 11), encodes the islet ATP-sensitive potassium channel Kir6.2, and*TCF7L2* (transcription factor 7–like 2) regulates proglucagon gene expression and thus the production of glucagon-like peptide-1. In addition, there is also a mutation to the Islet Amyloid Polypeptide gene that results in an earlier onset, more severe, form of diabetes.

Various hereditary conditions may feature diabetes, for example, myotonic dystrophy and Friedreich's ataxia. Wolfram's syndrome is an autosomal recessive neurodegenerative disorder that first becomes evident in childhood. It consists of diabetes insipidus, diabetes mellitus, optic atrophy, and deafness, hence the acronym DIDMOAD.

The search for Diabetes genes has predominantly been undertaken using the candidate gene approach. The case-control study design is generally employed and is appropriate for detecting both major and minor genes. The candidate gene approach requires a fair knowledge of the pathogenic mechanisms underlying Diabetes and this has benefitted from the many years of research in this field. Correspondingly, a host of genes involved in these pathways/processes have been treated as potential candidate genes. These genes include angiotensin-I converting enzyme (*ACE*), angiotensin II type 1 receptor (*AGTR1*), angiotensinogen (*AGT*), vascular endothelial growth factor (*VEGF*), aldose reductase (*AR2*), receptor for advanced glycation endproducts (*RAGE*), glucose transporter 1 (*GLUT1*), inducible and constitutive nitric oxide synthases (*NOS2A*, *NOS3*), transforming growth factor beta (*TGFbeta*), endothelin isoforms, and its cellular receptors, among others.

As is the current thought in the field of complex genetics, the effect sizes of these genetic factors are likely to be modest although major genes have been postulated to exist. Consequently, individual studies have, more often than not, yielded inconsistent and even conflicting findings. To circumvent this issue, meta-analyses have been undertaken to pinpoint the few genes for which there might be cumulative evidence for an association with Diabetes.

(i) Aldose Reductase (AKR1B1, Human Chromosome 7q35)

Aldose reductase (AKR1B1) is the rate-limiting enzyme of the polyol pathway, which catalyzes NADPH-dependent reduction of glucose to sorbitol. AKR1B1 has been reported in human pericytes, and activation of this pathway has been strongly implicated in the pathogenesis of DR. Notably, retinal vascular changes such as microaneurysm formation and degeneration of retinal pericytes may be induced in rats and dogs that have been made hyperglycemic by a diet rich in galactose, the latter being reduced by AKR1B1 to form galactitol. The search for pharmacological inhibitors of this enzyme for use in the treatment of DR is ongoing. In a recent meta-analysis of the various polymorphisms in *AKR1B1*, the Z-2 allele of the (C A)n microsatellite located at the 5□ end of the gene showed the most significant association with diabetic retinopathy (OR = 2.33, 95% CI = 1.49–3.64, *P* = . 0 0 0 2), independently of the type of diabetes present.

(ii) Vascular Endothelial Growth Factor (VEGF, Human Chromosome 6p12)

Growth factor mediation — particularly vascular endothelial growth factor (VEGF) — is part of retinopathy. PDR appears to be driven by ischemia in the retina. Growth factors in the eye lead to neovascularization.

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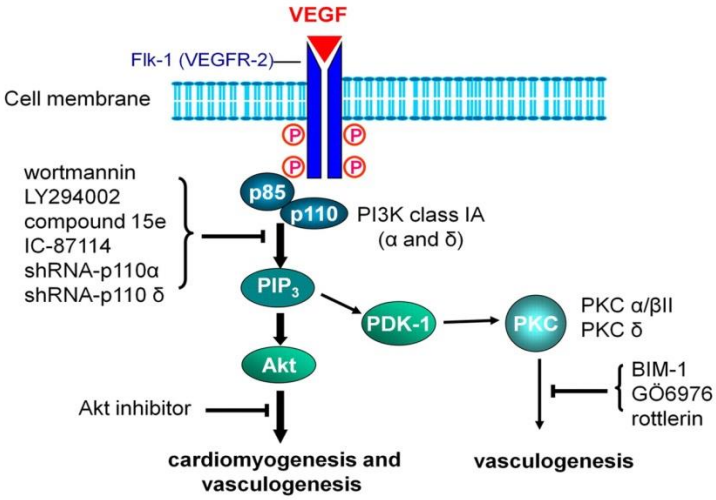
VEGF is a 46 kDa homodimeric glycoprotein that was first identified in vascularized tumors. VEGF is produced by many ocular cell types and is induced by hypoxia. It binds to high-affinity receptors on retinal endothelial cells and stimulates retinal endothelial cell growth. VEGF promotes vascular permeability and induces disassociation of tight junction components. Retinal VEGF levels are elevated in diabetes and VEGF can cause diabetic retinopathy-like changes in the eye. Data shows that VEGF plays a key role in mediating retinal neovascularization and vascular permeability under ischemic retinal conditions, such as diabetic retinopathy.
If an agent was able to block VEGF, we might be able to prevent diabetic complications in the eye. VEGF inhibition in mice and primate models was shown to be successful.

VEGF is an important growth factor involved in causing vascular permeability. High vitreous levels have been repeatedly detected in eyes of patients undergoing vitrectomy operations for PDR and diabetic macular edema. The cellular effects of VEGF are mediated primarily through two closely related receptor tyrosine kinases VEGFR-1 (Flt1) and VEGFR-2 (KDR/Flk1). Regulation of target genes such as heptocyte growth factor (HGF), urinary and tissue plasminogen activator (uPA, tPA), matrix metalloproteinase-9 (MMP9) is then achieved through complex signaling pathways, including through protein kinase C (PKC). VEGF inhibition has been shown to ameliorate retinal changes including retinal neovascularization and breakdown of the blood-retinal barrier. A total of six polymorphisms (rs25648, rs1570360, rs3095039, rs35569394, rs699947 and rs2010963) in *VEGF* have been examined and of these, the G allele of rs2010963 was significantly associated with a reduced risk of NPDR in patients with type 2 diabetes (OR = 0.62, 95% CI = 0.48–0.81, *P* = . 0 0 0 5). Considering that VEGF has been implicated in neovascularization, it might appear surprising that none of the polymorphisms so far including rs2010963 has been significantly associated with PDR.



VEGF and PKC Association

(iii) Angiotensin-I Converting Enzyme (ACE, Human Chromosome 17q23)

Among the DR candidate genes, ACE is the most widely studied. The well-known insertion deletion (I/D) polymorphism in ACE which results from the insertion/deletion of a 287 bp Alu sequence in intron 16 accounts for half the variance of serum enzyme levels. Individuals who are homozygous for the insertion allele (II genotype) have significantly lower levels of ACE compared to carriers of the deletion allele (ID and DD genotypes). A meta-analysis of six studies on this polymorphism in patients with type 1 diabetes and seven studies in patients with type 2 diabetes suggested that there was no statistically significant association of this polymorphism and the development of any form of diabetic retinopathy. A second recent independent meta-analysis corroborated this finding but suggested that ACE I/D may be associated with PDR (OR = 1.37, 95% CI = 1.02–1.84) under a dominant genetic model assuming either fixed or random effects.

iv) Protein Kinase C

Protein kinase-C (PKC) activation appears to be a key player in many of the microvascular disease pathways. PKC inhibition may help ameliorate hyperglycemia-induced cellular dysfunction. The PKC

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enzyme modulates molecules in the body and the beta isoform is primarily involved in the complications of diabetes and other vascular issues. In the state of hyperglycemia, diaclyglycerol is synthesized, which in turn activates PKC. Therefore, the fundamental problem is that PKC is activated in the diabetic condition.

The level of PKC activity then correlates to the severity of diabetic retinopathy. PKC-beta activation plays a key role in mediating early retinal changes in diabetes. Even when PKC-beta was overexpressed in nondiabetic mice, retinal abnormalities were seen. Based on the methods like clustalw and phylogenetic tree construction several proteins involved in the pathogenesis of diabetic retinopathy are identified and shows that BDNF**,** aldose reductase, nitric oxide synthase has role in diabetes and its complications**.** On scrutinizing we tend to take PKCβ and its role in diabetic retinopathy. In hypoglycemic conditions, DAG-PKC pathway plays a major role by which increase within the levels of DAG leads to PKC activation. DAG is derived from the hydrolysis of phosphatidylinositol 4-5 bisphosphate, by a membrane bound enzyme phospholipase C - (PLC)**.** DAG–PKC pathway can also be activated by hyperglycemia induced oxidants such as H2O2 which are known to activate PKC either directly or by increasing DAG production**.** In early stages increase in DAG may activate PKC and in advanced stages, when VEGF levels are elevated PKC plays an important role in modulation of VEGF action and as a stimulator of VEGF expression. PKC activation induced by hyperglycemia could alter the expression of various growth factors like VEGF that induces retinal vessel permeability. PKCβ activation affects VEGF expression through the mRNA-stabilizing human embryonic lethal abnormal vision (ELAV) protein, HuR , in the retina**.** Previous work additionally explains that in order to test the linearity of the multiple sequences at a time for a number of proteins general regression model technique algorithm (GRMT1) can be used and high accuracy sequence clustering can also be done by using a clustering algorithm**.** Inhibition of PKCβ leads to prevention of glucose induced increase in VEGF expression (Figure1)**.** Hence oral administration of PKCβ inhibitor LY333531 will forestall or reverse blood retinal barrier breakdown by inhibiting VEGF expression. Ruboxistaurin or LY333531 could be a competitive inhibitor that acts by interacting with the ATP binding site which shows selectivity towards PKC and hence found to be an important therapeutic agent for diabetic retinopathy.

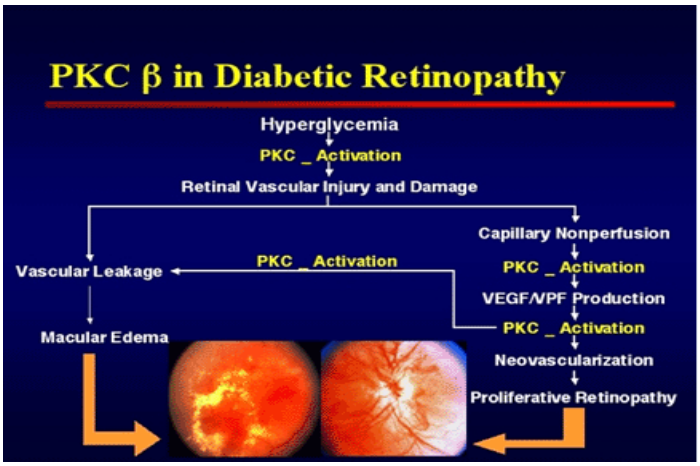

Fig 3: Role of PKCβ in Diabetic Retinopathy

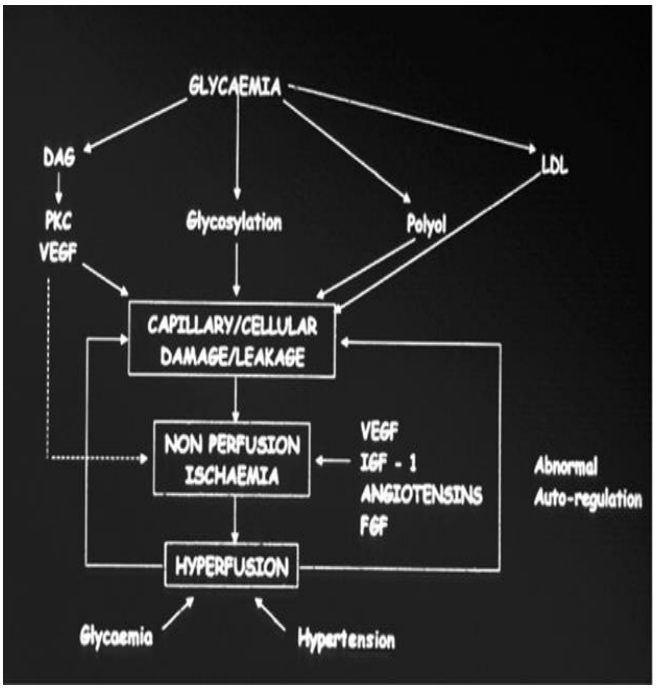

Fig 4: Proteins' action linkage in DR

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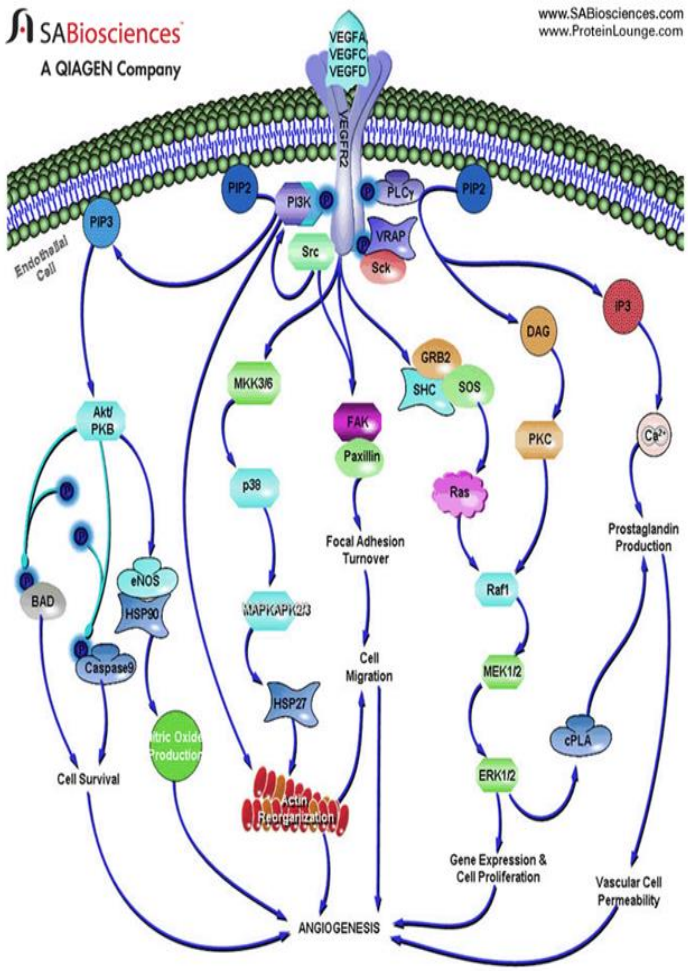
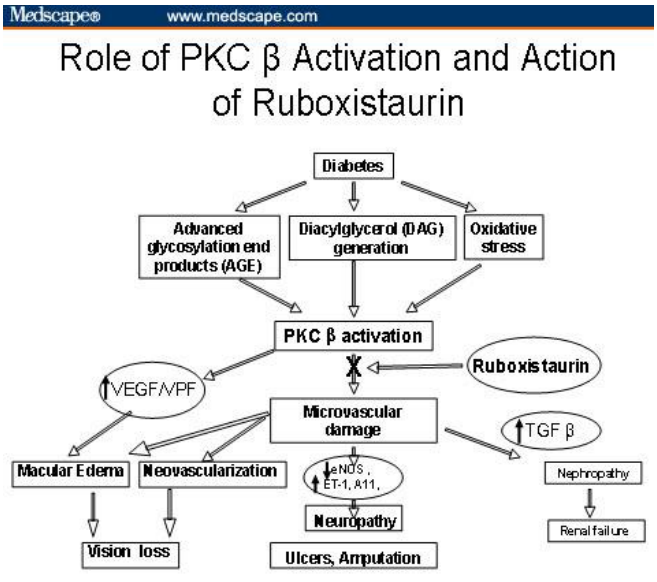


Fig 5: Showing PKC Activation Pathway



Role of PKCβ activation and action of Ruboxistaurin

V) PPARg

Peroxisome proliferator activated receptor gamma is a pleotropic transcriptional factor belongs to the class of nuclear receptors subfamily 1, group c, and member 3. This protein is involved in the regulation of several major metabolic pathways like lipid metabolism, adipogenesis and insulin sensitivity. This nuclear receptor controls the energy balance of the system through its involvement in various inputs and outputs of metabolic pathways. Retinoid X Receptors are the obligate heterodimeric partners for PPAR action. PPAR ligands activate the PPAR protein which then forms the dimer with RXR thus activating the target gene expression by binding to the Peroxisome proliferator responsive element (PPRE). PPARg palys a critical role in energy balance including glucose homeostasis. Any disregulation of these processes due to PPARg emphasizes its role in causing diabetes. PPARg expression in the retina has a beneficial role in the modulation of inflammation, angiogenesis and apoptosis in retinal and endothelial cells thereby ameliorating the retinal and endothelial damage caused by high glucose induced prolonged inflammation. Therefore PPARg can be used as one of the potential target in the treatment of Diabetic retinopathy. PPARg expression is found in a suppressed state in the experimental models of diabetes. Use of PPAR activators can be one of the rescue treatments for improving the condition of diabetic retinopathy.

VI) Butyrylcholine esterase

- **Butyrylcholine esterase** (also known as pseudo cholinesterase, plasma cholinesterase, **BCHE**, or **BuChE**) is a non-specific cholinesterase enzyme that hydrolyses many different choline esters. In humans, it is found primarily in the liver and is encoded by the *BCHE* gene.

It is very similar to the neuronal acetylcholinesterase, which is also known as RBC or erythrocyte cholinesterase. The term "serum cholinesterase" is generally used in reference to a clinical test that reflects levels of both of these enzymes in the blood.

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Butyrylcholine is a synthetic compound and does not occur in the body naturally. It is used as a tool to distinguish between acetyl- and butyryl cholinesterase.

- **Clinical significance**

Pseudo cholinesterase deficiency results in delayed metabolism of only a few compounds of clinical significance, including the following:succinylcholine, mivacurium, procaine, hero in and cocaine. Of these, its most clinically important substrate is the depolarizing neuromuscular blocking agent, succinylcholine, which the pseudo cholinesterase enzyme hydrolyzes to succinylmonocholine and then to succinic acid.

In individuals with normal plasma levels of normally functioning pseudo cholinesterase enzyme, hydrolysis and inactivation of approximately 90-95% of an intravenous dose of succinylcholine occurs before it reaches the neuromuscular junction. The remaining 5-10% of the succinylcholine dose acts as an acetylcholine receptor agonist at the neuromuscular junction, causing prolonged depolarization of the postsynaptic junction of the motor-end plate. This depolarization initially triggers fasciculation of skeletal muscle. As a result of prolonged depolarization, endogenous acetylcholine released from the pre synaptic membrane of the motor neuron does not produce any additional change in membrane potential after binding to its receptor on the myocyte. Flaccid paralysis of skeletal muscles develops within 1 minute. In normal subjects, skeletal muscle function returns to normal approximately 5 minutes after a single bolus injection of succinylcholine as it passively diffuses away from the neuromuscular junction. Pseudo cholinesterase deficiency can result in higher levels of intact succinylcholine molecules reaching receptors in the neuromuscular junction, causing the duration of paralytic effect to continue for as long as 8 hours. This condition is recognized clinically when paralysis of the respiratory and other skeletal muscles fails to spontaneously resolve after succinylcholine is administered as an adjunctive paralytic agent during anesthesia procedures. In such cases respiratory assistance is required.

In 2008, an experimental new drug was discovered for the potential treatment of cocaine abuse and overdose based on the pseudocholiesterase structure. It was shown to remove cocaine from the body 2000 times as fast as the natural form of BChE. Studies in rats have shown that the drug prevented convulsions and death when administered cocaine overdoses. This enzyme also metabolizes succinylcholine which accounts for its rapid degradation in the liver and plasma. There may be genetic variability in the kinetics of this enzyme that can lead to prolonged muscle blockade and potentially dangerous respiratory depression that needs to be treated with assisted ventilation.

Mutant alleles at the BCHE locus are responsible for suxamethonium sensitivity. Homozygous persons sustain prolonged apnea after administration of the muscle relaxant suxamethonium in connection with surgical anesthesia. The activity of pseudocholinesterase in the serum is low and its substrate behavior is atypical. In the absence of the relaxant, the homozygote is at no known disadvantage.

Finally, pseudocholinesterase metabolism of procaine results in formation of paraaminobenzoic acid (PABA). If the patient receiving procaine is on sulfonamide antibiotics such as bactrim the antibiotic effect will be antagonized by providing a new source of PABA to the microbe for subsequent synthesis of folic acid.

1.12. Health Complications of Diabetes

The National Institute of Health has identified some of the complications that can arise from diabetes:[63]

(i) Heart Disease and Stroke

- In 2004, heart disease was noted on 68 percent of diabetes-related death certificates among people aged 65 years or older.
- In 2004, stroke was noted on 16 percent of diabetes-related death certificates among people aged 65 years or older.[64]
- Adults with diabetes have heart disease death rates about two to four times higher than adults without diabetes.
- The risk for stroke is two to four times higher among people with diabetes.

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(ii) **High Blood Pressure**

- In 2003 to 2004, 75 percent of adults with self-reported diabetes had blood pressure greater than or equal to 130/80 millimeters of mercury (mm Hg) or used prescription medications for hypertension.

(iii) **Blindness**

- Diabetes is the leading cause of new cases of blindness among adults aged 20 to 74 years.
- Diabetic retinopathy causes 12,000 to 24,000 new cases of blindness each year.[62]

(iv) **Kidney Disease**

- Diabetes is the leading cause of kidney failure, accounting for 44 percent of new cases in 2005.
- In 2005, 46,739 people with diabetes began treatment for end-stage kidney disease in the United States and Puerto Rico.
- In 2005, a total of 178,689 people with end-stage kidney disease due to diabetes were living on chronic dialysis or with a kidney transplant in the United States and Puerto Rico.

(v) **Nervous System Disease**

- About 60 to 70 percent of people with diabetes have mild to severe forms of nervous system damage. The results of such damage include impaired sensation or pain in the feet or hands, slowed digestion of food in the stomach, carpal tunnel syndrome, erectile dysfunction, or other nerve problems.
- Almost 30 percent of people with diabetes aged 40 years or older have impaired sensation in the feet—for example, at least one area that lacks feeling.
- Severe forms of diabetic nerve disease are a major contributing cause of lower-extremity amputations.

(vi) **Amputations**

- More than 60 percent of nontraumatic lower-limb amputations occur in people with diabetes.
- In 2004, about 71,000 nontraumatic lower-limb amputations were performed in people with diabetes.

(vii) **Dental Disease**

- Periodontal, or gum, disease is more common in people with diabetes. Among young adults, those with diabetes have about twice the risk of those without diabetes.
- Persons with poorly controlled diabetes (A1C > 9 percent) were nearly three times more likely to have severe periodontitis than those without diabetes.
- Almost one-third of people with diabetes have severe periodontal disease with loss of attachment of the gums to the teeth measuring 5 millimeters or more.

(viii) **Complications of Pregnancy**

- Poorly controlled diabetes before conception and during the first trimester of pregnancy among women with type 1 diabetes can cause major birth defects in 5 to 10 percent of pregnancies and spontaneous abortions in 15 to 20 percent of pregnancies.
- Poorly controlled diabetes during the second and third trimesters of pregnancy can result in excessively large babies, posing a risk to both mother and child.

(ix) Other Complications

- Uncontrolled diabetes often leads to biochemical imbalances that can cause acute life-threatening events, such as diabetic ketoacidosis and hyperosmolar, or nonketotic, coma.
- People with diabetes are more susceptible to many other illnesses and, once they acquire these illnesses, often have worse prognoses. For example, they are more likely to die with pneumonia or influenza than people who do not have diabetes.
- Persons with diabetes aged 60 years or older are two to three times more likely to report an inability to walk a quarter of a mile, climb stairs, do housework, or use a mobility aid

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compared with persons without diabetes in the same age group.

1.13. Diabetic Neuropathies Nerve Damage

1.13.1. Nerve Disorders

Diabetic neuropathies are a family of nerve disorders caused by diabetes. People with diabetes can, over time, have damage to nerves throughout the body. Neuropathies lead to numbness and sometimes pain and weakness in the hands, arms, feet, and legs. Problems may also occur in every organ system, including the digestive tract, heart, and sex organs. People with diabetes can develop nerve problems at any time, but the longer a person has diabetes, the greater the risk.[65]

An estimated 50 percent of those with diabetes have some form of neuropathy, but not all with neuropathy have symptoms. The highest rates of neuropathy are among people who have had the disease for at least 25 years.

Diabetic neuropathy also appears to be more common in people who have had problems controlling their blood glucose levels, in those with high levels of blood fat and blood pressure, in overweight people, and in people over the age of 40. The most common type is peripheral neuropathy, also called distal symmetric neuropathy, which affects the arms and legs.

1.13.2. Causes

The causes are probably different for different varieties of diabetic neuropathy. Researchers are studying the effect of glucose on nerves to find out exactly how prolonged exposure to high glucose causes neuropathy. Nerve damage is likely due to a combination of factors:

- Metabolic factors, such as high blood glucose, long duration of diabetes, possibly low levels of insulin, and abnormal blood fat levels
- Neurovascular factors, leading to damage to the blood vessels that carry oxygen and nutrients to the nerves
- Autoimmune factors that cause inflammation in nerves
- Mechanical injury to nerves, such as carpal tunnel syndrome
- Inherited traits that increase susceptibility to nerve disease
- Lifestyle factors such as smoking or alcohol use.

1.13.3. Symptoms

Symptoms depend on the type of neuropathy and which nerves are affected. Some people have no symptoms at all. For others, numbness, tingling, or pain in the feet is often the first sign. A person can experience both pain and numbness. Often, symptoms are minor at first, and since most nerve damage occurs over several years, mild cases may go unnoticed for a long time. Symptoms may involve the sensory or motor nervous system, as well as the involuntary (autonomic) nervous system. In some people, mainly those with focal neuropathy, the onset of pain may be sudden and severe.

Symptoms may include:

- Numbness, tingling, or pain in the toes, feet, legs, hands, arms, and fingers
- Wasting of the muscles of the feet or hands
- Indigestion, nausea, or vomiting
- Diarrhea or constipation
- Dizziness or faintness due to a drop in postural blood pressure
- Problems with urination
- Erectile dysfunction (impotence) or vaginal dryness
- Weakness

In addition, the following symptoms are not due to neuropathy but nevertheless often accompany it:

- Weight loss
- Depression

1.13.4. Types of Diabetic Neuropathy

Diabetic neuropathies can be classified as peripheral, autonomic, proximal, and focal. Each affects different parts of the body in different ways.

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- Peripheral neuropathy causes either pain or loss of feeling in the toes, feet, legs, hands, and arms.
- Autonomic neuropathy causes changes in digestion, bowel and bladder function, sexual response, and perspiration. It can also affect the nerves that serve the heart and control blood pressure. Autonomic neuropathy can also cause hypoglycemia (low blood sugar) unawareness, a condition in which people no longer experience the warning signs of hypoglycemia.
- Proximal neuropathy causes pain in the thighs, hips, or buttocks and leads to weakness in the legs
- Focal neuropathy results in the sudden weakness of one nerve, or a group of nerves, causing muscle weakness or pain. Any nerve in the body may be affected.

Neuropathy affects the nerves throughout the body can this includes:

Peripheral Neuropathy

- Toes
- Feet
- Legs
- Hands
- Arms

Autonomic Neuropathy

- Heart and blood vessels
- Digestive system
- Urinary tract
- Sex organs
- Sweat glands
- Eyes

Proximal Neuropathy

- Thighs
- Hips
- Buttocks

Focal Neuropathy

- Eyes
- Facial muscles
- Ears
- Pelvis and lower back
- Thighs
- Abdomen

1.13.4.1. Peripheral Neuropathy

This type of neuropathy damages nerves in the arms and legs. The feet and legs are likely to be affected before the hands and arms. Many people with diabetes have signs of neuropathy upon examination but have no symptoms at all. Symptoms of peripheral neuropathy may include:

- Numbness or insensitivity to pain or temperature
- A tingling, burning, or prickling sensation
- Sharp pains or cramps
- Extreme sensitivity to touch, even a light touch
- Loss of balance and coordination.[66]

These symptoms are often worse at night.

Peripheral neuropathy may also cause muscle weakness and loss of reflexes, especially at the ankle, leading to changes in gait (walking). Foot deformities, such as hammertoes and the collapse of the midfoot, may occur. Blisters and sores may appear on numb areas of the foot because pressure or injury goes unnoticed. If foot injuries are not treated promptly, the infection may spread to the bone, and the foot may then have to be amputated. Some experts estimate that half of all such amputations are preventable if minor problems are caught and treated in time.

1.13.4.2. Autonomic Neuropathy

Autonomic neuropathy affects the nerves that control the heart, regulate blood pressure, and control blood glucose levels. It also affects other internal organs, causing problems with digestion, respiratory function, urination, sexual response, and vision. In addition, the system that restores blood glucose levels to normal after a hypoglycemic episode may be affected, resulting in loss of the warning signs of hypoglycemia such as sweating and palpitations.

Normally, symptoms such as shakiness occur as blood glucose levels drop below 70 mg/dL. In people with autonomic neuropathy, symptoms may not occur,

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making hypoglycemia difficult to recognize. However, other problems can also cause hypoglycemia unawareness so this does not always indicate nerve damage.

The heart and circulatory system are part of the cardiovascular system, which controls blood circulation. Damage to nerves in the cardiovascular system interferes with the body's ability to adjust blood pressure and heart rate. As a result, blood pressure may drop sharply after sitting or standing, causing a person to feel light-headed—or even to faint. Damage to the nerves that control heart rate can mean that it stays high, instead of rising and falling in response to normal body functions and exercise.[67][68]

(i) **Digestive System** - Nerve damage to the digestive system most commonly causes constipation. Damage can also cause the stomach to empty too slowly, a condition called gastroparesis. Severe gastroparesis can lead to persistent nausea and vomiting, bloating, and loss of appetite. Gastroparesis can make blood glucose levels fluctuate widely as well, due to abnormal food digestion.

Nerve damage to the esophagus may make swallowing difficult, while nerve damage to the bowels can cause constipation alternating with frequent, uncontrolled diarrhea, especially at night. Problems with the digestive system may lead to weight loss.

(ii) **Urinary Tract and Sex Organs** - Autonomic neuropathy most often affects the organs that control urination and sexual function. Nerve damage can prevent the bladder from emptying completely, allowing bacteria to grow in the bladder and kidneys and causing urinary tract infections. When the nerves of the bladder are damaged, urinary incontinence may result because a person may not be able to sense when the bladder is full or control the muscles that release urine.

Neuropathy can also gradually decrease sexual response in men and women, although the sex drive is unchanged. A man may be unable to have erections or may reach sexual climax without ejaculating normally. A woman may have difficulty with lubrication, arousal, or orgasm.

(iii) **Sweat Glands** - Autonomic neuropathy can affect the nerves that control sweating. When nerve damage prevents the sweat glands from working properly, the body cannot regulate its temperature properly. Nerve damage can also cause profuse sweating at night or while eating.

(iv) **Eyes** - Finally, autonomic neuropathy can affect the pupils of the eyes, making them less responsive to changes in light. As a result, a person may not be able to see well when the light is turned on in a dark room or may have trouble driving at night.

1.13.4.3. Proximal Neuropathy

Proximal neuropathy, sometimes called lumbosacral plexus neuropathy, femoral neuropathy, or diabetic amyotrophy, starts with pain in the thighs, hips, buttocks,legs, usually on one side of the body. This type of neuropathy is more common in those with type 2 diabetes and in older people. It causes weakness in the legs, manifested by an inability to go from a sitting to a standing position without help. Treatment for weakness or pain is usually needed. The length of the recovery period varies, depending on the type of nerve damage.[66]

1.13.4.4. Focal Neuropathy

Occasionally, diabetic neuropathy appears suddenly and affects specific nerves, most often in the head, torso, or leg. Focal neuropathy may cause:

- Inability to focus the eye
- Double vision
- Aching behind one eye
- Paralysis on one side of the face (Bell's palsy)
- Severe pain in the lower back or pelvis
- Pain in the front of a thigh
- Pain in the chest, stomach, or flank

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- Pain on the outside of the shin or inside the foot
- Chest or abdominal pain that is sometimes mistaken for heart disease, heart attack, or appendicitis

Focal neuropathy is painful and unpredictable and occurs most often in older people. However, it tends to improve by itself over weeks or months and does not cause long-term damage.

People with diabetes also tend to develop nerve compressions, also called entrapment syndromes. One of the most common is carpal tunnel syndrome, which causes numbness and tingling of the hand and sometimes muscle weakness or pain. Other nerves susceptible to entrapment may cause pain on the outside of the shin or the inside of the foot.[69]

1.13.4.5. Preventing Diabetic Neuropathy

The best way to prevent neuropathy is to keep blood glucose levels as close to the normal range as possible and maintaining safe blood glucose levels protects nerves throughout the body.

Neuropathy is diagnosed on the basis of symptoms and a physical exam. During the exam, the doctor may check blood pressure and heart rate, muscle strength, reflexes, and sensitivity to position, vibration, temperature, or a light touch.[70]

The doctor may also do other tests to help determine the type and extent of nerve damage.

- A comprehensive foot exam assesses skin, circulation, and sensation. The test can be done during a routine office visit. To assess protective sensation or feeling in the foot, a nylon monofilament (similar to a bristle on a hairbrush) attached to a wand is used to touch the foot. Those who cannot sense pressure from the monofilament have lost protective sensation and are at risk for developing foot sores that may not heal properly. Other tests include checking reflexes and assessing vibration perception, which is more sensitive than touch pressure.
- Nerve conduction studies check the transmission of electrical current through a nerve. With this test, an image of the nerve conducting an electrical signal is projected onto a screen. Nerve impulses that seem slower or weaker than usual indicate possible damage. This test allows the doctor to assess the condition of all the nerves in the arms and legs.
- Electromyography (EMG) shows how well muscles respond to electrical signals transmitted by nearby nerves. The electrical activity of the muscle is displayed on a screen. A response that is slower or weaker than usual suggests damage to the nerve or muscle. This test is often done at the same time as nerve conduction studies.
- Quantitative sensory testing (QST) uses the response to stimuli, such as pressure, vibration, and temperature, to check for neuropathy. QST is increasingly used to recognize sensation loss and excessive irritability of nerves.
- A check of heart rate variability shows how the heart responds to deep breathing and to changes in blood pressure and posture.
- Ultrasound uses sound waves to produce an image of internal organs. An ultrasound of the bladder and other parts of the urinary tract, for example, can show how these organs preserve a normal structure and whether the bladder empties completely after urination.
- Nerve or skin biopsy involves removing a sample of nerve or skin tissue for examination by microscope. This test is most often used in research settings.

1.14. Treatment

The first step is to bring blood glucose levels within the normal range to prevent further nerve damage. Blood glucose monitoring, meal planning, exercise, and oral drugs or insulin injections are needed to control blood glucose levels. Although symptoms may get worse when blood glucose is first brought under control, over time, maintaining lower blood glucose levels helps lessen neuropathic symptoms.

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Importantly, good blood glucose control may also help prevent or delay the onset of further problems.

Additional treatment depends on the type of nerve problem and symptom, as described in the following sections.

People with neuropathy need to take special care of their feet. The nerves to the feet are the longest in the body and are the ones most often affected by neuropathy. Loss of sensation in the feet means that sores or injuries may not be noticed and may become ulcerated or infected. Circulation problems also increase the risk of foot ulcers.

More than half of all lower limb amputations in the United States occur in people with diabetes—86,000 amputations per year. Doctors estimate that nearly half of the amputations caused by neuropathy and poor circulation could have been prevented by careful foot care. The recommended steps to follow are:[71]

- Clean feet daily, using warm—not hot—water and a mild soap. Avoid soaking the feet. Dry them with a soft towel; dry carefully between the toes.
- Inspect feet and toes every day for cuts, blisters, redness, swelling, calluses, or other problems. Use a mirror (laying a mirror on the floor works well) or get help from someone else if they cannot see the bottoms of the feet.
- Moisturize feet with lotion, but avoid getting it between the toes.
- After a bath or shower, file corns and calluses gently with a pumice stone.
- Each week or when needed, cut toenails to the shape of the toes and file the edges with an emery board.
- Always wear shoes or slippers to protect feet from injuries. Prevent skin irritation by wearing thick, soft, seamless socks.
- Wear shoes that fit well and allow the toes to move. Break in new shoes gradually by wearing them for only an hour at a time at first.
- Before putting shoes on, look them over carefully and feel the insides with the hand to make sure they have no tears, sharp edges, or objects in them that might injure the feet.
- If they need help taking care of their feet, they should make an appointment to see a foot doctor (podiatrist).

1.14.1. Pain Relief

To relieve pain, burning, tingling, or numbness, the doctor may suggest aspirin, acetaminophen, or nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen. (People with renal disease should use NSAIDs only under a doctor's supervision.) A topical cream called capsaicin is another option. Tricyclic antidepressant medications such as amitriptyline, imipramine, and nortriptyline, or anticonvulsant medications such as carbamazepine or gabapentin may relieve pain in some people. Codeine may be prescribed for a short time to relieve severe pain. Also, mexiletine, used to regulate heartbeat, has been effective in treating pain in several clinical trials.

Other pain treatments include transcutaneous electronic nerve stimulation (TENS), which uses small amounts of electricity to block pain signals, as well as hypnosis, relaxation training, biofeedback, and acupuncture.

Walking regularly or using elastic stockings may also help leg pain.

1.14.2. Gastrointestinal Problems

To relieve mild symptoms of gastroparesis—indigestion, belching, nausea, or vomiting—doctors suggest eating small, frequent meals, avoiding fats, and eating less fiber. When symptoms are severe, the doctor may prescribe erythromycin to speed digestion, metoclopramide to speed digestion and help relieve nausea, or other drugs to help regulate digestion or reduce stomach acid secretion.

To relieve diarrhea or other bowel problems, the doctor may prescribe an antibiotic such as tetracycline, or other medications as appropriate.

1.14.3. Dizziness and Weakness

Sitting or standing slowly may help prevent the light-headedness, dizziness, or fainting associated with

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blood pressure and circulation problems. Raising the head of the bed or wearing elastic stockings may also help. Some people may benefit from increased salt in the diet and treatment with salt-retaining hormones. Others may benefit from high blood pressure medications. Physical therapy can help when muscle weakness or loss of coordination is a problem.

1.14.4. Urinary and Sexual Problems

To clear up a urinary tract infection, the doctor will probably prescribe an antibiotic. Drinking plenty of fluids will help prevent another infection. People who have incontinence should try to urinate at regular intervals (every 3 hours, for example) since they may not be able to tell when their bladder is full.

To treat erectile dysfunction in men, the doctor will first do tests to rule out a hormonal cause. Several methods are available to treat erectile dysfunction caused by neuropathy, including taking oral drugs, using a mechanical vacuum device, or injecting a drug called a vasodilator into the penis before sex. The vacuum and vasodilator raise blood flow to the penis, making it easier to have and maintain an erection. Another option is to surgically implant an inflatable or semirigid device in the penis. A constriction ring or penile sling may be helpful.

Vaginal lubricants may be useful for women when neuropathy causes vaginal dryness. To treat problems with arousal and orgasm, the doctor may refer the woman to a gynecologist.

1.14.5. Summary Points

- Diabetic neuropathies are nerve disorders caused by many of the abnormalities common to diabetes, such as high blood glucose.
- Neuropathy can affect nerves throughout the body, causing numbness and sometimes pain in the hands, arms, feet, or legs, and problems with the digestive tract, heart, and sex organs.
- Treatment first involves bringing blood glucose levels within the normal range. Good blood glucose control may help prevent or delay the onset of further problems.
- Foot care is another important part of treatment. People with neuropathy need to inspect their feet daily for any injuries. Untreated injuries increase the risk of infected foot sores and amputation.
- Treatment also includes pain relief and other medications as needed, depending on the type of nerve damage.
- Smoking significantly increases the risk of foot problems and amputation. If they smoke, it is advised that they ask their health care provider for help in quitting.

1.14.6. Preventing Diabetes Complications

Diabetes can affect many parts of the body and can lead to serious complications such as blindness, kidney damage, and lower-limb amputations. Working together, people with diabetes, their support network, and their health care providers can reduce the occurrence of these and other diabetes complications by controlling the levels of blood glucose, blood pressure, and blood lipids and by receiving other preventive care practices in a timely manner.[72]

1.14.7. Glucose Control

- Studies in the United States and abroad have found that improved glycemic control benefits people with either type 1 or type 2 diabetes. In general, every percentage point drop in A1C blood test results—for example, from 8.0 to 7.0 percent—can reduce the risk of microvascular complications—eye, kidney, and nerve diseases—by 40 percent.
- In patients with type 1 diabetes, intensive insulin therapy has long-term beneficial effects on the risk of cardiovascular disease.

1.14.8. Blood Pressure Control

- Blood pressure control reduces the risk of cardiovascular disease—heart disease or stroke—among persons with diabetes by 33 to 50 percent, and the risk of microvascular complications—eye, kidney, and nerve diseases—by approximately 33 percent.
- In general, for every 10 mm Hg reduction in systolic blood pressure, the risk for any complication related to diabetes is reduced by 12 percent.

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1.14.9. Control of Blood Lipids

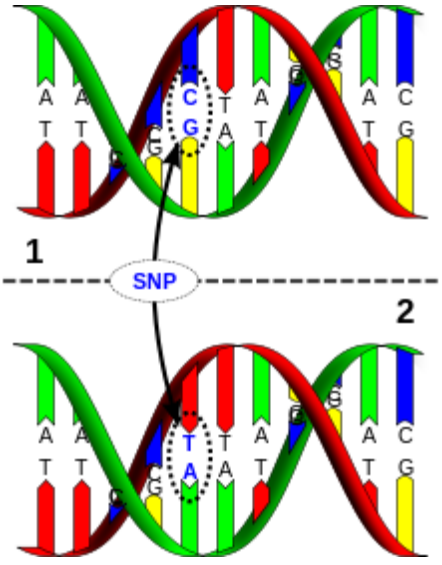
- Improved control of LDL cholesterol can reduce cardiovascular complications by 20 to 50 percent.

1.14.10. Preventive Care Practices for Eyes, Feet, and Kidneys

- Detecting and treating diabetic eye disease with laser therapy can reduce the development of severe vision loss by an estimated 50 to 60 percent.
- Comprehensive foot care programs can reduce amputation rates by 45 to 85 percent.
- Detecting and treating early diabetic kidney disease by lowering blood pressure can reduce the decline in kidney function by 30 to 70 percent. Treatment with angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) are more effective in reducing the decline in kidney function than other blood pressure lowering drugs.

In addition to lowering blood pressure, ARBs reduce proteinuria, a risk factor for developing kidney disease, by 35 percent—similar to the reduction achieved by ACE inhibitors.

1.15. Single-Nucleotide Polymorphism (SNP)



DNA molecule 1 differs from DNA molecule 2 at a single base-pair location (a C/T polymorphism).

A **single-nucleotide polymorphism** is a DNA sequence variation occurring when a single nucleotide — A, T, C or G — in the genome differs between members of a biological species or paired chromosomes in a human. For example, two sequenced DNA fragments from different individuals, AAGCCTA to AAGCTTA, contain a difference in a single nucleotide. In this there are two *alleles*. Almost all common SNPs have only two alleles. The genomic distribution of SNPs is not homogenous; SNPs usually occur in non-coding regions more frequently than in coding regions or, in general, where natural selection is fixating the allele of the SNP that constitutes the most favorable genetic adaptation. Other factors, like genetic recombination and mutation rate, can also determine SNP density.

SNP density can be predicted by the presence of microsatellites: AT microsatellites in particular are potent predictors of SNP density, with long (AT)(n) repeat tracts tending to be found in regions of significantly reduced SNP density and low GC content.

Within a population, SNPs can be assigned a minor allele frequency — the lowest allele frequency at a locus that is observed in a particular population. This is simply the lesser of the two allele frequencies for single-nucleotide polymorphisms. There are variations between human populations, so a SNP allele that is common in one geographical or ethnic group may be much rarer in another.

These genetic variations between individuals (particularly in non-coding parts of the genome) are exploited in DNA fingerprinting, which is used in forensic science. Also, these genetic variations underlie differences in our susceptibility to disease. The severity of illness and the way our body responds to treatments are also manifestations of genetic

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variations. For example, a single base mutation in the APOE (apolipoprotein E) gene is associated with a higher risk for Alzheimer disease.

1.15.1. Types

Single-nucleotide polymorphisms may fall within coding sequences of genes, non-coding regions of genes, or in the intergenic regions (regions between genes). SNPs within a coding sequence do not necessarily change the amino acid sequence of the protein that is produced, due to degeneracy of the genetic code.

SNPs in the coding region are of two types, synonymous and non synonymous SNPs. Synonymous SNPs do not affect the protein sequence while non synonymous SNPs change the amino acid sequence of protein. The non synonymous SNPs are of two types: mis sense and nonsense.

SNPs that are not in protein-coding regions may still affect gene splicing, transcription factor binding, messenger RNA degradation, or the sequence of non-coding RNA. Gene expression affected by this type of SNP is referred to as an e SNP (expression SNP) and may be upstream or downstream from the gene.

1.15.2. Use and importance

Variations in the DNA sequences of humans can affect how humans develop diseases and respond to pathogens, chemicals, drugs, vaccines, and other agents. SNPs are also critical for personalized medicine. However, their greatest importance in biomedical research is for comparing regions of the genome between cohorts (such as with matched cohorts with and without a disease) in genome-wide association studies.

The study of SNPs is also important in crop and livestock breeding program.

SNPs are usually biallelic and thus easily assayed. A single SNP may cause a Mendelian disease. For complex diseases, SNPs do not usually function individually; rather, they work in coordination with other SNPs to manifest a disease condition as has been seen in Osteoporosis.

SNPs have been used in genome-wide association studies (GWAS), e.g. as high-resolution markers in gene mapping related to diseases or normal traits. The knowledge of SNPs will help in understanding pharmacokinetics (PK) or pharmacodynamics, i.e. how drugs act in individuals with different genetic variants. A wide range of human diseases, i.e. Sickle–cell anemia, β Thalassemia and Cystic fibrosis result from SNPs. Diseases with different SNPs may become relevant pharmacogenomic targets for drug therapy. Some SNPs are associated with the metabolism of different drugs.

1.15.3. Examples

rs6311 and rs6313 are SNPs in the HTR2A gene on human chromosome 13.
A SNP in the *F5* gene causes a hyper coagulability disorder with the variant Factor V Leiden.
rs3091244 is an example of a triallelic SNP in the CRP gene on human chromosome 1.
TAS2R38 codes for PTC tasting ability, and contains 6 annotated SNPs.
rs148649884 and rs138055828 in the *FCN1* gene encoding M- ficolin crippled the ligand-binding capability of the recombinant M-ficolin.

1.15.4. Databases

As there are for genes, bioinformatics databases exist for SNPs. *db SNP* is a SNP database from the National Center for Biotechnology Information (NCBI). *SNP edia* is a wiki-style database supporting personal genome annotation, interpretation and analysis. The *OMIM* database describes the association between polymorphisms and diseases (e.g., gives diseases in text form), the Human Gene Mutation Database provides gene mutations causing or associated with human inherited diseases and functional SNPs, and GWAS Central allows users to visually interrogate

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the actual summary-level association data in one or more genome-wide association studies. The International SNP Map working group mapped the sequence flanking each SNP by alignment to the genomic sequence of large-insert clones in Gene bank. These alignments were converted to chromosomal coordinates

"Single nucleotide polymorphisms and recombination rate in humans

"Heterogeneous distribution of SNPs in the human genome: Microsatellites as predictors of nucleotide diversity and divergence".

1.16. BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF)

1.16.1. INTRODUCTION:

Brain-derived neurotrophic factor, also known as **BDNF**, is a secreted protein that, in humans, is encoded by the *BDNF* gene. BDNF is a member of the "neurotrophin" family of growth factors, which are related to the canonical "nerve growth factor", NGF. Neurotrophic factors are found in the brain and the periphery.

(i) Neurotrophin

Neurotrophins are a family of proteins that induce the survival, development, and function of neurons.

They belong to a class of growth factors, secreted proteins that are capable of signaling particular cells to survive, differentiate, or grow. Growth factors such as neurotrophins that promote the survival of neurons are known as neurotrophic factors. Neurotrophic factors are secreted by target tissue and act by preventing the associated neuron from initiating programmed cell death - thus allowing the neurons to survive. Neurotrophins also induce differentiation of progenitor cells, to form neurons.

Although the vast majority of neurons in the mammalian brain are formed prenatally, parts of the adult brain (for example, the hippocampus) retain the ability to grow new neurons from neural stem cells, a process known as neurogenesis. Neurotrophins are chemicals that help to stimulate and control neurogenesis.

(ii) Terminology

The term *neurotrophin* may be used as a synonym for *neurotrophic factor*, but the term *neurotrophin* is more generally reserved for four structurally related factors: nerve growth factor (NGF),brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4), with *neurotrophic factor* additionally referring to the GDNF family of ligands and ciliary neurotrophic factor(CNTF).

During the development of the vertebrate nervous system, many neurons become redundant (because they have died, failed to connect to target cells, etc.) and are eliminated. At the same time, developing neurons send out axon outgrowths that contact their target cells. Such cells control their degree of innervation (the number of axon connections) by the secretion of various specific neurotrophic factors that are essential for neuron survival. One of these is nerve growth factor (NGF or beta-NGF), a vertebrate protein that stimulates division and differentiation of sympathetic and embryonic sensory neurons. NGF is mostly found outside the central nervous system (CNS), but slight traces have been detected in adult CNS tissues, although a physiological role for this is unknown. It has also been found in several snake venoms.

In the peripheral and central neurons, neurotrophins are important regulators for survival, differentiation, and maintenance of nerve cells. They are small proteins that secrete into the nervous system to help keep nerve cells alive. There are two distinct classes of glycosylated receptors that can bind to neurotrophins. These two proteins are p75 (NTR), which binds to all neurotrophins, and subtypes of Trk, which are each specific for different neurotrophins.

The reported structure above is a 2.6 Å-resolution crystal structure of neurotrophin-3 (NT-3) complexed to the ectodomain of glycosylated (NRT), forming a symmetrical crystal structure.

There are two classes of receptors for neurotrophins: the "Trk" family of Tyrosine kinases receptors.

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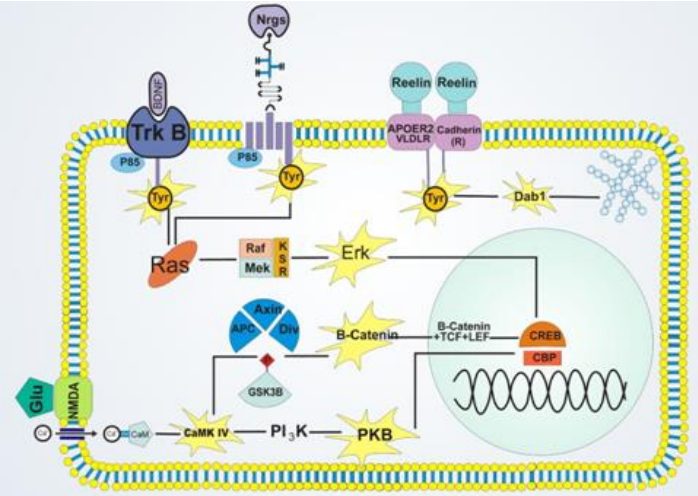

1.16.2. Types

(i) Nerve growth factor

Nerve growth factor (NGF), the prototypical growth factor, is a protein secreted by a neuron's target cell. NGF is critical for the survival and maintenance of sympathetic and sensory neurons. NGF is released from the target cells, binds to and activates its high affinity receptor TrkA on the neuron, and is internalized into the responsive neuron. The NGF/TrkA complex is subsequently trafficked back to the neuron's cell body. This movement of NGF from axon tip to soma is thought to be involved in the long-distance signaling of neurons

(ii) Brain-derived neurotrophic factor

Brain-derived neurotrophic factor (BDNF) is a neurotrophic factor found originally in the brain, but also found in the periphery. To be specific, it is a protein that has activity on certain neurons of the central nervous system and the peripheral nervous system; it helps to support the survival of existing neurons, and encourage the growth and differentiation of new neurons and synapses through axonal and dendritic sprouting. In the brain, it is active in the hippocampus, cortex, cerebellum, and basal forebrain — areas vital to learning, memory, and higher thinking. BDNF is the second neurotrophic factor to be characterized, after NGF and before neurotrophin-3.

BDNF is one of the most active substances to stimulate neurogenesis. Mice born without the ability to make BDNF suffer developmental defects in the brain and sensory nervous system, and usually die soon after birth, suggesting that BDNF plays an important role in normal neural development.

Despite its name, BDNF is actually found in a range of tissue and cell types, not just the brain. Expression can be seen in the retina, the CNS, motor neurons, the kidneys, and the prostate. Exercise has been shown to increase the amount of BDNF and therefore serve as a vehicle for neuroplasticity.

(iii) Neurotrophin-3

Neurotrophin-3, or NT-3, is a neurotrophic factor, in the NGF-family of neurotrophins. It is a protein growth factor that has activity on certain neurons of the peripheral and central nervous system; it helps to support the survival and differentiation of existing neurons, and encourages the growth and differentiation of new neurons and synapses. NT-3 is the third neurotrophic factor to be characterized, after NGF and BDNF.

NT-3 is unique among the neurotrophins in the number of neurons it has potential to stimulate, given its ability to activate two of the receptor tyrosine kinase neurotrophin receptors (TrkC and TrkB). Mice born without the ability to make NT-3 have loss of proprioceptive and subsets of mechanoreceptive sensory neurons.

(iv) Neurotrophin-4

Neurotrophin-4 (NT-4) is a neurotrophic factor that signals predominantly through the TrkB receptor tyrosine kinase. It is also known as NT4, NT5, NTF4, and NT-4/5.

1.16.3. Function

BDNF acts on certain neurons of the central nervous system and the peripheral nervous system, helping to support the survival of existing neurons, and encourage the growth and differentiation of new neurons and synapses. In the brain, it is active in the hippocampus, cortex, and basal forebrain—areas

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vital to learning, memory, and higher thinking BDNF itself is important for long-term memory. BDNF was the second neurotrophic factor to be characterized after nerve growth factor (NGF).

Although the vast majority of neurons in the mammalian brain are formed prenatally, parts of the adult brain retain the ability to grow new neurons from neural stem cells in a process known as neurogenesis. Neurotrophins are chemicals that help to stimulate and control neurogenesis, BDNF being one of the most active. Mice born without the ability to make BDNF suffers developmental defects in the brain and sensory nervous system, and usually dies soon after birth, suggesting that BDNF plays an important role in normal neural development.

In addition to its production and functions in the brain and nervous system, BDNF secreted by contracting muscle has been found to play a role in muscle repair, regeneration, and differentiation. This is supplementary to its well-known functions in neurobiology. BDNF can therefore now be identified as a myokine that plays a role in peripheral metabolism, myogenesis, and muscle regeneration.

(i) Tissue distribution

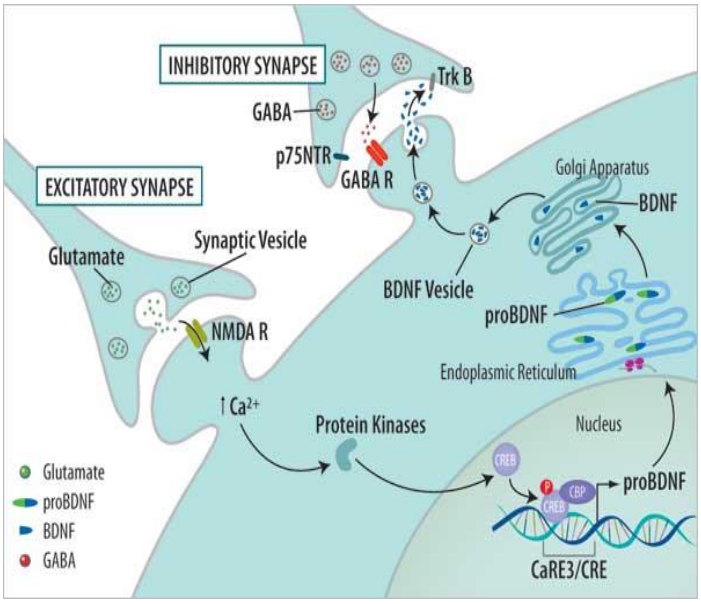
Counter intuitively, BDNF is actually found in a range of tissue and cell types, not just in the brain. It is also expressed in the retina, the central nervous system, motor neurons, the kidneys, and the prostate. BDNF is present in high concentration in hippocampus and cerebral cortex. BDNF is also found in human saliva.

(ii) Mechanism of action

BDNF binds at least two receptors on the surface of cells that are capable of responding to this growth factor, TrkB and the LNGFR (for *low-affinity nerve growth factor receptor*, also known as p75). It may also modulate the activity of various neurotransmitter receptors, including the α_7 nicotonic receptor.

TrkB is a receptor tyrosine kinase (meaning it mediates its actions by causing the addition of phosphate molecules on certain tyrosines in the cell, activating cellular signaling). There are other related Trk receptors, TrkA and TrkC. Also, there are other neurotrophic factors structurally related to BDNF: NGF (for Nerve Growth Factor), NT-3 (for Neurotrophin-3) and NT-4 (for Neurotrophin-4). While TrkB is the primary receptor for BDNF and NT-4, TrkA is the receptor for NGF, and TrkC is the primary receptor for NT-3. NT-3 binds to TrkA and TrkB as well, but with less affinity (thus the caveat "primary receptor").

The other BDNF receptor, plays a somewhat less clear role. Some researchers have shown that the NTR binds and serves as a "sink" for neurotrophins. Cells that express both the NTR and the Trk receptors might, therefore, have a greater activity, since they have a higher "micro concentration" of the neurotrophin. It has also been shown, however, that the NTR may signal a cell to die via apoptosis; so, therefore, cells expressing the NTR in the absence of Trk receptors may die rather than live in the presence of a neurotrophin



(iii) Secretion

BDNF is made in the endoplasmic reticulum and secreted from dense-core vesicles. It binds carboxy peptidase E (CPE), and the disruption of this binding has been proposed to cause the loss of sorting of BDNF into dense-core vesicles. The phenotype for BDNF knockout mice can be severe, including postnatal lethality. Other traits include sensory neuron losses that affect coordination, balance, hearing, taste, and breathing. Knockout mice also exhibit cerebellar

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abnormalities and an increase in the number of sympathetic neurons.

Exercise has been shown to increase the secretion of BDNF as a myokine at the mRNA and protein levels in the rodent hippocampus, suggesting the potential increase of this neurotrophin after exercise in humans. It is well known that BDNF increases in brain tissue in response to acute exercise and exercise training and may account for the effect of exercise in the protection against neurodegenerative diseases such as dementia. Recent studies have thus confirmed that exercise induces an expression of BDNF in skeletal muscle, as well as in the brain.

Caffeine improves recognition memory, and this effect may be related to an increase of the BDNF and TrkB immune content in the hippocampus.

(iv) Genetics

The BDNF protein is coded by the gene that is also called *BDNF*. In humans this gene is located on chromosome 11. Val66Met (rs6265) is a single nucleotide polymorphism in the gene where adenine and guanine alleles vary, resulting in a variation between valine and methionine at codon 66.

As of 2008, Val66Met is probably the most investigated SNP of the *BDNF* gene, but, besides this variant, other SNPs in the gene are C270T, rs7103411, rs2030324, rs2203877, rs2049045 and rs7124442.[The polymorphism Thr2Ile may be linked to congenital central hypoventilation syndrome.

In 2009, variants close to the BDNF gene were found to be associated with obesity in two very large genome wide-association studies of body mass index (BMI).

(v) Disease linkage

Various studies have shown possible links between BDNF and conditions such as depression, bipolar disorder, schizophrenia, obsessive-compulsive disorder, Alzheimer's disease, Huntington's disease, Rett syndrome, and dementia, as well as anorexia nervosa and bulimia nervosa.

(vi) Depression

Exposure to stress and the stress hormone corticosterone has been shown to decrease the expression of BDNF in rats, and, if exposure is persistent, this leads to an eventual atrophy of the hippocampus. Atrophy of the hippocampus and other limbic structures has been shown to take place in humans suffering from chronic depression. In addition, rats bred to be heterozygous for BDNF, therefore reducing its expression, have been observed to exhibit similar hippocampal atrophy. This suggests that an etiological link between the development of depression and BDNF exists. Supporting this, the excitatory neurotransmitter glutamate, voluntary exercise, caloric restriction, intellectual stimulation, curcumin and various treatments for depression (such as antidepressants and electroconvulsive therapy and sleep deprivation) increase expression of BDNF in the brain.

(vii) Eczema

High levels of BDNF and Substance P have been found associated with increased itching in eczema.

(viii) Epilepsy

Epilepsy has also been linked with polymorphisms in BDNF because of BDNF's vital role in the development of the brain.Levels of both BDNF mRNA and BDNF protein are known to be up-regulated in epilepsy. BDNF modulates excitatory and inhibitory synaptic transmission by inhibiting GABAA-receptor-mediated post-synaptic currents. This provides a potential mechanism for the observed up-regulation.

(ix) Alzheimer's disease

Post mortem analysis has shown lowered levels of BDNF in the brain tissues of people with Alzheimer's disease, although the nature of the connection remains unclear. Studies suggest that neurotrophic factors have a protective role against amyloid beta toxicity. A connection between depression and dementia has been suggested to be mediated by BDNF. Depression causes shrinkage of the hippocampus. When antidepressants are administered, the levels of BDNF are raised to protect and increase the volume of hippocampal and other cells. In Alzheimer's, the hippocampus is also damaged, lowering levels of the neurotrophic factor. Another possible link between BDNF and dementia is through fitness, since exercise can release BDNF and preserve cognition in older people.

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(x) Drug addiction

BDNF is a critical regulator of drug dependency. Animals chronically exposed to drugs of abuse show increased levels of BDNF in the ventral tegmental area (VTA) of the brain, and when BDNF is injected directly into the VTA of rats, the animals act as if they are dependent on opiates.

1.17. Obesity and type 2 diabetes

Obesity and type 2 diabetes are diseases that can substantially decrease life expectancy, diminish quality of life and increase healthcare costs. The incidence of obesity and diabetes continues to rise by epidemic proportions. The term "diabesity" has been coined to describe obesity-dependent diabetes.

Diabetes is a disease characterized by high levels of blood glucose resulting from defects in insulin production, insulin action or both. Type 1 diabetes develops when the body's immune system destroys pancreatic beta cells, the only cells in the body that make the hormone insulin that regulates blood glucose. This form of diabetes usually strikes children and young adults, although disease onset can occur at any age.

1.17.1. *Risk of Type 2 Diabetes*

The risk of developing type 2 diabetes is determined by some factors that can be modified and others that cannot. Some people are at higher risk for developing type 2 diabetes because of their genes. A first-degree relative of a person with types 2 diabetes has a risk five to 10 times higher than a person without a family history.

Another factor that may increase the risk of type 2 diabetes is low birth weight. Intrauterine growth restriction leading to low birth weight seems to be associated with increased risk in adulthood of insulin resistance, glucose intolerance and type 2 diabetes

At all ages, the risk of type 2 diabetes rises with increasing body weight. The prevalence of type 2 diabetes is three to seven times higher in those who are obese than in normal weight adults, and is 20 times more likely in those with a body mass index (BMI) greater than 35 kg/m 2.

1.17.2. *Obesity's Role in the Development of Type 2 Diabetes*

It is not known for sure why some people develop insulin resistance, but it is known that obesity and lack of physical activity make it worse. The development of insulin resistance is an important component in the development of type 2 diabetes. The connection is also seen in the fact that weight-loss can improve control or cure type 2 diabetes. In addition to the degree of obesity, where the excess body fat is deposited is important in determining the risk of type 2 diabetes.

1.17.3. *Treating Obesity Will Treat Type 2 Diabetes*

Weight-loss is an important goal for overweight or obese persons, particularly those with type 2 diabetes. Moderate and sustained weight-loss (five percent to 10 percent of body weight) can improve insulin action, decrease fasting glucose concentrations and reduce the need for some diabetes medications. A program of diet, exercise and behavior modification can successfully treat obesity, but pharmacotherapy and/or surgery may be warranted.

1.17.4. *Prevention*

Preventing and treating obesity will help in the prevention and treatment of diabetes. Promoting a healthy lifestyle in children and adolescents will put them on a path that will decrease their risk of diabetes and its complications. Helping adults at high risk for diabetes to change their diet and lifestyle may prevent them from developing diabetes and its consequences.

2. REVIEW OF LITERATURE

2.1. Single nucleotide polymorphisms and recombination rate in humans.

Nachman MW.

Abstract

Levels of heterozygosity for single nucleotide polymorphisms vary by more than one order of magnitude in different regions of the human genome. Regional differences in the rate of recombination explain a substantial fraction of the variation in levels

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of nucleotide polymorphism, consistent with the widespread action of natural selection at the molecular level.

2.2. ***A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms***.

Sachidanandam R, Weissman D, Schmidt SC, Kakol JM, Stein LD, Marth G, Sherry S, Mullikin JC, Mortimore BJ, Willey DL, Hunt SE, Cole CG, Coggill PC, Rice CM, Ning Z, Rogers J, Bentley DR, Kwok PY, Mardis ER, Yeh RT, Schultz B, Cook L, Davenport R, Dante M, Fulton L, Hillier L, Waterston RH, McPherson JD,Gilman B, Schaffner S, Van Etten WJ, Reich D, Higgins J, Daly MJ, Blumenstiel B, Baldwin J, Stange-Thomann N, Zody MC, Linton L, Lander ES,

Abstract

We describe a map of 1.42 million single nucleotide polymorphisms (SNPs) distributed throughout the human genome, providing an average density on available sequence of one SNP every 1.9 kilobases. These SNPs were primarily discovered by two projects: The SNP Consortium and the analysis of clone overlaps by the International Human Genome Sequencing Consortium. The map integrates all publicly available SNPs with described genes and other genomic features. We estimate that 60,000 SNPs fall within exon (coding and untranslated regions), and 85% of exons are within 5 kb of the nearest SNP. Nucleotide diversity varies greatly across the genome, in a manner broadly consistent with a standard population genetic model of human history. This high-density SNP map provides a public resource for defining haplotype variation across the genome, and should help to identify biomedically important genes for diagnosis and therapy.

2.3. ***Identification of base pairs in single-nucleotide polymorphisms by MutS protein-mediated capillary electrophoresis***.

Drabovich AP, Krylov SN.

Abstract

Single-nucleotide polymorphisms (SNPs) are widespread genomic variations, which are associated with serious health disorders and drug resistance. Multiple clinical applications and studies of global population genetics require fast and informative analysis of SNPs. Most of conventional methods sense the presence of the SNP but cannot identify the base pair in it. Our approach is based on the unique ability of MutS protein to bind different single-nucleotide mismatches in DNA with different affinities. Conceptually, the DNA in question is mixed with reference DNA, melted, and reannealed. If the DNA in question has an SNP, the products of reannealing will have two different single-nucleotide mismatches, which provide a base-pair-specific signature of the SNP. The products of reannealing are mixed with MutS, equilibrated, and separated by equilibrium capillary electrophoresis of equilibrium mixtures with MutS in the run buffer. The pattern of migration times of DNAs with mismatches is used for unequivocal identification of the base pair in the SNP. In addition to its ability to identify base pairs in SNPs, the new analytical approach is fast, simple, highly sensitive, and requires no quantitation. It will find applications in studies of heterogeneity of base pairs in known SNPs in large human populations.

2.4. Genetic identification by mass spectrometric analysis of single-nucleotide polymorphisms: ternary encoding of genotypes.

Griffin TJ, Smith LM.

Abstract

An approach to genetic identification using biallelic single-nucleotide polymorphism (SNP) genetic markers is described in which the three possible genotypes, AA, Aa, or aa, where "A" and "a" represent the two SNP alleles, are assigned a ternary (base 3) digit of 0, 1, or 2, respectively. Genotyping an individual over a panel of separate SNP markers produces a composite ternary genetic code that can be converted to an easily stored, decimal (base 10) genetic identification number. The unambiguous identification of 11 individuals is demonstrated using

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ternary genetic codes generated from MALDI-TOF mass spectrometric genotyping data from 7 different SNP markers.

2.5. neurotrophin-regulated signalling pathways

Eric J Huang[1] and Louis F Reichardt[2]

ABSTRACT

Neurotrophins are a family of closely related proteins that were identified initially as survival factors for sensory and sympathetic neurons, and have since been shown to control many aspects of survival, development and function of neurons in both the peripheral and the central nervous systems. Each of the four mammalian neurotrophins has been shown to activate one or more of the three members of the tropomyosin-related kinase (Trk) family of receptor tyrosine kinases (TrkA, TrkB and TrkC). In addition, each neurotrophin activates p75 neurotrophin receptor (p75NTR), a member of the tumour necrosis factor receptor super family. Through Trk receptors, neurotrophins activate Ras, phosphatidyl inositol-3 (PI3)-kinase, phospholipase C-γ1 and signalling pathways controlled through these proteins, such as the MAP kinases. Activation of p75NTR results in activation of the nuclear factor-κB (NF-κB) and Jun kinase as well as other signalling pathways. Limiting quantities of neurotrophins during development control the number of surviving neurons to ensure a match between neurons and the requirement for a suitable density of target innervation. The neurotrophins also regulate cell fate decisions, axon growth, dendrite growth and pruning and the expression of proteins, such as ion channels, transmitter biosynthetic enzymes and neuropeptide transmitters that are essential for normal neuronal function. Continued presence of the neurotrophins is required in the adult nervous system, where they control synaptic function and plasticity, and sustain neuronal survival, morphology and differentiation. They also have additional, subtler roles outside the nervous system. In recent years, three rare human genetic disorders, which result in deleterious effects on sensory perception, cognition and a variety of behaviours, have been shown to be attributable to mutations in brain-derived neurotrophic factor and two of the Trk receptors.

2.6. Clinical relevance of the neurotrophins and their receptors.

Allen SJ, Dawbarn D.

Abstract

The neurotrophins are growth factors required by discrete neuronal cell types for survival and maintenance, with a broad range of activities in the central and peripheral nervous system in the developing and adult mammal. This review examines their role in diverse disease states, including Alzheimer's disease, depression, pain and asthma. In addition, the role of BDNF (brain-derived neurotrophic factor) in synaptic plasticity and memory formation is discussed. Unlike the other neurotrophins, BDNF is secreted in an activity-dependent manner that allows the highly controlled release required for synaptic regulation. Evidence is discussed which shows that sequestration of NGF (nerve growth factor) is able to reverse symptoms of inflammatory pain and asthma in animal models. Both pain and asthma show an underlying pathophysiology linked to increases in endogenous NGF and subsequent NGF-dependent increase in BDNF. Conversely, in Alzheimer's disease, there is a role for NGF in the treatment of the disease and a recent clinical trial has shown benefit from its exogenous application. In addition, reductions in BDNF, and changes in the processing and usage of NGF, are evident and it is possible that both NGF and BDNF play a part in the aetiology of the disease process. This highly selective choice of functions and disease states related to neurotrophin function, although in no way comprehensive, illustrates the importance of the neurotrophins in the brain, the peripheral nervous system and in non-neuronal tissues. Ways in which the neurotrophins, their receptors or agonists/antagonists may act therapeutically are discussed.

2.7. The distribution of the BDNF mRNA in the adult mouse brain

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M Hofer, S R Pagliusi, A Hohn, J Leibrock, and Y A Barde

Abstract
Brain-derived neurotrophic factor (BDNF) is a protein that allows the survival of specific neuronal populations. This study reports on the distribution of the BDNF mRNA in the adult mouse brain, where the BDNF gene is strongly expressed, using quantitative Northern blot analysis and in situ hybridization. All brain regions examined were found to contain substantial amounts of BDNF mRNA, the highest levels being found in the hippocampus followed by the cerebral cortex. In the hippocampus, which is also the site of highest nerve growth factor (NGF) gene expression in the central nervous system (CNS), there is approximately 50-fold more BDNF mRNA than NGF mRNA. In other brain regions, such as the granule cell layer of the cerebellum, the differences between the levels of BDNF and NGF mRNAs are even more pronounced. The BDNF mRNA was localized by in situ hybridization in hippocampal neurons (pyramidal and granule cells). These data suggest that BDNF may play an important role in the CNS for a wide variety of adult neurons.

2.8. Novel Neurotrophin-1/B Cell-Stimulating Factor-3 (Cardiotrophin-Like Cytokine) Stimulates Corticotroph Function via a Signal Transducer and Activator of Transcription-Dependent Mechanism Negatively Regulated by Suppressor of Cytokine Signaling-3

, Nicola B. Isele, Florian B. Kopp, Gerald Spoettl, Neziha Cengic, Matthias M. Weber, Giorgio Senaldi, and Dieter Engelhardt

Abstract
Novel neurotrophin-1/B cell-stimulating factor-3 (NNT-1/BSF-3) is a recently cloned gp130 cytokine, acting through the tripartite ciliary neurotrophic factor receptor (CNTFR) α/leukemia inhibitory factor receptor (LIFR)/gp130 receptor complex. The aim of the current study was to investigate the role of NNT-1/BSF-3 in corticotroph cell function and further characterize NNT-1/BSF-3 signaling pathways. Using RT-PCR, expression of ciliary neurotrophic factor receptor α, leukemia inhibitory factor receptor, and gp130 could be demonstrated in mRNA derived from murine corticotroph AtT-20 cells and murine pituitary tissue. Incubation of AtT-20 cells with 10 ng/ml recombinant human NNT-1/BSF-3 rapidly induced tyrosine-phosphorylation of signal transducer and activator of transcription (STAT)3 and STAT1 at 5 and 10 min. Proopiomelanocortin promoter activity and suppressor of cytokine signaling (SOCS)-3 promoter activity were significantly stimulated by NNT-1/BSF-3 4.0 ± 0.3- and 5.9 ± 0.2-fold, respectively. In comparison with untreated control, NNT-1/BSF-3 significantly stimulated ACTH secretion at 24 and 48 h 1.7 ± 0.2-fold and 1.5 ± 0.1-fold above baseline. In comparison with mock-transfected cells, stable over expression of SOCS-3 in AtT-20 cells abolished NNT-1/BSF-3-induced STAT1 and STAT3 phosphorylation and almost completely inhibited STAT-dependent proopiomelanocortin promoter and SOCS-3 promoter activities. In addition, NNT-1/BSF-3-induced ACTH secretion at 48 h was significantly attenuated by SOCS-3 over expression. In summary, we have shown that NNT-1/BSF-3 is a modulator of corticotroph cell function, which is negatively regulated by SOCS-3. Our data indicate that the activation of the Jak-STAT cascade is essential for corticotroph NNT-1/BSF-3 signaling. Further studies will have to investigate the possible*in vivo* role of NNT-1/BSF-3 as a neuro immunoendocrine modulator of hypothalamus-pituitary-adrenal axis stress response.

2.9. Brain-derived neurotrophic factor.

Binder DK, Scharfman HE.

Abstract
Since the purification of BDNF in 1982, a great deal of evidence has mounted for its central roles in brain development, physiology, and pathology. Aside from its importance in neural development and cell survival, BDNF appears essential to molecular mechanisms of synaptic plasticity. Basic activity-related changes in the central nervous system are thought to depend on BDNF modification of synaptic transmission, especially in the hippocampus and neocortex.

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Pathologic levels of BDNF-dependent synaptic plasticity may contribute to conditions such as epilepsy and chronic pain sensitization, whereas application of the trophic properties of BDNF may lead to novel therapeutic options in neurodegenerative diseases and perhaps even in neuropsychiatric disorder

2.10. Brain-derived neurotrophic factor/TrkB signaling in memory processes.

Yamada K, Nabeshima T.

Abstract

Activity-dependent changes in synaptic strength are considered mechanisms underlying learning and memory. Brain-derived neurotrophic factor (BDNF) plays an important role in activity-dependent synaptic plasticity such as long-term potentiation. Recent experimental evidence supports the role of BDNF in memory processes: Memory acquisition and consolidation are associated with an increase in BDNF mRNA expression and the activation of its receptor TrkB. Genetic as well as pharmacologic deprivation of BDNF or TrkB impairs learning and memory. In a positively motivated radial arm maze test, activation of the TrkB/phosphatidylinositol-3 kinase (PI3-K) signaling pathway in the hippocampus is associated with consolidation of spatial memory through an activation of translational processes. In a negatively motivated passive avoidance test, mitogen-activated protein kinase (MAPK) is activated during acquisition of fear memory. Furthermore, recent findings suggest the importance of interaction between BDNF/TrkB signaling and NMDA receptors for spatial memory. A Src-family tyrosine kinase, Fyn plays a role in this interaction by linking TrkB with NR2B. These findings suggest that BDNF/TrkB signaling in the hippocampus plays a crucial role in learning and memory.

2.11.Variant BDNF (Val66Met) impact on brain structure and function.

Bath KG, Lee FS.

Abstract

Neurotrophins, such as brain-derived neurotrophic factor (BDNF), are a unique family of polypeptide growth factors that influence differentiation and survival of neurons in the developing nervous system. In adults, BDNF is important in regulating synaptic plasticity and connectivity in the brain. Recently, a common single-nucleotide polymorphism in the human BDNF gene, resulting in avaline to methionine substitution in the prodomain (Val66Met), has been shown to lead to memory impairment and susceptibility to neuropsychiatric disorders. An understanding of how this naturally occurring polymorphism affects behavior, anatomy, and cognition in adults is an important first step in linking genetic alterations in the neurotrophin system to definable biological outcomes in humans. We review the recent literature linking this BDNF polymorphism to cognitive impairment in the context of in vitro and transgenic animal studies that have established BDNF's central role in neuronal functioning in the adult brain.

2.12.Brain-derived neurotrophic factor in neurodegenerative diseases.

Zuccato C, Cattaneo E.

Abstract

Changes in the levels and activities of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), have been described in a number of neurodegenerative disorders, including Huntington disease, Alzheimer disease and Parkinson disease. It is only in Huntington disease, however, that gain-of-function and loss-of-function experiments have linked BDNF mechanistically with the underlying genetic defect. Altogether, these studies have led to the development of experimental strategies aimed at increasing BDNF levels in the brains of animals that have been genetically altered to mimic the aforementioned human diseases, with a view to ultimately influencing the clinical treatment of these conditions. In this article, we will review the current knowledge about the involvement of BDNF in a number of neurodegenerative diseases, with particular emphasis on Huntington disease, and will provide the

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rationale for and discuss the problems in proposing BDNF treatment as a beneficial and feasible therapeutic approach in the clinic.

2.13. *Alterations of Serum Levels of BDNF-Related mi-RNAs in Patients with Depression.*

You-Jie Li, Zong-Hua Gao, , Zhen Yue, Yan-Xia Zhang,Xin-Xin Li, Can Zhang, Shu-Yang Xie, Ping-Yu Wang

ABSTRACT

Depression is a serious and potentially life-threatening mental disorder with unknown etiology. Emerging evidence shows that brain-derived neurotrophic factor (BDNF) and microRNAs (miRNAs) play critical roles in the etiology of depression. Here this study was aimed to identify and characterize the roles of BDNF and its putative regulatory mi-RNAs in depression. First, we identified that miR-182 may be a putative mi-RNA that regulates BDNF levels by bioinformatic studies, and characterized the effects of miR-182 on the BDNF levels using cell-based studies, side by side with miR-132 (a known mi-RNA that regulates BDNF expression). We showed that treatment of miR-132 and miR-182 respectively decreased the BDNF protein levels in a human neuronal cell model, supporting the regulatory roles of miR-132 and miR-182 on the BDNF expression. Furthermore, we explored the roles of miR-132 and miR-182 on the BDNF levels in depression using human subjects by assessing their serum levels. Compared with the healthy controls, patients with depression showed lower serum BDNF levels (via the enzyme-linked immunosorbent assays) and higher serum miR-132 and miR-182 levels (via the real-time PCR). Finally, the Pearson's (or Spearman's) correlation coefficient was calculated to study whether there was a relationship among the Self-Rating Depression Scale score, the serum BDNF levels, and serum BDNF-related mi-RNA levels. Our results revealed that there was a significant negative correlation between the SDS scores and the serum BDNF levels, and a positive correlation between the SDS scores and miR-132 levels. In addition, we found a reverse relationship between the serum BDNF levels and the miR-132/miR-182 levels in depression. Collectively, we provided evidence supporting that miR-182 is a putative BDNF-regulatory mi-RNA, and suggested that the serum BDNF and its related mi-RNAs may be utilized as important biomarkers in the diagnosis or as therapeutic targets of depression.

3. MATERIALS AND METHODS

3.1. Material and Methods:

- 10 Blood samples of the DMT2 patients were collected in EDTA vials and DNA isolation was performed using Bunce method.
- The Isolated DNA was subjected to Agarose Gel Electrophoresis.
- Primer was designed for the Target gene BDNF using Primer BLAST, and the gene was amplified using thermo cycler ABI 9700.
- The sequencing of the amplified gene was performed to identify the SNPs.

3.2. Anti Coagulated Blood Genomic DNA isolation by BUNCE METHOD

Chemicals list:

1. Solution –A: cell lysis buffer (100ml):

1M-Tris Hcl =1ml (pH 7.8) → maintain biological pH.

Sucrose =10.0 gms → It binds with WBC to form a protective layer.

MgCl2 = 47 mgs → lysis of RBC.

Dissolve all the components in 50ml of distilled water. Adjust the pH to 8.0, and make up the volume to 100ml with distilled water.

2. Solution-B: WBC lysis buffer (100 ml):

1M-Tris Hcl = 40ml (pH 7.8)

0.5M EDTA =12ml, pH 8.0 (chelating agent, It's used for preventing DNA Fragmentation by stopping the activity of DNase).

NaCl = 0.876gms → lysis of WBC.

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Dissolve all the components in 50ml of distilled water. Adjust the pH to 8.0, and make up the volume to 100ml with distilled water.

3. 3M Sodium acetate =650µl → Neutralizing the DNA against free radicals of the cytoplasm and keeping it save in acidic environment.

4. **Ice cold Chloroform** = 650µl → Removes the pigmentary molecules like Chlorophyll.

5. **Iso propanol** = equal volume of the supernatant→ DNA Precipitation.

6. **70% Ethanol** = 1ml → Removing remaining impurities/proteins.

7. **TE-buffer** = Preserve the DNA Pellets.

3.3. Protocol for genomic DNA

Take 1ml of anti coagulated blood (EDTA) & add 3ml of solution-A.
↓
Shake it slowly for 10 mints
↓
Incubate at 37°C/5 mints
↓
Centrifuge at 6000 rpm/5mints
↓
Take pellet & add 2 volume of solution-B. Shake it
↓
Incubate at 37°C/30 mints
↓
Add 650µl of 3M Sodium acetate
↓
Incubate at 65°C/20 mints (water bath)
↓
Add 650µl of Ice cold chloroform
↓
Shake it for 60mints
↓
Centrifuge at 6000rpm/10mints
↓
Take the supernatant and add equal volume of ice cold Isopropanol
↓
Incubate at -20°c /30 mints (keep at 4°C / overnight)
↓
Centrifuge at 12000rpm/5mints
↓
Wash the pellet with 70% Ethanol
↓
Centrifuge at 10000rpm/5mints
↓
Discard the supernatant and air dry the pellet
↓
Dissolve the DNA pellet in 100 µl of TE buffer.

3.4. Agarose Gel Electrophoresis Protocol:

1) 40ml of 0.8% Agarose gel was prepared by dissolving 0.32gm of Agarose in 40ml of TAE buffer.
2) The solution was kept in microwave oven at power level 800v for 2mints for proper dissolving and to get a clear transparent solution.
3) The agarose solution was allowed to cool at room temperature and 5µl of Ethidium Bromide was dissolved.
4) The Gel casting tray, Chamber & combs were wiped and cleaned with 70% Ethanol.
5) The boundaries of the tray were sealed with cello tape carefully.
6) The Agarose gel was poured into the tray, comb was placed properly and the gel was allowed to solidify for about 20-30 mints.
7) After solidification the comb and tape were removed carefully.
8) The loading samples were prepared by mixing 10µl of the extracted DNA and 5µl of loading dye.

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9) The samples were loaded in the corresponding wells made by removing the comb.
10) The gel was allowed to run for 45 mints to 1 hr at 100 volts.
11) The DNA bands were observed under U.V. Transilluminator.

3.5. Polymerase Chain Reaction

The BDNF gene for was amplified using Thermo cycler/Polymerase Chain Reaction.

3.5.1. PRINCIPLE: The double stranded DNA is denatured to separate into two single strands and allowed to hybridize with a primer and then forms the primer template molecule used for the synthesis of DNA by Taq DNA polymerase enzyme. The PCR mainly involved 3 reactions based on the temperature gradient. They are

1. Denaturation
2. Annealing
3. Synthesis

3.5.2. Materials required

Genomic DNA/Template DNA=2µl (sample)
Two primers i.e. forward primer= 2µl and Reverse primer=2µl
PCR buffer=4µl
DNTPs =4µl
Taq-DNA polymerase enzyme =0.2µl
MgCl2 =2µl
Nuclease free water for making volume upto 25 µl

3.5.3. Roles of Components in PCR

1. Genomic DNA (sample) acts as template on which complimentary DNA has be synthesized to make the multiple copies.
2. PCR buffer maintains biological pH of the sample at different conditions within the Thermo Cycler.
3. Forward/Reverse primers are used for amplification of specific regions/sequences/genes.
4. Taq-DNA polymerase enzyme is used for the synthesis of new strands of DNA by using dNTPs present in the PCR mixtures. It catalyzes the dNTPs and facilitates chain elongation. It is a Thermo stable DNA polymerase enzyme isolated from the Bacterium **"Thermus aquaticus"** and is resistant up to 95°C.
5. MgCl2 acts as a co-factor for Taq-DNA polymerase to initiate the reaction.

3.5.4. Procedure

1. Pre-denaturation: The pre-denaturation was performed at 94°C for 5 mins in which the tightly coiled complementary double helical strands of DNA get unwinded and other small ions or particles attached to DNA are degraded/ removed.
2. Denaturation: Denaturation was performed at 94°C 1 mint. In Denaturation step the two strands of the genomic DNA get denatured in which the Hydrogen bonds between them is broken down exposing the two separate DNA Templates.
3. Annealing: The annealing temperature was set at 55°C for 1min wherein the forward primer and Reverse primer will bind to the complementary sequences present on both strands of template DNA. These primers help in the synthesis of the new strands by using DNA –polymerase enzyme.
4. Extension/Elongation: Extension step is performed at 72°C for 2mints.In this reaction a new strand of complementary strands is synthesized by Taq- DNA polymerase enzyme by utilizing dNTPs present in the sample. The steps of Denaturation, Annealing and Extension are run for 30 cycles to yield enough amplicons that can be subjected for sequencing.
5. Final Extension: Final extension is performed at 72°C for 10 mins**.** In final extension step proof reading takes place wherein any mis match/mis pairing are repaired.
6. Soak temperature/preservation temperature: The Hold temperature/soak temperature was set at 4°C due to which the amplicons remain safe at an optimum temperature of 4°C until next use.
7. The amplicons were run on 1% agarose gel electrophoresis as a qualitative check.
8. The amplicons were purified by washing with 1Msodium acetate and 70% of ethanol and subjected for sequencing.

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4. RESULTS DISCUSSION AND CONCLUSION

4.1. Agarose Gel Electrophoresis of Genomic DNA

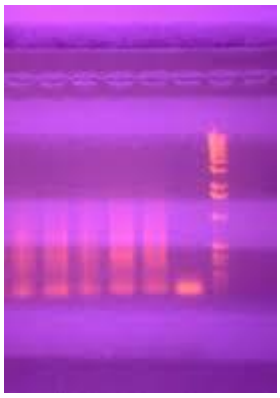

Fig: Genomic DNA Bands of Blood

4.2. SNP Study of BDNF by dbSNP

rs6265 *[Homo sapiens]*
ATCATTGGCTGACACTTTCGAACAC[A/G]TGAT
AGAAGAGCTGTTGGATGAGGA
Chromosome:11:27658369

Ancestral Allele:	**G**

From the sequence of BDNF of Homo sapiens the desired region containing position of SNP was selected as query sequence or reference sequence to be compared with the sequences obtained upon sequencing of 10 diabetic samples. This query sequence was retrieved from NCBI. Primer was selected by Primer BLAST and synthesized for this query sequence.

4.3. Retrieval of target region of BDNF from NCBI

4.3.1. Homo sapiens chromosome 11, GRCh38 Primary Assembly

Showing 501 bp region from base 27658350 to 27658850.
>gi|568815587:c27658850-27658350 Homo sapiens chromosome 11, GRCh38 Primary Assembly
TTGCACTTGCTTAGAAGCCATCTAATCTCAGG
TTTATATGCTAGATCTTGGGGGAAACACTGCA
TGTCTCTGGTTTATATTAAACCACATACAGCA
CACTACTGACACTGATTTGTGTCTGGTGCAGC
TGGAGTTTATCACCAAGACATAAAAAAACCTT
GACCCTGCAGAATGGCCTGGAATTACAATCAG
ATGGGCCACATGGCATCCCGGTGAAAGAAAG
CCCTAACCAGTTTTCTGTCTTGTTTCTGCTTTCT
CCCTACAGTTCCACCAGGTGAGAAGAGTGATG
ACCATCCTTTTCCTTACTATGGTTATTTCATAC
TTTGGTTGCATGAAGGCTGCCCCCATGAAAGA
AGCAAACATCCGAGGACAAGGTGGCTTGGCCT
ACCCAGGTGTGCGGACCCATGGGACTCTGGAG
AGCGTGAATGGGCCCAAGGCAGGTTCAAGAG
GCTTGACATCATTGGCTGACACTTTCGAACAC
GTGATAGAAGAGCTGTTGGA

4.3.2. Primer designing by Primer BLAST

Forward Primer: GCAGCCTTCATGCAACCAAA
Reverse Primer: TGGCATCCCGGTGAAAGAAA

4.4. Agarose Gel Electrophoresis of amplicons of Target region of BDNF

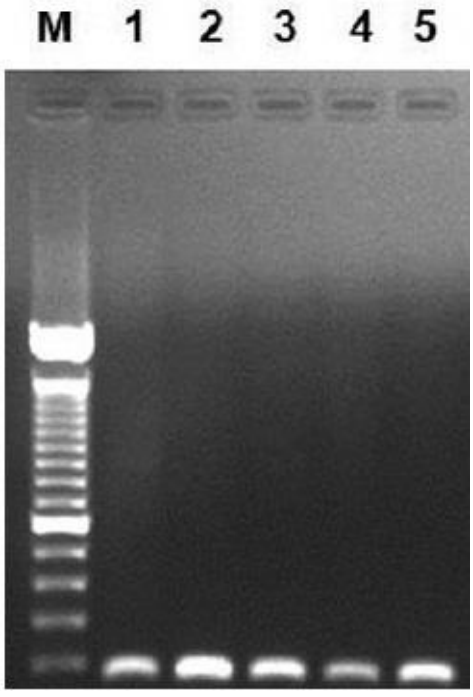

Fig: Amplicons Bands of Target region of BDNF

4.4.1. Sequences of 10 Diabetic Samples as obtained

1. TTGCACTTGCTTAGAAGCCATCTAAT
CTCAGGTTTATATGCTAGATCTTGGGGGAAAC

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ACTGCATGTCTCTGGTTTATATTAAACCACATA
CAGCACACTACTGACACTGATTTGTGTCTGGT
GCAGCTGGAGTTTATCACCAAGACATAAAAAA
ACCTTGACCCTGCAGAATGGCCTGGAATTACA
ATCAGATGGGCCACATGGCATCCCGGTGAAAG
AAAGCCCTAACCAGTTTTCTGTCTTGTTTCTGC
TTTCTCCCTACAGTTCCACCAGGTGAGAAGAG
TGATGACCATCCTTTTCCTTACTATGGTTATTT
CATACTTTGGTTGCATGAAGGCTGCCCCCATG
AAAGAAGCAAACATCCGAGGACAAGGTGGCT
TGGCCTACCCAGGTGTGCGGACCCATGGGACT
CTGGAGAGCGTGAATGGGCCCAAGGCAGGTT
CAAGAGGCTTGACATCATTGGCTGACACTTTC
GAACACGTGATAGAAGAGCTGTTGGA

2. TTGCACTTGCTTAGAAGCCATCTAAT
CTCAGGTTTATATGCTAGATCTTGGGGGAAAC
ACTGCATGTCTCTGGTTTATATTAAACCACATA
CAGCACACTACTGACACTGATTTGTGTCTGGT
GCAGCTGGAGTTTATCACCAAGACATAAAAAA
ACCTTGACCCTGCAGAATGGCCTGGAATTACA
ATCAGATGGGCCACATGGCATCCCGGTGAAAG
AAAGCCCTAACCAGTTTTCTGTCTTGTTTCTGC
TTTCTCCCTACAGTTCCACCAGGTGAGAAGAG
TGATGACCATCCTTTTCCTTACTATGGTTATTT
CATACTTTGGTTGCATGAAGGCTGCCCCCATG
AAAGAAGCAAACATCCGAGGACAAGGTGGCT
TGGCCTACCCAGGTGTGCGGACCCATGGGACT
CTGGAGAGCGTGAATGGGCCCAAGGCAGGTT
CAAGAGGCTTGACATCATTGGCTGACACTTTC
GAACACGTGATAGAAGAGCTGTTGGA

3. TTGCACTTGCTTAGAAGCCATCTAAT
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CAGCACACTACTGACACTGATTTGTGTCTGGT
GCAGCTGGAGTTTATCACCAAGACATAAAAAA
ACCTTGACCCTGCAGAATGGCCTGGAATTACA
ATCAGATGGGCCACATGGCATCCCGGTGAAAG
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CATACTTTGGTTGCATGAAGGCTGCCCCCATG
AAAGAAGCAAACATCCGAGGACAAGGTGGCT
TGGCCTACCCAGGTGTGCGGACCCATGGGACT
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CAAGAGGCTTGACATCATTGGCTGACACTTTC
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4. TTGCACTTGCTTAGAAGCCATCTAAT
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ACCTTGACCCTGCAGAATGGCCTGGAATTACA
ATCAGATGGGCCACATGGCATCCCGGTGAAAG
AAAGCCCTAACCAGTTTTCTGTCTTGTTTCTGC
TTTCTCCCTACAGTTCCACCAGGTGAGAAGAG
TGATGACCATCCTTTTCCTTACTATGGTTATTT
CATACTTTGGTTGCATGAAGGCTGCCCCCATG
AAAGAAGCAAACATCCGAGGACAAGGTGGCT
TGGCCTACCCAGGTGTGCGGACCCATGGGACT
CTGGAGAGCGTGAATGGGCCCAAGGCAGGTT
CAAGAGGCTTGACATCATTGGCTGACACTTTC
GAACACATGATAGAAGAGCTGTTGGA

5. TTGCACTTGCTTAGAAGCCATCTAAT
CTCAGGTTTATATGCTAGATCTTGGGGGAAAC
ACTGCATGTCTCTGGTTTATATTAAACCACATA
CAGCACACTACTGACACTGATTTGTGTCTGGT
GCAGCTGGAGTTTATCACCAAGACATAAAAAA
ACCTTGACCCTGCAGAATGGCCTGGAATTACA
ATCAGATGGGCCACATGGCATCCCGGTGAAAG
AAAGCCCTAACCAGTTTTCTGTCTTGTTTCTGC
TTTCTCCCTACAGTTCCACCAGGTGAGAAGAG
TGATGACCATCCTTTTCCTTACTATGGTTATTT
CATACTTTGGTTGCATGAAGGCTGCCCCCATG
AAAGAAGCAAACATCCGAGGACAAGGTGGCT
TGGCCTACCCAGGTGTGCGGACCCATGGGACT
CTGGAGAGCGTGAATGGGCCCAAGGCAGGTT
CAAGAGGCTTGACATCATTGGCTGACACTTTC
GAACACGTGATAGAAGAGCTGTTGGA

6. TTGCACTTGCTTAGAAGCCATCTAAT
CTCAGGTTTATATGCTAGATCTTGGGGGAAAC
ACTGCATGTCTCTGGTTTATATTAAACCACATA
CAGCACACTACTGACACTGATTTGTGTCTGGT
GCAGCTGGAGTTTATCACCAAGACATAAAAAA

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TGATGACCATCCTTTTCCTTACTATGGTTATTT
CATACTTTGGTTGCATGAAGGCTGCCCCCATG
AAAGAAGCAAACATCCGAGGACAAGGTGGCT
TGGCCTACCCAGGTGTGCGGACCCATGGGACT
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CAAGAGGCTTGACATCATTGGCTGACACTTTC
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7. TTGCACTTGCTTAGAAGCCATCTAAT
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ACTGCATGTCTCTGGTTTATATTAAACCACATA
CAGCACACTACTGACACTGATTTGTGTCTGGT
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CATACTTTGGTTGCATGAAGGCTGCCCCCATG
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TGGCCTACCCAGGTGTGCGGACCCATGGGACT
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8. TTGCACTTGCTTAGAAGCCATCTAAT
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ACTGCATGTCTCTGGTTTATATTAAACCACATA
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CATACTTTGGTTGCATGAAGGCTGCCCCCATG
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9. TTGCACTTGCTTAGAAGCCATCTAAT
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ACTGCATGTCTCTGGTTTATATTAAACCACATA
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CATACTTTGGTTGCATGAAGGCTGCCCCCATG
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TGGCCTACCCAGGTGTGCGGACCCATGGGACT
CTGGAGAGCGTGAATGGGCCCAAGGCAGGTT
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10. TTGCACTTGCTTAGAAGCCATCTAAT
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CATACTTTGGTTGCATGAAGGCTGCCCCCATG
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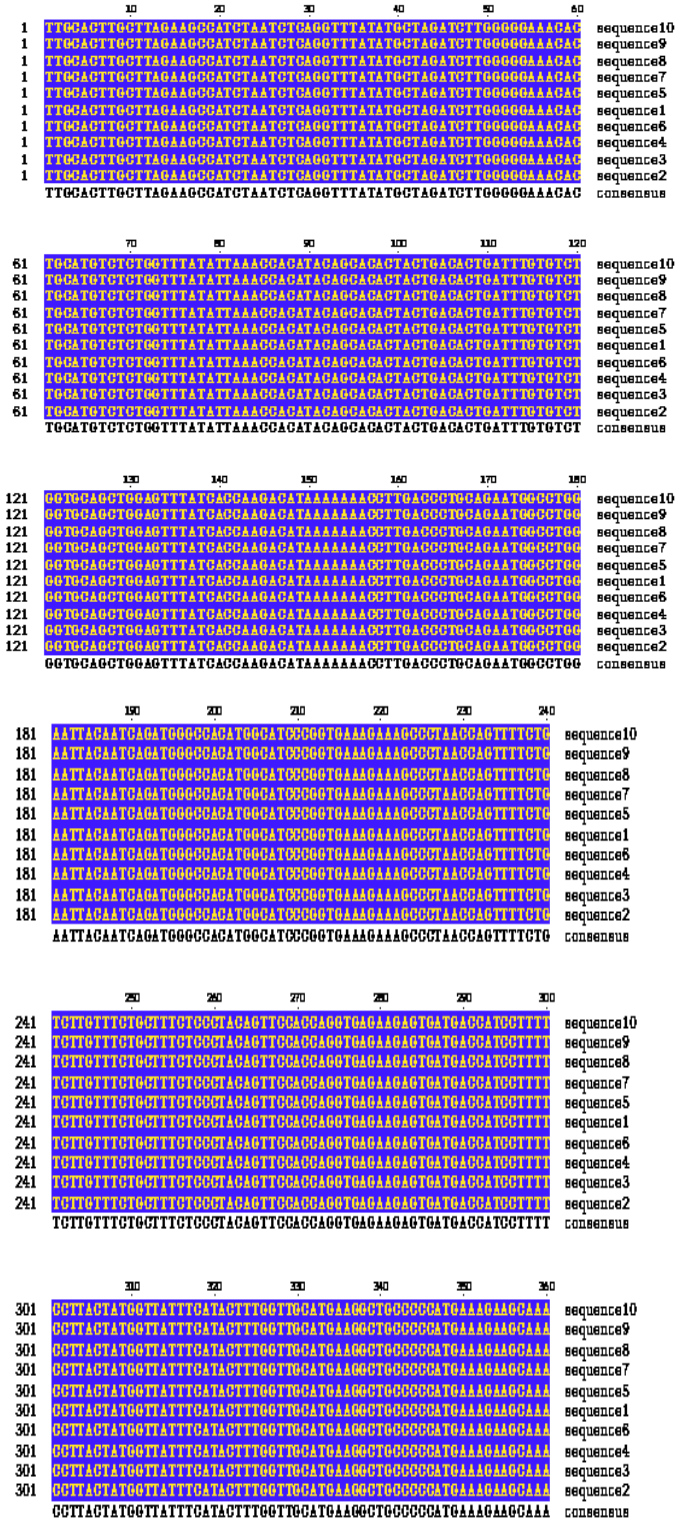
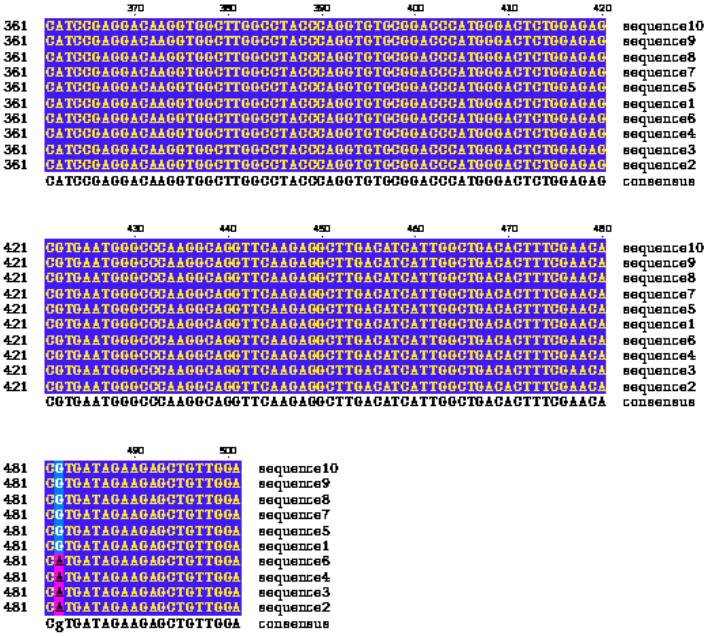
4.4.2. SNP Study of obtained sequences by Tex Shade





4.5. DISCUSSION AND CONCLUSION

The present work initiated with the aim of checking the frequency of any reported SNP of a gene in DMT2 Patients of Telangana region. Samples were collected from Diagnostic Centres. Coincidently all the 10 samples collected were showing a case history of neurological disorders also. It became pertinent to select and study such a gene that is common to DMT2 and Neurological disorder. BDNF was found to be one of such genes. Owing to this great significance of BDNF it was selected as target gene to be studied in terms of its SNPs. A list of reported SNPs in BDNF were searched in dbSNP and one of them **rs6265** at position 27,658,369 on chromosome 11 was selected for further studies. The aim was to check the frequency of this G to A Polymorphism in the test samples. For this, the primer set was designed and amplification was performed on 10 diabetic samples. No control was taken as the purpose was to check the frequency of SNP in DMT2 samples only and not to compare with Non diabetic ones. The sequencing result when analyzed by Bioinformatics tool clearly indicated that four diabetic samples (Sample number 2, 3, 4 and 6) were found to have A instead of G (rs6265) at position

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(chr 11: 27,658,369) that corresponds to val66met polymorphism.

However this study does not confirm the universal invariable presence of the SNP in the diabetic population rather its existence is limited to the test sample set only. The hypothesis of the occurrence of both the variants in diabetic as well as non diabetic samples can not be ruled out.

Hence a further study on a wider and large sample set is recommended. As a future aspect of the report the work can be further extended on proteomic level wherein the corresponding change occurred due to the mutation in the protein can be further detected at structural and functional level.

REFERENCES

1. "Diabetes Blue Circle Symbol". International Diabetes Federation. 17 March
2. Shoback, edited by David G. Gardner, Dolores (2011). *Greenspan's basic & clinical endocrinology* (9th ed.). New York: McGraw-Hill Medical. pp. Chapter 17. ISBN 0-07-162243-8.
3. "Diabetes - Overview". NHS. Retrieved 2013-07-14.
4. *Williams textbook of endocrinology* (12th ed.). Philadelphia: Elsevier/Saunders. pp. 1371–1435. ISBN 978-1-4377-0324-5.
5. Lambert, P.; Bingley, P. J. (2002). "What is Type 1 Diabetes?". *Medicine* **30**: 1–5.doi:10.1383/medc.30.1.1.28264. Diabetes Symptomsedit
6. Rother KI (April 2007). "Diabetes treatment—bridging the divide". *The New England Journal of Medicine* **356** (15): 1499–501. doi:10.1056/NEJMp078030.PMID 17429082.
7. "Diabetes Mellitus (DM): Diabetes Mellitus and Disorders of Carbohydrate Metabolism: Merck Manual Professional". Merck Publishing. April 2010. Retrieved 2010-07-30.
8. Dorner M, Pinget M, Brogard JM (May 1977). "Essential labile diabetes". *MMW Munch Med Wochenschr* (in German)**119** (19): 671–4. PMID 406527.
9. Lawrence JM, Contreras R, Chen W, Sacks DA (May 2008). "Trends in the prevalence of preexisting diabetes and gestational diabetes mellitus among a racially/ethnically diverse population of pregnant women, 1999–2005".*Diabetes Care* **31** (5): 899–904. doi:10.2337/dc07-2345.PMID 18223030.
10. Handelsman Y, MD. "A Doctor's Diagnosis: Prediabetes".*Power of Prevention* **1** (2).
11. "Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications" (PDF). World Health Organisation. 1999.
12. Cooke DW, Plotnick L (November 2008). "Type 1 diabetes mellitus in pediatrics". *Pediatr Rev* **29** (11): 374–84; quiz 385. doi:10.1542/pir.29-11-374. PMID 18977856.
13. Emerging Risk Factors Collaboration (2010). "Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: A collaborative meta-analysis of 102 prospective studies". *The Lancet* **375** (9733): 2215–22.
14. Boussageon R, Bejan-Angoulvant T, Saadatian-Elahi M, Lafont S, Bergeonneau C, Kassaï B, Erpeldinger S, Wright JM, Gueyffier F, Cornu C (2011). "Effect of intensive glucose lowering treatment on all cause mortality, cardiovascular death, and microvascular events in type 2 diabetes: meta-analysis of randomised controlled trials". .
15. Cukierman, T (8 Nov 2005). "Cognitive decline and dementia in diabetes—systematic overview of prospective observational studies". Springer-Verlag. Retrieved 28 Apr 2013.
16. Washington R.E., Andrews R.M., Mutter R.L. Emergency Department Visits for Adults with Diabetes, 2010. HCUP Statistical Brief #167. November 2013. Agency for Healthcare Research and Quality, Rockville,

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International Journal & Magazine of Engineering, Technology, Management and Research
A Peer Reviewed Open Access International Journal

17. Risérus U, Willet W (January 2009). "Dietary fats and prevention of type 2 diabetes". *Progress in Lipid Research*
18. Malik, VS; Popkin, BM, Bray, GA, Després, JP, Hu, FB (2010-03-23). "Sugar Sweetened Beverages, Obesity, Type 2 Diabetes and Cardiovascular Disease risk"..
19. Malik, VS; Popkin, BM, Bray, GA, Després, JP, Willett, WC, Hu, FB (November 2010). "Sugar-Sweetened Beverages and Risk of Metabolic Syndrome and Type 2 Diabetes: A meta-analysis".
20. Hu, EA; Pan, A, Malik, V, Sun, Q (2012-03-15). "White rice consumption and risk of type 2 diabetes: meta-analysis and systematic review". *BMJ (Clinical research ed.)*
21. Lee, I-Min; Shiroma, Eric J; Lobelo, Felipe; Puska, Pekka; Blair, Steven N; Katzmarzyk, Peter T (1 July 2012). "Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy".
22. Unless otherwise specified, reference is: Table 20-5 inMitchell, Richard Sheppard; Kumar, Vinay; Abbas, Abul K.; Fausto, Nelson. *Robbins Basic Pathology*. Philadelphia: Saunders
23. Sattar N, Preiss, D, Murray, HM, Welsh, P, Buckley, BM, de Craen, AJ, Seshasai, SR, McMurray, JJ, Freeman, DJ (February 2010). "Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials".
24. *Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation*. Geneva: World Health Organization.
25. Vijan, S (March 2010). "Type 2 diabetes". *Annals of Internal Medicine*
26. ""Diabetes Care" January 2010". *American Diabetes Association*. Retrieved 2010-01-29.
27. Saydah SH, Miret M, Sung J, Varas C, Gause D, Brancati FL (August 2001). "Postchallenge hyperglycemia and mortality in a national sample of U.S. adults". *Diabetes Care***24** (8): 1397–402.
28. *Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia : report of a WHO/IDF consultation*. World Health Organization. 2006.
29. Santaguida PL, Balion C, Hunt D, Morrison K, Gerstein H, Raina P, Booker L, Yazdi H. "Diagnosis, Prognosis, and Treatment of Impaired Glucose Tolerance and Impaired Fasting Glucose". *Summary of Evidence Report/Technology Assessment, No. 128*. Agency for Healthcare Research and Quality. Retrieved 2008-07-20.
30. Selvin E, Steffes MW, Zhu H, Matsushita K, Wagenknecht L, Pankow J, Coresh J, Brancati FL (2010). "Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults". *N. Engl. J. Med.* **362** (9): 800–11.
31. Nathan DM, Cleary PA, Backlund JY, Genuth SM, Lachin JM, Orchard TJ, Raskin P, Zinman B; Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group (December 2005). "Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes".*The New England Journal of Medicine* **353** (25): 2643–53.
32. . "The effect of intensive diabetes therapy on the development and progression of neuropathy. The Diabetes Control and Complications Trial Research Group". *Annals of Internal Medicine* **122** (8): 561–8.
33. National Institute for Health and Clinical Excellence.*Clinical guideline 66: Type 2 diabetes*. London, 2008.
34. Cavanagh, P. R. (2004). "Therapeutic footwear for people with diabetes". *Diabetes/Metabolism Research and Reviews***20**: S51–S55.
35. Adler AI, Stratton IM, Neil HA, Yudkin JS, Matthews DR, Cull CA, Wright AD, Turner

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International Journal & Magazine of Engineering, Technology, Management and Research
A Peer Reviewed Open Access International Journal

RC, Holman RR (August 2000)."Association of systolic blood pressure with macrovascular and microvascular complications of type 2 diabetes (UKPDS 36): prospective observational study". *BMJ* **321** (7258): 412

36. Ripsin CM, Kang, H, Urban, RJ (2009). "Management of blood glucose in type 2 diabetes mellitus". *American family physician* **79** (1): 29–36.
37. Pignone M, Alberts MJ, Colwell JA, Cushman M, Inzucchi SE, Mukherjee D, Rosenson RS, Williams CD, Wilson PW, Kirkman MS; American Diabetes Association; American Heart Association; American College of Cardiology Foundation (June 2010). "Aspirin for primary prevention of cardiovascular events in people with diabetes: a position statement of the American Diabetes Association, a scientific statement of the American Heart Association, and an expert consensus document of the American College of Cardiology Foundation". *Diabetes Care* **33** (6): 1395–402.
38. Polisena J, Tran K, Cimon K, Hutton B, McGill S, Palmer K (2009). "Home telehealth for diabetes management: a systematic review and meta-analysis". *Diabetes Obes Metab* **11** (10): 913–30.
39. Wild S, Roglic G, Green A, Sicree R, King H (2004). "Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030". *Diabetes Care* **27** (5): 1047–53.
40. Ripoll, Brian C. Leutholtz, Ignacio (2011-04-25). *Exercise and disease management* (2nd ed.). Boca Raton: CRC Press. p. 25.
41. editor, Leonid Poretsky, (2009). *Principles of diabetes mellitus* (2nd ed.). New York: Springer. p. 3
42. Konstantinos Laios *et al.*; Karamanou, M; Saridaki, Z; Androutsos, G (2012). "Aretaeus of Cappadocia and the first description of diabetes". *Hormones* **11** (1): 109–113.
43. Oxford English Dictionary. *diabetes*. Retrieved 2011-06-10.
44. Harper, Douglas (2001–2010). "Online Etymology Dictionary. *diabetes.*". Retrieved 2011-06-10.
45. Dallas, John (2011). "Royal College of Physicians of Edinburgh. Diabetes, Doctors and Dogs: An exhibition on Diabetes and Endocrinology by the College Library for the 43rd St. Andrew's Day Festival Symposium"."MyEtimology. *mellitus.*". Retrieved 2011-06-10.
46. Theodore H. Tulchinsky, Elena A. Varavikova (2008). *The New Public Health, Second Edition*. New York: Academic Press. p. 200. ISBN 0-12-370890-7.
47. National Diabetes Information Clearinghouse, *What Diabetes Is* [Website] January 6, 2009 http://www.cdc.gov/diabetes/faq/basics.htm#5
48. Pozzilli P, Buzzetti R. A new expression of diabetes: double diabetes. Trends Endocrinol Metab 2007; 18: 52-7.
49. Tuomi T. Type 1 and type 2 diabetes: what do they have in common? Diabetes 2005; 54 Suppl 2: S40-5.
50. van Deutekom AW, Heine RJ, Simsek S. The islet autoantibody titres: their clinical relevance in latent autoimmune diabetes in adults (LADA) and the classification of diabetes mellitus. Diabet Med 2008; 25: 117-25.
51. Lin J, Zhou ZG, Wang JP, Zhang C, Huang G. From Type 1, through LADA, to type 2 diabetes: a continuous spectrum? Ann N Y Acad Sci 2008; 1150: 99- 102.
52. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2009; 87: 4-14.
53. Diagnosis and classification of diabetes

ISSN No: 2348-4845
International Journal & Magazine of Engineering, Technology, Management and Research
A Peer Reviewed Open Access International Journal

mellitus. Diabetes Care 2011; 34 Suppl 1: S62-9.

54. McCarthy, M. I. (December 2010). "Genomics, Type 2 Diabetes, and Obesity". In Feero, W. G.; Guttmacher, A. E. *The New England Journal of Medicine* **363** (24): 2339–50.doi:10.1056/NEJMra0906948. PMID 21142536.

55. CDC, *Treating Diabetes (insulin and oral medication use)* [Website] January 15, 2009. http://www.cdc.gov/diabetes/statistics/treating_national.htm

56. Walley AJ, Blakemore AI, Froguel P (October 2006). "Genetics of obesity and the prediction of risk for health". *Human Molecular Genetics*. 15 Spec No 2: R124–30. doi:10.1093/hmg/ddl215.PMID 16987875.

57. UKPDS Group. Intensive blood glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33)Lancet. 1998;352:837–853.

58. National Diabetes Information Clearinghouse, *National Diabetes Statistics* [Website] January 9, 2009. http://diabetes.niddk.nih.gov/dm/pubs/statistics/#allages

59. Barrett TG (September 2001). "Mitochondrial diabetes, DIDMOAD and other inherited diabetes syndromes". *Best Practice & Research. Clinical Endocrinology & Metabolism* **15** (3): 325–43.doi:10.1053/beem.2001.0149. PMID 11554774.

60. Sasaki A, Uehara M, Horiuchi N, Hasegawa K. A long-term follow-up study of diabetic patients in Osaka, Japan: mortality and causes of death. Tohoku J Exp Med.1983;141(suppl):639–644.

61. *Williams textbook of endocrinology*. (12th ed. ed.). Philadelphia: Elsevier/Saunders. pp. 1371–1435. ISBN 978-1-4377-0324-5.

62. . CDC, *Treating Diabetes (insulin and oral medication use)* [Website] January 15, 2009. http://www.cdc.gov/diabetes/statistics/treating_national.htm

63. Gonzalez EL, Johansson S, Wallander MA, Rodriguez LA. Trends in the prevalence and incidence of diabetes in the UK: 1996-2005. J Epidemiol Community Health 2009; 63: 332-6.

64. McCarthy MI. Genomics, type 2 diabetes, and obesity. N Engl J Med 2010; 363: 2339-50.

65. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998; 15: 539- 53

66. Camastra S, Bonora E, Del Prato S, Rett K, Weck M, Ferrannini E (December 1999). "Effect of obesity and insulin resistance on resting and glucose-induced thermogenesis in man. EGIR (European Group for the Study of Insulin Resistance)". *Int. J. Obes. Relat. Metab. Disord.* **23** (12): 1307–13.doi:10.1038/sj.ijo.0801072. PMID 10643689.

67. Permutt MA, Wasson J, Cox N. Genetic epidemiology of diabetes. J Clin Invest 2005; 115: 1431-9.

68. Eckel RH, Kahn SE, Ferrannini E, et al. Obesity and type 2 diabetes: what can be unified and what needs to be individualized?

ISSN No: 2348-4845
International Journal & Magazine of Engineering, Technology, Management and Research
A Peer Reviewed Open Access International Journal

Diabetes Care 2011; 34: 1424-30.

69. Wang J, Luben R, Khaw KT, Bingham S, Wareham NJ, Forouhi NG. Dietary energy density predicts the risk of incident type 2 diabetes: the European Prospective Investigation of Cancer (EPIC)-Norfolk Study. Diabetes Care 2008; 31: 2120-5.

70. Hectors TL, Vanparys C, van der Ven K, et al. Environmental pollutants and type 2 diabetes: a review of mechanisms that can disrupt beta cell function. Diabetologia 2011; 54: 1273-90.

71. Anderson JW, Kendall CW, Jenkins DJ. Importance of weight management in type 2 diabetes: review with meta-analysis of clinical studies. J Am Coll Nutr 2003; 22: 331-9.

72. Schulze MB, Heidemann C, Schienkiewitz A, Bergmann MM, Hoffmann K, Boeing H. Comparison of anthropometric characteristics in predicting the incidence of type 2 diabetes in the EPIC-Potsdam study. Diabetes Care 2006; 29: 1921-3.

73. Pierce M, Keen H, Bradley C. Risk of diabetes in offspring of parents with non-insulin-dependent diabetes. Diabet Med 1995; 12: 6-13.

74. Shai I, Jiang R, Manson JE, Stampfer MJ, Willett WC, Colditz GA, Hu FB. Ethnicity, obesity, and risk of type 2 diabetes in women: a 20-year follow-up study. Diabetes Care 2006; 29: 1585-90.

75. Nichols GA, Hillier TA, Brown JB. Progression from newly acquired impaired fasting glusose to type 2 diabetes. Diabetes Care 2007; 30: 228-33.

76. Gress TW, Nieto FJ, Shahar E, Wofford MR, Brancati FL. Hypertension and antihypertensive therapy as risk factors for type 2 diabetes mellitus. Atherosclerosis Risk in Communities Study. N Engl J Med 2000; 342: 905-12.

77. Mooradian AD. Dyslipidemia in type 2 diabetes mellitus. Nat Clin Pract Endocrinol Metab 2009; 5: 150-9.

78. Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG, Willett WC. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. N Engl J Med 2001; 345: 790-7.

79. Kim C, Newton KM, Knopp RH. Gestational diabetes and the incidence of type 2 diabetes: a systematic review. Diabetes Care 2002; 25: 1862-8.

80. Whincup PH, Kaye SJ, Owen CG, et al. Birth weight and risk of type 2 diabetes: a systematic review. JAMA 2008; 300: 2886-97.

81. Legro RS. Type 2 diabetes and polycystic ovary syndrome. Fertil Steril 2006; 86 Suppl 1: S16-7.

82. DECODE Study Group: Age- and sex-specific prevalences of diabetes and impaired glucose regulation in 13 European cohorts. Diabetes Care 2003; 26: 61-9.

83. Dechenes CJ, Verchere CB, Andrikopoulos S, Kahn SE. Human aging is associated with parallel reductions in insulin and amylin release. Am J Physiol 1998; 275: E785-91.

84. Corona, Erik. [geneworld.stanford.edu "Geneworld"] Check |url= scheme (help). *World Wide Patterns of Genetic Risk for Disease*. Stanford University. Retrieved 17 November 2011

85. Cotran, Kumar, Collins; **Robbins Pathologic Basis of Disease,**Saunders Sixth Edition,

1999; 913-926.

86. Lyssenko V, Jonsson A, Almgren P, *et al.* (November 2008). "Clinical risk factors, DNA variants, and the development of type 2 diabetes". *The New England Journal of Medicine* **359** (21): 2220–32.doi:10.1056/NEJMoa0801869. PMID 19020324.

87. Gibbons, Ann (4). "Diabetes Genes Decline Out of Africa". *Science* **334** (6056): 583.doi:10.1126/science.334.6056.583

88. Jones CW. Gestational diabetes and its impact on the neonate. Neonatal Netw 2001; 20: 17-23.