Investigation of copy number variations in the development of rare Mendelian diseases

DOCTORAL (PHD) THESIS

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Introduction

Technological innovations in the second half of the 20th century and the early 21st century have contributed greatly to the development of many areas of science, including human genetics. In order to characterize copy number variations in patients suffering from neurofibromatosis or Marfan syndrome, modern methodologies, including multiplex ligation-dependent probe amplification (MLPA) and array comparative genomic hybridization (aCGH) were applied in our research work. Furthermore, genotype-phenotype analysis was set up based on the demonstrated results and clinical data collection. During our thesis, examination of the correlation between the course and severity of the disease and the presented genetic variations was carried out. Moreover, an investigation of the association between the detected large CNVs and the severity of the cardiovascular manifestations in Marfan syndrome was performed and presented in our research. Exploration of the role of regulatory elements, especially focusing on transcription factor binding sites located within the *FBN1* gene was applied. In addition, breakpoints of a large *de novo* deletion in this gene were investigated and a molecular mechanism behind the formation of this non-recurrent copy number variation (CNV) was proposed.

Copy number variations

Abnormal copy number variation is a type of structural variation (SV) appearing quite often in an individual's genome. SVs are a group of genomic rearrangements affecting long stretches of a nucleotide sequence. They can be characterized into fine-, intermediate-, and large-scale SVs. CNV is defined as a copy number change involving a DNA segment that is 1kb or larger. Mutation rates widely vary between different loci and appear much higher for CNVs than for SNPs. CNVs contribute to genomic diversity between individuals and play a significant role in evolution.

Types of copy number variations

Two major groups can be differentiated by breakpoint analyses of CNVs known as recurrent and non-recurrent CNVs. Recurrent CNVs are more or less located on the same genomic location with identical breakpoints, thus affecting similar sizes of DNA segments among unrelated individuals. In contrast, non-recurrent CNVs are detected at different locations with an observable difference in their breakpoints and sizes. A third group can be differentiated,

called non-recurrent CNVs with grouping. In this case, one side of the CNVs is localized into a broadly similar genome location, while the breakpoint at the other side varies.

Mechanism of copy number variation formation

The formation of CNVs can happen during recombination- and replication-based mechanisms, as well. In addition, many cases show the contribution of transposable elements in the formation of numerous CNVs. Unequal meiotic recombination-based mechanisms are non-allelic homologous recombination (NAHR), non-homologous end joining (NHEJ) and microhomology-mediated end joining (MMEJ). Proposed mechanisms based on replication errors are serial replication slippage (SRS), fork stalling and template switching (FoSTeS) and microhomology-mediated break-induced replication (MMBIR). From previous studies, it seems that certain conditions or agents lead to replications stress, which could potentially form harmful CNVs.

One of the most known mechanisms is NAHR, which contributes to most of the recurrent CNVs. The CNVs are often found in close proximity to segmental duplications or LCRs, although other long stretches of homology can also be responsible for NAHR, such as Alu or L1 elements. NHEJ is one of the main repair mechanisms to restore double-strand breaks (DSBs), especially in G0 and G1 phases. MMEJ is a more error-prone, independent, alternative form of NHEJ. According to the FoSTeS model, the replication fork stops due to some event, and then switches to a different template by annealing to a complementary microhomologous region on a replication fork in close proximity and consequently continues replication. MMBIR is a proposed, specific form of break-induced replication (BIR) that repairs single dsDNA ends coming from collapsed replication forks. MMBIR uses short microhomology coming from another replication fork in close proximity for template switching. SRS are basically multiple rounds of forward and backward replication slippage, which often generate smaller complex rearrangements. MEs, also known as transposable elements (TEs), are a type of genetic material, which is capable of relocating themself in and across genomes, therefore make up a significant part of the human genome.

Rare Diseases

By the definitions of the European Commission on Public health, rare diseases are “life-threatening or chronically debilitating diseases which are of such low prevalence that special combined efforts are needed to address them”. The prevalence number is specified as less than

1/2000 people. Currently around 7000 rare diseases are acknowledged. The disorders can be inherited from parents or generated *de novo*. To our current knowledge ca. 50% of rare diseases affect children. Both disease progression and the manifestation of the disorder are different amongst patients.

Marfan syndrome

Marfan syndrome (MFS; OMIM #154700) is an autosomal dominant, multi-systemic disease with high clinical heterogeneity, affecting mainly the skeletal, ocular and cardiovascular systems. The most life-threatening complication in MFS is connected to the cardiovascular system, including dilatation of the aortic root and ascending aorta, which can result in aortic dissection and sudden death. The disease is caused by mutations in the fibrillin-1 (*FBN1*) gene, which consists of 65 coding exons and is located on the long arm of chromosome 15 (15q21.1). It encodes a major component of microfibrils in the extracellular matrix, called fibrillin-1. Both single, multiple exons and whole *FBN1* deletions have been reported so far. In the case of *FBN1*, 2-7% of MFS patients have been reported to carry a copy number variation.

Neurofibromatosis

Neurofibromatosis has multiple distinct types, although the three most frequent are neurofibromatosis type 1 and 2 (NF1 and NF2) and schwannomatosis. The most common type, the neurofibromatosis 1 (NF1; MIM#162200), is an autosomal dominant disorder caused by genetic alterations in the gene called *NF1*. The main clinical features of NF1 are the hyperpigmented skin macules, called café-au-lait spots (CALS), and the pathognomonic neurofibromas. Many features increase in frequency with aging and show age-dependent penetrance. NF1 is a tumor suppressor gene, localized on chromosome 17 (17q11.2), which encodes a Ras-specific GTPase-activating protein, called neurofibromin. Present day nearby 5-11% of NF1 patients have copy number variations (CNVs), specifically deletions encompassing the *NF1* and contiguous genes. Currently, there are 4 types of microdeletions, called types 1, 2, 3 and atypical. The main difference between them is the breakpoint location, the size involved, and the affected region, specifically the affected genes inside the deletions range. Type 1, 2 and 3 are caused by interchromosomal recombination, known as NAHR during either meiosis (type 1, type 3), or mitosis (type 2). In the case of atypical microdeletions, the causes are heterogeneous.

Aims

Our aim was to

1. determine the frequency and the type of copy number variations among patients with type 1 neurofibromatosis;
2. explore the genotype-phenotype correlation between different types of copy number variations in the NF1 microdeletion patient cohort;
3. compare the differences in the clinical course of the intragenic and microdeletion patient cohort suffering from type 1 neurofibromatosis ;
4. reveal an association between the detected large *FBN1* deletions so far and the severity of the cardiovascular manifestations;
5. investigate the contribution of the deletion of regulatory elements in the clinical course of Marfan syndrome;
6. explore the mechanism underlying the large deletion of *FBN1*.

Materials and methods

Patients with suspected syndromes for Marfan syndrome or neurofibromatosis were referred for genetic testing at our institute (Department of Medical Genetics). Our research included 41 patients with suspected Marfan syndrome or a related connective tissue disorder. Preliminary analysis of the *FBN1*, *TGFBR1* and *TGFBR2* genes were performed by Sanger sequencing with negative results. As a control, 15 patients with intragenic *FBN1* mutations were enrolled into the study, as well. All of the patients fulfilled the revised Ghent criteria.

Our research included 640 unrelated patients with suspected neurofibromatosis. After Sanger sequencing of the *NF1* gene or NGS analyses of *NF1*, *NF2*, *KIT*, *PTPN11*, *RAF1*, *SMARCB1*, and *SPRED1* genes no disease-causing mutations have been identified in 252 patients. Of these, 17 patients with large *NF1* deletion were identified by MLPA. The patient cohort consisted of mainly children (14 out of 17) with the ages between 2 and 17 years. As a control, 33 patients with intragenic *NF1* mutations were enrolled into the study, as well.

DNA was isolated from peripheral blood leukocytes with E.Z.N.A.® Blood DNA Maxi kit, MLPA and Whole Genome aCGH analysis was performed. In silico CNV interpretation and somatic mosaicism determination was carried out. Long-range PCR and subsequent Sanger

sequencing with targeted primers were applied with the help of in silico analysis to confirm the *FBN1* deletion and determine the breakpoints. Regulatory elements were analysed within the *FBN1* gene with the help of the UCSC genome browser. Statistical analysis (Two-tailed Fisher's exact test) was applied to measure differences in the frequencies of clinical features between patients with copy number variations (*NF1* microdeletion and *FBN1* large deletion) and patients with intragenic mutations. A difference with $p<0.05$ was considered as significant.

Results

Marfan syndrome

During MLPA analysis a novel large deletion encompassing exons 46-47 was identified in a 22-year old female and her 1-year-old son. As a consequence, the 31st and 32nd calcium binding EGF-like domains of the fibrillin-1 protein are deleted which contributes to the development of the Marfan syndrome. Identification of the exact breakpoints of the *FBN1* deletion revealed the loss of a 4916 nucleotide long sequence with the insertion of 'TG' nucleotides.

Investigation of the association between the severity of cardiovascular manifestations and CNVs

Cardiovascular (CV) symptoms were classified into two distinct groups, called minor and major CV. The former includes annulus mitralis calcification (age of onset, <40y), pulmonary artery dilatation, mitral valve prolapse, aorta descendens or aorta abdominalis dilatation or dissection (age of onset, <50y). Major symptoms include aorta ascendens, aortic ascendens dilatation with or without aortic regurgitation and involvement of the sinuses of Valsalva.

A great portion of the patients carrying single-exon deletion showed major CV symptoms (10 out of 16; 63%), in addition, one patient had minor symptoms, and two patients had no manifestations in the cardiovascular system. Patients with multiple exon deletions showed much higher frequency of major CV symptoms (16 out of 19; 84%). Furthermore, 11 patients had minor symptoms besides their major CV symptoms. In case of whole gene deletion, 11 out of 16 patients (69%) displayed major CV symptoms, where eight patients belong to two families.

Among our control patients (intragenic mutations) six patients showed major (40%), four patients displayed minor CV manifestations (mitral valve prolapse only, 27%) and five patients

did not have any CV symptoms. The observed frequencies of the major CV manifestations demonstrated a significant difference (73 vs 40%, respectively; p=0.031) between patients with large deletion and the control patient cohort. In case of patients with multiple exon deletions the results were quite similar (84% vs 40%; p=0.012). Finally, no significant difference was presented between the patients carrying single exon deletion and the patients with an intragenic *FBN1* mutation.

Analyses of regulatory elements within *FBN1* gene focusing on transcription factor binding sites

Numerous transcription factor binding sites (TFBS) have been found in the region of *FBN1* gene affected by different CNVs. According to the preliminary in silico analysis among the presented TFBS, STAT3 shows a potential correlation with CV symptoms and supposed to play a role in the development of cardiovascular manifestations. Five cases presented a deletion involving STAT3 binding sites. Out of them, four patients developed aortic dilatations and one patient suffered from an acute dissection of the ascending aorta and right coronary artery as well. Furthermore, several regulatory elements (promoters and/or enhancers) known to be active in the aorta have been found in the region of *FBN1* gene affected by different CNVs.

Neurofibromatosis

17 patients showed heterozygous deletions of the entire *NF1* gene and several contiguous genes in its flanking regions. The MLPA analysis revealed twelve type-1 and five atypical deletions. An aCGH analysis was applied in ten patients for the confirmation of the MLPA results. Altogether twelve type-1, one type-2 and four atypical deletions (three distinct novel atypical deletions) were identified in our patient cohort. Besides, no type-3 microdeletion was detected. Out of the ten patients examined by aCGH, one patient with atypical deletion showed somatic mosaicism with an extent of ca. 30%.

Clinical characterization of our patients with different types of *NF1* microdeletion

Seven major categories (Dysmorphic features, skin manifestations, neurofibromas and other tumours, skeletal anomalies, ocular manifestations, neuropsychological symptoms, connective tissue anomalies and cardiac abnormalities) were determined and selected for genotype-phenotype association analysis.

9 out of 17 patients (53%) presented facial dysmorphism. A similar prevalence was recognized in case of hypertelorism. Coarse facial appearance and large hands and feet seem to be characteristic dysmorphic features of *NF1* microdeletion patients, because it was frequent in our type-1 deletion patients (8 out of 12, 67%), and both symptoms were also noted in the type-2 deletion patient. On contrary, dysmorphic traits were rare event in our intragenic *NF1* patient population.

Regardless of the type of deletion, café-au-lait spots (CALs) were observed in all patients. However axillary and inguinal freckling was absent in the type-2 deletion patient, they showed high frequency in type-1 (10 out of 12; 83%) and atypical (3 out of 4; 75%) deletion groups. Excess soft tissue in hands and feet was presented among our patients, though at a lower frequency. Skin manifestations, including CALs (30 out of 33; 91%) and axillary and inguinal freckling (17 out of 33; 52%) are characteristic of intragenic NF1 patients as well.

According to our results, subcutaneous neurofibromas were the most common among types of neurofibromas, although it is worth mentioning that whole-body and spinal MRI is not part of the routine procedure in our patient management and 14 out of 17 patients were children, furthermore 10 out of 14 were under 10 years old at the age of examination. Optic pathway glioma (OPG) was detected by MRI in four patients and it was not symptomatic in any of these cases. Among the control patients two symptomatic and two asymptomatic OPG were observed.

Malignant peripheral nerve sheath tumours (MPNST) were observed in two of our patients, one was adult and one was nearly adult (36 years and 17 years old, respectively), and both belonged to type-1 deletion group. It is noteworthy to mention that MPNST show age-related penetrance. Among our intragenic NF1 patients, subcutaneous fibromas were found with 30% frequency, the occurrence of cutaneous and plexiform neurofibromas were 18% and 6 %, respectively. Spinal neurofibromas were observed in 3% of our patients. Furthermore, 4 out of 33 (12%) of the control patient cohort developed OPG, and no MPNST was observed.

Majority of our patients demonstrated some form of anomalies of the skeletal system (16 out of 17; 94%). Macrocephaly was the most frequent. The intragenic NF1 patient group demonstrated skeletal anomalies less frequently (33%).

Ocular manifestations were observed in 7 of 17 of our patients (41%). Even though Lisch nodule is a characteristic feature for type 1 neurofibromatosis, it was observed in 3 out of 12 patients with type-1 deletion and in the patient with type-2 deletion. Somewhat similar frequencies were observed in the intragenic NF1 patient cohort.

Significant delay in cognitive development and general learning difficulties (75%), and speech difficulties (67%) were observed with relatively high frequency in type-1 patients. The majority of our patients presented T2 hyperintensities (76%). Neuropsychological manifestations were rare in the NF1 intragenic patient cohort. T2 hyperintensities had the highest prevalence with 39% (13 out of 33 patients).

Discussion

Marfan syndrome

Investigating the association between the detected large FBN1 deletions and the severity of the cardiovascular manifestations

In our patient cohort a novel large deletion, affecting exons 46 and 47, was identified in the *FBN1* gene by MLPA. The *de novo* origin was revealed and confirmed by molecular genetic testing of our primary case and her parents.

Besides full *FBN1* gene deletions, there are 34 various CNVs, affecting single or multiple exons. Detailed clinical evaluation of the presented cases revealed severe cardiovascular manifestations (dilatation and/or dissection of the thoracic aorta) in the majority (26 of 34) of the patients. In six cases no clinical data or no clear clinical information was available. In our primary case only mitral valve prolapse was seen and her 1-year old infant's cardiovascular system was intact. Thereby, apart from CNVs, other factors supposedly play a role in the development of severe cardiovascular manifestations. Interpretation of the results revealed that cardiovascular manifestations are more severe and frequent in the patients affected by CNVs compared to the patients suffering from intragenic *FBN1* gene mutations.

We hypothesize that the deletion of TB domains (namely TB6, TB7 and TB8) in the patients causes the release of active TGF-β into ECM in the aortic wall which in turn overactivates the canonical TGF-β signaling pathway. This effect then may superimpose to the microfibril degeneration and finally together lead to severe cardiovascular manifestations (i.e. aortic dilatation and aortic dissection) in these cases.

After comprehensive evaluation, our *in silico* analysis of *FBN1* gene demonstrated the presence of potential transcription binding sites for STAT3 in a number of cases. We suppose that in CNV patients carrying a deletion involving STAT3 binding sites, the deletion itself has an effect

on STAT3 signaling pathways which may superimpose on the *FBN1* gene defect and together they lead to a severe cardiovascular manifestation in these patients.

Discussion of the mechanism underlying the large FBN1 deletion

According to our hypotheses a potential dinucleotide insertion ('TG') created a 'CCTTGCCTTG' direct repeat sequence, which might interrupt the replication machinery. The insertion itself or the generated repeat potentially caused the replication fork to slow down, stall and eventually collapse. Presumably, this event resulted in a single DSB, where a 5' to 3' resection generated a sequence with a short 3' overhang. As a result of the resection, a DNA segment was exposed to another DNA segment with possible microhomology in close proximity. As a consequence, a D-loop was formed with the 3' overhang part of the dsDNA invading the microhomologous region, where annealing and restarting of the synthesis occurred. On the other hand, we suggest a simultaneous adenine-to-guanine substitution due to an erroneous DNA repair, which at that position creates a microhomology on the other DNA segment, therefore eventually creating the final sequence with the ~5 kilobase long deletion supplemented by a 'TG' dinucleotide insertion at the breakpoints.

Hereby we suggest that MMBIR were responsible for the formation of the CNV in our case, which is supported by the fact that MMBIR is often associated with small stretches (1-4bp) of microhomology.

Neurofibromatosis

In order to reveal genotype-phenotype correlations, we compared the clinical characterization of our patients with the published data on microdeletion and intragenic *NF1* patients. Remarkable difference was observed in several manifestations, such as dysmorphic features, subcutaneous neurofibromas, skeletal anomalies and neurobehavior problems. Although significant differences were recognized in certain clinical features between cases with large *NF1* microdeletion published previously and in our microdeletion patient cohort, it is noteworthy to mention that particular manifestations are age dependent.

Dysmorphic features are characteristics for patients with *NF1* microdeletion, especially in individuals with type-1 deletions. In our type-1 patient cohort, 67% of the affected individuals possess this feature. The represented data imply that it is a very frequent symptom in patients with type-1 deletions.

Large hands and feet in our patient cohort showed a slightly higher frequency of (67%) compared to the observed percentage (46%) demonstrated earlier. Previous studies established an early-onset of neurofibromas among *NF1* microdeletion patients. Besides the close frequency observed in our patients and others (58% and 76%, respectively) of the detected subcutaneous neurofibromas, the occurrence of cutaneous or plexiform neurofibromas was greatly lower in our patients compared to other patient groups (8% vs. 86%, 17% vs 76%, respectively). Patients with subcutaneous neurofibromas possess a higher risk for the development of MPNSTs, and the presence of plexiform neurofibromas involves risk for the development of malignant tumour. A significant delay in cognitive development was found more frequently in our type-1 patients, but the prevalence of intellectual disability was less pronounced. Type-1 deletion harbors fourteen protein coding genes and four microRNA genes. Haploinsufficiency of certain co-deleted genes with *NF1* might influence some clinical manifestations, and it may contribute to the severity of the disease.

In contrast to previous cases, neurobehavioral problems, cardiac manifestations, freckling, hyperflexibility of the joints and externally observable neurofibromas were absent in our type-2 patient's phenotype. Interestingly, the whole clinical picture is dominated by skeletal anomalies.

Atypical deletions are observed in around 8-10% among patients with *NF1* microdeletions. They form a heterogeneous group with their various localization and affected size and the presented diverse clinical picture. In our patient cohort we observed a higher frequency (23%) and only one patient showed mosaicism. During our research three distinct, novel deletions were found.

Characteristic hallmarks of NF1 microdeletions, such as facial dysmorphia, facial asymmetry, large hands and feet and coarse face, were observed in the majority of patients with type-1 NF1 microdeletion, and presented at least in half of the atypical cases identified so far. However, in our patient cohort only one patient showed facial dysmorphia and another had hypertelorism.

Neuropsychological manifestations were absent in our patients, only one showed significant delay in the cognitive development.

According to previous studies, a lower cognitive ability was revealed in patients with *NF1* microdeletion compared to the patients with intragenic mutations. Co-deletion of genes *RNF135* and *OMG* (oligodendrocyte myelin) is assumed to play a role in the development of decreased cognitive ability. *OMG*, which plays an important role in early brain development,

was connected to certain neuropsychiatric disorders, and to the progression of intellectual disability. However, our patients encompassing *RNF135* and *OMG* hardly displayed neuropsychiatric symptoms, so further factors might be also necessary for the development of these manifestations in patients with *NF1* microdeletions.

Several genes (*ATAD5, COPRS, UTP6* and *SUZ12*) in the 17q11.2 region were supposed to be involved in tumorigenesis, therefore they may be accounted for an increased risk for high tumour load.

Conclusion

1. Three types of *NF1* microdeletion (type-1, type-2 and atypical) were identified in our NF1 patient cohort. Among the detected 17 microdeletion, altogether twelve type-1 (~70%), one type-2 (~6%) and four atypical deletions (~24%) were identified. Three distinct novel atypical deletions and no type-3 microdeletion were detected.

2. Genotype-phenotype analyses among our patients revealed that specific clinical manifestations, such as dysmorphic facial features, macrocephaly, large hands and feet, delayed cognitive development and/or learning difficulties, speech difficulties, subcutaneous neurofibromas and overgrowth are characteristic for the patient group with type-1 NF1 microdeletion. Our patient with non-mosaic type-2 NF1 large deletion had only a few of the typical clinical symptoms observed in NF1 microdeletion: macrocephaly, large hands and feet, as well as learning difficulties, moreover our patient with atypical NF1 microdeletion demonstrated facial dysmorphism, presence of the subcutaneous neurofibromas, delayed cognitive development and macrocephaly.

3. We observed that patients with *NF1* large deletion presented more severe clinical phenotype compared to individuals with intragenic *NF1* mutations, possibly due to the affected gene contents and/or the loss of other regulatory DNA elements.

4. We demonstrated, with the help of the literature data and our results, that large various CNVs are often associated with severe cardiovascular manifestations in Marfan syndrome.

5. An association between severe cardiovascular symptoms and the large deletions of the *FBN1* gene was supposed, and we found that involvements of regulatory elements (lack of transcrioption binding site for STAT3) may play a role in the development of cardiovascular symptoms.

6. Breakpoint characterization of the large deletion detected in *FBN1* gene presented a 4916 nucleotide long deletion, with a TG dinucleotide insertion. With the help of previous models and bioinformatic analysis, we proposed that a rare mechanism, termed microhomology-mediated break induced replication, might be responsible for the large deletion.

Papers on which the thesis is based

1. Genotype-Phenotype Associations in Patients With Type-1, Type-2, and Atypical NF1 Microdeletions

G Büki, A Zsigmond, M Czakó, R Szalai, G Antal, V Farkas, G Fekete, D Nagy, M Széll, Tihanyi, B Melegh, K Hadzsiev, Judit Bene

Frontiers in Genetics 2021 Jun 8;12:673025. doi: 10.3389/fgene.2021.673025. eCollection 2021.

Impact factor: 4.772

2. Microhomology-Mediated Break-Induced Replication: A Possible Molecular Mechanism of the Formation of a Large CNV in FBN1 Gene in a Patient with Marfan Syndrome

G Buki, K Hadzsiev, J Bene

Current Molecular Medicine April 2022 DOI: 10.2174/1566524022666220428111943

Impact factor: 2.616

3. Neurofibromatosis-1 microdeletiós szindróma: Molekuláris genetika és klinikai heterogenitás

G Buki, A Till, A Zsigmond, J Bene, K Hadzsiev

Orvosi Hetilap 2022; 163(51): 2041–2051. DOI:10.1556/650.2022.32673

Impact factor: 0.707

Summed impact factor: 8.095

4. Potential association between large FBN1 deletions and severe cardiovascular phenotype in Marfan syndrome due to probable tissue specific enhancers abolishment

G Buki, R Szalai, A Pinter, K Hadzsiev, B Melegh, T Rauch, J Bene

Under publication

Impact factor: -

Other Publications

1. **Identification, presence, and possible multifunctional regulatory role of invertebrate gonadotropin-releasing hormone/corazonin molecule in the great pond snail (Lymnaea stagnalis)**

I Fodor, Z Zrinyi, R Horváth, P Urbán, R Herczeg, G Büki, J M Koene, P-S Tsai, Z Pirger

Gen Comp Endocrinol. 2020 Dec 1;299:113621. doi: 10.1016/j.ygcen.2020.113621. Epub 2020 Sep 20.

Impact factor: 2.63

2. Revealing the impact of the Caucasus region on the genetic legacy of Romani people from genome-wide data

Z Bánfai, V Ádám, E Pöstyéni, G Büki, M Czakó, A Miseta, B Melegh

PLoS One. 2018 Sep 10;13(9):e0202890. doi: 10.1371/journal.pone.0202890. eCollection 2018.

Impact factor: 3.24

3. A rare form of ion channel gene mutation identified as underlying cause of generalized epilepsy

Á Till, R Szalai, M Hegyi, E Kövesdi, G Büki, K Hadzsiev, B Melegh

Orv Hetil. 2019 May;160(21):835-838. doi: 10.1556/650.2019.31404.

Impact factor: 0.41

4. Genome-Wide Marker Data-Based Comparative Population Analysis of Szeklers From Korond, Transylvania, and From Transylvania Living Non-Szekler Hungarians

V Ádám, Z Bánfai, K Sümegi, G Büki, A Szabó, L Magyari, A Miseta, M Kásler, B Melegh

Front Genet. 2022 Mar 28;13:841769. doi: 10.3389/fgene.2022.841769. eCollection 2022.

Impact factor: 4.599

Summed impact factor: 10,879

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