A novel heterozygous *ZBTB18* missense mutation in a family with non-syndromic intellectual disability and structural brain abnormalities

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Research

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Abstract

Background

Intellectual Disability (ID) is a characterized by significantly impaired adaptive behavior and cognitive capacity. Currently, high throughput sequencing approaches have led to the identification of genetic causes for ID in 25-50% of cases, while the causes of inherited ID are less well known. Here, we have investigated the genetic cause for non-syndromic ID in a Han Chinese family.

Methods

Whole genome sequencing was performed on identical twin sisters diagnosed with ID, their respective children as well as their asymptomatic parents. Data was filtered for rare variants and in silico prediction tools used to establish pathogenic alleles. Candidate mutations were validated by Sanger sequencing. Molecular modelling was conducted to establish the effects of the mutation on the protein encoded by a candidate coding gene.

Results

A novel heterozygous variant in the *ZBTB18* gene c.1323C>G (p.His441Gln) was identified. This variant co-segregated with affected individuals in an autosomal dominant pattern and was not detected in asymptomatic family members. Molecular studies reveal that a p.His441Gln substitution disrupts the coordination of a zinc ion within the second zinc finger and disrupts the capacity for ZBTB18 to bind DNA.

Conclusions

c.1323C>G mutation in *ZBTB18* gene on 1 chromosome may be related with the phenotype of intellectual disability in this family. WGS is an efficient method to perform molecular diagnosis for hereditary ID. This is the first report of an inherited *ZBTB18* mutation for ID and suggests that mutations that disrupt C2H2 motifs underlie human neurodevelopmental disorder.

Background

Intellectual disability (ID) is a generalized neurodevelopmental disorder characterized by substantial impairment in intellectual capacity (such as reasoning, learning, problem solving) as well as adaptive behaviours (such with conceptual, social and practical skills), and affects 1–3% of children [1, 2]. ID can be diagnosed in isolation or in combination with congenital malformations, as well as with additional neurological features such as epilepsy, sensory impairment and autism spectrum disorder (ASD). The severity of ID can range from mild, moderate, severe and profound [3, 4]. Individuals living with an ID

diagnosis face significant medical, financial, and psychological challenges. Equally the burden of ID on families, the community and on the health-care system of nations is considerable [5]. The genetic and phenotypic presentation of ID is extremely heterogeneous [3], with both environmental as well as genetic causes documented. For example, fetal exposure to alcohol and other teratogens, as well as prenatal infections, traumatic brain injury, neurologic/brain disorders, nutritional deficiencies, and inborn errors of metabolism are all known environmental causes for ID [3, 6]. On the other hand, the genetic causes for ID include chromosomal abnormalities, copy number variation (CNV), as well as single gene mutations that cause disease in a dominant or recessive fashion.

Traditional testing methods for genetic diagnosis of ID including karyotype, microarray, targeted polymerase chain reaction (PCR), Fragile X DNA testing for repeat sequences, fluorescent in situ hybridization (FISH) and mitochondrial DNA testing, have been met with great success [7]. More recently, Chromosomal microarray analysis (CMA), comparative genomic hybridization (CGH) or SNP arrays, have been successfully applied as first-tier tests to diagnose genetic causes of childhood ID [2, 8]. Previous studies on the genetic etiologies of ID have effectively utilized CMA [9–11]. However, intrinsic limitations are evident to each of these approaches. For example, karyotype and microarray testing only surveys the genome at low resolution, while PCR, Fragile X testing, FISH, and mitochondrial DNA testing all focus only on distinct candidate genomic loci for mutation testing. More recently, the rapid development of next generation sequencing (NGS) approaches for clinical diagnostic applications, such as whole-exome sequencing (WES) and whole-genome sequencing (WGS) have facilitated a broad and high-resolution discovery platform to characterize ID genes and their causal mutations [12–21]. To date, over 700 genes and 130 rare CNVs have been identified as putative genetic causes of X-linked, autosomal-dominant as well as autosomal-recessive ID and IDassociated disorders [2, 22]. Notably, inherited genetic forms of ID are rare in occurrence, detected in approximately 5% of cases [23].

In this study, we performed whole genome sequencing of family members with non-syndromic ID to identify a novel mutation to the *ZBTB18* gene that shows an autosomal-dominant pattern of inheritance.

Methods

Subjects Clinical Samples

A non-consanguinous family from the Sichuan province in China with two generations of individuals with ID was recruited following informed consent (Figure 1). Two affected (III:1, III:2) members received full clinical evaluation. The blood samples of four affected subjects(II:2, II:3, III:1, III:2) and the parents of the affected twins (I:1,I:2) were collected for analysis. Clinical records and radiographic images were published under the patients' written permission. The study was approved by the Ethics Committee of West China Second University Hospital, Sichuan University (No: 2015011) and adheres to the principles stated in the Declaration of Helsinki.

DNA extraction and whole genome sequencing

Genomic DNA was isolated from peripheral blood leukocytes, collected from participants using DNA QIAamp mini kit (Qiagen, Hilden, Germany) according to instructions of the manufacturer. WGS was performed on samples from 4 affected patients (II:2,II:3,III:1,III:2) and 2 normal family members (I:1,I:2). Sequencing was carried out with a BGI-seq 500 with 50bp paired-end reads.

Bioinformatics analysis

Sequencing data was analysed using SOAPnuke package [24] to remove adapter sequences and low-quality reads, following which reads were mapped to the human genome reference (UCSC GRCh37/hg19) through the Burrows-Wheeler aligner (BWA-MEM, version 0.7.10) [25]. Variant calling was performed using the Genome Analysis Tool Kit (GATK, version 3.3) [26]. Variant Effect Predictor (VEP) was used to annotate and classify all the variants [27]. We then screened variants based on their frequency in public and internal databases (1000 genome, GnomAD, and data not shown) [28], retaining only variants with a Minor Allele Frequency (MAF) <0.005. Variants with subsequently filtered based on the ID inheritance model of the pedigree. Finally, pathogenic as well as likely pathogenic variants were identified using Sift [29] and PolyPhen2 [30].

Sanger sequence validation

Primers for PCR validation of sequence reads were designed using Primer 5.0. Putative candidate variants were verified by Sanger sequencing to exclude false positive variants. The six family members (affected individuals II:2,II:3,III:1,III:2; unaffected individuals I:1,I:2) were sequenced by bidirectional Sanger sequencing to determine co-segregation of the candidate mutations. Polymerase chain reaction (PCR) and sequencing primers are available upon request.

Molecular modelling

Schrodinger Suite 2018-3 was used for molecular modelling. The H441Q mutation was introduced into the homology model of the ZBTB18-*E1* protein-DNA complex [31] using Maestro. The mutated residue, as well as residues within 6.0 Å of the mutation, were energy minimised using Prime.

Results

Clinical features

Four individuals in this family have mild ID. The proband (III:1), was a 7-year-old male at the time of examination. A language impairment screening assessment was performed on the child to find that their overall language ability was at the level of an 11-12 month old. His speech related ability was at the level of a 9 month old, while auditory related expression was at the level of a 12-13 month old. His capacity for visual expression was at the level of a 12-13 month old. Magnetic resonance imaging (MRI) scan of the brain was carried out for the child at 31 months of age which revealed dysplasia of the corpus callosum. At 6 years of age, the posterior horns of both lateral ventricles were enlarged, the corpus callosum was

abnormal, and the hippocampus was small. Electroencephalogram (EEG) examination suggested abnormality.

In the case of subject III:2, a general examination was performed at 19 months, with MRI scans revealing mild paraventricular white matter softening. Application tof the Intelligence Development Diagnostic Scale test showed a total DQ of 47.2, and the overall level of intelligence development was significantly lower than that of a typical child of the same age. The ability to cope with people was evaluated at that of a 9 month old, while the ability to cope with things was evaluated at equivalent to an 8 month old, while gross motor ability was at equivalent to a 12 month old. The ability of fine motor speech was equivalent to an 8 month old. Currently, both III:1 and III:2 are attending special schools for intellectually disabled children.

Clinical diagnostic data is not available for subjects II:2 and II:3, whom are parents of the affected twins. However, according to their account of early childhood when both had been weaned and were at 1 year of age, both twins often developed fever with bouts of convulsions lasting three to four cycles. Convulsions occurred when the body temperature was only 37.5°C, and this was diagnosed as epilepsy. Anti-convulsion medication Diazepan was effective to reduce the incidence of epileptic episodes. As adults, both II:2 and II:3 are restricted to ordinary housework, such as cooking, washing dishes, sweeping the floor. Both are unable to perform more complicated tasks alone, such as shopping and paying for goods and handling money.

Mutation Detection

To identify the causative variants in the ID family, we performed WGS as described (see Methods). High-quality data was obtained, with mean coverage in excess of 90%, and with average read depth at > 40X (Table 1).

Table 1
Average depth and coverage of WGS

Sample	Q20	Q30	Average depth	Coverage (%)
I:1	98.05	90.90	44.41	92.35
I:2	98.36	92.17	45.28	91.83
II:2	98.40	92.05	45.72	91.81
II:3	98.44	92.19	43.09	91.82
III:1	98.47	92.42	43.97	92.35
III:2	98.00	90.65	45.27	91.81

Bioinformatics analysis identified a *de novo* mutation in both II:2 and II:3 defined as c.1323C>G, in reference to RefSeq transcript NM_205768.2, located within Exon2 of the *ZBTB18* gene. This variant

segregated perfectly in affected family members, and was not detected in unaffected individuals, where tested. In addition, Sanger sequencing was also to confirm co-segregation of the heterozygous variant in these affected members in the pedigree (Figure 2). This variant has not been reported in HGMD database [32], gnomAD or ClinVar [33] databases, suggesting it is a novel *ZBTB18* missense variant. Variant calling analysis also did not identify/identifies other candidate gene mutations.

The ZBTB18 gene encodes a transcription factor protein that binds DNA in a sequence-specific manner to influence gene expression during fetal nervous system development [34–38]. The c.1323C>G mutation detected in this family pedigree results in an amino acid substitution from histidine to glutamine at position 441 (p.His441Gln) of the polypeptide sequence. This variant is predicted to be probably damaging (Polyphen score 0.997) and deleterious (SIFT score 0). Sequence alignment with vertebrate orthologues revealed codon 441 of ZBTB18 to be highly conserved from humans to frogs to fish (Figure 3), further suggesting a mutation at this codon may have an impact on the function of the polypeptide. Moreover, we performed molecular modelling to find that His441 is a zinc-coordinating residue within the second zinc finger motif of ZBTB18 [31]. As such, a H441Q substitution likely disrupts zinc coordination critical to the formation of this zinc finger protein folding motif that, in turn, alters the structural stability of ZBTB18 and affect its capacity to bind DNA for (Fig. 4). Given the putative role of His441 in protein folding, it is plausible that a p.His441Gln substitution could also alter steady-state levels of the ZBTB18 protein variant, leading to cellular dysfunction.

Discussion

Here, we report a novel heterozygous missense mutation (c.1323C>G, p.His441Gln) to the *ZBTB18* (NM_205768.2) gene in a Han Chinese family that segregates with ID in an autosomal dominant fashion. The variant was detected as a *de novo* mutation in a monozygotic twin female pair, but not their parents. Each child of the twin females inherited this variant, and they were born with brain developmental disorder and ID. To our knowledge, this is the first report of an inherited ZBTB18 mutation for ID.

ZBTB18 is essential to the development of the mammalian brain [39]. The expression and function of ZBTB18 in neural stem cells and their postmitotic progeny is crucial to the formation and differentiation of appropriate numbers of neurons and astrocytes within fetal brain [34]. During embryonic cerebral cortex development, ZBTB18 mediates multipolar-to-bipolar transition of migrating cortical neurons by negative inhibition of the expression downstream target genes such as *Neurog2* and *Rnd2* [37, 38]. Moreover, ZBTB18 is essential for the growth and organization of the cerebellum and regulates the development of both GABAergic and glutamatergic neurons [35]. It has been reported that ZBTB18 acts to repress the expression of *pax6*, *ngn2* and *neuroD1*, since expression of these three sequential pro-neurogenic genes can signal intermediate neurogenic progenitors (INP) within the embryonic mouse cerebellum to differentiate into neurons and initiate migration [36]. Given such critical roles for ZBTB18 in mammalian brain development, it is perhaps unsurprising that mutations to this gene lead to ID and human brain disorder.

The *ZBTB18* gene encodes a transcriptional repressor protein comprising an N-terminal BTB (broad complex tramtrack bric-brac) domain mediating protein-protein interaction, as well as four Cys_2His_2 (C2H2) type zinc fingers for DNA-binding within its C-terminus, respectively. In humans, genetic mutations to *ZBTB18*, including Copy Number Variation, microdeletions, microduplications, as well as Single Nucleotide Variants (SNVs), are associated with structural brain abnormalities, neuronal migration disorder and ID [12, 14, 16, 17, 40–53]. Interestingly, while disease-associated nonsense and frameshift mutations are documented across the coding sequence, the overwhelming majority of disease-associated missense mutations are mapped to the C-terminal, C2H2 zinc finger domain, while a small proportion of such variants map to the BTB domain. Within the coding sequence of the C2H2 zinc finger domain, the majority of disease-associated ZBTB18 missense variants are clustered map to the second, third and fourth zinc fingers, corresponding with the importance of these three motifs for DNA-binding [31]. Missense variation to residues within zinc fingers have been shown to impair the capacity of ZBTB18 to bind DNA and disrupt its function as a site-specific transcriptional repressor [31, 46]. In addition, disease-associated ZBTB18 variants could have a dominant-negative effect by disrupting the DNA-binding by circulating wildtype protein in the presence of the mutant protein [46]. Thus, growing evidence indicates that alterations to DNA-binding and disruptions to transcriptional regulation could represent pathological mechanisms through which *ZBTB18* missense variants cause disease in humans [31].

The novel missense mutation c.1323C>G (p.His441Gln) of *ZBTB18* is anticipated to be pathogenic based on the following evidence. Firstly, this variant is not detected in the general population (gnomAD) nor the National Center for Biotechnology Information (NCBI) single nucleotide polymorphism database (dbSNP) [54]. Secondly, the mutation leads to substitution of a highly conserved amino acid (His441) with basic properties, to glutamine that features polar, neutral side chains. Furthermore, two independent bioinformatics algorhithms (SIFT and PolyPhen2) described this variant as pathogenic. Thirdly, our molecular modelling studies reveal that His441 is critical as a zinc-coordinating reside in the second zinc-finger motif. As all four zinc fingers are essential for ZBTB18 to bind DNA [31, 55], a His441Gln mutation could destabilize the protein, disrupt sequence-specific binding, alter transcriptional regulation in cells, or all of the above. Indeed, recent studies of a p.Asn461Ser mutation detected in a child with ID [46] demonstrated that such a variant not only resulted in reduced steady-state levels of the mutant protein, but also compromised sequence-specific DNA binding and transcriptional repression [31].

The accurate detection of disease-causing mutations in subjects with ID is necessary to provide appropriate genetic counseling, as well as to inform potential future gene-specific therapies to alleviate clinical symptoms. Given the extensive genetic heterogeneity of ID, a genome-wide diagnostic approach is crucial to be able to detect of all types of causative genetic variants, whether such variants are detected in coding or noncoding loci. Given the ever-improving cost-effectiveness of WGS in clinical diagnostic sequencing, it is increasingly being used to detect causative mutations [13, 18, 19, 21].

Our study demonstrates that WGS is efficient for detecting genetic causes of hereditary ID. Our finding of a c.1323C>G (p.His441Gln) mutation to *ZBTB18* expands the spectrum of mutations that cause inherited forms of ID, and demonstrates that WGS is highly efficient to provide a precise genetic diagnosis.

Abbreviations

ID
intellectual disability
CNVs
copy number variants
CMA
chromosomal microarray analysis
NGS
next generation sequencing
WES
whole-exome sequencing
WGS
whole-genome sequencing
MR
magnetic resonance
MRI
magnetic resonance imaging.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of West China Second University Hospital, Sichuan University (No: 2015011) and adhered to the tenets of the Declaration of Helsinki.

Consent for publication

The parental consent (I:1 and I:2) was attained for this study.

Availability of data and materials

All data are contained in the article and details of sequencing primers for validation of ZBTB18 mutant alleles is available upon request.

Competing interests

The authors declare that they have no conflicts of interest with the contents of this article.

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Authors' contributions

Nana Li and Ping Yu developed the study design. Nana Li, Lu Li, Meixian Wang, Julian Heng and Ping Yu conducted the experiment and drafted the manuscript. Yanna Zou, Zhen Liu, Ying Deng and Hong Kang assisted in analyzing the genomic data. Yanna Zou, Hong Qin and Xiaoqiong Qiu participated in the clinical evaluation of the patients. Mark Agostino and Julian I-T Heng performed molecular modeling studies. Yanping Wang, Jun Zhu and Julian I-T Heng participated in critical review and revision of the manuscript. All authors have read and approved the final manuscript.

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Figures

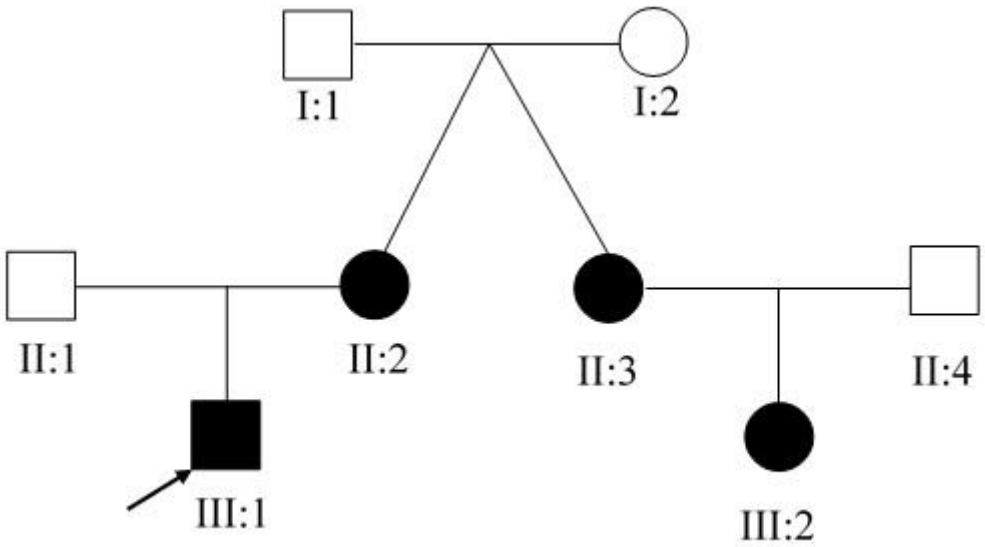

Figure 1

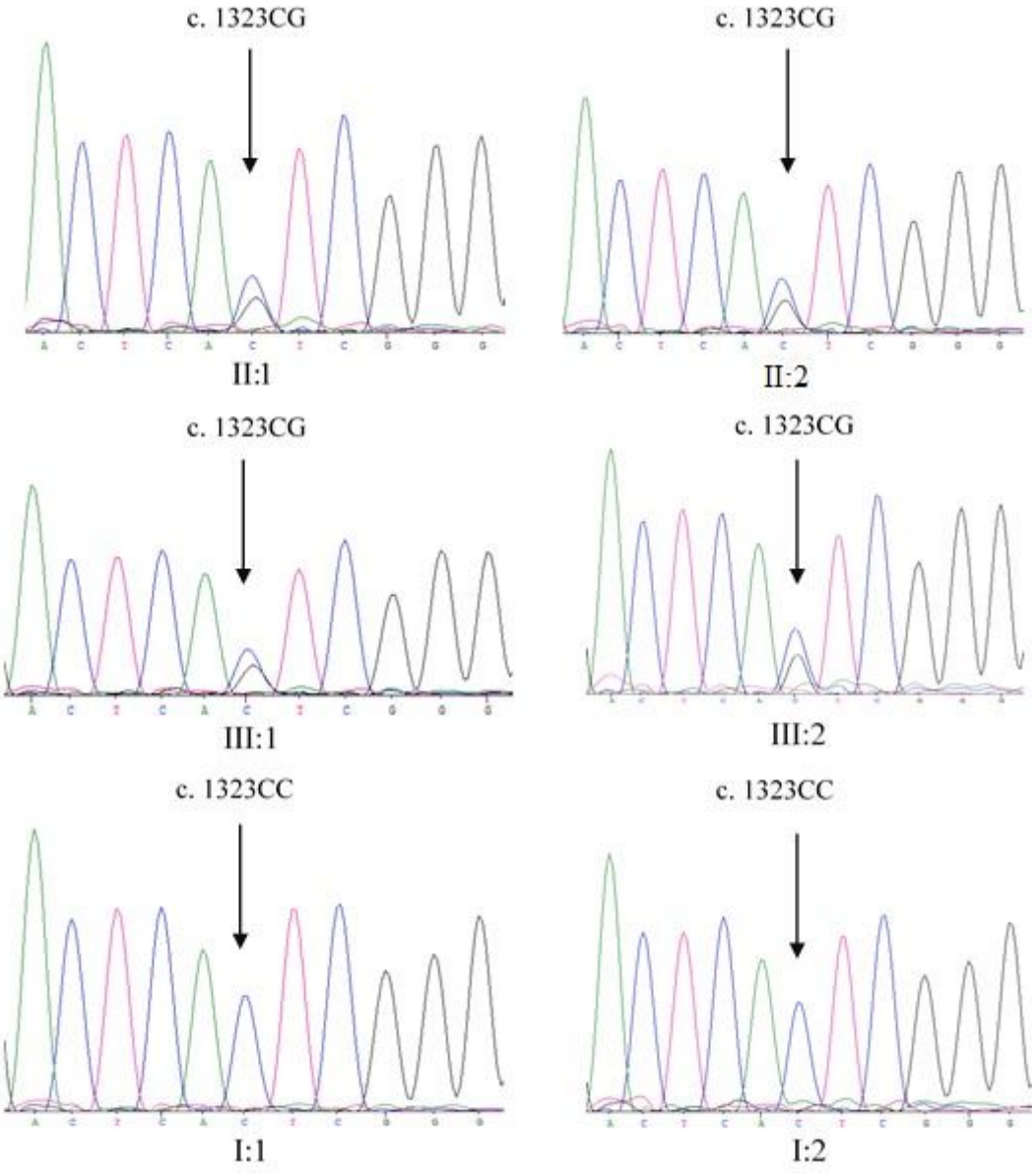

Figure 2

Figure 3

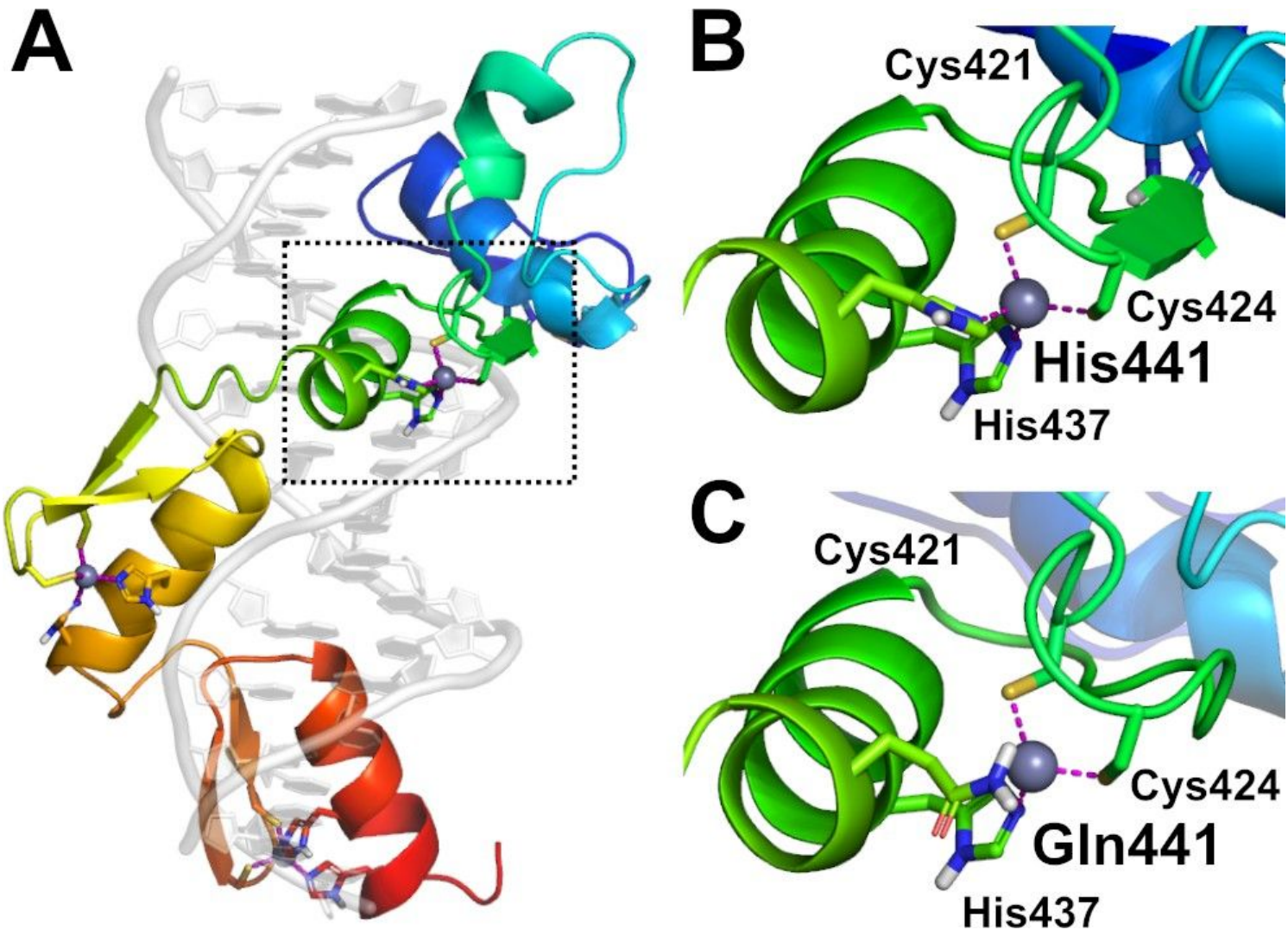
A
B
Cys421
Cys424
His441
His437
C
Cys421
Cys424
Gln441
His437

Figure 4