Involvement of polymorphisms of the nerve growth factor and its receptor encoding genes in the etiopathogenesis of ischemic stroke

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Abstract

Background: Despite the important role of the nerve growth factor in the survival and maintenance of neurons in ischemic stroke, data regarding the relationships between variations in the encoding gene and stroke are lacking. In the present study, we evaluated the association of the functional polymorphisms in NGF (rs6330) and NGFR (rs2072446 and rs734194) genes with ischemic stroke in an Armenian population.

Methods: In total, 370 unrelated individuals of Armenian nationality were enrolled in this study. Genomic DNA samples of patients and healthy controls were genotyped using polymerase chain reaction with sequence-specific primers.

Results: The results obtained indicate that the minor allele of rs6330 ($P_{corr} = 2.4E-10$) and rs2072446 ($P_{corr} = 0.02$) are significantly overrepresented in stroke group, while the minor allele of rs734194 ($P_{corr} = 8.5E-10$) was underrepresented in diseased subjects. Single nucleotide polymorphisms in NGF gene (rs6330) and NGFR gene (rs2072446 and rs734194) are associated with the disease. Furthermore, it was shown that the carriage of the NGF rs6330*T minor allele is associated with increased infarct volume and higher risk of recurrent stroke.

Conclusions: In conclusion, our findings suggest that the NGF rs6330*T and NGFR rs2072446*T minor alleles might be nominated as a risk factor for developing ischemic stroke and NGFR rs734194*G minor allele as a protective against this disease at least in Armenian population.

Keywords: Ischemic stroke, Nerve growth factor, Nerve growth factor receptor, NGF, NGFR, Single nucleotide polymorphism
which neurons can be protected from cell death. Likewise, it was found that intraventricular NGF ameliorated the development of delayed neuronal death [8]. NGF is a known activator for ERK5 (mitogen-activated protein kinase 5) which is key modulator of neuroprotection [9]. Furthermore, NGF up-regulates the expression of the KLF4 transcription factor that plays important role in initiating an anti-apoptotic and anti-inflammatory response [10]. Thus, recent studies suggest ERK5/KLF4 cascade as a common downstream signaling pathway for NGF-induced neuroprotection against oxidative stress [11]. Hence NGF is involved in several crucial processes related to IS etiopathogenesis and it is a good candidate to influence the susceptibility to IS, which is considered to be a complex polygenic disease [12]. There are number of single nucleotide polymorphisms (SNPs) in genes encoding NGF and NGFR that may alter their function and expression levels [13]. Thus, we have used a candidate polymorphism study approach to identify the possible association of the several functional SNPs (Fig. 1) in the genes encoding NGF (rs6330 and rs4839435) and NGFR (rs11466155, rs2072446 and rs734194) with IS in Armenian population. These polymorphisms were selected based on previous studies implicating them in several neurodegenerative, psychological, neurological and neurovascular diseases [13–15].

**Methods**

**Subjects**

In total, 170 patients with IS (males/females: 88/82; mean age ± SD: 55 ± 9.7 years) and 200 healthy subjects (males/females: 109/91; mean age ± SD: 49 ± 9.2 years) were enrolled in this study. All subjects were unrelated Caucasians of Armenian ancestry.

Patients were hospitalized in the neurological departments of medical centers of the Ministry of Health of Republic of Armenia (MH RA). Diagnosis of IS was based on clinical history and neurological examination of patients and was confirmed by brain computer tomography (CT) imaging and basal laboratory tests. According to the CT used for the quantitative assessment of the volume of the ischemic area, in 42.5% of IS patients the volume of infarct area was less than 60cm³ and in 57.5% of patients the volume of infarct area was more than 60cm³. Stroke subtype was classified according to TOAST definitions [16]. Stroke severity was scored using the National Institutes of Health Stroke Scale [17]. Among IS patients involved in this study 38 had cardioembolic stroke and 132 had atherothrombic stroke. Sixty six patients presented anatomically relevant CT hypodense areas in cortical-subcortical parts of cerebral right hemisphere, 97 in left hemisphere and 7 in brain stem. Among IS patients 112 had hyperlipidemia, 87 had arterial hypertension, 40 had atrial fibrillation and 66 had coronary artery disease. With regard to the lifestyle habits 110 patients were current smokers, and 59 were alcohol consumers. Seventy eight patients had positive family history of IS, including 48 maternal, 27 paternal and 3 biparental inheritance.

Healthy subjects (controls) without family history of IS and myocardial infarction were recruited among the blood donors of the Erebouni Medical Center of the MH RA and Blood Bank of Haematology Center after prof. R.Yeolyan. Healthy controls had no serious medical disorders, including coronary artery disease, atrial fibrillation, arterial

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**Fig. 1** The localization of the selected 5 SNPs in NGF gene and NGFR gene
hypertension, and hyperlipidemia, or treatment during the past 12 months. No special studies have been performed to assess the progress of atherosclerotic process in healthy subjects group. Exclusion criteria for all subjects included past or present history of neuropsychiatric metabolic disorders, myocardial infarction, as well as oncological and immune system diseases.

**Collection of blood samples and extraction of genomic DNA**

About 5 ml of venous blood was collected from each study subject by venipuncture in EDTA-containing tubes. Blood samples of IS patients were collected on days 1–4 of stroke onset. Genomic DNA was isolated from fresh blood samples according to the standard phenol-chloroform method and stored at −30 °C until further use [18].

**Selection of the SNPs in NGF and NGFR genes**

According to the National Center of Biotechnology Information (NCBI) databases [http://www.ncbi.nlm.nih.gov/], from 6132 SNPs found in NGF gene and from 2714 SNPs in NGFR gene the 120 and 178 SNPs are non-synonymous, respectively. From these SNPs we selected those which: 1) have MAFs > 0.05; 2) have reported association with several neurological and neurovascular diseases; 3) presumably have influence on structure and/or function of NGF and NGFR proteins and 4) were linked to altered protein levels in serum [13, 19–26].

**Genotyping of selected SNPs in NGF and NGFR genes**

All DNA samples were genotyped for selected SNPs using polymerase chain reaction with sequence specific primers (PCR-SSP) using earlier described conditions [13, 18]. Detailed characterizations of the NGF rs6330 and rs4839435, as well as NGFR rs11466155, rs2072446 and rs734194 SNPs are shown in Fig. 1 and Table 2. All primers for the PCR-SSP were designed using the reference genomic sequences in the GenBank (http://www.ncbi.nlm.nih.gov). The primer sequences designed for mentioned SNPs and amplicons length are shown in Table 1.

The presence/absence of allele-specific amplicons in PCR products was visualized by electrophoresis in 2% agarose gel stained with ethidium bromide.

**Statistical analysis**

Distribution of genotypes for the mentioned SNPs was checked for correspondence to Hardy-Weinberg equilibrium (HWE) by Pearson χ² test. To reveal a potential association of these SNPs with IS, genotype, allele, and phenotype frequencies (carriage rates) in patients and controls were compared. The significance of differences between study groups in multiplicative, dominant and recessive models was determined using a Fisher’s exact test. With regard to additive model the differences in genotype distribution of the polymorphisms between case and control subjects were tested by logistic regression. The odds ratio (OR), 95% confidence interval (CI), and exact P value (Pnom) were calculated. Nominal P values (Pnom) were adjusted for multiple testing by Bonferroni correction (factor 5). Corrected P values (Pcorr) < 0.05 were considered significant [27]. The Mann-Whitney U (quantitative variables) and Fisher’s exact test (nominal and ordinal variables) were used to evaluate the possible differences of the clinical characteristics between minor allele carrier and non-carriers IS patients.

**Table 1** The primer sequences and amplicons size of the studied SNPs

| Gene | Accession | SNP ID | Primer type | Nucleotide sequence of primer (5′ → 3′) | Amplicon size, bp |
|------|-----------|-------|-------------|----------------------------------------|------------------|
| NGF  | NG_007944 | rs6330 | reverse for standard C allele | CTGAAGTGTAGTCCAGTGGG | 187 |
|      |           |       | reverse for minor T allele | CTGAAGTGTAGTCCAGTGA | |
|      |           |       | forward constant | CTGACATTAGTACTCCATGAA | |
|      |           | rs4839435 | forward for standard G allele | TGGGTGCCAAAAAGCTTGGC | 188 |
|      |           |       | forward for minor A allele | TGGGTGCCAAAAAGCTTGGT | |
|      |           |       | reverse constant | GCAGCTCCTGCAATTATCCA | |
|      |           | rs11466155 | reverse for standard C allele | AGGCTATGTAGGCCAACAAGG | 210 |
|      |           |       | reverse for minor T allele | AGGCTATGTAGGCCAACAAGA | |
|      |           |       | forward constant | CAGAGGGCTGGACAGCACA | |
|      |           | rs2072446 | forward for standard C allele | GTCCACACCCCCAGGGGCTC | 190 |
|      |           |       | forward for minor T allele | GTCCACACCCCCAGGGGCTT | |
|      |           |       | reverse constant | AGCAGCCAGGATGGAGCAAT | |
|      |           | rs734194 | forward for standard T allele | GCTGGAGCTGGCGTCGTCG | 186 |
|      |           |       | forward for minor G allele | GCTGGAGCTGGCGTCGTCG | |
|      |           |       | reverse constant | CTAGAGCTGGAGAAATCCC | |

Stepanyan et al. BMC Medical Genetics (2018) 19:33
Results

The genotyping analysis showed that genotype distribution of the NGF gene rs6330 and rs4839435 and the NGFR gene rs11466155, rs2072446 and rs734194 SNPs in study groups were concordant with HWE ($P > 0.05$) (Table 2). Furthermore, the observed minor allele frequencies (MAFs) of the studied SNPs (Table 2) in Armenian population were compared from those reported in major ($n = 2,504$) population of the 1000 Genomes Project. The results showed significant difference between MAFs of the NGF gene rs4839435 as well as NGFR gene rs2072446 and rs734194 SNPs in Armenian population compared to 1000 Genomes Project data, whereas there was no difference for other two studied SNPs (NGF gene rs6330 and NGFR gene rs11466155) (Table 2). SNP analysis revealed that rs6330 variation of the NGF gene is associated with IS in the multiplicative model ($P_{corr} = 3.6E-11$), the dominant model ($P_{corr} = 2.4E-10$), the recessive model ($P_{corr} = 0.001$), and the additive model ($P_{corr} = 0.0005$) (Table 3). Moreover, the frequency and carriage (dominant model) of NGF rs6330*T minor allele were 1.98 (0.406 vs. 0.205; $P_{corr} = 3.6E-11$) and 1.85 (0.647 vs. 0.35; $P_{corr} = 2.4E-10$) times respectively increased in IS patients in comparison with healthy controls (Table 3). In IS patients group, Mann-Whitney U test revealed statistically significant difference of the frequencies of the rs2072446 polymorphism CC, CT and TT genotypes (additive model) of T minor allele carriers compared to non-carriers (CT + TT vs. CC; 188 ± 234 cm$^3$ vs. 72 ± 128 cm$^3$; $P = 0.023$). Furthermore, 27% of all patients enrolled in study experienced second or third IS; and also there were significantly higher number of recurrent IS events in T minor allele carriers compared to non-carriers (CT + TT vs. CC, 39.3% vs. 10.5%; OR = 5.71; 95% CI: 2.68–12.2; $P = 1.0E-6$).

According to the data obtained for the genotyping of the NGF gene rs2072446 SNP, the frequency and carriage (multiplicative and dominant models) of T minor allele were 1.29 (0.378 vs. 0.293; $P_{corr} = 0.04$) and 1.26 (0.6 vs. 0.475; $P_{corr} = 0.02$) times respectively increased in IS patients compared to controls. Further, there were statistically significant difference of the frequencies of the rs2072446 polymorphism CC, CT and TT genotypes (additive model) between patients and controls groups ($P_{corr} = 0.0005$).

A strong negative association was detected between IS and NGFR gene rs734194 genetic variation in the all studied models (multiplicative $P_{corr} = 1.1E-11$; dominant $P_{corr} = 8.5E-10$; recessive $P_{corr} = 4.8E-4$; additive $P_{corr} = 0.0005$) (Table 3). Particularly, NGFR rs734194*G minor allele frequency and carriage were 2.73 (0.1 vs. 0.273; $P_{corr} = 1.1E-11$) and 2.42 (0.188 vs. 0.455; $P_{corr} = 8.5E-10$) times respectively decreased in IS patients in comparison with controls.

Finally, no association with IS were observed for rs4839435 SNP of the NGF gene and the rs11466155 SNP of the NGFR genes (Table 3).

According to LD analysis results (Table 4) NGF gene rs6330 and rs4839435, as well as NGFR gene rs11466155, rs2072446 and rs734194 SNPs are not in strong LD among all studied groups. Furthermore, LD between selected SNPs in NGFR gene in Armenian population differs from LD in population samples from the HapMap Project [28]. As for obtained linkage between NGF gene rs6330 and rs4839435 SNPs in Armenian population it is almost the same as in HapMap (Table 4).

In order to evaluate an interaction between the significant alleles at the two loci (NGF rs6330/NGFR rs2072446) we compared the relative risk of IS in patients carrying one of the risk alleles with those who have two risk alleles at both loci. Increased relative risk for IS was observed in double risk allele carrier patients compared to carriers of either NGF rs6330*T or NGFR rs2072446*T alleles (Table 5).

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Table 2: HWE and MAFs of the selected SNPs in the studied groups and in major population

| Gene SNP ID | Minor allele | MAF (frequency/number) | HWE (%) |
|-------------|--------------|------------------------|---------|
|             | IS patients  | Controls               |         |
| NGF rs6330  | T            | 0.406/138               | 0.06    |
| NGF rs4839435| A            | 0.344/117               | 0.0001  |
| NGFR rs11466155| T          | 0.282/96                | 0.025   |
| NGFR rs2072446| T            | 0.378/125               | 0.0001  |
| NGFR rs734194| G            | 0.1/34                  | 0.0001  |

MAF: minor allele frequency, HWE: Hardy–Weinberg equilibrium
Discussion
In the current study, we observed positive association of the NGF gene rs6330 and NGFR gene rs2072446 SNPs with IS. In contrast, rs734194 genetic variant of the NGFR gene showed negative association with IS susceptibility. We have also observed that the carriage of the T minor allele of NGF gene rs6330 polymorphism is associated with increased infarct volume and higher risk of recurrent IS.

Recent GWAS reported number of loci which are associated with IS risk [29–31]. Despite the populations included in these studies were almost the same, still differences in reported results were present. Thus the study by Ikram et al. [31] identified two intergenic SNPs within 11 kb of the gene NINJ2 associated with IS. Two recent GWAS studies [29, 30] did not replicate this finding, however, partially confirmed several loci reported from other studies [32, 33] as well as identified new ones. Despite the fact that latter studies were performed by same consortium, only four loci were overlapping. The general disparity of the results might be partly explained by different quality control filters before imputation as well as methods for assessing population structure in GWAS or due to genetic differences between populations included in these studies. For example, an association of the PRKCH locus with stroke identified in a GWAS in Japanese participants was not found in European populations [30].

Genomic loci that are associated with increased stroke susceptibility and were reported by recent GWAS in European, non-Hispanic black and Hispanic ancestry samples do not include NGF and NGFR genes [29, 30]. Inconsistency of our data with the published results might be explained due to a long period of isolation of Armenian population since the Bronze Age and subsequently a unique profile of rare disease alleles [34]. Furthermore, recent studies reported very restricted genetic affinities of Armenians with European populations [35, 36]. From the other side the genetic structure of Armenian population is still largely unknown, which forces us to employ candidate gene approach to study NGF and NGFR, since their involvement in neuroprotection after IS and several pathogenic processes contributing to the development of IS is well documented [5–9].

NGF rs6330 and NGFR rs2072446 functional SNPs lead to non-synonymous amino acid substitutions and are possibly involved in the gene expression and protein secretion [13, 19]. The first one is described as a C104 → T exchange in exon 1 of the NGF gene and

| Gene SNP (M/m) | Groups | Genotypes (number/frequency) | Model | OR (95%CI) | P<sub>nom</sub> |
|---------------|--------|-----------------------------|-------|------------|---------------|
| NGF rs6330 (C/T) | IS patients | MM 60 (0.35) Mm 82 (0.48) mm 28 (0.175) | multiplicative | 2.65 (1.996–3.5) | 7.3E-12 |
|                | Controls | 130 (0.65) 58 (0.29) 12 (0.06) | recessive | 3.07 (1.65–5.72) | 0.0002 |
| NGF rs4839435 (G/A) | IS patients | MM 73 (0.43) Mm 77 (0.45) mm 20 (0.12) | multiplicative | 1.05 (0.81–1.37) | 0.45 |
|                | Controls | 85 (0.425) 97 (0.485) 18 (0.09) | recessive | 1.35 (0.76–2.39) | 0.3 |
| NGF rs1146615S (C/T) | IS patients | MM 86 (0.505) Mm 72 (0.424) mm 12 (0.071) | multiplicative | 1.11 (0.84–1.46) | 0.48 |
|                | Controls | 110 (0.55) 75 (0.375) 15 (0.075) | recessive | 1.06 (0.54–2.07) | 0.86 |
| NGF rs2072446 (C/T) | IS patients | MM 68 (0.4) Mm 79 (0.465) mm 23 (0.135) | multiplicative | 1.43 (1.09–1.86) | 0.008 |
|                | Controls | 105 (0.525) 73 (0.365) 22 (0.11) | recessive | 1.3 (0.76–2.23) | 0.3 |
| NGF rs734194 (T/G) | IS patients | MM 138 (0.81) Mm 30 (0.18) mm 2 (0.01) | multiplicative | 3.36 (2.36–4.78) | 2.3E-12 |
|                | Controls | 109 (0.545) 73 (0.365) 18 (0.09) | recessive | 7.95 (2.35–26.89) | 9.7E-5 |

"M/m" indicates major/minor alleles; multiplicative model indicates "M vs. m"; dominant model indicates "Mm + mm vs. MM"; recessive model indicates "MM + Mm vs. mm"; additive model indicates "MM vs. Mm vs. mm".
leads to amino acid substitution of alanine to valine at position 35 (Ala35Val). Some authors suppose that the increase in amino acid size at this position could modify the tertiary structure of NGF, leading to altered interaction and signaling via the NGFR [20]. Therefore, T minor allele could be a risk factor for IS through its possible involvement in increased cell death and consequently, decreased neuronal survival after ischemia. This is consistent with our findings on association of rs6330*T minor allele with increased lesion volume after IS. With regard to NGFR rs2072446 polymorphism, the C739→T nucleotide transition in exon 4 leads to serine to leucine substitution at the amino acid position 205 (Ser205Leu) and the change of polar (hydrophilic) amino acid to nonpolar (hydrophobic) subsequently, causes alteration of the NGFR protein structure and function [21]. In addition, rs734194 is located on 3’ UTR and thereby it could be engaged in regulating the mRNA stability and translational efficiency. Hence, it is possible that this SNP alters the expression of NGFR or the binding of NGF, which can decrease neuronal apoptosis rate [22]. All together, these mechanisms may partially explain the involvement of the NGF rs6330 and the NGFR rs2072446 in the higher risk of IS, and the protective role of NGFR rs734194 against this disease observed in our study.

Recent studies reported that rs6330, rs2072446 and rs734194 genetic variants are associated with number of neurovascular and psychiatric diseases, which might predispose to stroke and increase its likelihood [23, 24]. Particularly, it was shown that NGF rs6330*T minor allele is positively associated with the risk of migraine, schizophrenia and Alzheimer’s disease [13, 14, 25]. As for NGFR rs2072446, it was observed as being associated with depression and schizophrenia [13, 15]. Furthermore, inherited rs734194 polymorphism of NGFR gene was associated with the decreased risk of obsessive-compulsive

### Table 4
LD values (the absolute D’ and r^2) IS patients (b), and r^2 values calculated from the data obtained for Armenian population involved in this study and for the results available on HapMap (c)

|                   | NGF rs6330 | NGF rs4839435 | NGFR rs11466155 | NGFR rs2072446 | NGFR rs734194 |
|-------------------|------------|---------------|-----------------|----------------|---------------|
| r^2/|D’|                  |               |                 |                 |                 |
| NGF rs6330        | –          | 0.01867       | –               | –              | –             |
| NGF rs4839435     | 0.18280    | –             | –               | –              | –             |
| NGFR rs11466155   | –          | –             | 0.01859         | 0.10206        | –             |
| NGFR rs2072446    | –          | –             | 0.14499         | –              | 0.01801       |
| NGFR rs734194     | –          | –             | 0.34901         | 0.13786        | –             |

For each pair of SNPs r^2 and |D’| values are shown above and below the diagonal, respectively

### Table 5
Relative risk of IS among carriers of NGF rs6330*T, NGFR rs2072446*T and TT (rs6330/rs2072446) alleles

|                   | NGF rs6330*T | NGF rs2072446*T | TT(rs6330/rs2072446) |
|-------------------|--------------|-----------------|--------------------|
| Relative risk     | 1.85         | 1.26            | 2.16               |
| 95% CI            | 1.48–2.3     | 1.05–1.53       | 1.46–3.17          |
| Significance level| P < 0.0001   | P = 0.001       | P = 0.0001         |
disease, schizophrenia and Alzheimer’s disease [13, 22, 26].

According to the data of Cerebrovascular Disease Knowledge Portal most recent GWAS have discovered associations between the studied sequence variants in NGF and NGFR gene and cerebrovascular disease or related traits. Thus, NGF gene rs2072446 polymorphism was associated with IS of TOAST undetermined etiology \((p = 0.037)\), large artery atherosclerosis \((p = 0.03)\), height \((p = 0.01)\) and waist-hip ratio \((p = 0.013)\) [29, 37–40]. Regarding NGFR gene rs734194 SNP, its minor allele showed association with TOAST small artery occlusion \((p = 0.02)\), Type 2 diabetes \((p = 0.019)\), height \((p = 0.019)\), serum cystatin C \((p = 0.016)\) [39, 41–44]. However, this is the first study to evaluate the association of the genetic variations of the NGF and the NGFR genes with IS, infarction size and recurrence of the disease in Armenian population. Thus, our findings indicate that mentioned above SNPs could play a potential role in the etiopathogenesis of IS. We suggest that further studies are required to clarify the functional consequences of the mentioned SNPs as well as NGF and NGFR genes in the etiology and the molecular pathomechanisms of IS. Our results are preliminary and they need to be confirmed in studies with larger sample size and in different ethnic groups.

Conclusions

Our findings suggest that the NGF rs6330*T and NGFR rs2072446*T minor alleles might be nominated as a risk factor for developing IS and NGF rs734194*G minor allele as a protective against this disease in Armenian population.

Abbreviations

CI: Confidence interval; CT: Computer tomography; HWE: Hardy-Weinberg equilibrium; IS: Ischemic stroke; MAF: Minor allele frequency; NGF: Nerve growth factor; NGFR: Nerve growth factor receptor; OR: Odds ratio; PCR-SSP: Polymerase chain reaction with sequence specific primers; SNP: Single nucleotide polymorphism

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Availability of data and materials

The sequences of all primers for the PCR-SSP were designed using the genomic sequences in the GenBank [http://www.ncbi.nlm.nih.gov] with the accession codes NG_007944 and AC006487.

The MAFs data of the investigated SNPs in major population that support the findings of this study were reported by 1000 Genomes Project and are available in The National Center for Biotechnology Information (NCBI) [https://www.ncbi.nlm.nih.gov/] with the identifiers rs4839435, rs2072446, rs734194, rs6330, rs11466155.

The authors declare that all other data supporting the findings of this study are available within the article.

Authors’ contributions

All authors contributed extensively to the work presented in this paper. AS performed the experiments, analyzed the data and wrote the manuscript, RZ designed the primers and jointly performed the experiments, ArS participated in the collection of blood samples helped with the analysis of the clinical histories of the patients, GT jointly wrote the manuscript and helped to evaluate and edit the manuscript. AA designed and supervised the experiments, as well as helped with the data analysis and interpretation. All authors revised and approved the final version of the manuscript.

Ethics approval and consent to participate

All subjects or their legal representatives gave their informed consent to participate in the study, which was approved by the Ethical Committee of the Institute of Molecular Biology of the National Academy of Sciences RA (IRB00004079).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Johnson W, Onuma O, Owolabi M, Sachdev S. Stroke: a global response is needed. Bull World Health Organ. 2016;94(9):634–64A.
2. Khachatryan S, Andreasyan D, Bazarchyan A, Simonyan S, Muradyan G, Torosyan A, Chamanyan A, Matevosyan M, Kostandy M, Simonyan A, Tongoyan A. Health and Health care yearbook, RA 2016. National institute of health, named after S. Avakianyan, Mih, RA. 2016. p. 93–95.
3. Bracci-Laudiero L, De Stefano ME. NGF in early embryogenesis, differentiation, and pathology in the nervous and immune systems. Curr Top Behav Neurosci. 2016;29:125–52.
4. Aloe L, Rocco ML, Babamino B0, Micera A. Nerve growth factor: role in growth, differentiation and controlling cancer cell development. J Exp Clin Cancer Res. 2016;35(1):116.
5. Spero TB, Neff PS, Hankins GR, Tuttle JB. Mechanisms of increased NGF production in vascular smooth muscle of the spontaneously hypertensive rat. Exp Cell Res. 1998;241(1):186–93.
6. Chaldakov GN, Stankulov IS, Fiore M, Gheniev PI, Aloe L. Nerve growth factor levels and mast cell distribution in human coronary atherosclerosis. Atherosclerosis. 2001;159(1):57–66.
7. Villolada P, Hauser SL, Bartke I, Unger J, Heald N, Rosenberg D, Cheung SW, Mobley WC, Fisher S, Genain CP. Human nerve growth factor protects common marmosets against autoimmune encephalomyelitis by switching the balance of T helper cell type 1 and 2 cytokines within the central nervous system. J Exp Med. 2000;191(10):1799–806.
8. Shigeno T, Mima T, Takakura K, Graham DI, Nato G, Hashimoto Y, Furukawa S. Amelioration of delayed neuronal death in the hippocampus by nerve growth factor. J Neurosci. 1991;11(9):2914–9.
9. Wang YM, Yang F, Zhang YX. Preconditioning-induced activation of ERKs is dependent on moderate Ca2+ influx via NMDA receptors and contributes to ischemic tolerance in the hippocampal CA1 region of rats. Life Sci. 2006;79(19):1839–46.
10. Ohnesorge N, Viemann D, Schmidt N, Czymai T, Spiering D, Schmolke M, Ludwig S, Roth J, Goebeler M, Schmidt M. Erk activation elicits a vasoprotective endothelial phenotype via induction of Kruppel-like factor 4 (KLF4). J Biol Chem. 2013;288(34):26190–20.  
11. Su C, Sun F, Cunningham RL, Rybalchenko N, Singh M. ERK5/KLF4 signaling as a common mediator of the neuroprotective effects of both nerve growth factor and hydrogen peroxide preconditioning. Age (Dordr). 2014;36(4):6985.  
12. TitoV BV, Matveeva NA, Martynov MY, Favorova OO. Ischemic stroke as a complex polygenic disease. Mol Biol. 2015;49(2):224–48.  
13. Zakharyan R, Atshemyan S, Gevorgyan A, Boyajan A. Nerve growth factor and its receptor in schizophrenia. BBA Clin. 2014;21:14–9.  
14. Coskun S, Varol S, Ozdemir HH, Agacayak E, Aydin B, Kapan O, Camkurk M, Tunc S, Cevik M. Association of brain-derived neurotrophic factor and nerve growth factor gene polymorphisms with susceptibility to migraine. Neuropsychiatr Dis Treat. 2016;12:1779–85.  
15. Colle R, Defesseille E, Martin S, David DJ, Hardy P, Tararu A, Falissard B, Verstuyft C, Comble E. BDNF/TRKBP7/5NTR polymorphisms and their consequences on antidepressant efficacy in depressed patients. Pharmacogenomics. 2015;16(9):997–1013.  
16. Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Leary BB, Gordon DL, Marsh MC, Galimberti D, Scarpini E, Zanetti O, Gennarelli M, Tabaton M. Possible association between genetic variants related to glutamatergic, dopaminergic and neurodevelopment pathways and white matter microstructure in child and adolescent patients with obsessive-compulsive disorder. J Affect Disord. 2015;186:284–92.  
17. Muir KW, Weir CJ, Murray GD, Povey C, Lees KR. Comparison of neurological cognitive impairment. J Alzheimers Dis. 2012;29(3):699–705.  
18. Sambrook J, Russell DW. Molecular Cloning: A Laboratory Manual, 4th edition. New York: Cold Spring Harbor Laboratory Press; 2001. p. 455–541. https://www.cshprotocols.com/published/2013/MC4/MC4FM.pdf.  
19. Szczepankiewicz A, Rachel M, Sobkowiak P, Kycler Z, Wojsyk-Banaszak I, Roks G, de Kort PL, Taylor KD, de Lau LM, Oostra BA, Uitterlinden AG, Rotter J, Boeninkc E, Piaty BM, Mosley TH, van Duijn CM, Brefett MM, Longstreth WT Jr, Wolf PA. GenOMewise association studies. N Engl J Med. 2009;360(17):1718–27.  
20. Holliday EG, Maguire JM, Evans TJ, Kolbar SA, Jannesi J, Sturm JW, Hankey GJ, Baker R, Golledge J, Parsons MW, Malik R, McEvoy M, Bios LS, Lewis MD, Linz LF, Peet R, Oldmeadow C, Smith W, Muscato P, Barlea S, Bevan S, Bis JC, Boeninkc E, Brescegate GB, Brott TG, Brown RD Jr, Cheng YC, Cole JW, Cotracic I, Devan WJ, Forrage M, Fuike RL, Göttersdott S, Gschwendtner A, Ikarra MA, Longstreth WT Jr, Mesfia JA, Mitchell BD, Mosley TH, Nalls MA, Panati EA, Piaty BM, Sharma P, Stefansson K, Thorleifsson G, Thorsteinsson D, Traylor M, Verhaeren BF, Wiggins KL, Worrell BA, Sudlow C, Rothwell PM, Faramar M, Dichtt M, Rosand J, Markus HS, Scott RJ, Leivo C, Attern J, Australian Stroke Genetics Collaborative. International stroke Genetics Consortium; Wellcome Trust Case Control Consortium 2. Common variants at 6p21 are associated with large artery atherosclerotic stroke. Nat Genet. 2012;44(10):1147–51.  
21. Traylor M, Faramar M, Holliday EG, Sudlow C, Hopekew JC, Cheng YC, Farnoge M, Ikarra MA, Malik R, Bevan S, Thorsteinsdott S, Nalls MA, Longstreth W, Wiggins KL, Yadav S, Pariati EA, Distefano AM, Worrell BM, Kitten SJ, Khan MS, Reiner AP, Helgadott S, Acherberc S, Fernandez-Cadenas I, Abboud S, Schmidt R, Walters M, Chen WM, Ringelstein EB, Dointments M, Ho Wk, Pea L, Jemmms R, Norwing B, Higgins P, Benn M, Sale M, Kuhlenbäumer G, Doney AS, Vicentz AE, Delavaran H, Algra A, Davies G, Oliveira SA, Palmer CN, Deary I, Schmidt H, Pandolfo M, Montaner J, Carty C, de Babker PI, Kostulas K, Ferro JM, van Zuydam NR, Valdimarsson E, Nordestgaard BG, Lindgren A, Thyn P, Stovik S, Salehen D, Pael G, Berger K, Thorleifsson G, Australo stroke genetics collaborative. Wellcome Trust case control consortium 2 (WTCCC2), Hofman A, Mosley TH, Mitchell BD, Furie K, Clarke R, Levi S, Cethadi S, Gschwendtner GB, Boncoraglio GB, Sharma P, Bis JC, Göttersoddot S, Piaty BM, Rothwell PM, Rosand J, Mesfia JA, Stefansson K, Dichtt M, Markus HS. International stroke genetics consortium. Genetic risk factors for ischaemic stroke and its subtypes (the METAstroke collaboration): a meta-analysis of genome-wide association studies. Lancet Neurol. 2012;11(11):951–62.  
22. Haber M, Mezzavilla M, Xue Y, Comas D, Gasparini P, Zollaua P, Tyler-Sim C, Genetc evidence for an origin of the Armenians from bronze age mixing of multiple populations. Eur J Hum Genet. 2016;24(6):931–6.  
23. Herrera KJ, Lowery RK, Hadden L, Calderon S, Chouc I, Ysipsokulc P, Reginocu M, Underhill PA, Herrera R. Neolithic patirnnial signals indicate that the Armenian plateau was repopulated by agriculturalists. Eur J Hum Genet. 2012;20(3):313–20.  
24. Marganayc A, Denenick M, Hovhannissianc H, Marialyc H, Beller R, Khashatychan Z, Avetisycn P, Bataladyan R, Bobholtycy A, Melikyanc V, Saryangc G, Piliposycan A, Simonyanc H, Mtkrchtyanc R, Deniscyc G, Ysipsokulyc P, Willersleyc E, Allento M. Eight millennia of matrilineal genetic continuity in the south Caucasian. Curr Biol. 2017;27(30):2323–8.  
25. Cerebrovascular Disease Knowledge Portal, NINDS grant #1R24NS092983. 2018.02.13. http://cerebrovascularportal.org/varia/variantinfo/variationinfo/ n2374464A.  
26. Devette S, Kamanati Y, Metso TM, Kloss M, Chauhan G, Engelsber TG, Pezzini A, Thys V, Markus H, Dichtt M, Wolf C, Dittric R, Touez E, Southerlanc AM, Samson Y, Abboud S, Bejyt Y, Caso V, Bersano A, Gschwendtncr GB, Sessia M, Cole J, Larny C, Medercos E, Beretta S, Bonati LH, Grau AJ, Michel P, Majerski JI, Sharma P, Kalashnkovc L, Nazarorc M, Dobynbca L, Bartelsc E, Guillotc B, van den Hersc K, Fernandez-Cadenasc J, Jood K, Nalls MA, De Leeuc FE, Jernc C, Cheng YC, Werner IM, Metso AJ, Liycy C, Lyerc PA, Brandtc A, Boncoraglici GB, Wichmunc H, Geigerc C, Johnson AD, Bötttcherc T, Castellanc M, Arveiller D, Ikarra MA, Breltec MM, Padovanc A, Meschica JF, Kuhlenbcner G, Rolfs A, Worrellc B. Common variation in PHACTRI is...
associated with susceptibility to cervical artery dissection. Nat Genet. 2015; 47(17):83–83. International stroke genetics consortium, Ringelstein EB, Zelenika D, Tatsiakou M, Leys D, Amouyel P, Dallongeville J; CADSiP group.

39. Young KL, Painter J, Powell JE, Lehtimäki T, Mangino M, Laakso M, Bouchard C, Rauramaa R, Sørensen TIA, Tuomilehto J, Salomaa V, Penninx BWJH, de Uitterlinden AG, Pérusse L, Wilson JF, Hayward C, Polasek O, Cucca F, Lemarchand L, Gyllensten U, Ohlsson C, Hofman A, Rivadeneira F, Walker M, Wild S, Wong A, Wright AF, Zillikens MC, Zubair N, Haiman CA, Vestergaard H, van Vliet-Ostaptchouk JV, Vohl MC, Völker U, Waeber G, Swift AJ, Tammelin T, Tan ST, Thorand B, Thuillier D, Vandenput L, Šilbernagel G, Smith BH, Smith JA, Snieder H, Stan O, Rankinen T, Rasmussen-Torvik LJ, Rawal R, Ridker PM, Rose LM, Rudan I, Montgomery GW, De Moor MHM, Mulas A, Müller-Nurasyid M, Musk AW, Hottenga JJ, Huang J, Huang T, Hui J, Huth C, Hutri-Kähönen N, James AL, Grammer TB, Grarup N, van Grootheest G, Harald K, Hastie ND, Havulinna A, Delgado GE, Dueker N, Dörr M, Ebeling T, Eiriksdottir G, Esko T, Faul JD, Fu J, Busonero F, Buyske S, Campbell H, Chines PS, Collins FS, Corre T, Smith GD, Bertoni AG, Blangero J, Bonnefond A, Bonnycastle LL, Borja JB, Brage S, Adair LS, Amin N, Balkau B, Auvinen J, Beilby J, Bergman RN, Bergmann S, Pasko D, Johansson Å, Snitker S, Cheng YC, Eriksson J, Lim U, Aadahl M, PJ, Jackson AU, Bragg-Gresham JL, Vitart V, Marten J, Navarro P, Bellis C, Fornage M, Hassinen M, Bielak LF, Cadby G, Tanaka T, Mägi R, van der Most P, Huppertz C, Willemsen G, Peyrot WJ, Wu Y, Kristiansson K, Demirkan A, Lohman K, Rivera NV, Whitfield JB, Zhao JH, Stringham HM, Liu CT, Hirschhorn JN, Johnson AD, Borecki IB, Province MA, Wareham NJ, Tardif JC, Khaw KT, van Duijn CM, Boreckia IB, Province M, de Andrade M, Turner J, Martin NG, Kuh D, Liu Y, Linneberg A, März W, Strauch K, Kivimäki M, Harris TB, Gudnason V, Völzke H, Qi L, Jarvelin MR, Chambers JC, Kooper JS, Frouget P, Koopman C, Vollenweider P, Hallmans G, Hansen T, Pedersen O, Metspalu A, Wareham NJ, Langenbrand C, Weir DR, Porteous DJ, Boekevink E, Chamman DJ; CHARGE Consortium; EPIC-InterAct Consortium; PAGE Consortium, Abeba DR, Barroso I, McCarthy MI, Fragu F, Turner J, O’Connell JR, van Duijn CM, Boehnke M, Heid IM, Mohlke KL, Strachan DP, Fox CS, Liu CT, Hirschhorn JN, Klein RJ, Johnson AD, Boreckia IB, Franks PW, North KE, Cupples LA, Loos RJF, Käppelinen T. Geno-me-wide physical activity interactions in adiposity – A meta-analysis of 200, 452 adults. PLoS Genet. 2017;13(4)10.1101/655282.

40. Graff M, Scott RA, Justice AE, Young KL, Feitosa MF, Barata L, Winkler TW, Yang J, Loos RJ, Powell JE, Medland SE, Speliotes EK, Chasman DI, Rose LM, Stepansy et al. BMC Medical Genetics 2012;490(7419):267–268.}

41. Stepanyan et al. BMC Medical Genetics (2018) 19:33
Parker AN, Ridker PM, Kardia SL, Blankenberg S, Liu Y, Curhan GC, Franke A, Rochat T, Paulweber B, Prokopenko I, Wang W, Gudnason V, Shuldiner AR, Coresh J, Schmidt R, Ferrucci L, Shlipak MG, van Duijn CM, Borecki I, Krämer BK, Rudan I, Gyllensten U, Wilson JF, Witteman JC, Pramstaller PP, Rettig R, Hastie N, Chasman DI, Kao WH, Heid IM, Fox CS. New loci associated with kidney function and chronic kidney disease. Nat Genet. 2010;42(5):376–84.