ANALYSIS OF CLINICAL MANIFESTATIONS OF NEUROFIBROMATOSIS TYPE 1 IN PATIENTS WITH NONSENSE PATHOGENIC VARIANTS IN THE *NF1* GENE FROM THE REPUBLIC OF BASHKORTOSTAN

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Abstract. *Relevance*: neurofibromatosis type 1 (NF1) is a hereditary tumor syndrome occurring with a frequency of 1:3164. NF1 is characterized by severe clinical manifestations with multiple cutaneous and subcutaneous tumors, plexiform neurofibromas, skeletal abnormalities and cognitive disorders. The study of the genetic causes of NF1 can become the basis for prenatal diagnosis and the use of new methods of treating the disease. *Materials and methods:* clinical and epidemiological study of NF1 patients in the Republic of Bashkortostan, sequencing the *NF1* gene in their DNA samples as well as whole genome sequencing using the WGS method. To search for the pathogenic variants we found in publications by other authors, we analyzed the Scopus, WoS, ClinVar, PubMed, and SNP databases. *Results*: the frequency of occurrence of NF1 in the republic is 1:7407. 23 nonsense pathogenic variants in 21 exons of the *NF1* gene were identified in 39 NF1 patients from 26 families. *Discussion and conclusion*: the frequency of development of the main clinical manifestations of NF1 in patients from the republic corresponds to data from around the world, however, plexiform neurofibromas, optic nerve gliomas, short stature and decreased intelligence were detected significantly less frequently. Of the 23 nonsense pathogenic variants we identified, 16 pathogenic variants were previously described by researchers from various countries, and 7 pathogenic variants: NM_001042492.3:c.55G>T (NP_001035957.1:p.Glu19Ter), NM_001042492.3:c.2806A>T (NP_001035957.1:p.Lys936Ter), NM_001042492.3:c.3284T>A (NP_001035957.1:p.Leu1095Ter), NM_001042492.3:c.5014G>T (NP_001035957.1:p.Gly1672Ter), NM_001042492.3:c.5242C>T (NP_001035957.1:p.Arg1748Ter), NM_001042492.3:c.7365T>G (NP_001035957.1:p.Tyr2455Ter) and NM_001042492.3:c.7482G>A (NP_001035957.1:p.Trp2494Ter) have been identified for the first time in the word. Patients with nonsense pathogenic variants have significantly higher rates of brain tumors and epilepsy compared to all NF1 patients in the republic.

Keywords: *NF1* gene, genotype-phenotypic correlations, treatment, neurofibromatosis type 1, nonsense pathogenic variants.

List of Abbreviations

CALM – café au lait macules
MPNST – malignant peripheral nerve sheath tumor
NF1 – neurofibromatosis type 1
NF1 – neurofibromatosis type 1 gene
RB – the Republic of Bashkortostan

Introduction

Neurofibromatosis type 1 (NF1) is one of the most common hereditary tumor syndromes with an autosomal dominant type of inheritance, occurring worldwide with an average frequency of 1 in 3164 population (varies in different countries from 1:2132 to 1:4712) (Lee *et al.*, 2023). NF1 is caused by heterozygous pathogenic variants in the tumor suppressor gene *NF1*, which encodes neurofibromin, a GTPase-activating protein consisting of 2818 amino acids and containing a domain that negatively regulates the activity of Ras proto-oncogenes, called GRD (GAP-related domain) (Chai *et al.*, 2019). In addition, neurofibromin contains cysteine/serine rich domain (CSRD), Sec14 homology-like, pleckstrin homology-like and syndecan-2 binding domains (Frayling *et al.*, 2019). The *NF1* gene is located on 17q11.2 (Fahsold *et al.*, 2000) and consists of 57 exons (Jeong *et al.*, 2006). According to the results of independent scientific studies of the *NF1* gene

in patients with NF1, disease-causing pathogenic variants are distributed across all 57 exons, and there are no hot spots of mutagenesis in the gene. Therefore, NGS and Sanger sequencing methods are used for molecular genetic identification of the cause of NF1 (Ars *et al.*, 2006; Barretina *et al.*, 2010; Cali *et al.*, 2017; Chai *et al.*, 2019; Crona *et al.*, 2013; Fahsold *et al.*, 2000; Frayling *et al.*, 2019; Jeong *et al.*, 2006; Griffiths *et al.*, 2007; Heim *et al.*, 1995; Ko *et al.*, 2013; Lee *et al.*, 2006; Messiaen *et al.*, 2000; Palma Milla *et al.*, 2018; Park *et al.*, 2000; Pros *et al.*, 2010; Sabbagh *et al.*, 2013; Toonen *et al.*, 2016; Upadhyaya *et al.*, 2008; Vuralli *et al.*, 2016).

NF1 is manifested by the development of characteristic café-au-lait macules (CALM) with a diameter of more than 5 mm in prepuberty and more than 15 mm in postpuberty – 99%), Lisch nodules, cutaneous and subcutaneous neurofibromas, optic nerve gliomas, and plexiform neurofibromas. Clinically, a diagnosis of NF1 (according to the National Institutes of Health (NIH) criteria) is made when a patient has two or more of these features, or one feature and a family history of NF1 (Anderson & Gutmann, 2020). The presence of CALM alone is not sufficient to diagnose NF1, as 19.5% to 57.1% of people with this feature do not have NF1 (Bernier *et al.*, 2016). According to the world scientific literature, CALM is determined in 96.5% of patients with NF1, freckling of the axillary and groin areas – in 90% of patients (Miraglia *et al.*, 2020), neurofibromas – y 50% (Anderson & Gutmann, 2020) – 78,1% (Miraglia *et al.*, 2020) (on average = 64%) patients. Neurofibromas are found in an average of 50% of patients with NF1, Lisch nodules in 70%, and plexiform neurofibromas in 40% (Anderson & Gutmann, 2020). Optic nerve gliomas are detected in 27% of patients with NF1, brain tumors in 10% of patients, and hydrocephalus in 7.7% of patients with NF1 worldwide (Glomova *et al.*, 2019).

Premature mortality in patients with NF1 is due to the development of plexiform nephrofibromas in 40% of patients, which can compress vital organs (Anderson & Gutmann, 2020), as well as an increased incidence of malignant neoplasms. NF1-specific malignant peripheral nerve sheath tumors (MPNSTs), previously described as neurogenic sarcoma, neurofibrosarcoma, malignant neurilemoma, or malignant schwannoma (Lim *et al.*, 2024), are very rare pathologies in the general population worldwide. MPNST occurs in the general population at a rate of 1.46 cases per million person-years (1182 cases of MPNST were detected in the US population from 1973 to 2009, of which 165 were in individuals aged 0 to 19 years) (Bates *et al.*, 2014). For their diagnosis, equivalent methods are MRI and PET/CT with 2-(18F)-fluoro-2-deoxy-d-glucose (Ko & Kim, 2024). However, in patients with NF1, the incidence of MPNST is extremely high and amounts to 13%. Usually, these tumors arise as a result of the degeneration of already existing plexiform neurofibromas. Moreover, in patients with NF1, these tumors turned out to be more aggressive with an increased risk of mortality (Lim *et al.*, 2024).

In addition to the tumor syndrome, patients with NF1 show signs of brain damage, which is characterized by a decrease in intelligence and the development of epilepsy. According to a 2022 meta-analysis, children with NF1 show a statistically significant decrease in IQ compared to healthy peers (Al-Farsi *et al.*, 2022). Intellectual disabilities are found in 48% of NF1 cases, with an average IQ of 85 (Anderson & Gutmann, 2020). According to the conducted meta-analyses, seizures are determined in 8.1% of patients with NF1 (of which generalized tonic-clonic seizures in 16.8%, focal seizures in 54.2%; against the background of 1-2 anticonvulsant drugs, the absence of seizures is determined in 68.5%; median age is 3.5-12 years) (Wu *et al.*, 2024).

NF1 patients are characterized by musculoskeletal system involvement. According to a meta-analysis, 26.6% of patients with NF1 have scoliosis. It usually develops in early childhood, most often affecting the thoracic spine. No reliable correlation was found between scoliosis and the NF1 genotype (Wang *et al.*, 2024). A recent meta-analysis showed that NF1 is associated with decreased bone mineral density at the lumbar spine and femur, with increased blood levels of parathyroid hormone

and C-telopeptide of type 1 collagen, and decreased alkaline phosphatase, calcium, vitamin D, osteocalcin, and markers of bone formation compared to healthy individuals (Kaspiris *et al.*, 2024). On average, 5% of NF1 patients worldwide have pseudoarthrosis (Ly & Blakeley, 2019) and 24% have short stature (Virdis *et al.*, 2003). Chest wall deformity is detected in 3.5% of patients with NF1, which is significantly higher than in the general population (0.3%) (Francis *et al.*, 2016).

Material and Methods

A retrospective analysis of patients with NF1 from the Republic of Bashkortostan (RB) was conducted based on data on those registered with a geneticist at the Republican Medical Genetic Center with an established diagnosis of NF1. With the consent of the patients, 7 ml of blood samples were taken from them, from which DNA was isolated using the phenol-chloroform extraction method. These samples were used for further studies. To identify intragenic nonsense pathogenic variants, the *NF1* gene (57 exons) for 23 patients with NF1 was sequenced using an automatic sequencer ABI PRISM model 310 (Applied Biosystems, USA) with the SYEnamicTMET fluorescent labeling kit according to the Amersham Pharmacia Biotech DYEnamicTM ET Terminator Cycle Sequencing Kit protocol. The BioEdit v.5.0.9 application was used to read the nucleotide sequences. For another 16 NF1 patients, whole genome sequencing (WGS) was performed using the DNBSEQ-T7RS platform. Genomic DNA was isolated from the blood-containing plates using a MGIEasy Magnetic Beads Genomic DNA Extraction Kit (MGI, Shenzhen, China) according to the manufacturer's protocol. The extracted DNA was quantified using a Qubit™ dsDNA Quantification Assay Kit (ThermoFisherScientific, USA). Each gDNA sample (1000 ng) was used to construct a genomic DNA library using the MGIEasy Fast PCR-FREE FS DNA Library Prep Set V2.0 (MGI, Shenhzen, China) according to the manufacturer's instructions. DNA was fragmented by enzymatic fragmentation with size select step using magnetic beads. DNA end-repair and adapter ligation were conducted using the MGIEasy UDB PF Adapters-96 Kit (MGI, Shenzhen, China). The products were run on the 4200 TapeStation using the Agilent D1000 ScreenTape (Agilent, Santa Clara, CA, USA) to assess the size distribution of the libraries. They were also quantified using a Qubit™ dsDNA Quantification Assay Kit. The DNA fragments were circularized and 75 fmol of ssCirDNA were amplified using rolling-circle amplification to generate DNA nanoball-based libraries, which were loaded onto a DNBSEQ-T7RS Sequencing flow cell with a DNBSEQ-T7RS High-throughput Sequencing Kit (MGI, Shenzhen, China). The library was run on a DNBSEQ-T7RS (MGI, Shenzhen, China) platform at paired-end 150 bp reads. To search for the pathogenic variants we found in publications by other authors, the Scopus, WoS, ClinVar, PabMed, and SNP databases were analyzed. For high-quality binary data, statistical processing was performed using an interactive 2×2 contingency table with the calculation of association statistics (Pearson's $\chi 2$ criterion) with the Yates's correction for continuity developed by V.P. Leonov (http://www.biometrica.tomsk.ru/freq2.htm), analysis of four-field contingency tables on the website https://medstatistic.ru/calculators/calchi.html. The clinical manifestations of the disease (frequency of CALM, cutaneous and subcutaneous neurofibromas, plexiform neurofibromas, optic nerve gliomas, brain cysts and tumors, skeletal abnormalities) were analyzed in all patients with NF1, as well as in patients with identified nonsense pathogenic variants. The frequency of clinical signs of NF1 in patients from RB was compared with world data. A search for possible genotypic correlations in patients with the nonsense pathogenic variants we identified was also conducted. All studies were conducted in compliance with biomedical ethics and meet GCP (Good Clinical Practice) standards.

Results

In the Republic of Bashkortostan, 544 patients with NF1 from 433 families are registered; the frequency of occurrence of NF1 is 1:7407 people. 299 sporadic (55%) and 45% fa-

milial cases of NF1 were identified. The distribution of patients by ethnicity corresponds to the regional characteristics, the male to female ratio is 1:1 (52% female and 48% male). CALM were identified in all patients with NF1; cutaneous or subcutaneous neurofibromas were detected in 323 patients (59%), and cognitive impairment was found in 78 patients with NF1 (14%). In some patients, brain damage was detected, causing hydrocephalus in 3.3%, brain cysts in 3.8%, brain tumors in 4%, and epilepsy - optic nerve gliomas in 5.3% of all patients with NF1. Plexiform neurofibromas were described in only 34 patients (6%). Scoliosis was detected in 93 patients with NF1 (17%), short stature in 71 (13%), chest deformity in 28 (5%), and pseudoarthrosis of the shin bones in 16 (3%) patients with NF1.

A comparative analysis of clinical manifestations of NF1 in patients from RB (Table 1) indicates a statistically significant lower incidence of plexiform neurofibromas, optic nerve gliomas, short stature and intellectual disabilities compared to NF1 data worldwide (Anderson & Gutmann, 2020; Glomova *et al.*, 2019; Virdis *et al.*, 2003). The incidence of CALM, cutaneous and subcutaneous neurofibromas, brain tumors, hydrocephalus, epilepsy and skeletal abnormalities in patients with NF1 from our republic is consistent with the data of other authors (Anderson & Gutmann, 2020; Chelleri *et al.*, 2021; Glomova *et al.*, 2019; Ly & Blakeley, 2019; Miraglia *et al.*, 2020; Wang *et al.*, 2024; Wu *et al.*, 2024).

As a result of sequencing of DNA samples from patients with NF1 from the Republic of Bashkortostan, the following nonsense pathogenic variants were identified (Table 2). Of the results presented in the table, 8 pathogenic variants

■ (NM_001042492.3:c.910C>T: NP_001035957.1:p.R304Ter, NM_001042492.3:c.1318C>T: NP_001035957.1:p.R440Ter, NM_001042492.3:c.1713G>A: NP_001035957.1:p.W571Ter, NM_001042492.3:c.2994T>A: NP_001035957.1:p.Tyr998Ter, NM_001042492.3:c.3301C>T: NP_001035957.1:p.Q1101Ter, NM_001042492.3:c.5902C>T: NP_001035957.1:p.R1968Ter, NM_001042492.3:c.6349C>T: NP_001035957.1:p.Q2117Ter, NM_001042492.3:c.7909C>T: NP_001035957.1:p.R2637Ter) ■

were detected by whole genome sequencing using the NGS method.

A comparative analysis of 39 patients with the nonsense pathogenic variants we identified was carried out with the general group of NF1 patients from the Republic of Bashkortostan (Table 3). As a result, a significantly higher frequency of detection of epilepsy and brain tumors was determined in patients with nonsense pathogenic variants. When comparing the clinical manifestations of NF1 according to the world scientific literature with the characteristics of the disease in patients with the pathogenic variants we identified (Table 4), a significantly rarer detection of plexiform neurofibromas, plexiform neurofibromas and intellectual disabilities was determined. Of the 23 nonsense pathogenic variants in the *NF1* gene that we identified, 16 pathogenic variants had been previously described by researchers from various countries (Ars *et al.*, 2006; Barretina *et al.*, 2010; Cali *et al.*, 2017; Chai *et al.*, 2019; Crona *et al.*, 2013; Fahsold *et al.*, 2000; Frayling *et al.*, 2019; Griffiths *et al.*, 2007; Heim *et al.*, 1995; Jeong *et al.*, 2006; Kang *et al.*, 2020; Ko *et al.*, 2013; Lee *et al.*, 2006; Messiaen *et al.*, 2000; Palma Milla *et al.*, 2018; Park *et al.*, 2000; Pros *et al.*, 2010; Sabbagh *et al.*, 2013; Toonen *et al.*, 2016; Upadhyaya *et al.*, 2008; Vuralli *et al.*, 2016).

Table 1

Comparison of manifestations of neurofibromatosis type 1 in patients from the Republic of Bashkortostan with world data

Clinical manifestations	Frequency of occurrence in patients with nonsense pathogenic variants from RB in %	Frequency of occurrence in patients worldwide in % (author)	χ2 test; p-value at 1 degree of freedom
Neurofibromas	59%	64% (Anderson & Gutmann, 2020; Miraglia *et al.*, 2020)	$\chi^2 = 0.528$; $p = 0.468$
Plexiform neurofibromas	6%	40% (Anderson & Gutmann, 2020)	$\chi^2 = 32.637$; $p < 0.001$
Optic nerve gliomas	5.3%	27% (Glomova *et al.*, 2019)	$\chi^2 = 18.006$; $p < 0.001$
Brain tumors	4.6%	10% (Glomova *et al.*, 2019)	$\chi^2 = 1.802$; $p = 0.18$
Hydrocephalus	3.8%	7.7% (Glomova *et al.*, 2019)	$\chi^2 = 1.418$; $p = 0.234$
Epilepsy	3.3%	8.1% (Wu *et al.*, 2024)	$\chi^2 = 2.4$; $p = 0.121$
Scoliosis	17%	26.6% (Wang *et al.*, 2024)	$\chi^2 = 2.914$; $p = 0.088$
Short stature	13%	24% (Virdis *et al.*, 2003)	$\chi^2 = 4.013$; $p = 0.046$
Chest deformity	5%	3.5% (Chelleri *et al.*, 2021)	$\chi^2 = 0.116$; $p = 0.734$
Pseudoarthrosis	3%	5% (Ly & Blakeley, 2019)	$\chi^2 = 0.521$; $p = 0.471$
Cognitive impairment	14%	48% (Anderson & Gutmann, 2020)	$\chi^2 = 27.022$; $p < 0.001$

Table 2

Clinical manifestations of NF1 in patients from the Republic of Bashkortostan with identified nonsense pathogenic variants

№	Family	Patient's gender/age (sporadic case or inheritance)	Detected pathogenic variants/ exon number	Clinical manifestations	Description earlier in literature (country)
1	1	Female/ 10 (sporadic)	NM_001042492.3:c.55G>T (NP_001035957.1:p.Glu19Ter)/ 1	CALM, optic nerve glioma	not described earlier
2	2	Female/ 61 (sporadic)	NM_001042492.3:c.910C>T (NP_001035957.1:p.Arg304Ter)/ 9	CALM, multiple cutaneous and subcutaneous neurofibromas, epilepsy, brain tumor	UK (Upadhyaya *et al.*, 2008), Spain (Pros *et al.*, 2010), USA (Barretina *et al.*, 2010), Korea (Ko *et al.*, 2013), Sweden (Crona *et al.*, 2013), France (Sabbagh *et al.*, 2013)
3		Male/ 39 (from mother)	NM_001042492.3:c.910C>T (NP_001035957.1:p.Arg304Ter)/ 9	CALM	
4		Female/ 33 (from mother)	NM_001042492.3:c.910C>T (NP_001035957.1:p.Arg304Ter)/ 9	CALM, multiple cutaneous and subcutaneous neurofibromas, low stature, scoliosis of 2 st., chest deformity, flat feet, facial dysmorphic disorder	
5	3	Female/ 31 (from father)	NM_001042492.3:c.1278G>A (NP_001035957.1:p.Trp426Ter)/ 12	CALM, multiple cutaneous neurofibromas, grade 2 scoliosis, normal intelligence (normal height and intelligence)	UK (Griffiths *et al.*, 2007)
6		Male/ 65 (sporadic)	NM_001042492.3:c.1278G>A (NP_001035957.1:p.Trp426Ter)/ 12	CALM, multiple cutaneous and subcutaneous neurofibromas, brain tumor, normal intelligence, brain tumor (normal growth and intelligence)	
7	4	Female/ 65 (sporadic)	NM_001042492.3:c.1318C>T (NP_001035957.1:p.Arg440Ter)/ 12	CALM, multiple subcutaneous neurofibromas, short stature	USA (Heim *et al.*, 1995), Germany (Fahsold *et al.*, 2000)
8		Male/ 37 (from mother)	NM_001042492.3:c.1318C>T (NP_001035957.1:p.Arg440Ter)/ 12	CALM, focal subcutaneous neurofibromas on the trunk, scoliosis, pseudoarthrosis of the lower leg, facial dysmorphism, short stature	
9	5	Male/ 1 year (sporadic)	NM_001042492.3:c.1318C>T (NP_001035957.1:p.Arg440Ter)/ 12	CALM, epilepsy	
10	6	Female/ 63 (from mother)	NM_001042492.3:c.1318C>T (NP_001035957.1:p.Arg440Ter)/ 12	CALM, multiple cutaneous neurofibromas	
11	7	Male/ 31 (sporadic)	NM_001042492.3:c.1713G>A (NP_001035957.1:p.Trp571Ter)/ 15	CALM, focal cutaneous neurofibromas on the trunk, short stature, grade 3 scoliosis, Arnold-Chiari malformation	UK (Upadhyaya *et al.*, 2008)
12	8	Female/ 29 (from mother)	NM_001042492.3:c.2041C>T (NP_001035957.1:p.Arg681Ter)/ 17	CALM, focal large subcutaneous neurofibromas on head and trunk, plexiform neurofibroma of iliac region, short stature	USA (Toonen *et al.*, 2016)
13		Female/ 32 (from mother)	NM_001042492.3:c.2041C>T (NP_001035957.1:p.Arg681Ter)/ 17	CALM, multiple cutaneous and subcutaneous neurofibromas, epilepsy	

Continuation of table 2

№	Family	Patient's gender/age (sporadic case or inheritance)	Detected pathogenic variants/ exon number	Clinical manifestations	Description earlier in literature (country)
14		Male/ 11 (from mother)	NM_001042492.3:c.2041C>T (NP_001035957.1:p.Arg681Ter)/ 17	CALM, epilepsy, delayed psychomotor development, MRI - foci of a lowered plane of the brain	
15	9	Male/ 20 (sporadic)	NM_001042492.3:c.2806A>T (NP_001035957.1:p.Lys936Ter)/ 21	CALM, multiple cutaneous and subcutaneous neurofibromas, retrocerebellar cyst and brain tumor	not described
16	10	Female/ 43 (from mother)	NM_001042492.3:c.2994T>A (NP_001035957.1:p.Tyr998Ter)/ 23	CALM, focal subcutaneous and cutaneous neurofibromas on the trunk and arms, grade 4 scoliosis, short stature	Germany (Fahsold *et al.*, 2000), Taiwan (Lee *et al.*, 2006)
17	11	Male/ 13 (sporadic)	NM_001042492.3:c.3158C>G (NP_001035957.1:p.Ser1053Ter)/ 24	CALM, on MRI of the brain, gliosis of the basal ganglia, brainstem and cerebellum, in dynamics with an increase in size	UK (Frayling *et al.*, 2019)
18	12	Female/ 34 (from father)	NM_001042492.3:c.3284T>A (NP_001035957.1:p.Leu1095Ter)/ 25	CALM, multiple cutaneous and subcutaneous neurofibromas	not described
19		Male/ 6 (from mother)	NM_001042492.3:c.3284T>A(NP_001035957.1:p.Leu1095Ter)/ 25	CALM, focal subcutaneous neurofibromas on the body, asymmetry of the cerebral ventricles, brain cyst, scoliosis, flat feet	
20		Male/ 3 (from mother)	NM_001042492.3:c.3284T>A (NP_001035957.1:p.Leu1095Ter)/ 25	CALM, focal subcutaneous neurofibromas on the body	
21	13	Female/ 22 (sporadic)	NM_001042492.3:c.3301C>T (NP_001035957.1:p.Gln1101Ter)/ 25	CALM, multiple subcutaneous neurofibromas, scoliosis grade 2, hydrocephalus (normal intelligence)	Spain (Palma Milla *et al.*, 2018)
22	14	Male/ 15 (sporadic)	NM_001042492.3:c.4084C>T (NP_001035957.1:p.Arg1362Ter)/ 30	CALM, multiple subcutaneous neurofibromas	Germany (Fahsold *et al.*, 2000; Kluwe *et al.*, 2003), Korea (Ko *et al.*, 2013)
23	15	Female/ 54 (sporadic)	NM_001042492.3:c.4537C>T (NP_001035957.1:p.Arg1513Ter)/ 34	CALM, multiple cutaneous and subcutaneous neurofibromas (normal body growth and intelligence)	Germany (Fahsold *et al.*, 2000), Belgium (Messiaen *et al.*, 2000), Korea (Jeong *et al.*, 2006), Spain (Ars *et al.*, 2006)
24		Female/ 26 (from mother)	NM_001042492.3:c.4537C>T (NP_001035957.1:p.Arg1513Ter)/ 34	CALM	
25	16	Male/ 32 (sporadic)	NM_001042492.3:c.4600C>T (NP_001035957.1:p.Arg1534Ter)/ 35	CALM, multiple subcutaneous neurofibromas, brain tumor	China (Chai *et al.*, 2019)
26	17	Female/ 19 (sporadic)	NM_001042492.3:c.5014G>T (NP_001035957.1:p.Gly1672Ter)/ 36	CALM, hydrocephalus, cerebellar tumor	not described

End of table 2

№	Family	Patient's gender/age (sporadic case or inheritance)	Detected pathogenic variants/ exon number	Clinical manifestations	Description earlier in literature (country)
27	18	Female/ 26 (sporadic)	NM_001042492.3:c.5242C>T (NP_001035957.1:p.Arg1748Ter)/ 37	CALM, multiple subcutaneous and cutaneous neurofibromas	not described
28		Male/ 2 (from mother)	NM_001042492.3:c.5242C>T (NP_001035957.1:p.Arg1748Ter)/ 37	CALM	
29	19	Male/ 84 (sporadic)	NM_001042492.3:c.5902C>T (NP_001035957.1:p.Arg1968Ter)/ 40	multiple cutaneous neurofibromas, CALM and strabismus	Korea (Park *et al.*, 2000)
30		Female/ 42 (from father)	NM_001042492.3:c.5902C>T (NP_001035957.1:p.Arg1968Ter)/ 40	CALM, multiple subcutaneous neurofibromas and short stature	
31		Female/ 46 (from father)	NM_001042492.3:c.5902C>T (NP_001035957.1:p.Arg1968Ter)/ 40	CALM, multiple subcutaneous and cutaneous neurofibromas, Lisch nodules, decreased intelligence, severe myopia, brain cyst, optic nerve glioma	
32	20	Male/ 19 (from father)	NM_001042492.3:c.6349C>T (NP_001035957.1:p.Gln2117Ter)/ 41	CALM, multiple cutaneous and subcutaneous neurofibromas, scoliosis and flat feet (epilepsy, profound psychomotor retardation, asymmetry of the lateral horns of the brain on MRI)	UK (Frayling *et al.*, 2019)
33		Male/ 57 (from father)	NM_001042492.3:c.6349C>T (NP_001035957.1:p.Gln2117Ter)/ 41	CALM, multiple cutaneous and subcutaneous neurofibromas	
34	21	Female/ 24 (sporadic)	NM_001042492.3:c.6792C>A (NP_001035957.1:p.Tyr2264Ter)/ 45	CALM, pseudoarthrosis of the right leg, funnel chest, hydrocephalus (decreased intelligence)	Spain (Ars *et al.*, 2006), Germany (Fahsold *et al.*, 2000), Belgium (Messiaen *et al.*, 2000), Korea (Jeong *et al.*, 2006)
35	22	Male/ 14 (from mother)	NM_001042492.3:c.6792C>A (NP_001035957.1:p.Tyr2264Ter)/ 45	CALM, plexiform neurofibroma on the lumbar region, signs of encephalopathy of the brain	
36	23	Female/ 7 (sporadic)	NM_001042492.3:c.7102G>T (NP_001035957.1:p.Glu2368Ter) / 47	CALM, psychomotor retardation	Korea (Kang *et al*., 2020)
37	24	Male/ 18 (sporadic)	NM_001042492.3:c.7365T>G (NP_001035957.1:p.Tyr2455Ter)/ 49	CALM, plexiform neurofibroma on the arm, scoliosis grade 3, hallux valgus, psychomotor retardation, brain tumor	not described
38	25	Male/ 1 year (sporadic)	NM_001042492.3:c.7482G>A (NP_001035957.1:p.Trp2494Ter)/ 50	CALM	not described
39	26	Female/ 25 (sporadic)	NM_001042492.3:c.7846C>T (NP_001035957.1:p.Arg2616Ter)/ 53	CALM, scoliosis (no neurofibromas, normal intelligence, CT scan of the brain without pathology)	Türkiye (Vuralli *et al.*, 2016), Italy (Cali *et al.*, 2017)

Table 3

Comparison of NF1 manifestations in patients with nonsense pathogenic variants with the general group of all NF1-patients from the Republic of Bashkortostan

Clinical manifestations	Frequency of occurrence in patients with nonsense pathogenic variants from RB (%) n = 39	Frequency of occurrence in patients from RB (%) n = 544	χ2 test; p-value at 1 degree of freedom
Neurofibromas	21 (54%)	318 (58.6%)	$\chi^2 = 0.318$; $p = 0.573$
Plexiform neurofibromas	3 (7.7%)	34 (6%)	$\chi^2 = 0.13$; $p = 0.985$
Optic nerve gliomas	2 (5%)	33 (6%)	$\chi^2 = 0.057$; $p = 0.812$
Brain tumor	6 (15%)	25 (4.6%)	$\chi^2 = 8.414$; $p = 0.004$
Brain cyst	3 (7.7%)	22 (4%)	$\chi^2 = 1.18$; $p = 0.278$
Hydrocephalus	3 (7.7%)	21 (3.8%)	$\chi^2 = 1.354$; $p = 0.245$
Epilepsy	5 (13%)	18 (3.3%)	$\chi^2 = 8.688$; $p = 0.004$
Scoliosis	10 (26%)	93 (17%)	$\chi^2 = 1.827$; $p = 0.177$
Short stature	7 (18%)	71 (13%)	$\chi^2 = 0.753$; $p = 0.386$
Chest deformity	2 (5%)	28 (5%)	$\chi^2 = 0.137$; $p = 0.712$
Pseudoarthrosis	2 (5%)	16 (3%)	$\chi^2 = 0.582$; $p = 0.446$
Cognitive impairment	5 (13%)	77 (14%)	$\chi^2 = 0.054$; $p = 0.817$

Table 4

Comparison of NF1 manifestations in patients with nonsense pathogenic variants from the Republic of Bashkortostan with world data

Clinical manifestations	Frequency of occurrence in patients with nonsense pathogenic variants from RB in %	Frequency of occurrence in patients worldwide in % (author)	χ2 test; p-value at 1 degree of freedom
Neurofibromas	54%	64% (Anderson & Gutmann, 2020; Miraglia *et al.*, 2020)	$\chi^2 = 2.067$; $p = 0.151$
Plexiform neurofibromas	7.7%	40% (Anderson & Gutmann, 2020)	$\chi^2 = 28.07$; $p < 0.001$
Optic nerve gliomas	5%	27% (Glomova *et al.*, 2019)	$\chi^2 = 37.5$; $p < 0.001$
Brain tumor	15%	10% (Glomova *et al.*, 2019)	$\chi^2 = 0.627$; $p = 0.429$
Hydrocephalus	7.7%	7.7% (Glomova *et al.*, 2019)	not applicable
Epilepsy	13%	8.1% (Wu *et al.*, 2024)	$\chi^2 = 1.33$; $p = 0.249$
Scoliosis	26%	26.6% (Wang *et al.*, 2024)	not applicable
Short stature	18%	24% (Virdis *et al.*, 2003)	$\chi^2 = 1.085$; $p = 0.298$
Chest deformity	5%	3.5% (Chelleri *et al.*, 2021)	$\chi^2 = 0.521$; $p = 0.471$
Pseudoarthrosis	5%	5% (Ly & Blakeley, 2019)	not applicable
Cognitive impairment	13%	48% (Anderson & Gutmann, 2020)	$\chi^2 = 28.895$; $p < 0.001$

Discussion

23 nonsense pathogenic variants in 21 exons were identified in 39 patients from 26 families. Of the identified pathogenic variants, 7 were previously not described in the scientific literature. There were 20 sporadic cases of NF1 in RB, inheritance from the mother – 13, inheritance from the father – 6, that is, newly occurring cases accounted for 51%. A study of the clinical features of NF1 in patients with the nonsense pathogenic variants we identified showed that CALM characteristic of NF1 were identified in all patients, multiple subcutaneous and cutaneous neurofibromas were detected in 13 patients, multiple subcutaneous in 2, multiple cutaneous in 3, focal subcutaneous in 2, and focal cutaneous neurofibromas in 1 patient. Table 2 presents the characteristics of specific clinical signs of NF1 in patients from RB. Analysis of the features of clinical manifestations of NF1 depending on the type of pathogenic variants did not reveal genotypic correlations, which is consistent with data from 20 scientific articles (Ars *et al.*, 2006; Barretina *et al.*, 2010; Cali *et al.*, 2017; Chai *et al.*, 2019; Crona *et al.*, 2013; Fahsold *et al.*, 2000; Frayling *et al.*, 2019; Jeong *et al.*, 2006; Griffiths *et al.*, 2007; Heim *et al.*, 1995; Ko *et al.*, 2013; Lee *et al.*, 2006; Messiaen *et al.*, 2000; Palma Milla *et al.*, 2018; Park *et al.*, 2000; Pros *et al.*, 2010; Sabbagh *et al.*, 2013; Toonen *et al.*, 2016; Upadhyaya *et al.*, 2008; Vuralli *et al.*, 2016).

The 7 previously undescribed nonsense pathogenic variants in the *NF1* gene that we identified that cause the development of NF1 are innovative data for the world literature. In addition, molecular genetic confirmation of NF1 can become the basis for the use of modern targeted therapy for the disease. In 2024, a meta-analysis of the efficacy and safety of treating plexiform neurofibromas with selumetinib in patients with NF1 was conducted. The combined improvement rate was 75.3% for pain and 77.8% for movement disorders (Han *et al.*, 2024). For the treatment of cutaneous neurofibromas in patients with NF1, 0.5% cutaneous gel NFX-179, containing a metabolically labile MEK inhibitor, has proven its effectiveness. These methods were developed specifically for the treatment of NF1, so confirming the diagnosis by finding the pathogenic variants is especially important, especially since multiple tumors on the patient's body cause serious psychological trauma to patients and their relatives (Sarin *et al.*, 2024).

Conclusion

We conducted a clinical and epidemiological study of patients with NF1 from the Republic of Bashkortostan. As a result, the prevalence of the disease was determined to be 1:7407, which is significantly less than the world average (1:3164). The ratio of male to female patients with NF1 was 1:1, sporadic cases accounted for 55%. The clinical characteristics of patients with NF1 from the Republic of Bashkortostan with world data indicate a comparable frequency of occurrence of neurofibromas, brain tumors, hydrocephalus, epilepsy, scoliosis, chest deformity and pseudoarthrosis. A statistically significantly lower incidence of plexiform neurofibromas, optic nerve gliomas, short stature and decreased intelligence was determined in patients with NF1 from the Republic of Bashkortostan. Molecular genetic study of DNA samples from patients with NF1 from the Republic of Bashkortostan allowed us to identify 23 nonsense pathogenic variants in 39 patients from 26 families. The identified pathogenic variants are distributed across 21 exons of the *NF1* gene (Fig. 1), which corresponds to the data of the world scientific literature on the absence of mutagenesis hot spots in the gene. Analysis of clinical manifestations did not reveal geno-phenotypic correlations, which corresponds to the data of scientific literature worldwide. However, comparison of the frequency of occurrence of individual symptoms showed more frequent development of epilepsy and brain tumors in patients with nonsense pathogenic variants compared to all patients with NF1 in the Republic of Bashkortostan. Of the 23 nonsense pathogenic variants in the *NF1* gene that we identified, 7 had not previously been described in the scientific literature. These include

■ NM_001042492.3:c.55G>T (NP_001035957.1:p.Glu19Ter) in exon 1, NM_001042492.3:c.2806A>T (NP_001035957.1:p.Lys936Ter) in exon 21, NM_001042492.3:c.3284T>A (NP_001035957.1:p.Leu1095Ter) in exon 25, NM_001042492.3:c.5014G>T (NP_001035957.1:p.Gly1672Ter) in exon 36, NM_001042492.3:c.5242C>T (NP_001035957.1:p.Arg1748Ter) in exon 37, NM_001042492.3:c.7365T>G (NP_001035957.1:p.Tyr2455Ter) in exon 49, and NM_001042492.3:c.7482G>A (NP_001035957.1:p.Trp2494Ter) in exon 50. ■

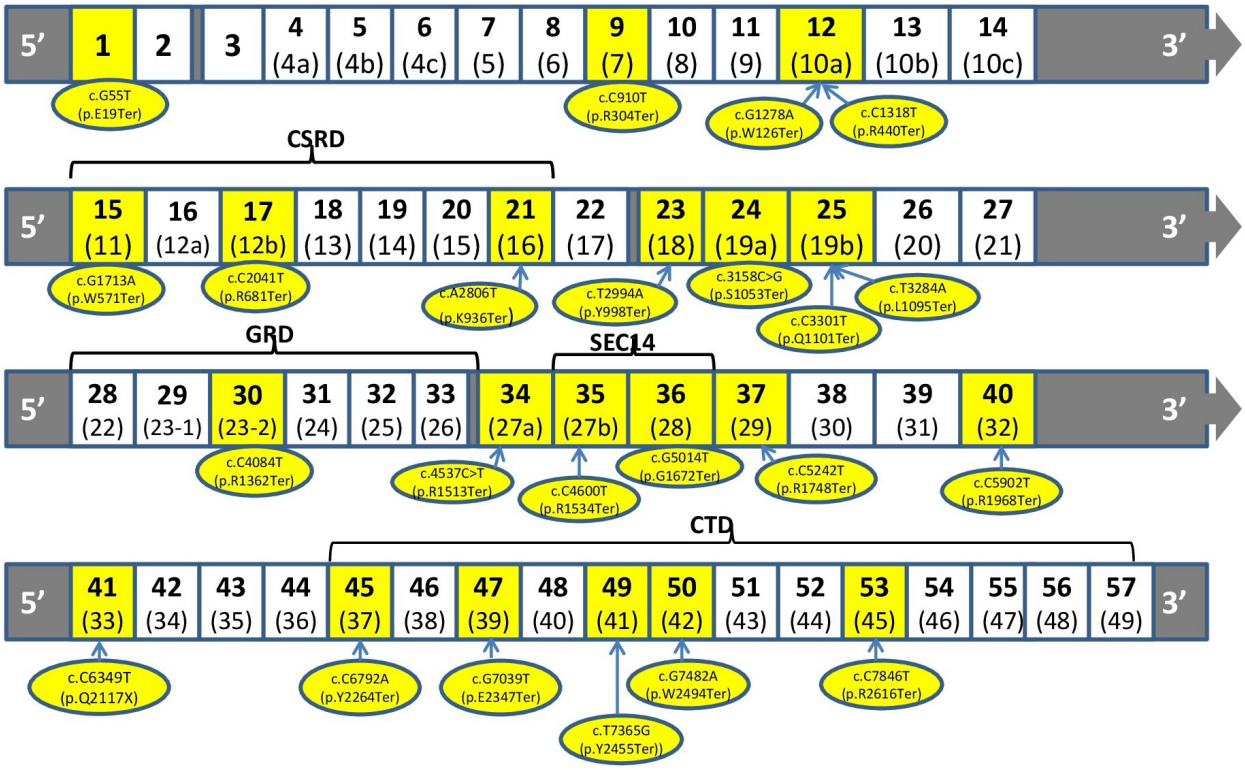

Fig. 1. Distribution of nonsense pathogenic variants in the *NF1* gene identified in NF1 patients from the Republic of Bashkortostan (curly brackets indicate exons encoding the corresponding domains in the neurofibromin protein (CSRD, GRD, SEC14, CTD); numbers in brackets indicate the old designation of exons)

The obtained results are innovative and will allow updating the database of pathogenic variants in the *NF1* gene. In addition, confirmation of NF1 at the molecular genetic level will allow using modern methods of targeted therapy of the disease in patients from the Republic of Bashkortostan, as well as preventing this hereditary pathology at the level of the Republican Medical Genetic Center.

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