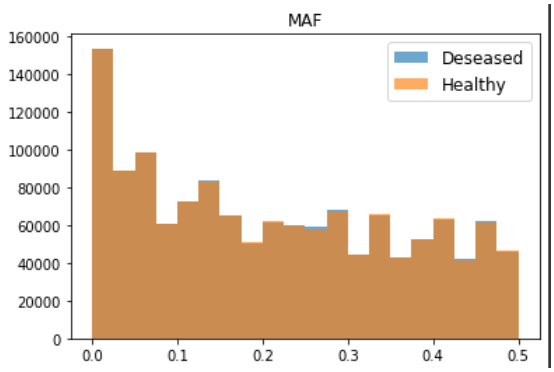
EKSPERIMENT 25 – IDENTIFIKACIJA NASLEDNE BOLESTI I PROBLEMATIČNOG GENA

- U studiji je učestvovalo 200 subjekata, 100 zdravih i 100 obolelih.
- U populaciji je posmatrano 1340823 mutacija, broj mutacija po hromozomu je prikazan ispod:

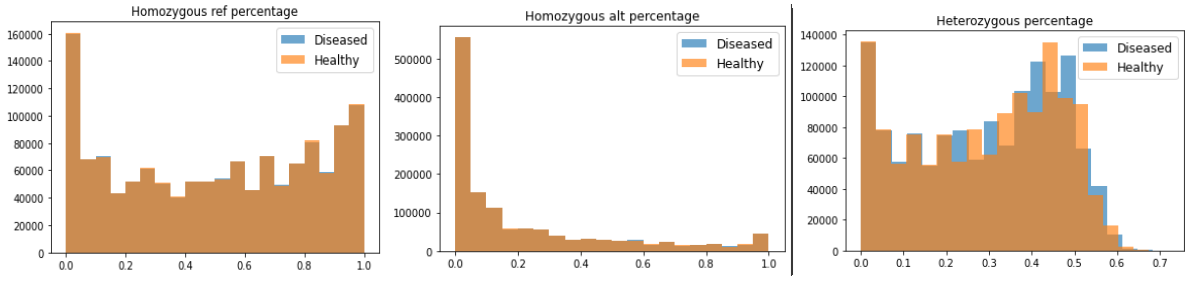
Chromosome	Number of mutations
1	102842
2	114870
3	92888
4	85336
5	86532
6	95476
7	74002
8	76756
9	65374
10	72903
11	67554
12	66884
13	56428
14	44790
15	38680
16	37876
17	30975
18	41882
19	19172
20	33382
21	18402
22	17819

Total number of mutations: 1340823

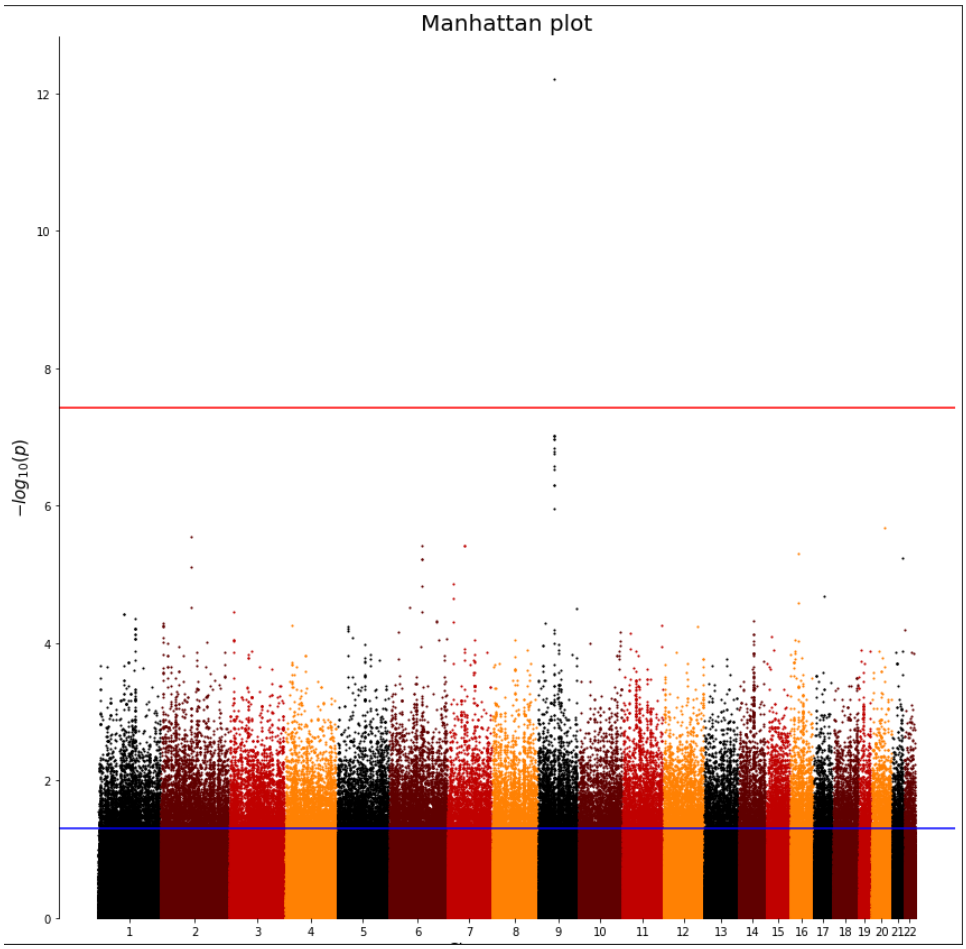
- Frekvencijeređih alela (Minor allele frequency, MAF) za svaku mutaciju:



- Procenat homozigotnih referentnih, alternativnih i heterozigotnih uzoraka za svaku mutaciju:



- Primenom hi kvadrat testa sa Bonferoni korekcijom dobijamo sledeći Menhetnov grafikon, problematičan gen je u 9. hromozomu na poziciji 35749845 (p-vrednost hi kvadrat testa za tu mutaciju je 6.080356967693687e-13).
  Bonferoni korekcija je potrebna zato što kada radimo sa velikom količinom podataka, može se desiti da veliki broj mutacija budu u prihvatajućoj p vrednosti (0.05). Zbog toga primenjujemo Bonferoni korekciju, tako što 0.05 podelimo sa ukupnim brojem mutacija, i tada pomeramo granicu zadovoljavajućih p vrednosti.



- Primenom hi kvadrat testa na preostali gen, dolazimo do zaključka da ne odstupa od Hardi-Vajnberg ekvilibrijuma.
  U ovoj situaciji nije potrebno koristiti Bonferoni korekciju, zbog toga što razmatramo samo jedan gen. P-vrednost testa je 0.9835412651281092.
- Korišćenjem UCSC Genome browser-a nalazimo problematičan gen (GBA2)
- Uz pomoć OMIM baze nalazimo da GBA2 gen prouzrokuje autozomno recesivnu spastičnu paraplegiju tipa 46.

- Autozomno recesivna spastična paraplegija tipa 46 (SPG46) je retka, složena vrsta nasledne spastične paraplegije, koju karakteriše početak, u ranom detinjstvu ili u detinjstvu, tipičnim znakovima spastične paraplegije (tj. spastičnog hoda i slabosti donjih ekstremiteta) povezanih sa raznim dodatnim manifestacijama, uključujući spastičnost i slabost gornjih ekstremiteta, pseudobulbarnu dizartriju, disfunkciju mokraćne bešike, cerebelarnu ataksiju, kataraktu i kognitivna oštećenja, koja mogu napredovati do demencije. Imidžing mozga može pokazati stanjivanje corpus callosum i blagu atrofiju cerebruma i cerebeluma. SPG46 je rezultat mutacija u GBA2 genu (9p13.2), koji kodira ne-lizozomalnu glukozilceramidazu.

CLINICAL FEATURES:

Boukhris et al. (2008) reported a consanguineous Tunisian family (TUN35) in which 5 individuals had a form of early-onset complicated spastic paraplegia. The patients had insidious onset of stiffness and weakness of the lower limbs between 2 and 10 years of age. On physical examination at ages 30 to 35 years, there was a mild to moderate handicap, with only 1 patient requiring a walking aid. All patients showed typical signs of spastic paraplegia, such as spastic gait and weakness of the lower limbs with brisk reflexes and bilateral extensor plantar responses. More variable features included upper limb spasticity and weakness, pseudobulbar dysarthria (in 3), and bladder dysfunction (in 2). Intellectual development was normal in early childhood, but mild cognitive decline appeared progressively as gait difficulties worsened. All also had upper limb dysmetria, suggestive of cerebellar dysfunction, as well as congenital bilateral cataract. Four patients had pes cavus, 2 had scoliosis, and 1 had decreased vibration sense. Brain imaging of 2 patients showed thinning of the corpus callosum and mild cerebellar and cerebral atrophy. Linkage to SPG11 (604360), SPG15 (270700), and other known SPG loci was excluded.

Martin et al. (2013) restudied the Tunisian family reported by Boukhris et al. (2008) and presented 3 additional families with SPG46. There were 11 patients in all. The phenotype was homogeneous: patients presented with progressive difficulty walking due to lower limb spasticity in infancy or childhood (range, 1-16 years), resulting in a need for a cane in their twenties and a wheelchair in their fifties. All developed cerebellar ataxia and cataracts. All patients also had mild to moderate mental impairment, which progressed to dementia in older age. Three patients had hearing loss, and 3 had an axonal neuropathy. Brain MRI showed atrophy of the cerebrum, cerebellum, and corpus callosum. Two affected males had testicular hypotrophy with normal hormone function. Semen analysis of 1 of these men showed severe spermatozoid head abnormalities with necrospermia and reduced velocity, consistent with infertility.

INHERITANCE:

The transmission pattern of spastic paraplegia in the family reported by Boukhris et al. (2008) was consistent with autosomal recessive inheritance.

MAPPING:

By genomewide linkage analysis followed by fine mapping in the Tunisian family reported by Boukhris et al. (2008), Boukhris et al. (2010) found linkage to a 15.4-cM (45.1-Mb) region between markers rs9103 and D9S1799 on chromosome 9p21.2-q21.12 (maximum 2-point lod score of 3.27 at D9S304 under stringent conditions). Multipoint analysis using markers in the candidate interval generated a significant lod score of 5.21. The locus was designated SPG46. Sequencing of exons and intron/exon boundaries of 3 candidate genes, CNTFR (118946), DNAJB5 (611328), and FBXO10 (609092), failed to identify any pathogenic mutations.

MOLECULAR GENETICS:

In 11 patients from 4 unrelated families with autosomal recessive hereditary spastic paraplegia-46, Martin et al. (2013) identified 4 different biallelic mutations in the GBA2 gene (609471.0001-609471.0004). Three of the mutations were truncating, and 1 was a missense mutation that was shown to result in complete loss of enzyme function. The mutations were found by exome sequencing of the candidate region identified by linkage analysis.

In 10 patients from 4 unrelated Tunisian families with autosomal recessive cerebellar ataxia and spasticity, Hammer et al. (2013) identified 3 different homozygous mutations in the GBA2 gene (609471.0005-609471.0007). The first 2 mutations were identified by homozygosity mapping and exome sequencing.

In 3 sibs, born of consanguineous Cypriot parents, with SPG46, Votsi et al. (2014) identified a homozygous mutation in the GBA2 gene (D594H; 609471.0008).