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Accessing the GAS for the variant TNF (rs1800629) in diabetic nephropathy using STREGA statement

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Thesis
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Αξιολόγηση κατά STREGA των Γενετικών Μελετών για το ρόλο του TNF (rs1800629) στη διαβητική νεφροπάθεια

Supervisor:
Prof. Stefanidis I.

My thanks

to all the professors of this postgraduate program for their offer to my knowledge and especially to the professor Stefanidis I. for his guidance to this project and finally to the personnel of the program for the assistance during the course.

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Abstract

The occurrence of diabetic nephropathy is related to various factors including genetic factors such as the Tumor Necrosis Factor – a, the role of which attracts much attention. In order to examine this role have been conducted genetic association studies (GAS).

This thesis is an effort to assess the quality of the GAS for the variant TNF (RS1800629) in diabetic nephropathy according to the recommendations of STrengthening the REporting of Genetic Association Studies (STREGA) statement. For this purpose have been selected five representative studies from the international bibliography and have been commented point by point to the 22 proposed items of the STREGA statement and provided an overall assessment.

The solid conclusion is that the way the researchers design, conduct and present their studies may vary but the most important elements are usually taken into consideration.

Genetic Association Study, STREGA statement, Tumor Necrosis Factor – alpha, diabetic nephropathy, variant, polymorphism

Introduction

Genetic association studies (GAS) are used to find candidate genes or genome regions that contribute to a specific disease by testing for a correlation between disease status and genetic variation.[1] Genetic association studies could be characterized as analogous to traditional epidemiological studies.

A **Single nucleotide polymorphism (SNP)** is a variation in a single nucleotide that occurs at a specific position in the genome, where each variation is present to some appreciable degree within a population (e.g. > 1%) [2] SNPs may be within genes -coding SNP- but the most of them are outside of the genes –non coding. SNPs are responsible for 90% of all human genetic variation (almost 12 million SNPs in the NCBI SNP database). [3] Thus they are the most commonly studied genetic variable. The majority of SNPs isn't responsible for a disease.

The **tumor necrosis factor (TNF)** superfamily refers to a group of multifunctional proniflammatory cytokines that can cause cell death (apoptosis).The first two members of the family are TNF-alpha (TNF-α) and TNF-beta (TNF-β). The TNF-alpha, is a monocyte-derived cytotoxin that has been implicated in tumor regression, septic shock, and cachexia. [2] Tumor necrosis factor alpha (TNF –a) is coded by TNF gene located on chromosome 6 in human leukocyte antigen (HLA) region class III and has been found to be a crucial component of the pro-inflammatory cytokines, mainly produced by macrophages and adipocytes, and TNF-a can induce the apoptosis of insulin-producing cells and mediating the inflammatory process in the islets of Langerhans or result in insulin resistance by direct inhibition of phosphorylation of insulin receptor and substrates and by reduction of glucose uptake by peripheral tissues.[4]

The tumor necrosis factor-alpha gene, **rs1800629**, is also known as the TNF-308 SNP. [5] Occasionally the rs1800629 (A) allele is referred to as 308.2 or TNF2, with the more common (G) allele being 308.1 or TNF1. [4] The (A) allele is associated with higher levels of TNF expression. [5] This SNP has been linked to a wide variety of conditions. [5]

Diabetic nephropathy is a clinical syndrome characterized by persistent albuminuria (>300 mg/d or >200 μg/min), progressive decline in the glomerular filtration rate (GFR) and elevated arterial blood pressure. [5] The pathophysiologic event in diabetic nephropathy is a progressive thickening of the basement membrane. Diabetic nephropathy occurs in 15-40% of the people with type 1 diabetes, with a peak incidence at 15-20 years disease duration. The prevalence in type 2 diabetes is 5-20% according to the international bibliography.

STrengthening the REporting of Genetic Association Studies (STREGA) has been built on the STrengthening the Reporting of OBservational Studies in Epidemiology (**STROBE**) Statement with additions to 12 of the 22 items by a multi-institutional team. The STREGA statement describes the guidelines that allow a better framework to report genetic association studies. The STREGA recommendations do not prescribe or dictate how a genetic association study should be designed but seek to enhance the transparency of its reporting, regardless of choices made during design, conduct, or analysis. [6] The additions concern population stratification, genotyping errors, modelling haplotype variation, Hardy-Weinberg equilibrium, replication, selection of participants, rationale for choice of genes and variants, treatment effects in studying quantitative traits, statistical methods, relatedness, reporting of descriptive and outcome data, and the volume of data issues that are important to consider in genetic association studies. [6] The STREGA recommendations are available at www.strega-statement.org

Have been selected five genetic association studies from the international literature which have been conducted by different researchers, concerning different populations and taken place in different eras. The title and the link for each study are provided. Taking consideration the 22 items of the STREGA statement, have been commented the most important information and written an overall assessment of every study according to my judgment.

First Genetic Association Study

TNF-α-308G/A polymorphism associated with _TNF-α_ protein expression in patients with diabetic nephropathy

Yan Peng, Liu-Juan Li

Int J Clin Exp Pathol. 2015; 8(3): 3127–3131.
Published online 2015 Mar 1.

www.ncbi.nlm.nih.gov/pmc/articles/PMC4440137/

The first study is a case – control Genetic Association Study which analyzes the association of the TNF-a-308 G/A polymorphism with the TNF –a protein expression in patients with diabetic nephropathy and additionally examines whether the TNF-a-308 polymorphism is a genetic susceptibility factor for diabetic nephropathy.

Specifically, for TNF-α -308G/A, the G/A genotype could increase the risk for DN (OR = 2.15, 95% CI = 1.08-4.30). [7] The A allele frequency was found higher in cases compared with controls, which suggested that A allele served as a genetic-susceptibility factor for DN (OR = 1.89, 95% CI = 1.10-3.26). [7] Further analysis indicated that the level of TNF-α for individuals with mutant genotype (GA and AA) were higher than that of individuals with wild genotype ($P < 0.05$). [7] However, AA genotype showed no effects on DN susceptibility (OR = 2.08, 95% CI = 0.56-7.33). [7]

STREGA statement - Title and abstract (1)

The title is merely informative about the study as there is no reference to the examined association between the TNF –a polymorphism and the occurrence of diabetic nephropathy. The abstract is quite informative and indicates sufficiently the study's design. The summary is also balanced.

STREGA statement – Introduction

Background rationale (2) & Objectives (3)

The introduction is in accordance with the proposed guidance of the STREGA statement about the background rational and the objectives but is insufficient if the study is the first report, a replication effort or both.

STREGA statement – Methods

Study design (4)

The study's design is described adequately.

Settings (5)

The presentation of the settings is deficit. There is no mention about the locations, the relevant dates, the recruitment periods and the data collection.

Participants (6)

The researchers of this case – control study gave the eligibility criteria, stated the age and gender match without mentioning the number of controls per case, or the rationale for the choice of cases and controls. Didn't report the number of individuals in whom genotyping was attempted and the number of individuals in whom genotyping was successful.

Variables (7)

About the variables, are clearly defined only the exposure outcomes and the diagnostic criteria. Several parameters of variable description have been omitted.

Data sources measurements (8)

That part of the study is well presented. Must mention that there's no statement about the laboratory/centre where the genotyping was done and whether genotypes were assigned using all of the data from the study simultaneously or in smaller batches.

Bias (9)

It is only mentioned that the subjects were unrelated Chinese, and the controls were matched to cases in age and gender. There was no significant effort to address potential sources of bias.

Study size (10)

There is no explanation how the study size was arrived at.

Quantitative variables (11)

There are no quantitative variables.

Statistical methods (12)

All the statistical methods were described, the software version was stated and the Hardy Weinberg equilibrium was considered by chi- square test. On the other hand, there was no comment about missing data, sensitivity analysis, if they have used methods to address multiple comparisons or to control the risk of false positive findings.

STREGA statement – Results

Participants (13)

This part of the study is inadequate according to the STREGA statement. There is only a table showing the genotype and allele frequencies of TNF-α-308 in case and control groups without any comment if the genotyping was attempted to all individuals and in whom was successful. No report about the participation and the completion of the study, no examina -

tion for eligibility, no flow diagram.

Descriptive data (14)

The researchers gave same information by genotype but no information concerning the demographic, clinical or social characteristics of the participants.

Outcome data (15) & Main Results (16)

Outcome data and main results have been adequately commented.

Other analyses (17)

There is no report if the researchers did any subgroup analyses or sensitivity analyses and is not stated if the detailed results are available elsewhere.

STREGA statement – Discussion

Key results (18)

The goal has been accomplished.

Limitations (19)

No discussion of the limitations.

Interpretation (20)

There is no cautious overall presentation, just a repetition of the findings

Generalizability (21)

No discussion in this part about the external validity of the study.

STREGA statement – Other Information

Funding (22)

There is no reference to the source of funding, only a comment about no conflict of interest.

Overall assessment

Taking into consideration all the STREGA recommendations, the assumption is that the well reported parts are the title, the introduction and the methods with some insufficiency. The results and the discussion part could have been more analytic. The information about the funding is missing. In my opinion, what needs improvement is the explanation about genotyping, the report of descriptive and the reference of outcome data.

Second Genetic Association Study

Association between tumor necrosis factor-a G-308A polymorphism and risk of nephropathy in obese Chinese type 2 diabetic patients

Ying Wang, Maggie C. Y. Ng, Wing-Yee So, Ronald Ma, Gary T. C. Ko, Peter C. Y. Tong and Juliana C. N. Chan

From the Division of Endocrinology, Department of Medicine & Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong SAR

Nephrol Dial Transplant (2005) 20: 2733–2738 doi:10.1093/ndt/gfi101 Advance Access publication 2 September 2005

http://www.ncbi.nlm.nih.gov/pubmed/16141456

The second study is a consecutive cohort study of 1281 Chinese type 2 diabetic patients. Is based on reported fact that the G-308A polymorphism in the promoter region of the tumor necrosis factor alpha (TNF-a) gene is associated with insulin resistance and obesity, both of which may increase the risk of diabetic nephropathy.[9]Thus examines the hypothesis that this polymorphism might interact with obesity to affect development of diabetic nephropathy.[9] In particular, by the study, stated that the G-308A polymorphism was not associated with either obesity or nephropathy and that the GG genotype of TNF-a G-308A polymorphism or a genetic variant in close linkage disequilibrium may interact with obesity to increase the risk of nephropathy in Chinese Type 2 diabetic patients.[9]

STREGA statement - Title and abstract (1)

The title and the abstract are fully harmonized with the STREGA recommendations.

STREGA statement – Introduction

Background rationale (2) & Objectives (3)

The authors gave a full explanation of the scientific background, rationale and the objectives. There is no statement if the study is the first report of a genetic association, a replication effort, or both.

STREGA statement – Methods

Study design (4)

The study design is presented extensively.

Settings (5)

The description of the settings, the eligibility criteria, the sources and methods of selection of participants for the cohort study are in detail.

Participants (6)

The information about the participants is sufficient, are given all the criteria and the methods of selection. All the outcomes, predictors, potential confounders and diagnostic criteria are defined.

Variables (7) & Data sources measurements (8)

These fields are explained in detail with the exception of the information whether genotypes were assigned using all of the data from the study simultaneously or in smaller batches.

Bias (9)

The researchers address the low frequency of the A allele in the study population as a potential source of bias.

Study size (10)

The study size is explained.

Quantitative variables (11)

There are no quantitative variables.

Statistical methods (12)

The statistical methods are commended extensively, including those used to control for confounding and for subgroups. The software version and the chosen options were stated. The HWE has been taken into consideration. The researchers applied the methods used to address multiple comparisons but there is no description of methods used to address and correct for relatedness among subjects.

STREGA statement – Results

Participants (13)

The parameters concerning the participants are given in detail, including the number of individuals at each stage of the study, number of potentially eligible, number of examined for eligibility, number of confirmed eligible. Also, included and analyzed the reasons for non-participation at each stage in the study.

Descriptive data (14)

The descriptive data are mentioned extensively, as there have been taken into consideration multiple clinical characteristics and potential confounders. Furthermore, the researchers added information by genotype.

Outcome data (15) Main Results (16)

This part is quite analytic according to the STREGA statement recommendations. There is a full report on outcomes (phenotypes) for each genotype category.

Main Results (16)

The main results are well presented, including unadjusted estimates, confounder-adjusted estimates, their precision (e.g., 95% confidence intervals) and made clear which confounders were adjusted for and why they were included. Results of adjustments for multiple comparisons are mentioned.

Other analyses (17)

All the analyses, including those of subgroups and genetic variants, are applied but no statement if detailed results are available elsewhere and if so, how they can be accessed.

STREGA statement – Discussion

Key results (18)

The authors provided a cautious summary of results with reference to study objectives.

Limitations (19)

The limitations have been commended sufficiently taking into account potential bias and imprecision.

Interpretation (20)

The interpretation part constitutes an overall interpretation of study's results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.

Generalizability (21)

There is a discussion of the external validity of the study results, the need for replication in other populations and in different ethnic groups.

STREGA statement – Other Information

Funding (22)

Is made known the study's funding and declared no conflict of interest.

Overall assessment

Despite the fact that the study conducted before the STREGA statement, fulfills almost all of the recommendations

Third Genetic Association Study

Association between LTA, TNF and AGER Polymorphisms and Late Diabetic Complications

Eero Lindholm,Ekaterina Bakhtadze, Corrado Cilio, Elisabet Agardh,Leif Groop, Carl-David Agardh

Published: June 25, 2008

http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0002546

The third study is a case – control study in Scandinavian subjects. Is based on the fact that several candidate genes on the short arm of chromosome 6 including the HLA locus, TNF,LTA and AGER could be associated with late diabetic complications. [10] This study aimed to explore whether polymorphisms (TNF -308 G→A, LTA T60N C→A and AGER -374 T→A) in these genes alone or together (as haplotypes) increased the risk for diabetic complications. [10]

The results concerning the TNF – a revealed that the allele frequencies of TNF -308 G→A polymorphisms were similar in type 1 diabetic patients with and without diabetic nephropathy. [10] No differences in allele or haplotype frequencies of the studied polymorphisms were observed between type 2 diabetic patients with and without diabetic nephropathy. [10]

STREGA statement - Title and abstract (1)

The title and the abstract are indicative of the study's design but not informative about all the findings.

STREGA statement – Introduction

Background rationale (2) & Objectives (3)

Both background rationale and objectives are explained extensively with the provision of adequate bibliography. The missing part is the statement if the study is the first report of a genetic association, a replication effort, or both.

STREGA statement – Methods

Study design (4)

Key elements of the study design were presented early in the study but the detailed methodology was in the end of the article.

Settings (5)

The settings are insufficient.

Participants (6)

The eligibility criteria, the sources, the methods of case ascertainment and the control selection are explained.

Variables (7)

The variables are described inadequate.

Data sources measurements (8)

This area is analytic.

Bias (9)

The small size and the lack of population stratification have been considered as bias.

Study size (10)

Full explanation of the study size.

Quantitative variables (11)

No quantitative variables.

Statistical methods (12)

The presentation of the statistical methods is according to the STREGA statement to the majority of the proposed items. It is specified where the HWE was considered and how.

STREGA statement – Results

Participants (13)

The researchers reported the numbers and percentages of individuals in whom genotyping was attempted, in whom genotyping was successful and numbers about re – genotyping.

Descriptive data (14)

Many clinical and social characteristics have taken into consideration and have been given some information by genotype. On the other hand, there is no indication of missing data for the variables of interest.

Outcome data (15) & Main Results (16)

According to the STREGA recommendations these areas are analytic and the numbers are also depicted in tables. As it concerns the main results, many confounders have been considered.

Other analyses (17)

Because of the nature of the study, there is a number of other analysis and reports about alleles, haplotypes and in addition about subgroups. No statement if detailed results are available elsewhere and f so, how they can be accessed.

STREGA statement – Discussion

Key results (18)

Good reference of key results and study objectives

Limitations (19)

The small sample size in type 1 diabetic patients and the lack of population stratification have been taken into account as sources of potential bias

Interpretation (20)

A cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence have been mentioned.

Generalizability (21)

There is a discussion about the luck of the external validity of the study due to the many limitations.

STREGA statement – Other Information

Funding (22)

The authors declared no existence of competing interest and mention the source of funding.

Overall assessment

This study is quite good study apart from the paradox presentation as the methods and the materials are mentioned in the end of the article.

Forth Genetic Association Study

Association of TGFβ1, TNF α, CCR2 and CCR5 gene polymorphisms in type-2 diabetes and renal insufficiency among Asian Indians

Pushplata Prasad,, Arun K Tiwari,, KM Prasanna Kumar,, AC Ammini,, Arvind Gupta,, Rajeev Gupta and BK Thelma

BMC Medical Genetics20078:20

https://www.researchgate.net/publication/5485188_Association_of_dopaminergic_pathway_gene_polymorphisms_with_chronic_renal_insufficiency_among_Asian_Indians_with_type-2_diabetes

Considering that cytokines play an important role in the development of diabetic chronic renal insufficiency the forth study tested the association of nine single nucleotide polymorphisms (SNPs) from TGFβ1, TNFα, CCR2 and CCR5 genes among individuals with type-2 diabetes with and without renal insufficiency using a case-control design. [11]

The researchers observed no allelic or genotypic association of G>A (-308) promoter SNP of the gene with CRI. [11]

STREGA statement - Title and abstract (1)

The title indicates the study's design using common terms and the abstract is a balanced summary of the article.

STREGA statement – Introduction

Background rationale (2) & Objectives (3)

The authors provide an extensive explanation of the scientific background and rationale. Specific objectives, including pre-specified hypotheses are stated. It is also mentioned that the study is part of an extensive analysis of genetic susceptibility to diabetic renal disease.

STREGA statement – Methods

Study design (4) & Settings (5)

The elements of the study design are presented early and the settings are described in detail including locations.

Participants (6)

The inclusion and exclusion criteria, the sources and methods of case ascertainment and control selection are mentioned. Demographic details and clinical profile of the study population are stated.

Variables (7)

The genetic variants were clearly defined.

Data sources measurements (8)

The researchers gave the sources of data and details of methods of assessment. Furthermore, stated the laboratory/centre where genotyping was done and the methods were described including those concerning the genotyping. On the other hand, didn't specify whether genotypes were assigned using all of the data from the study simultaneously or in smaller batches.

Bias (9)

No comment about bias.

Study size (10)

It is explained how the study size was arrived at.

Quantitative variables (11)

No quantitative variables.

Statistical methods (12)

The software version, the options, the HWE and methods to address multiple comparisons are referred. There is a lot of missing information. Missing information of significance, the number of individuals in whom genotyping was attempted and the number of individuals in whom genotyping was successful.

STREGA statement – Results

Participants (13)

The number of cases and controls is reported.

Descriptive data (14)

The descriptive data are commenced inadequate.

Outcome data (15) & Main Results (16)

The outcomes for each genotype and the results from the multiple comparisons are expresses but there is a lot of missing information, such as confounder -adjusted estimates and their precision.

Other analyses (17)

The analysis of the subgroups is commended. The detailed demographic and clinical characteristics of the study population are provided in an additional file.

STREGA statement – Discussion

Key results (18)

The key results are mentioned in brief.

Limitations (19)

The limitations are not discussed including sources of potential bias or imprecision.

Interpretation (20)

The authors gave an overall interpretation of the results considering objectives, results from similar studies and other relevant evidence.

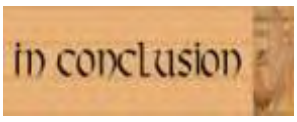
Generalizability (21)

No reference about external validity.

STREGA statement – Other Information

Funding (22)

The funding source is not mentioned. Declaration of no competing interests.



Overall assessment

The best written areas according to the STREGA statement are the title, the abstract and the introduction. Particularly, the background rationale and the objectives are commended extensively. The authors could have given more attention in areas such as the statistical methods, the bias, the presentation of the results, the information about the participants and the descriptive data.

Fifth Genetic Association Study

Predictive value of cytokine gene polymorphisms for the development of end-stage renal disease

Babel N1, Gabdrakhmanova L, Hammer MH, Schoenemann C, Skrypnikov V, Poliak N, Volk HD, Reinke P.

J Nephrol. 2006 Nov-Dec;19(6):802-7.

http://www.ncbi.nlm.nih.gov/pubmed/17173255

Based on the scientific background that cytokines play a crucial role in different immunopathological conditions and in addition that the cytokine secretion is reported to be determined by polymorphisms in the cytokine genes, this study explores the hypothesis that polymorphisms of TNF-α IL-10 and TGF-β1 genes may be possible genetic susceptibility factors for the progression of renal failure. [12] Is a case – control study (Caucasian population) in healthy subjects and in patients on hemodialysis due to type 2 diabetic nephropathy or chronic glomerulonephritis.

The study didn't detect significant differences in the TNF-α genotype distribution between healthy controls and patients with diabetic nephropathy- or glomerulonephritis-associated End State Renal Disease. [12]

STREGA statement - Title and abstract (1)

The names of the examined cytokines are omitted from the title. Apart from that the abstract provides an informative and balanced summary of the research.

STREGA statement – Introduction

Background rationale (2) & Objectives (3)

The scientific background is explained and the rationale for the investigation is reported. The researchers add information from the bibliography and declare preliminary study with unpublished data.

STREGA statement – Methods

Study design (4)

The elements of the study are presented early in the text.

Settings (5)

The recruitment centers are mentioned but no information about the relevant dates and the data collection.

Participants (6)

Eligibility criteria, sources and methods of case ascertainment and control selection are explained but the rationale for the choice of cases and controls is omitted. .

Variables (7)

Clear definition of all outcomes and predictors. The variants were defined and mentioned that the ethnicity may be confounding.

Data sources measurements (8)

Sources of data and details of methods of assessment (measurement) for each variable of interest were referred. The researchers provide adequate information about the laboratory methods, genotyping methods and platforms but not mentioned the laboratory/centre where genotyping was done or whether genotypes were assigned using all of the data from the study simultaneously or in smaller batches

Bias (9)

The authors address the small size of the study as potential source of bias and the ethnicity.

Study size (10)

The way the study size was arrived at is fully explained.

Quantitative variables (11)

There are no quantitative variables.

Statistical methods (12)

This area is described inadequate. Important information is not presented. The HWE was taken into consideration.

STREGA statement – Results

Participants (13)

The number of participants is mentioned and the eligibility. No report about any unsuccessful genotyping.

Descriptive data (14)

The demographic and clinical characteristics were available in a table. The ugly truth is that the offered information is little.

Outcome data (15) & Main Results (16)

The outcomes for each genotype were reported. These areas depict o good presentation according to the STREGA recommendations.

Other analyses (17)

Subgroup analysis is reported. No sensitivity analyses. No statement if the results are available elsewhere.

STREGA statement – Discussion

Key results (18)

The key results were summarized.

Limitations (19)

The small size and the ethnicity are considered as potential bias.

Interpretation (20)

The interpretation part is a good effort to discuss limitations of the study, taking into account sources of potential bias or imprecision and magnitude of potential bias**.**

Generalizability (21)

Have been provided little and not sufficient information about the external validity.

STREGA statement – Other Information

Funding (22)

No information about the funding or conflict of interest.

Overall assessment

The best described areas are the abstract, introduction and the discussion. In the title is not mentioned the names of the cytokines. Last but not least the methods have omitted seriously information about the statistical methods.

Quiz

Which research is better defined according to the STREGA statement?

Which items of the STREGA statement could be considered as the most important?

Which recommendation can be characterized as the absolute necessary information to be mentioned?

Assumption

Have been examined and commended five different in quality genetic association studies and what can be said is that every research team considers which components are important and valuable to be mentioned.

My solid conclusion is that apart from mentioning what are the pros and cons in every study, can't be declared which is the best according to the STREGA statement as we can't quantify the 22 items of the recommendation.

1. **Introduction to Genetic Association Studies (**Cathryn M. Lewis and Jo Knight)**Adapted from *Genetics of Complex Human Diseases* (ed. Al-Chalabi and Almasy). CSHL Press, Cold Spring Harbor, NY, USA, 2009**
2. **Wikipedia,** The Free Encyclopedia
3. **UCLA**, GENOSEQ, Genotyping & Sequensing
4. **Association of Tumor Necrosis Factor Alpha Promoter Polymorphism (TNF-a 238 G/A and TNF-a 308 G/A) with Diabetic Mellitus, Diabetic Retinopathy and Diabetic Nephropathy: A Meta-analysis** Nana Meng, Yue Zhang, Hao Li, Jinlan Ma, and Yi Qu *Department of Ophthalmology, Qilu Hospital of Shandong University, Jinan, China*
5. **SNPedia**
6. **Medscape**
7. **University of Ottawa,** http://www.medicine.uottawa.ca/public-health-genomics/web/eng/strega.htm
8. ***TNF-α*-308G/A polymorphism associated with *TNF-α* protein expression in patients with diabetic nephropathy (Yan Peng, Liu-Juan Li),** Int J Clin Exp Pathol. 2015;
9. **Association between tumour necrosis factor-a G-308A polymorphism and risk of nephropathy in obese Chinese type 2 diabetic patients** *(Ying Wang, Maggie C. Y. Ng, Wing-Yee So, Ronald Ma, Gary T. C. Ko, Peter C. Y. Tong and Juliana C. N. Chan)*
10. **Association between LTA, TNF and AGER Polymorphisms and Late Diabetic Complications** Eero Lindholm,Ekaterina Bakhtadze, Corrado Cilio, Elisabet Agardh,Leif Groop, Carl-David Agardh, (Published: June 25, 2008)
11. **Association of TGFβ1, TNFα, CCR2 and CCR5 gene polymorphisms in type-2 diabetes and renal insufficiency among Asian Indians** Pushplata Prasad**,** Arun K Tiwari**,** KM Prasanna Kumar**,** AC Ammini**,** Arvind Gupta**,** Rajeev Gupta and BK Thelma
12. **Predictive value of cytokine gene polymorphisms for the development of end-stage renal disease**Babel N1, Gabdrakhmanova L, Hammer MH, Schoenemann C, Skrypnikov V, Poliak N, Volk HD, Reinke P. (J Nephrol. 2006 Nov-Dec;19(6):802-7).