

Clinical and Molecular Characterization of a Rare KRAS p.Val14Ile Variant in Noonan Syndrome: Integrative Phenotypic Analysis and Molecular Dynamics Simulation

Xin Li

the People's Hospital of Dongying

Sanhua Yang

Lijin County Traditional Chinese Medicine Hospital

Xin Liu

the People's Hospital of Dongying

Chunqing Li

the People's Hospital of Dongying

Xiaotian Wang

the People's Hospital of Dongying

Shuping Wang

the People's Hospital of Dongying

Wentao Yang

ywentao2010@163.com

the People's Hospital of Dongying

Article

Keywords:

Posted Date: October 29th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10881419/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations:

No competing interests reported.

Tables 1 and 2 are available in the Supplementary Files section.

Clinical and Molecular Characterization of a Rare KRAS p.Val14Ile Variant in Noonan Syndrome: Integrative Phenotypic Analysis and Molecular Dynamics Simulation

Xin Li ¹, Sanhua Yang², Xin Liu ¹, Chunqing Li¹, Xiaotian Wang³, Shuping Wang^{1*}, Wentao Yang^{3*}

¹Department of Endocrinology, the People's Hospital of Dongying, Shandong 257091, China; ²Department of Pharmacy, Lijin County Traditional Chinese Medicine Hospital, Dongying, Shandong 257091, China; ³ Department of Emergency, the People's Hospital of Dongying, Shandong 257091, China;

Corresponding author: Wang Shuping(renzhendoctor@163.com)

Yang Wentao(ywentao2010@163.com)

Abstract

Noonan syndrome (NS) is a genetically heterogeneous RASopathy caused by dysregulation of the RAS/mitogen-activated protein kinase (RAS/MAPK) signaling pathway. Although PTPN11 is the most frequently implicated gene, KRAS variants are relatively rare, and the genotype–phenotype relationships and molecular consequences of individual KRAS variants remain incompletely understood. Here, we report a patient with NS harboring a rare *de novo* KRAS variant, NM_004985.4:c.40G>A (p.Val14Ile), and systematically investigated its clinical and potential molecular characteristics. A total of 57 patients with NS carrying KRAS variants were identified from the literature, including 10 patients with p.Val14Ile and 47 patients with other KRAS variants. Comparative clinical analysis showed broadly similar phenotypic profiles between the two groups across most RASopathy-associated organ systems. However, lymphatic and hematological abnormalities were significantly less frequent in the p.Val14Ile group than in the other KRAS variant group (25.00% vs. 86.67%, $P = 0.013$; OR = 0.05, 95% CI: 0.00–0.77), while differences were also observed in central nervous system and urinary system abnormalities. HPO-based analysis showed an overall trend toward lower odds of the

evaluated phenotypes in p.Val14Ile carriers, with a pooled OR of 0.63 (95% CI: 0.42–0.96) under the random-effects model and no significant heterogeneity ($I^2 = 0\%$, $P = 0.7938$). ACMG-based assessment, together with multiple in silico prediction tools, supported the pathogenic potential of the variant. Conservation analysis demonstrated that Val14 is highly conserved and located within the P-loop region of the KRAS G domain. Molecular docking showed weaker predicted GTP binding to p.Val14Ile KRAS than to wild-type KRAS (-7.829 vs. -8.387 kcal·mol⁻¹). Furthermore, 100-ns molecular dynamics simulations revealed increased local fluctuations, altered conformational sampling, reduced hydrogen-bonding interactions, and a less favorable MM/GBSA binding free energy for the mutant KRAS–GTP complex (-34.27 vs. -48.64 kcal·mol⁻¹). These findings suggest that the p.Val14Ile substitution may alter the conformational dynamics and interaction network of the KRAS–GTP complex without substantially disrupting the overall protein structure. Collectively, this study expands the clinical and molecular spectrum of KRAS -associated NS and provides a potential mechanistic explanation for the phenotypic characteristics of the rare p.Val14Ile variant.

Introduction

Noonan syndrome (NS) is a genetically heterogeneous multisystem disorder characterized by a broad and variable clinical phenotype. In 1962, Jacqueline Noonan first described nine patients with similar clinical features, including pulmonary valve stenosis, short stature, hypertelorism, intellectual disability, ptosis, cryptorchidism, and skeletal abnormalities. The condition was formally designated as Noonan syndrome in 1968 [1,2]. NS is predominantly inherited in an autosomal dominant manner, although a small proportion of cases follow an autosomal recessive pattern of inheritance [3]. The estimated incidence of NS is approximately 1 in 2,500 to 1 in 1,000 live births [4]. NS belongs to the group of RASopathies, a spectrum of developmental disorders caused by dysregulation of the RAS/mitogen-activated protein kinase (RAS/MAPK) signaling pathway. Accumulating evidence indicates that aberrant activation of the RAS/MAPK pathway contributes substantially to the

pathogenesis of NS [5]. The RAS/MAPK signaling cascade regulates a wide range of essential developmental processes, including cell proliferation, differentiation, migration, senescence, and apoptosis. Consequently, pathogenic variants (PVs) affecting components of this pathway can exert pleiotropic effects and lead to abnormalities involving multiple organ systems [6].

Molecular genetic studies have shown that variants in PTPN11 account for approximately 50% of NS cases, whereas the frequencies of variants in other disease-associated genes vary considerably. SOS1 variants account for approximately 13% of cases, while variants in RAF1 and RIT1 collectively account for approximately 3–17%. KRAS variants are identified in fewer than 5% of cases, whereas variants in NRAS, BRAF, and MAP2K1 each account for less than 1% of cases [3]. These genetic alterations can result in constitutive or dysregulated activation of the encoded proteins, ultimately leading to excessive activation of the RAS/MAPK signaling cascade, which represents a key molecular mechanism underlying the development of NS [7]. Although PTPN11 is the most frequently implicated gene in NS, KRAS also plays a critical role in the pathogenesis of this disorder.

KRAS is a key component of the RAS/MAPK signaling pathway and encodes a small GTPase that functions as a molecular switch in intracellular signal transduction [8]. Under physiological conditions, KRAS cycles tightly between an inactive GDP-bound state and an active GTP-bound state, thereby transmitting signals from upstream receptors to downstream signaling cascades [9]. Through this mechanism, KRAS regulates a broad range of cellular processes, including proliferation, differentiation, and survival. Characterizing the clinical phenotypes associated with KRAS variants and elucidating their underlying molecular mechanisms are therefore important for improving our understanding of the pathogenesis of RASopathies. However, previous studies of KRAS variants have focused predominantly on well-established recurrent or hotspot variants. Whether different KRAS variants are associated with distinct phenotypic spectra, and how variants at non-canonical sites produce variant-specific functional effects, remain incompletely understood.

In the present study, we identified a patient with Noonan syndrome carrying a rare missense variant in KRAS (NM_004985.4: c.40G>A, p.Val14Ile). To further characterize the clinical significance of this rare variant and systematically evaluate the phenotypic characteristics associated with genetic alterations affecting key components of the RAS/MAPK signaling pathway, we not only provide a detailed description of the patient's clinical features but also systematically reviewed the reported KRAS variants and their associated clinical phenotypes. We subsequently focused on the KRAS p.Val14Ile variant and integrated molecular docking with molecular dynamics simulations to investigate whether this variant may alter the conformational dynamics and GTP-binding characteristics of KRAS, thereby affecting its potential molecular function. These analyses were aimed at providing a potential molecular explanation for the distinctive clinical phenotype associated with this rare KRAS variant.

subject and methods

subjects

The research subject was a patient diagnosed with Noonan syndrome at Dongying People's Hospital in August 2024. The detailed clinical characteristics and genetic identification of this specific case were previously reported by our team in Chinese¹⁰. The proband was a 15-year and 2-month-old boy who presented to our clinic with delayed development of secondary sexual characteristics. His parents were healthy and non-consanguineous. The mother's pregnancy was uncomplicated, with no history of specific diseases or medication use, and the patient was delivered via cesarean section at 40 weeks of gestation. At birth, his length was 53 cm and his weight was 4,800 g. His developmental milestones were generally normal: he could sit at 6 months, crawl at 8 months, and walk at 13 months. Complementary foods were introduced at 7 months, and he was breastfed until 12 months of age. The patient exhibited poor academic performance. His dietary preferences included a high intake of meat and an aversion to wheat-based foods. Physical examination revealed a height of 149.6 cm (below the 3rd percentile) and a weight of 40.3 kg. Dysmorphic facial

features included a slightly high-arched palate, epicanthal folds, low-set ears, a low posterior hairline, and irregular dentition. There was a complete absence of axillary, pubic, and facial hair. Spontaneous nystagmus was observed. Thoracic examination revealed costal flaring, and palpable rachitic rosary-like changes were noted at the lower margins of the bilateral ribs. Hyperpigmentation was observed in the perineal region. Genital examination showed a left testicular volume of 1.62 ml, a right testicular volume of 1.92 ml, and a stretched penile length of approximately 3.8 cm. No other notable anomalies were found in the extremities or skeletal system.

Comprehensive laboratory and imaging evaluations were performed. Biochemical and endocrine assays revealed the following abnormalities: a decreased insulin-like growth factor-1 (IGF-1) level of 150.04 ng/ml (reference range: 235–988 ng/ml) and a peak growth hormone (GH) level of 3.128 ng/ml during the GH stimulation test. The triptorelin stimulation test demonstrated a peak luteinizing hormone (LH) level of 7.88 U/L, with a peak LH to peak follicle-stimulating hormone (FSH) ratio of 1.68. Radiographic assessment indicated that his bone age was delayed by 5 years and 2 months compared to his chronological age. Echocardiography revealed mild tricuspid regurgitation, and an electrocardiogram (ECG) indicated sinus rhythm with abnormal Q waves in leads V1 and V2. Other laboratory and imaging investigations, including 25-hydroxyvitamin D, parathyroid hormone, routine blood and urinalysis, liver and renal function, myocardial enzymes, electrolytes, thyroid function profiles, and pituitary magnetic resonance imaging (MRI), were all within normal limits.

methods

Genetic Sequencing

Parental authorization was secured prior to extracting 3mL of peripheral blood from the child patient and parents. Following the manufacturer's protocol, genomic DNA was isolated from blood samples utilizing the Tiangen Blood Genomic DNA Extraction Kit(DP318).The DNA samples were sent to Fuzhou Furui Medical Laboratory for whole exome sequencing(WES).The patient's guardian provided

signed informed consent before blood sampling commenced. The study protocol received approval from Dongying People's Hospital Ethics Committee (DYYX-2024-027).

Whole exome sequencing

The DNA samples were dispatched to Fuzhou Furui Medical Laboratory for WES. The genomic deoxyribonucleic acid (DNA) of the submitted samples was fragmented, ligated, amplified, and purified. The DNA library was prepared using a hybrid capture method, and human DNA was subsequently detected by means of a high-throughput sequencing platform.

In the course of the whole exome analysis, the exonic and flanking intronic regions (20bp) of 20,700 genes, as well as the complete mitochondrial ring genome sequence (16,569bp), were obtained. The sequencing data were aligned with the human genome hg19 (GRCh37) reference sequence, and the coverage of the target region and the quality of the sequencing were evaluated. The Next Gene V2.3.4 software was utilized to align the sequencing data with the UCSC hg19 human reference genome sequence and to identify genetic variations. Quality parameters, including the coverage of the target region and the average sequencing depth, were collected.

bioinformatics analysis

The bioinformatics analysis was conducted using the Next Gene V2.3.4 software and scripts developed by Fuzhou Furui Medical Laboratory. The annotation information included base and amino acid conservation (PhyloP: <http://compgen.cshl.edu/phast/phyloP-tutorial.php>), biological function prediction (PolyPhen2: <http://genetics.bwh.harvard.edu/pph2>), Mutation Taster v2021 software (<https://www.genecascade.org/MutationTaster2021/>), and SIFT (<https://sift.bii.a-star.edu.sg/>). The frequency of the general population was determined using ExAC (<https://gnomad.broadinstitute.org/>). The following databases were utilized: 1000 Genomes (<https://www.internationalgenome.org>), dbSNP (<https://www.ncbi.nlm.nih.gov/snp/>), a laboratory-built database, HGMD (<https://www.hgmlid.cf.ac.uk/ac/index.php>), Clinvar (<https://www.ncbi.nlm.nih.gov/clinvar/>), and OMIM

(<https://www.omim.org/>). The specific impact of the variants on SR protein binding was analyzed using ESE Finder 3.0 (<http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi>), with threshold values set at SF2/ASF:1.956, SC35: 2.383, SRp40: 2.67, and SRp55: 2.676. Conservation analysis of the variants was performed using Consurf (https://consurf.tau.ac.il/consurf_index.php). The Pymol software (<https://pymol.org>) was utilized to predict the secondary and tertiary structure of the KRAS protein. The pathogenicity of genetic variants was evaluated in accordance with the criteria and guidelines established by the American College of Medical Genetics and Genomics (ACMG).

Molecular dynamics (MD) simulations of the KRAS protein and its mutant complexes were performed using GROMACS 2024.4. The AMBER14SB force field was applied to describe the protein topologies, while the General AMBER Force Field 2 (GAFF2) was employed for the ligands. The complex systems were solvated in a cubic box using the TIP4P water model, ensuring a minimum distance of 1.2 nm between the complex and the periodic boundaries. Long-range electrostatic interactions were computed using the Particle Mesh Ewald (PME) method. To achieve system electroneutrality, an appropriate number of Na⁺ and Cl⁻ ions were added using a Monte Carlo ion placement method.

Prior to the production simulations, the systems were subjected to a rigorous energy minimization using the steepest descent algorithm for 50,000 steps, which was terminated when the maximum force fell below 1000 kJ/mol/nm. Subsequently, the systems were equilibrated in two stages. First, a 100ps NVT ensemble (50,000 steps with a 2 fs time step) was conducted to stabilize the temperature at 310 K. This was followed by a 100ps NPT ensemble to equilibrate the system pressure at 1 bar while maintaining the temperature at 310K. Upon completion of the minimization and equilibration phases, a 100ns unrestrained production MD simulation was carried out for each system with a time step of 2fs. Structural coordinates were saved every 10 ps for subsequent trajectory analysis.

Trajectory analyses, including Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), Radius of Gyration (Rg), Solvent Accessible

Surface Area (SASA), and the number of intermolecular hydrogen bonds, were calculated using the built-in tools of GROMACS. The relative free energy landscapes were also constructed to identify representative conformational states. Structural snapshots were extracted at 0, 25, 50, 75, and 100ns to compare the dynamic conformational changes. Finally, the binding free energies between the KRAS proteins and the ligands were calculated using the Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) method.

Literature Search Methodology

A comprehensive literature search was conducted across multiple electronic databases, including PubMed, Web of Science, Embase, and CNKI (China National Knowledge Infrastructure), from database inception up to August 2026. In addition, genetic variant databases including ClinVar and HGMD (Human Gene Mutation Database) were screened to ensure completeness.

A literature search was conducted using search terms including 'KRAS gene' and 'Noonan syndrome'. Language restrictions were limited to English and Chinese. Reference lists of relevant reviews and identified articles were manually searched to identify additional eligible studies. The detailed search strategy, including all MeSH terms and free-text keywords, is provided in Supplementary Text S1.

Statistical Analysis

For normally distributed continuous variables, data are presented as the mean $\pm$ standard deviation (SD), and group comparisons were performed using the independent samples t-test. For non-normally distributed continuous variables, data are presented as the median (interquartile range, IQR), and group comparisons were performed using the Mann–Whitney U test. Categorical variables are presented as frequencies and percentages. Group comparisons were performed using the chi-square test or Fisher exact test, as appropriate, depending on the expected cell frequencies. Univariate binary logistic regression was performed to evaluate the association between each KRAS gene (p.Val14Ile vs. others) as the binary outcome variable. Results are expressed as odds ratios (ORs) with 95% confidence intervals (95%

CIs). A two-sided P value of less than 0.05 was considered statistically significant. All statistical analyses were performed using R software (version 4.3.1).

Result

Genetic Sequencing Results

A heterozygous variant in the KRAS gene, NM_004985.4:c.40G>A, was identified in the patient. This variant results in the substitution of valine by isoleucine at amino acid position 14 (p.Val14Ile) and is classified as a missense variant. At the molecular level, the Val-to-Ile substitution may enhance intrinsic and guanine nucleotide exchange factor (GEF)-catalyzed nucleotide exchange, while also affecting GTP hydrolysis stimulated by GTPase-activating proteins (GAPs), potentially contributing to constitutive activation of the RAS/MAPK signaling pathway. The inheritance pattern was consistent with an autosomal dominant mode of inheritance. Sanger sequencing of the family members demonstrated that both parents were wild-type at this locus, indicating that the KRAS p.Val14Ile variant occurred *de novo* in the proband (Fig. 1).

Pathogenicity Analysis of Variants

According to the latest American College of Medical Genetics and Genomics (ACMG) guidelines [11], the KRAS variant NM_004985.4:c.40G>A was evaluated using an integrated, multidimensional approach. This heterozygous variant results in the substitution of valine by isoleucine at amino acid position 14 (p.Val14Ile). Based on the combined evidence, the variant was initially assessed as pathogenic according to the criteria PS2_VeryStrong + PS3 + PS4 + PM1 + PP2 + PM2_Supporting. The supporting evidence included the very strong pathogenicity criterion PS2_VeryStrong, based on the *de novo* occurrence of the variant [12–14]; functional evidence meeting PS3, as previous functional studies showed that the variant may enhance intrinsic and guanine nucleotide exchange factor (GEF)-catalyzed nucleotide exchange, while also affecting GTP hydrolysis stimulated by GTPase-activating proteins (GAPs) [15,16]; PS4, based on the available clinical and population-level evidence; PM1, indicating that the variant is located within a functionally important hotspot region [17]; PP2,

supporting the gene–phenotype relationship; and PM2_Supporting, based on its rarity in population databases. Furthermore, in silico analyses using MutationTaster, REVEL, PolyPhen-2, and SIFT consistently supported the pathogenic potential of this variant (Table 1).

Conservation analysis using ConSurf demonstrated that the affected residue is highly conserved across species (Fig. 2A). The variant is located within the P-loop (phosphate-binding loop) region of the G domain of KRAS (Fig. 2B). The P-loop of KRAS interacts with the phosphate groups of GTP or GDP and participates in interactions with GTPase activating proteins (GAPs), thereby contributing to the regulation of GTP hydrolysis and the intracellular molecular switch function of KRAS. Therefore, the substitution at this highly conserved site may interfere with normal GTP hydrolysis or alter the interaction between KRAS and GAPs, potentially favoring a constitutively active state of KRAS and resulting in aberrant RAS/MAPK signaling. Taken together, the bioinformatic analyses and the integrated ACMG evidence support the involvement of this variant in the molecular pathogenesis of Noonan syndrome. Based on the overall evidence, the variant was classified as pathogenic, providing important molecular evidence for clinical diagnosis and genetic counseling.

To further investigate a potential effect of the variant at the RNA level, an in silico splicing analysis was performed using ESEfinder 2.0 (Fig. 2C). The analysis predicted that the KRAS c.40G>A variant disrupts exonic splicing enhancer (ESE) motifs recognized by SF2/ASF (IgM-BRCA1) and SRp55. We therefore hypothesized that this alteration may impair the recruitment of the corresponding splicing factors by the spliceosome, potentially affecting the normal splicing of KRAS pre-mRNA, such as through exon skipping or alterations in splice-site selection. At the protein level, the variant results in the Val14Ile amino acid substitution. These findings raise the possibility that, in addition to the alteration of the KRAS amino acid sequence, aberrant pre-mRNA splicing may represent another potential mechanism contributing to the pathogenicity of this variant.

the Summary of the Results Found in the Extant Literature

The literature search identified 41 English-language publications reporting KRAS variants in patients with Noonan syndrome. After excluding systematic reviews, publications without reported KRAS variants, and studies focused exclusively on basic experimental research, 23 English-language publications were included in the final analysis. A separate search of the Chinese-language literature identified an additional 4 eligible publications, which were also included. Thus, a total of 27 publications were included in the literature-based analysis. The detailed characteristics of the included publications are summarized in Supplementary Table 1.

distribution of mutation Sites

A total of 57 patients with Noonan syndrome carrying KRAS variants were identified. To further characterize the distribution of specific variants across functionally important regions of the protein, we summarized and generated a domain-based variant map (Fig. 3). The map illustrates the distribution of recurrent and distinct amino acid substitution sites across the KRAS protein and their localization within specific functional domains.

KRAS gene mutation

A total of 57 patients carrying KRAS variants were included in this study. Patients harboring the p.Val14Ile variant were assigned to the study group (N = 10), whereas patients carrying other KRAS variants were assigned to the control group (N = 47). The detailed clinical characteristics are presented in Table 2. No statistically significant differences were observed between the two groups in terms of mean age or sex distribution, indicating good baseline comparability between the groups.

Comparison of patients carrying the p.Val14Ile variant with those carrying other KRAS variants demonstrated a high degree of similarity in the distribution of most common RASopathy-related clinical phenotypes, including facial features, craniofacial abnormalities, growth restriction, developmental and intellectual abnormalities, cardiovascular manifestations, skeletal abnormalities involving the trunk and limbs, skin and hair abnormalities, genitourinary abnormalities, and ear, nose, throat, and visual abnormalities. For most phenotypic categories, no statistically significant differences in prevalence were observed between the two groups ($P > 0.05$).

However, the p.Val14Ile group had a significantly lower frequency of lymphatic and hematological abnormalities than the other KRAS variant group (25.00% vs. 86.67%, $P = 0.013$; OR = 0.05, 95% CI: 0.00–0.77). Distinct distribution patterns were also observed for central nervous system abnormalities and urinary system abnormalities between the two groups ($P < 0.001$ and $P = 0.035$, respectively).

To further quantify the differences in phenotypic profiles between the two groups, Human Phenotype Ontology (HPO) terms were used to calculate the odds ratios (ORs) for individual system-specific phenotypes associated with the p.Val14Ile variant compared with other KRAS variants (Fig. 4).

The forest plot analysis showed an overall trend toward lower odds of several standardized HPO phenotypes in patients carrying the p.Val14Ile variant compared with those carrying other KRAS variants. Most of the HPO phenotypes included in the analysis had ORs below 1. The largest reductions in estimated odds were observed for hair abnormalities (OR = 0.10, 95% CI: 0.01–1.82), central nervous system abnormalities (OR = 0.14, 95% CI: 0.01–2.48), and urinary system abnormalities (OR = 0.22, 95% CI: 0.01–4.15). In contrast, the ORs for skeletal, ocular, and ear abnormalities were 1.22, 1.32, and 1.81, respectively. However, the 95% CIs for all individual HPO phenotypes crossed 1, and none of the individual phenotypes showed a statistically significant association with the p.Val14Ile variant.

Pooled analysis yielded an overall OR of 0.63 (95% CI: 0.40–1.01) under the common-effect model and 0.63 (95% CI: 0.42–0.96) under the random-effects model. These findings suggest that, compared with other KRAS variants, the p.Val14Ile variant was associated with lower overall odds of the evaluated phenotypic abnormalities. No significant heterogeneity was observed across the evaluated HPO phenotypes ($I^2 = 0\%$, $\tau^2 = 0$, $P = 0.7938$).

Overall, carriers of the p.Val14Ile variant showed a consistent trend toward lower odds of the evaluated HPO phenotypes compared with carriers of other KRAS variants. Although none of the individual HPO phenotype analyses reached statistical significance, the pooled analysis using the random-effects model indicated a statistically significant association between p.Val14Ile and lower overall odds of the

evaluated phenotypic abnormalities (OR = 0.63, 95% CI: 0.42–0.96). The absence of significant heterogeneity across the evaluated phenotypes ($I^2 = 0\%$, $P = 0.7938$) suggests that the direction of the overall effect was relatively consistent across the different HPO phenotype categories.

Molecular docking and molecular dynamics simulations of KRAS p.Val14Ile

Based on a systematic analysis of KRAS variants and their associated clinical phenotypes, we found that patients carrying the p.Val14Ile variant exhibited an overall trend toward lower odds of the evaluated HPO phenotypes compared with patients carrying other KRAS variants. Further analysis revealed that the KRAS variant identified in the present study, NM_004985.4:c.40G>A (p.Val14Ile), is located in the N-terminal region of the KRAS protein. Given that amino acid substitutions may affect local protein conformation, structural stability, and intramolecular interactions, thereby altering protein function, we hypothesized that the p.Val14Ile variant may contribute to the associated disease phenotype by altering the structural and conformational dynamics of KRAS. To further investigate the potential molecular mechanisms underlying this variant, molecular dynamics (MD) simulations were performed to comparatively evaluate the structural stability, conformational dynamics, and key intermolecular interactions of wild-type KRAS and the p.Val14Ile mutant protein. The results are presented below.

p.Val14Ile mutation reduces the predicted binding affinity of KRAS for GTP

In Fig. 5A, molecular docking analysis showed that the binding energies of GTP to wild-type KRAS and the p.Val14Ile mutant KRAS were -8.387 and -7.829 kcal·mol⁻¹, respectively. The more favorable binding energy observed for GTP binding to wild-type KRAS suggests a higher binding affinity than that of the p.Val14Ile mutant, indicating that the Val14Ile substitution may reduce the binding affinity of KRAS for GTP. However, molecular docking primarily represents a relatively static binding state and does not fully account for protein flexibility, temperature, pressure, or solvent effects. Therefore, to further assess the effects of the p.Val14Ile substitution on the binding affinity and stability of the KRAS–GTP

complex, 100-ns molecular dynamics (MD) simulations were performed for both the wild-type KRAS–GTP and p.Val14Ile mutant KRAS–GTP complexes.

As shown in Fig. 5B, the RMSD trajectory of the wild-type KRAS–GTP complex exhibited no substantial fluctuations and remained relatively stable at approximately 0.2 nm throughout the simulation. In contrast, the RMSD trajectory of the p.Val14Ile mutant KRAS–GTP complex showed noticeable changes around 35 ns and 80 ns, suggesting greater conformational fluctuations during the simulation. From the perspective of RMSD, the wild-type KRAS–GTP complex therefore exhibited greater structural stability than the p.Val14Ile mutant complex.

p.Val14Ile mutation alters the dynamic stability of the KRAS-GTP complex

As shown in Fig. 5C, the radius of gyration (Rg) of the wild-type KRAS–GTP complex was slightly more stable than that of the p.Val14Ile mutant KRAS–GTP complex, further suggesting a relatively higher conformational stability of the wild-type complex. Figure 5D shows that the solvent-accessible surface area (SASA) of the wild-type KRAS–GTP complex exhibited slightly smaller fluctuations than that of the mutant complex, with both remaining at approximately 90 nm² throughout the simulation. These findings indicate that the overall structural stability of KRAS remained relatively high and that the p.Val14Ile substitution did not result in a substantial disruption of the global protein structure. Taken together, these results suggest that p.Val14Ile primarily alters the dynamic behavior of the KRAS–GTP complex rather than causing substantial disruption of the overall protein structure.

p.Val14Ile mutation increases local flexibility of the KRAS protein

As shown in Fig. 5E, comparison of the RMSF profiles of wild-type and p.Val14Ile mutant KRAS revealed increased local fluctuations in the region spanning residues 30–40 following the substitution at residue 14. The amplitude of fluctuation in this region was slightly greater in the mutant protein than in the wild-type protein, suggesting that the p.Val14Ile substitution may reduce the local structural stability of KRAS. In contrast, the RMSF profiles of the remaining regions were largely comparable between the wild-type and mutant proteins, indicating that the p.Val14Ile substitution had minimal effects on the overall dynamic stability of other regions of

the KRAS protein.

p.Val14Ile mutation alters the conformational landscape of the KRAS-GTP complex

Structural superposition of the KRAS–GTP complexes at 0, 25, 50, 75, and 100 ns showed that GTP remained within the same binding pocket throughout the simulation in both the wild-type (WT) and p.Val14Ile systems, with no apparent large-scale positional displacement. These findings indicate that the overall binding mode of GTP was maintained during the simulation (Fig. 5F). The free-energy landscape (FEL) analysis of the wild-type KRAS–GTP complex revealed three relatively concentrated low-energy conformational clusters, whereas the p.Val14Ile mutant KRAS–GTP complex exhibited two distinctly dispersed low-energy clusters. This difference indicates that the low-energy conformational states of the mutant complex were more broadly distributed than those of the wild-type complex, suggesting that the p.Val14Ile substitution may alter the conformational sampling space and preferential conformational states of the KRAS–GTP complex (Fig. 5G).

p.Val14Ile mutation weakens the interaction network between KRAS and GTP

To investigate the hydrogen-bonding characteristics of the binding interface, we quantified the number of hydrogen bonds between GTP and the KRAS protein, which represent important stabilizing interactions within the complex. As shown in Fig. 5H, the number of hydrogen bonds between GTP and wild-type KRAS remained relatively stable at approximately 7–9 throughout the simulation, whereas the p.Val14Ile mutant KRAS–GTP complex maintained approximately 6–8 hydrogen bonds. The overall number of hydrogen bonds was slightly reduced following the p.Val14Ile substitution, consistent with the molecular docking results. These findings suggest that the p.Val14Ile substitution may modestly reduce the stability of the KRAS–GTP interaction.

As shown in Fig. 5I, GTP formed favorable interactions with the KRAS residues Asp119, Tyr32, Asp33, and Gly13, with estimated interaction energies of -5.7 , -3.0 , -2.1 , and -1.9 kcal·mol⁻¹, respectively. In the p.Val14Ile mutant KRAS, GTP formed

favorable interactions with Asp119, Gly15, and Phe28, with corresponding interaction energies of -5.8 , -2.4 , and -1.8 kcal·mol⁻¹, respectively. Comparison of the residue-level interaction energies indicated that the Val14Ile substitution altered the contribution of several residues to GTP binding. In particular, the interaction contribution of Tyr32 decreased from -3.0 kcal·mol⁻¹ in the wild-type complex to approximately -1.0 kcal·mol⁻¹ in the mutant complex, while that of Gly13 decreased from -1.9 kcal·mol⁻¹ to approximately -1.1 kcal·mol⁻¹. In contrast, the interaction contribution of Gly15 increased from -1.5 kcal·mol⁻¹ to -2.4 kcal·mol⁻¹ following the substitution. Taken together, these changes in interaction energies suggest that the p.Val14Ile substitution alters the interaction network and residue contribution pattern at the GTP-binding interface of KRAS.

p.Val14Ile mutation decreases the binding free energy of the KRAS-GTP complex

After equilibration of the complex systems, the mean binding free energies were calculated using the molecular mechanics/generalized Born surface area (MM/GBSA) method. As shown in Fig. 5J, the mean binding free energies of GTP binding to wild-type KRAS and p.Val14Ile mutant KRAS were -48.64 and -34.27 kcal·mol⁻¹, respectively. The less favorable binding free energy observed for the mutant complex suggests a reduced binding affinity of KRAS for GTP following the p.Val14Ile substitution. Collectively, these findings indicate that p.Val14Ile alters the conformational dynamics and interaction energy profile of the KRAS–GTP complex, potentially contributing to changes in its molecular behavior.

Discussion

In this study, we systematically investigated the phenotypic and molecular characteristics of the KRAS p.Val14Ile variant and compared its clinical phenotype spectrum with those associated with other KRAS variants. Forest plot analysis showed that the odds ratios (ORs) for most HPO phenotypes were below 1 in the p.Val14Ile group, suggesting an overall lower occurrence of the evaluated phenotypes. Although the confidence intervals for individual HPO categories crossed 1,

precluding definitive conclusions regarding specific phenotype associations, the pooled random-effects analysis yielded an OR of 0.63 (95% CI, 0.42–0.96). Notably, no significant heterogeneity was detected among the included HPO phenotypes ($I^2 = 0\%$). These findings suggest that p.Val14Ile may be associated with a phenotypic profile that differs from those of other KRAS variants. Importantly, the observed associations should be interpreted cautiously given the limited sample size and the uncertainty surrounding individual phenotype estimates.

KRAS is a central molecular switch in the RAS/MAPK pathway, and its activity is regulated by the GDP/GTP cycle. Increasing evidence indicates that germline KRAS variants are functionally heterogeneous and can perturb distinct aspects of nucleotide exchange, GTP hydrolysis, GAP/GEF regulation, and effector-protein interactions [18,19]. Consequently, the clinical consequences of KRAS variants cannot be predicted solely from the presence of a mutation; rather, the specific amino acid substitution and its structural context may determine the resulting biochemical and signaling properties [19–21]. This concept is particularly relevant to p.Val14Ile, which is located in the nucleotide-binding region of KRAS. Previous biochemical and cellular studies have demonstrated that V14I impairs intrinsic GTP hydrolysis and reduces responsiveness to GAP stimulation, while also altering growth-factor responses in hematopoietic progenitor cells [1]. Systematic functional analyses of germline KRAS variants have further shown that different substitutions can produce distinct combinations of altered nucleotide exchange, GTP hydrolysis, and effector interactions [19]. Thus, p.Val14Ile is unlikely to be adequately characterized by a simple gain- or loss-of-function framework.

To explore the structural basis underlying these functional differences, we combined molecular docking and molecular dynamics (MD) simulations. Molecular docking predicted a modest change in the GTP-binding energy following p.Val14Ile substitution, accompanied by a reduction in the number of hydrogen bonds between GTP and KRAS. Because docking provides a relatively static representation of ligand–protein interactions, these findings should not be interpreted as direct evidence of altered intracellular KRAS-GTP levels or signaling activity. Rather, they provided

an initial indication that the substitution may modify the local interaction environment of the nucleotide-binding region. This interpretation is consistent with the established biology of KRAS, in which functional output is determined not only by nucleotide binding but also by the dynamic interplay among nucleotide exchange, GTP hydrolysis, Mg^{2+} coordination, and interactions with GEFs, GAPs, and downstream effectors [2,5].

The subsequent 100-ns MD simulations provided evidence that p.Val14Ile primarily affects local rather than global structural dynamics. The mutant complex exhibited increased conformational fluctuations at approximately 35 and 80 ns, while RMSF analysis identified enhanced flexibility in the 30–40 residue region. This region encompasses Switch I, a critical structural element involved in nucleotide binding and recognition by downstream effectors [20–22]. In contrast, the relatively limited changes in Rg and the preservation of SASA values around 90 nm² suggest that the mutation does not substantially destabilize the overall protein architecture. These findings support a model in which p.Val14Ile induces localized dynamic perturbations that may propagate from the nucleotide-binding region toward functionally important Switch I residues. Such a mechanism is consistent with previous MD and enhanced-sampling studies showing that KRAS mutations can preserve the overall fold while substantially altering local flexibility, correlated motions, and conformational equilibria [20,23].

Analysis of the KRAS-GTP interaction network further supported this interpretation. Compared with the wild-type complex, the mutant maintained slightly fewer hydrogen bonds with GTP and displayed altered contributions from several key residues. In particular, the energetic contributions of TYR32 and GLY13 were altered, whereas GLY15 showed an increased contribution following the mutation. These changes suggest that p.Val14Ile does not simply weaken GTP binding but instead reorganizes the local noncovalent interaction network surrounding the nucleotide-binding site. Because Switch I residues are directly involved in effector recognition, even relatively subtle changes in their dynamic environment may influence the conformational states accessible to KRAS [20,23]. Importantly, the

persistence of GTP within the binding pocket throughout the simulation argues against complete disruption of nucleotide binding and instead favors a model involving altered interaction dynamics.

This interpretation was further supported by the free-energy landscape analysis. The wild-type KRAS-GTP complex displayed several relatively concentrated low-energy conformational clusters, whereas the mutant exhibited a more dispersed distribution of low-energy states. This finding suggests that p.Val14Ile may alter the conformational ensemble sampled by KRAS rather than inducing a single gross structural rearrangement. Such redistribution of conformational states is potentially important because KRAS signaling is intrinsically dependent on dynamic transitions among nucleotide- and effector-associated conformations. Previous studies have similarly shown that mutations in the P-loop and other functionally important regions can modify the conformational flexibility and free-energy distribution of the Switch I/II regions [23–25].

Consistent with these structural observations, MM/GBSA analysis showed that the calculated mean binding free energy of the KRAS-GTP complex changed from -48.64 kcal/mol for wild-type KRAS to -34.27 kcal/mol for p.Val14Ile. Although this result indicates a less favorable calculated binding free energy under the conditions of our simulation, it should not be equated with reduced KRAS activity. In particular, KRAS signaling cannot be inferred from GTP-binding affinity alone, because nucleotide exchange and hydrolysis, as well as interactions with regulatory proteins and downstream effectors, collectively determine the cellular signaling state [18,19]. Therefore, the MM/GBSA result is better interpreted as evidence that p.Val14Ile alters the thermodynamic characteristics of a specific KRAS-GTP conformational ensemble. Together with the docking, hydrogen-bond, residue-energy, and free-energy landscape analyses, these findings consistently point toward remodeling of the local interaction and conformational environment rather than global structural destabilization.

Taken together, our results support a mechanistic model in which p.Val14Ile induces a sequence of interconnected structural effects: local perturbation of the nucleotide-binding region, altered interaction networks around GTP, increased

flexibility of the adjacent Switch I region, and redistribution of the KRAS conformational ensemble. This model provides a potential structural explanation for the functional heterogeneity observed among germline KRAS variants. Notably, the conservative nature of the Val-to-Ile substitution does not necessarily imply a negligible functional effect. Because Val14 is positioned within a structurally and functionally important region, even a subtle change in side-chain properties may be propagated through dynamic coupling to neighboring residues and regulatory regions. This concept is consistent with previous evidence that distinct KRAS variants can produce different biochemical phenotypes despite preserving a highly similar overall protein structure [19–21,23,24].

The structural findings may also provide a potential explanation for the phenotypic differences observed in the present study. The lower overall frequency of HPO phenotypes in the p.Val14Ile group, together with the particularly low point estimate for hematologic/lymphatic abnormalities (OR, 0.29), raises the possibility that this variant produces a distinct signaling output compared with other KRAS variants. This interpretation is biologically plausible given previous evidence that V14I affects the behavior of hematopoietic progenitor cells [1]. However, the confidence interval for this phenotype crossed 1, and therefore the present data do not establish a protective effect or a causal relationship between p.Val14Ile and reduced hematologic/lymphatic abnormalities. Instead, the findings support the broader hypothesis that variant-specific alterations in KRAS conformational dynamics may contribute to the clinical heterogeneity of RASopathies.

Our study has several limitations. First, the relatively wide confidence intervals for individual HPO phenotypes indicate limited statistical power, and the pooled association should therefore be interpreted as evidence of an overall phenotypic trend rather than proof of a specific phenotype–variant relationship. Second, the 100-ns MD simulations may not adequately capture long-timescale conformational transitions or rare states of KRAS. Replicated longer-timescale simulations and enhanced-sampling approaches, such as GaMD, could provide more robust estimates of the conformational landscape and dynamic coupling associated with p.Val14Ile [23–25].

Third, molecular docking and MM/GBSA are computational approaches and cannot substitute for experimental measurements of KRAS biochemical activity. Future studies should therefore directly assess GDP/GTP exchange, intrinsic and GAP-mediated GTP hydrolysis, GEF/GAP responsiveness, KRAS-GTP abundance, and downstream RAF/MEK/ERK signaling. Cell-based and animal models will also be important for determining whether the structural effects identified here translate into altered tissue-specific phenotypes, particularly within the hematopoietic system.

In conclusion, our findings indicate that KRAS p.Val14Ile may exhibit a distinct clinical and molecular phenotype compared with other KRAS variants. Although the variant does not appear to disrupt the overall KRAS structure, it induces localized changes in conformational dynamics, particularly around the nucleotide-binding region and Switch I, accompanied by remodeling of GTP-associated interactions, redistribution of conformational states, and altered calculated binding free energy. These findings, together with previous biochemical evidence of abnormal nucleotide cycling and GTP hydrolysis, support a model in which p.Val14Ile produces a variant-specific alteration in the KRAS functional conformational landscape rather than a simple gain- or loss-of-function effect. Such a mechanism may contribute to the clinical heterogeneity associated with different KRAS variants and provides a structural framework for future experimental investigation of p.Val14Ile-associated disease mechanisms.

Acknowledgements

We are grateful to all the patients and their parents for the genuine interest in their metabolic health.

Author contributions

LX: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Methodology, Project administration, Supervision, Visualization, Writing-review & editing. YWT: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Writing-original draft. YSH: collect data and organize literature related to the article. LCQ: Collect data, retrieve and organize literature related to the manuscript, and cross-check the

details.LIX:Writing-review&editing.WXT:Funding acquisition and final approval of the manuscript.WSP:Investigation,Writing-review&editing.

Funding

This research was supported by Natural Science Foundation of Dongying City (Grant No.2025ZRWS068,2024ZRWS031), and Shandong Provincial Medical and Health Science and Technology Development Project (202103060606, 2018WS520). We are grateful for their financial support.

Data availability

Data Availability: The whole exome sequencing data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1540175 (BioSample SAMN64008568; SRA accession SRR31031147). The data are publicly available at <https://www.ncbi.nlm.nih.gov/sra/PRJNA1540175>.

Declarations

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

References

- [1]Roberts, A. E., Allanson, J. E., Tartaglia, M. et al. Noonan syndrome. *Lancet* 381, 333-342 (2013).
- [2]Noonan, J. A. Noonan syndrome. An update and review for the primary pediatrician. *Clin. Pediatr.* 33, 548-555 (1994).
- [3]Johnston, J. J., van der Smagt, J. J., Rosenfeld, J. A. et al. Autosomal recessive Noonan syndrome associated with biallelic LZTR1 variants. *Genet. Med.* 20, 1175-1185 (2018).
- [4]Mendez, H. M. & Opitz, J. M. Noonan syndrome: a review. *Am. J. Med. Genet.* 21, 493-506 (1985).
- [5]Tidyman, W. E. & Rauen, K. A. The RASopathies: developmental syndromes of Ras/MAPK pathway dysregulation. *Curr. Opin. Genet. Dev.* 19, 230-236 (2009).

- [6]Romano, A. A., Allanson, J. E., Dahlgren, J. et al. Noonan syndrome clinical features, diagnosis, and management guidelines. *Pediatrics* 126, 746-759 (2010).
- [7]Aoki, Y., Niihori, T., Banjo, T. et al. Gain-of-function mutations in RIT1 cause Noonan syndrome, a RAS/MAPK pathway syndrome. *Am. J. Hum. Genet.* 93, 173-180 (2013).
- [8]Simanshu, D. K., Nissley, D. V. & McCormick, F. RAS proteins and their regulators in human disease. *Cell* 170, 17-33 (2017).
- [9]Boriack-Sjodin, P. A., Margarit, S. M., Bar-Sagi, D. & Kuriyan, J. The structural basis of the activation of Ras by Sos. *Nature* 394, 337-343 (1998).
- [10]Li, X., Wang, Z., Qi, C. et al. A case of Noonan syndrome type 3 caused by KRAS variants and analysis of clinical phenotype spectrum. *Int. Med. Health Guid. News* 31, 3076-3080 (2025) (in Chinese).
- [11]Miller, D. T., Lee, K., Abul-Husn, N. S. et al. ACMG SF v3.2 list for reporting of secondary findings in clinical exome and genome sequencing: A policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genet. Med.* 25, 100866 (2023).
- [12]Costain, G., Walker, S., Marano, M. et al. Genome sequencing as a diagnostic test in children with unexplained medical complexity. *JAMA Netw. Open* 3, e2018109 (2020).
- [13]Lo, F. S., Lin, J. L., Kuo, M. T. et al. Noonan syndrome caused by germline KRAS mutation in Taiwan: report of two patients and a review of the literature. *Eur. J. Pediatr.* 168, 919-923 (2009).
- [14]Monies, D., Abouelhoda, M., Assoum, M. et al. Lessons learned from large-scale, first-tier clinical exome sequencing in a highly consanguineous population. *Am. J. Hum. Genet.* 104, 1182-1201 (2019).
- [15]Schubbert, S., Zenker, M., Rowe, S. L. et al. Germline KRAS mutations cause Noonan syndrome. *Nat. Genet.* 38, 331-336 (2006).
- [16]Gremer, L., Merbitz-Zahradnik, T., Dvorsky, R. et al. Germline KRAS mutations cause aberrant biochemical and physical properties leading to developmental disorders. *Hum. Mutat.* 32, 33-43 (2011).

- [17]Gelb, B. D., Cavé, H., Dillon, M. W. et al. ClinGen's RASopathy Expert Panel consensus methods for variant interpretation. *Genet. Med.* 20, 1334-1345 (2018).
- [18]Schubbert, S., Zenker, M., Rowe, S. L. et al. Germline KRAS mutations cause Noonan syndrome. *Nat. Genet.* 38, 331-336 (2006).
- [19]Gremer, L., Merbitz-Zahradnik, T., Dvorsky, R. et al. Germline KRAS mutations cause aberrant biochemical and physical properties leading to developmental disorders. *Hum. Mutat.* 32, 33-43 (2011).
- [20]Chen, C. C., Er, T. K., Liu, Y. Y. et al. Computational analysis of KRAS mutations: Implications for different effects on the KRAS p.G12D and p.G13D mutations. *PLoS One* 8, e55793 (2013).
- [21]Cirstea, I. C., Gremer, L., Dvorsky, R. et al. Diverging gain-of-function mechanisms of two novel KRAS mutations associated with Noonan and cardio-facio-cutaneous syndromes. *Hum. Mol. Genet.* 22, 262-270 (2013).
- [22]Schubbert, S., Shannon, K. & Bollag, G. Hyperactive Ras in developmental disorders and cancer. *Nat. Rev. Cancer* 7, 295-308 (2007).
- [23]Shi, S., Zheng, L., Ren, Y. & Wang, Z. Impacts of mutations in the P-loop on conformational alterations of KRAS investigated with Gaussian accelerated molecular dynamics simulations. *Molecules* 28, 2886 (2023).
- [24]Sayyed-Ahmad, A., Prakash, P. & Gorfe, A. A. Distinct dynamics and interaction patterns in H- and K-Ras oncogenic P-loop mutants. *Proteins* 85, 1618-1632 (2017).
- [25]Insight into structural dynamics involved in activation mechanism of full length KRAS wild type and P-loop mutants. *Heliyon* 10, e36161 (2024).
- [26]Conformational transformation of switch domains in GDP/K-Ras induced by G13 mutants: An investigation through Gaussian accelerated molecular dynamics simulations and principal component analysis.

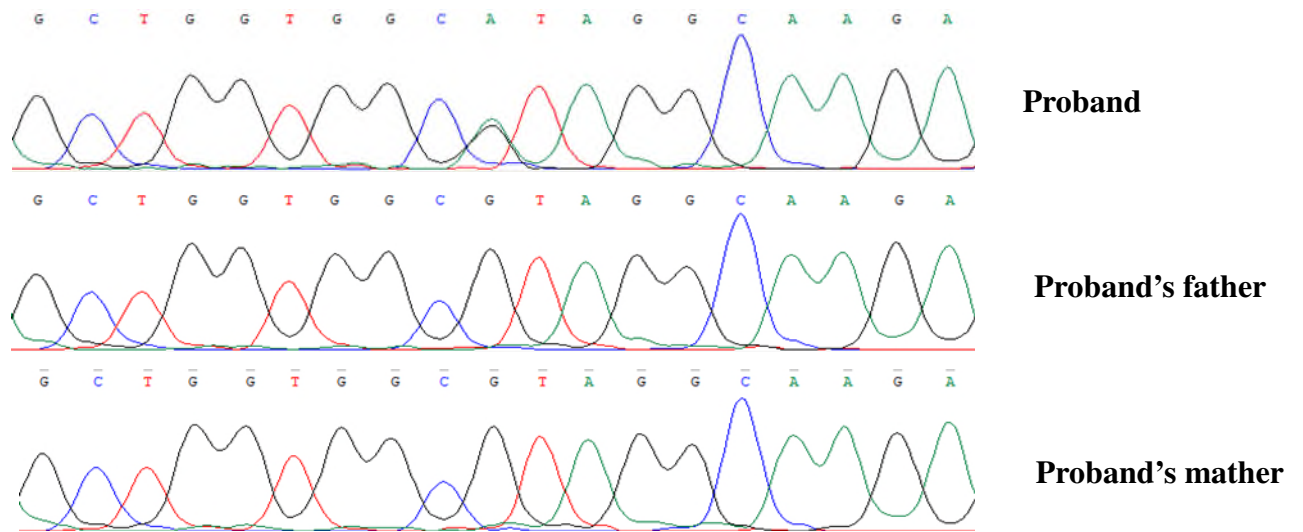
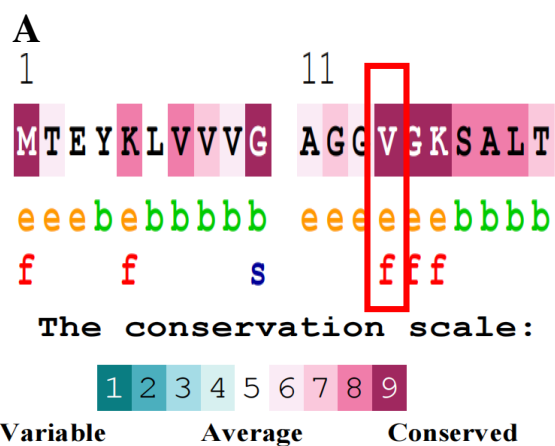
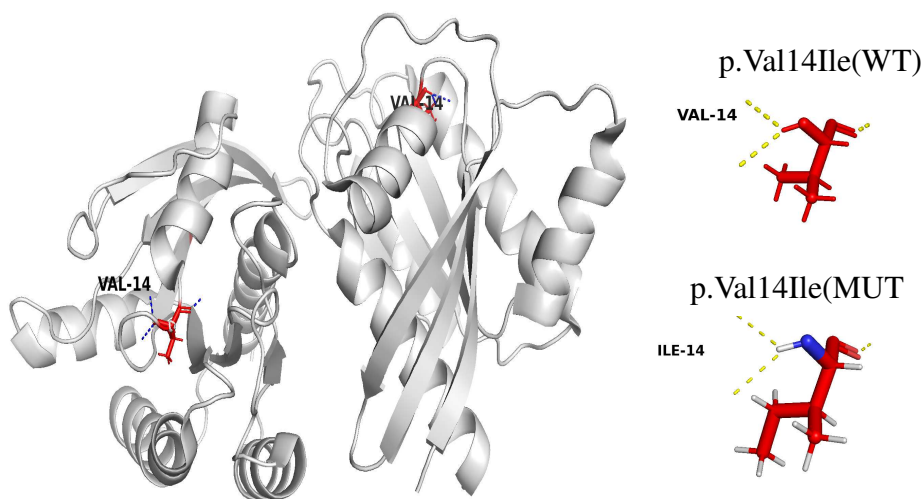


Figure 1. Sanger sequencing analysis of the patient and his/her parents

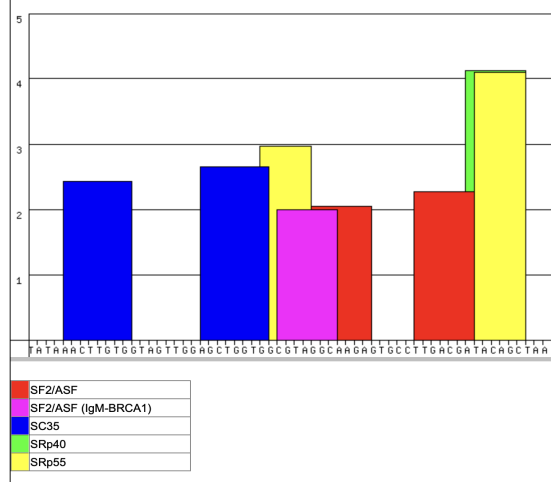


B



C

Graphical results



Graphical results

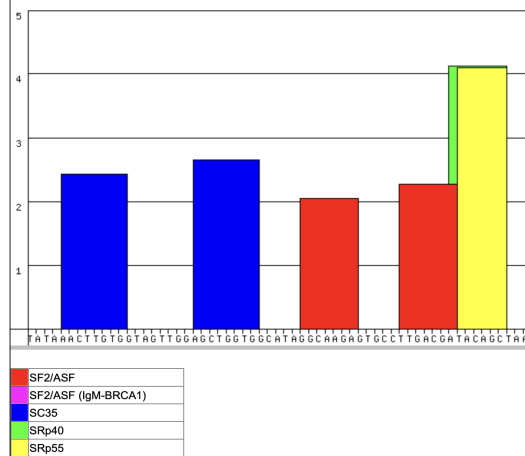


Figure 2. The bioinformatics analysis of c.40G>A. (A) ConSurf conservation analysis of c.40G>A, with deeper purple indicating higher conservation. The red box highlights the mutation sites. (B) ESE Finder 2.0 predicts that the c.40G>A mutation disrupts 1 SF2/ASF (IgM-BRCA1) binding motif and 1 SRp55 binding motif. The original SC35, SRp40, and standard SF2/ASF binding motifs remain intact post-mutation, and no new motifs are formed. (C) Positions of c.40G>A in the tertiary structure of the KRAS protein. Structural visualizations and comparative analyses of hydrogen bonding interactions were performed using PyMOL software (v2.5).

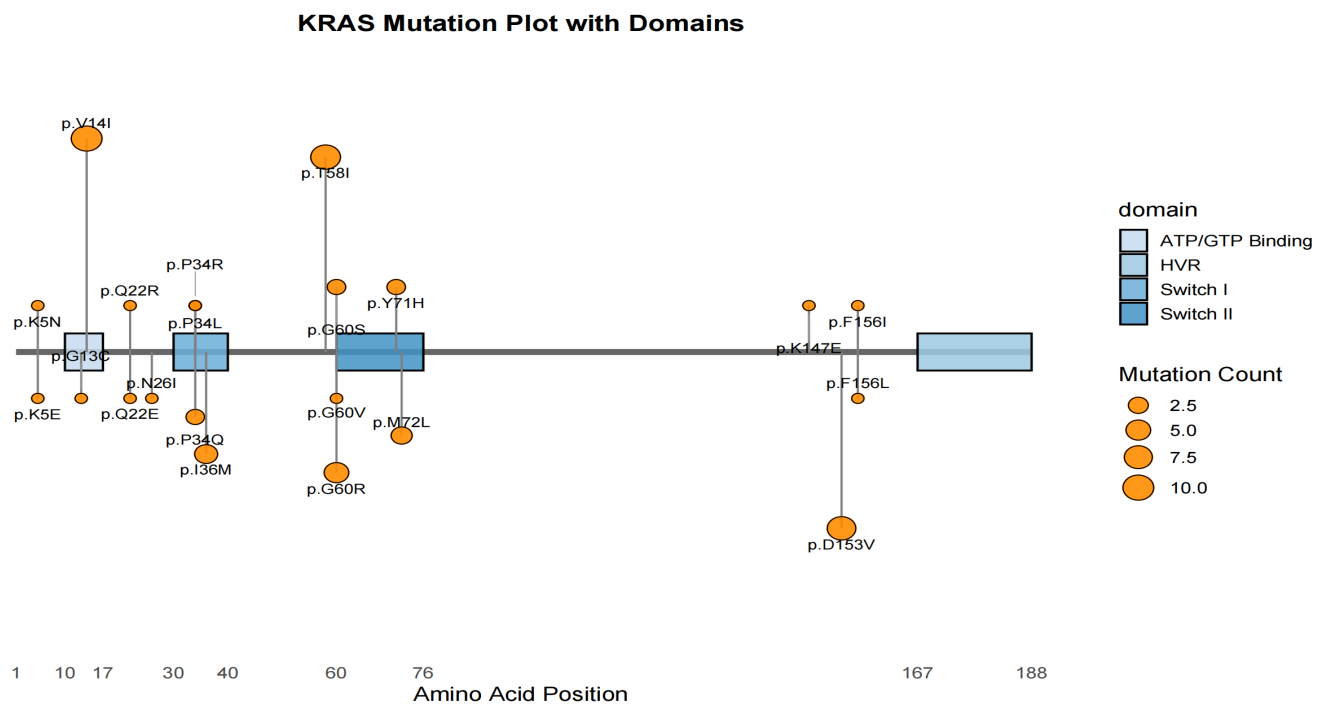


Figure 3. Locations of 21 different KRAS allelic mutations in the secondary structure.

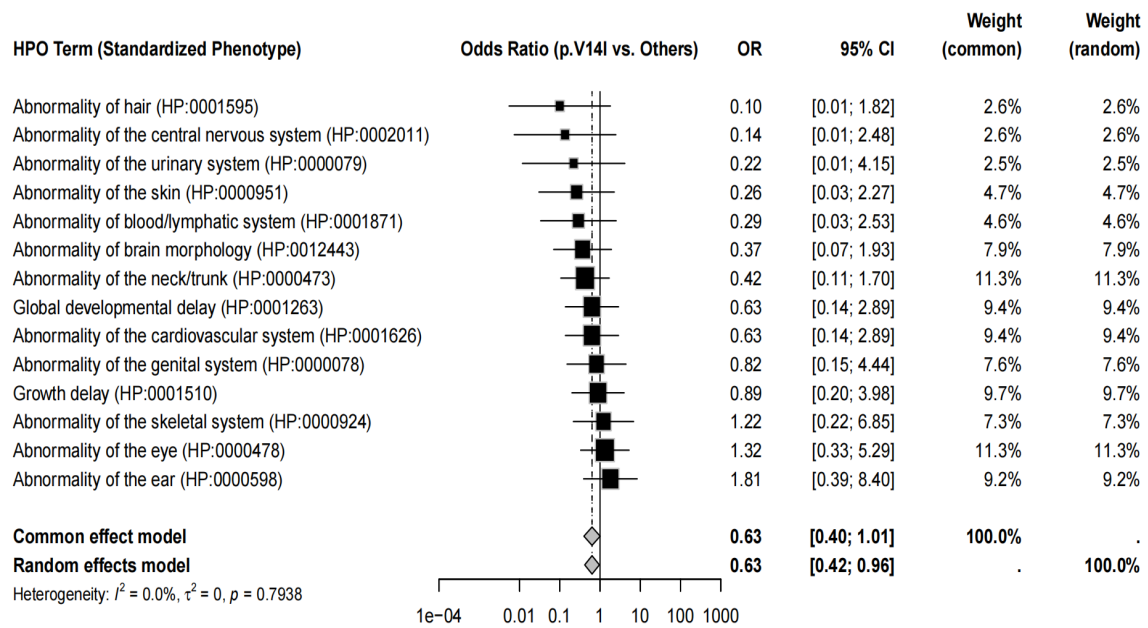
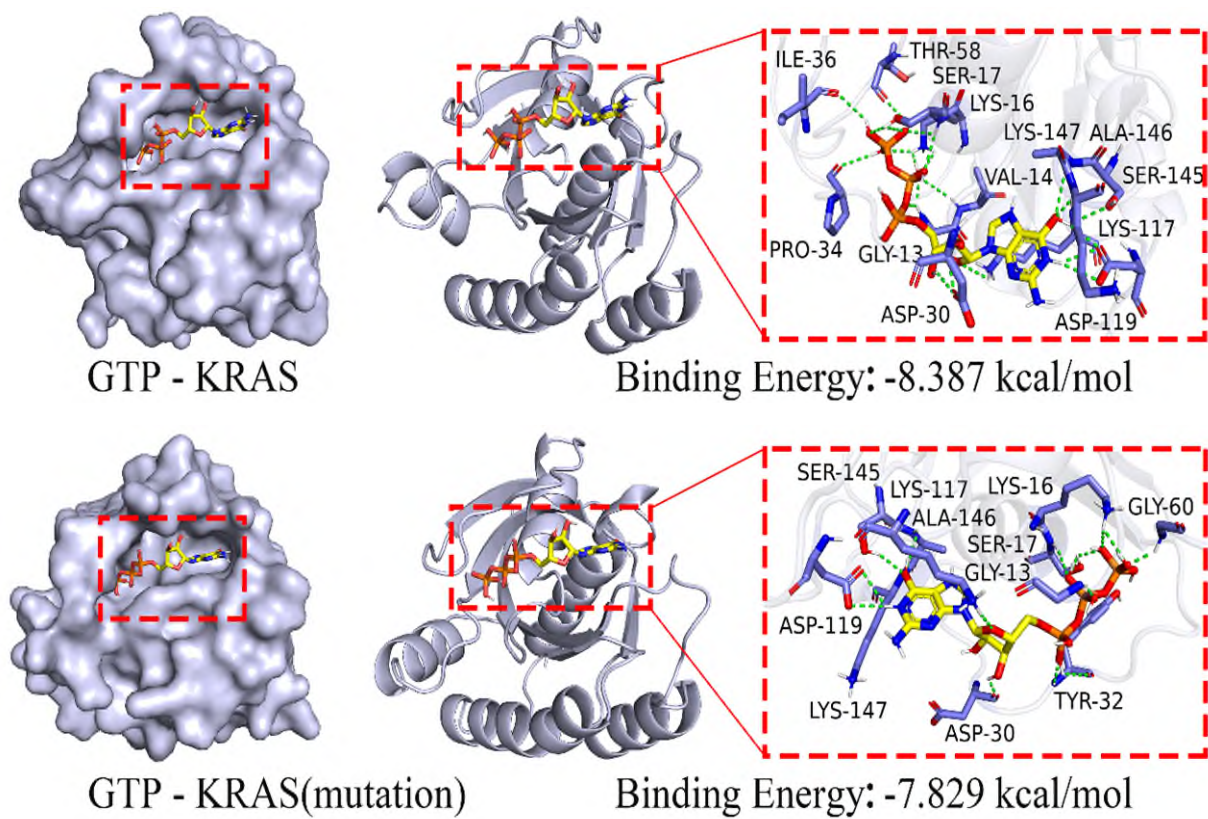
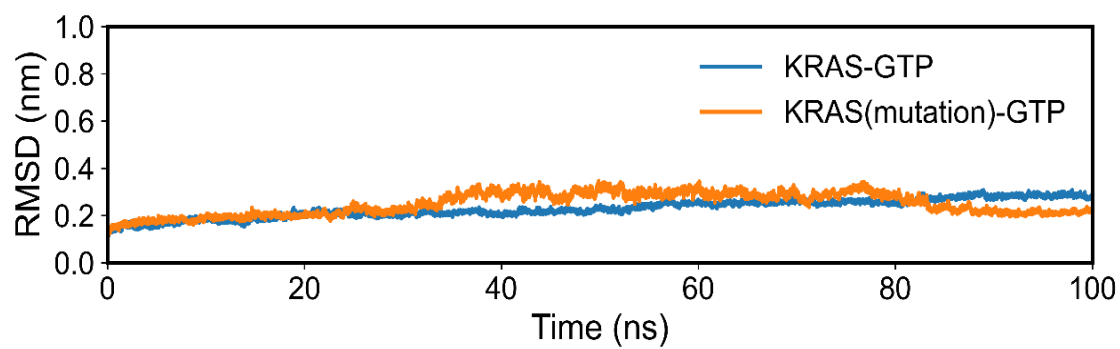
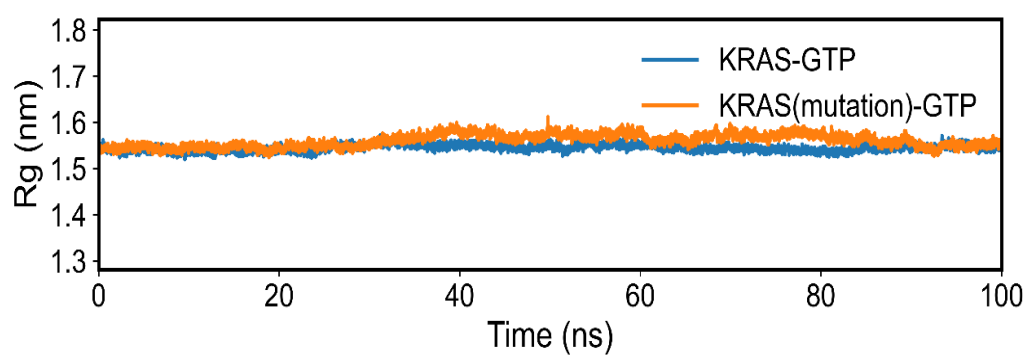
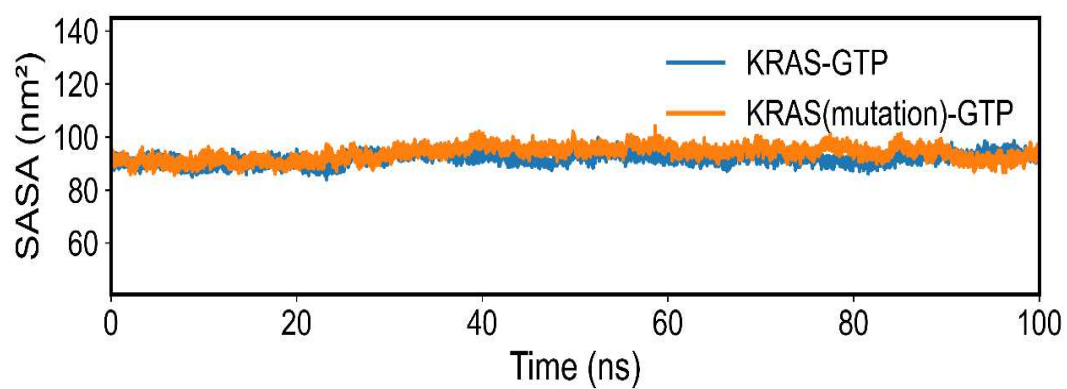
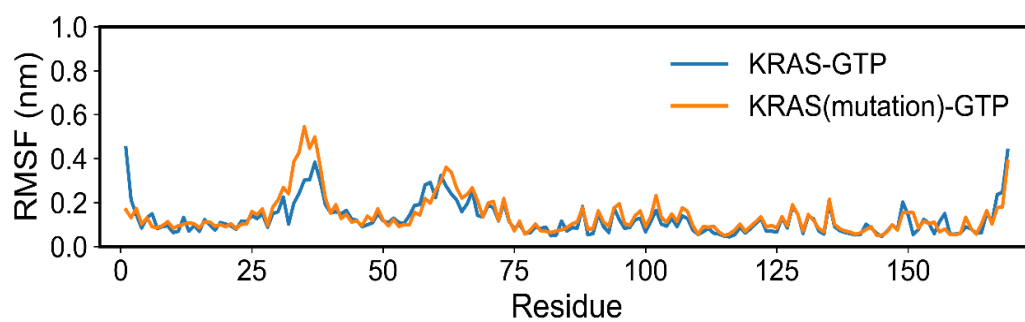
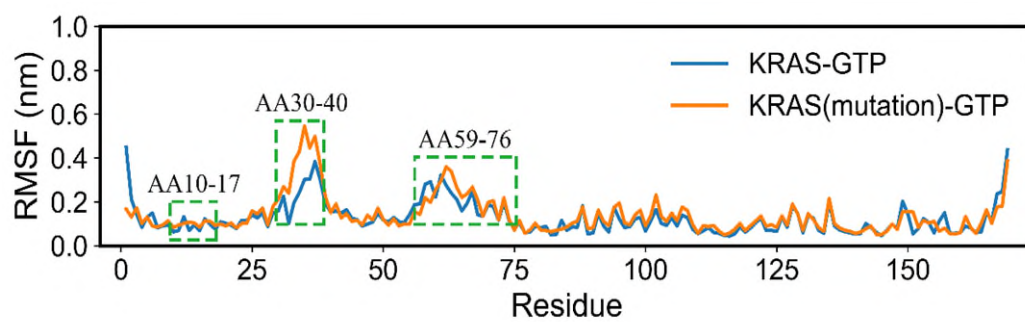
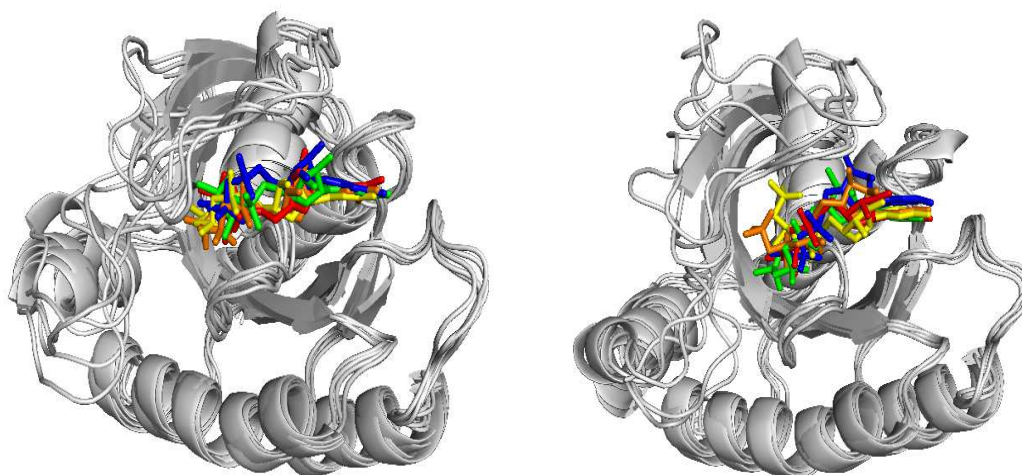
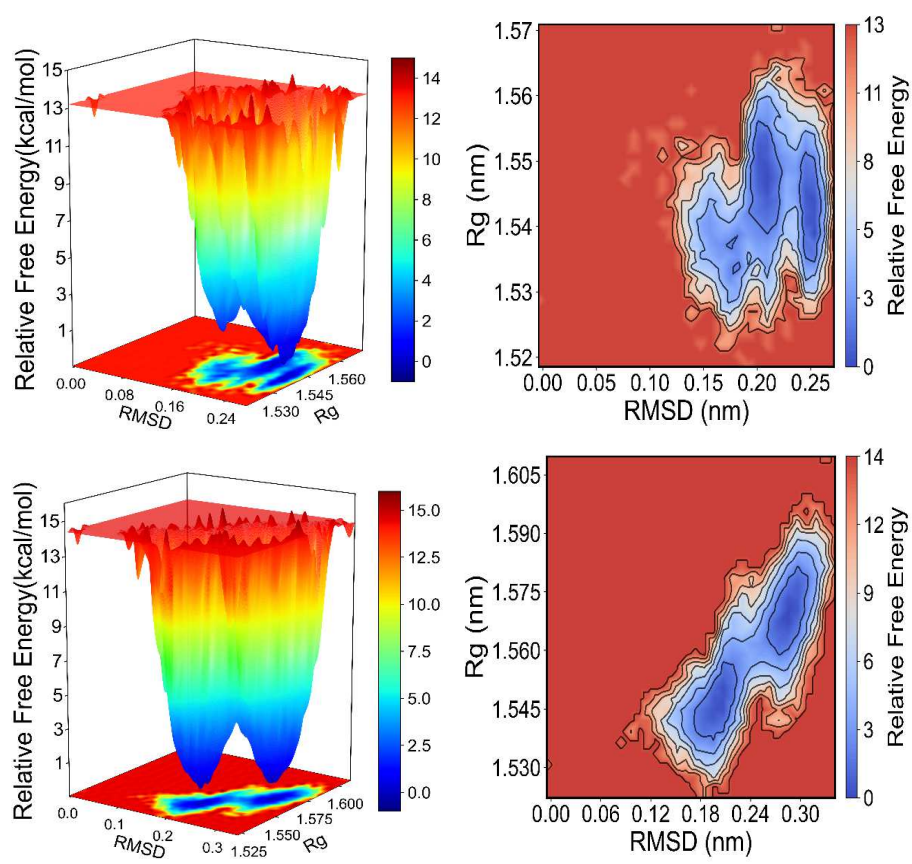


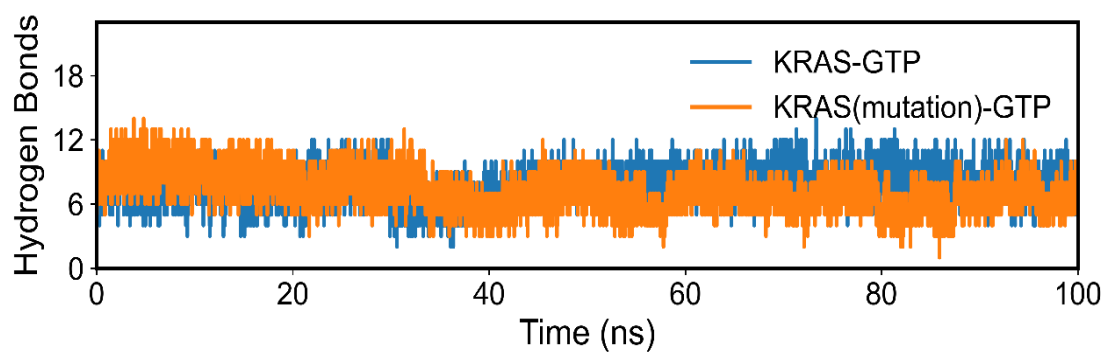
Figure 4.forest plot of odds ratios for standardized phenotypes (HPO terms) comparing p.V14I vs. others

A**B**

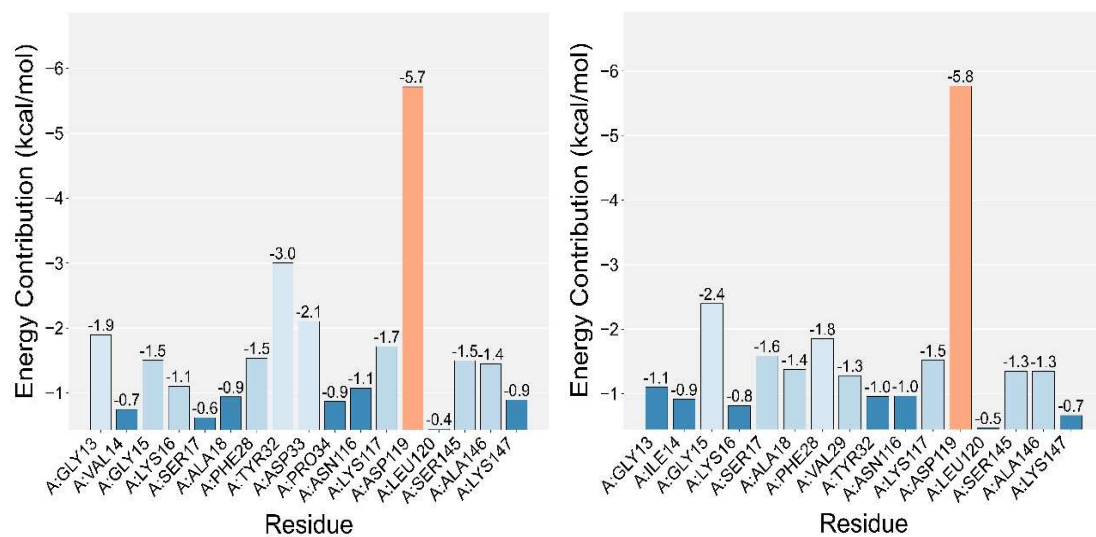
C**D****E**

F**G**

H



I



J

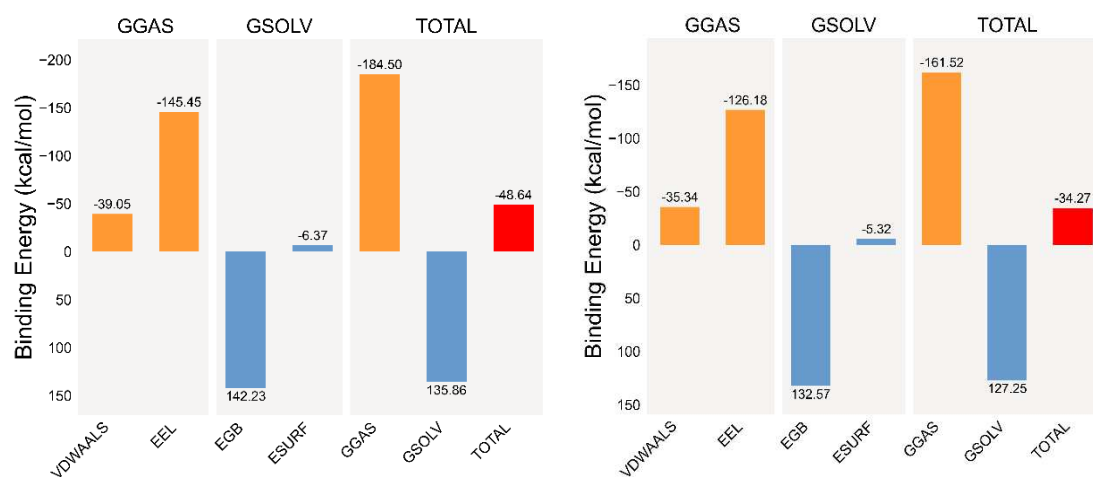


Figure 5.(A)Molecular docking visualization diagram;(B)Molecular dynamics simulation results: RMSD curve;(C)Molecular dynamics simulation results: Rg curve;(D)Molecular dynamics simulation results: SASA curve;(E)Molecular dynamics simulation results: RMSF curve;(F)Structural comparison from the molecular dynamics simulation at 0, 25, 50, 75, and 100 ns. The small molecules colored in red, green, blue, yellow, and orange correspond to the GTP structures at 0, 25, 50, 75, and 100 ns, respectively;(G)Molecular dynamics simulation results: Free energy landscape;(H)Molecular dynamics simulation results: Hydrogen bond number fluctuation curve;(I)Molecular dynamics simulation results: Per-residue energy contribution;(J)Molecular dynamics simulation results: Average binding free energy. VDWAALS, EEL, EGB, ESURF, GGAS, GSOLV, and TOTAL represent van der Waals energy, electrostatic energy, polar solvation energy, nonpolar solvation energy, gas-phase molecular mechanics energy, solvation free energy, and the total average binding free energy, respectively.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [table1.docx](#)
- [Table2.docx](#)
- [SupplementaryTable1.docx](#)
- [SupplementaryTextS1.docx](#)
- [ComputationalStructuralData.zip](#)