HHS Public Access

Author manuscript

Am J Med Genet A. Author manuscript; available in PMC 2019 December 28.

Published in final edited form as:

Am J Med Genet A. 2019 October ; 179(10): 2075–2082. doi:10.1002/ajmg.a.61306.

Phenotype Delineation of *ZNF462* related syndrome

Paul Kruszka[1], **Tommy Hu**[1], **Sungkook Hong**[1], **Rebecca Signer**[2], **Benjamin Cogné**[3], **Betrand Isidor**[3], **Sarah E. Mazzola**[4], **Jacques C. Giltay**[5], **Koen L.I. van Gassen**[5], **Eleina M. England**[6], **Lynn Pais**[6], **Charlotte W. Ockeloen**[7], **Pedro A. Sanchez-Lara**[8,9], **Esther Kinning**[10], **Darius J Adams**[11], **Kayla Treat**[12], **Wilfredo Torres-Martinez**[12], **Maria Francesca Bedeschi**[13], **Maria Iascone**[14], **Stephanie Blaney**[15], **Oliver Bell**[17], **Tiong Y. Tan**[18,19,20], **Marie-Ange Delrue**[21], **Julie Jurgens**[22], **Brenda J. Barry**[6,23], **Elizabeth C. Engle**[6,23,24], **Sarah K. Savage**[25], **Nicole Fleischer**[25], **Julian A. Martinez-Agosto**[2], **Kym Boycott**[16], **Elaine H. Zackai**[4], **Maximilian Muenke**[1]

[1.]Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA [2.]Department of Human Genetics, David Geffen School of Medicine, University of California, Los Angeles, CA, USA [3.]Service de génétique médicale, Hôtel-Dieu, CHU de Nantes, Nantes, France [4.]Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA, USA [5.]Department of Genetics, University Medical Center Utrecht, Utrecht, The Netherlands [6.]Center for Mendelian Genomics, Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA [7.]Department of Human Genetics, Radboud University Medical Center, Nijmegen, the Netherlands. [8.]Keck School of Medicine, University of Southern California, Los Angeles, CA, USA [9.]Department of Pediatrics, David Geffen School of Medicine, University of California, Los Angeles, CA, USA. [10.]West of Scotland Genetics Service, Queen Elizabeth Hospitals, Glasgow, Scotland [11.]Atlantic Health System, Morristown, NJ, USA [12.]Indiana University School of Medicine, Indianapolis, IN, USA [13.]Medical Genetic Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy [14.]Laboratorio di Genetica Medica, ASST Papa Giovanni XXIII, Bergamo, Italy [15.]Algoma Public Health, Sault Ste Marie, ON, Canada [16.]Children's Hospital of Eastern Ontario Research Institute, University of Ottawa, Ottawa, ON, Canada. [17.]Keck School of Medicine, University of Southern California, Los Angeles, CA [18.]Murdoch Children's Research Institute, Melbourne, Australia. [19.]Department of Paediatrics, University of Melbourne, Melbourne, Australia [20.]Victorian Clinical Genetics Services, Melbourne, Australia [21.]Département de pédiatrie, Service de génétique médicale, Centre Hospitalier Universitaire Ste-Justine, Université de Montréal, Montréal, Canada [22.]Department of Neurology, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA [23.]Howard Hughes Medical Institute, Chevy Chase, MD [24.]Department of Ophthalmology, Boston Children's Hospital, Harvard Medical School, Boston, MA [25.]FDNA Inc., Boston, MA, USA

Abstract

Correspondence: Paul Kruszka. paul.kruszka@nih.gov.

The authors have no conflict of interest to declare.

DATA SHARING

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Zinc finger protein 462 (ZNF462) is a relatively newly discovered vertebrate specific protein with known critical roles in embryonic development in animal models. Two case reports and a case series study have described the phenotype of 10 individuals with *ZNF462* loss of function variants. Herein, we present 14 new individuals with loss of function variants to the previous studies to delineate the syndrome of loss of function in *ZNF462*. Collectively, these 24 individuals present with recurring phenotypes that define a multiple congenital anomaly syndrome. Most have some form of developmental delay (79%) and a minority have autism spectrum disorder (33%). Characteristic facial features include ptosis (83%), down slanting palpebral fissures (58%), exaggerated Cupid's bow/wide philtrum (54%), and arched eyebrows (50%). Metopic ridging or craniosynostosis was found in a third of study participants and feeding problems in half. Other phenotype characteristics include dysgenesis of the corpus callosum in 25% of individuals, hypotonia in half, and structural heart defects in 21%. Using facial analysis technology, a computer algorithm applying deep learning was able to accurately differentiate individuals with *ZNF462* loss of function variants from individuals with Noonan syndrome and healthy controls. In summary, we describe a multiple congenital anomaly syndrome associated with haploinsufficiency of *ZNF462* that has distinct clinical characteristics and facial features.

Keywords

ZNF462; ptosis; developmental delay; autism spectrum disorders; corpus callosum; craniosynostosis

INTRODUCTION

Heterozygous loss of function variants in *ZNF462* present with a recognizable pattern of phenotype characteristics (Weiss et al., 2017). The first reported case was a reciprocal translocation t(2;9)(p24;q32) that disrupted both *ZNF462* and *ASXL2* (Ramocki et al., 2003; Talisetti et al., 2003). This individual presented with ptosis, agenesis of the corpus callosum, ventricular septal defect, periventricular nodular heterotopia, retina and iris colobomas, and a dysplastic left ear and hearing loss. *ASXL2* was subsequently associated with Shashi-Pena syndrome which presents as macrocephaly, retrognathia, low set ears, hypertelorism, arched eyebrows, intellectual disability, scoliosis, congenital heart disease, and hypotonia (Shashi et al., 2016). The phenotype of the individual in this case report likely resulted from the loss of function of both *ZNF462* and *ASXL2*. Over a decade later, Weiss et al. described 6 individuals from four families with putative loss of function variants and two unrelated individuals with deletions involving adjacent genes (Weiss et al., 2017). The individuals described by Weiss et al. presented with ptosis (100%), trigonocephaly or metopic ridging (83%), and developmental delay or autism spectrum disorder (33%) (Weiss et al., 2017). Subsequently, Cosemans et al. described an individual with a *de novo* translocation that disrupted *ZNF462* and *KLF12* who presented with clinical features similar to those described by Weiss et al. (Cosemans et al., 2018; Weiss et al., 2017).

Zinc finger protein 462 (ZNF462) is a C2H2 type zinc finger transcription factor of unknown function (Nagase, Nakayama, Nakajima, Kikuno, & Ohara, 2001). Although the specific function of this molecule is unknown, animal studies have shown that it plays a vital

role in embryonic development. In *Xenopus laevis*, knockdown expression of *Zfp462* disturbs early embryonic development and results in altered cell division during the cleavage stage; this phenotype is rescued with human *ZNF462* mRNA (Laurent et al., 2009). In the mouse model, Zfp462 knockout (KO) mice were prenatal lethal and heterozygous knockout mice ($Zfp462^{+/-}$) had developmental delay, low body and brain weights, and anxiety-like behaviors with excessive self-grooming behavior (Wang et al., 2017).

In this report, we describe 14 new individuals in addition to the 10 previously reported cases in the medical literature with truncating variants in *ZNF462*, collectively review the clinical presentation of this syndrome, and test facial analysis technology's ability to diagnose this syndrome.

METHODS

Clinical

The study was approved by National Human Genome Research Institute Institutional Review Board (IRB). Thirteen new individuals in this report with loss of function variants in *ZNF462* were diagnosed using whole exome sequencing (WES) in multiple research and commercial labs including GeneDx and Ambry, and one individual (patient 5) was diagnosed by whole genome sequencing. Nine of the fourteen individuals were ascertained through GeneMatcher (Sobreira, Schiettecatte, Valle, & Hamosh, 2015).

Facial analysis technology

We performed two binary classification experiments using the Face2Gene Research application (FDNA Inc., Boston, MA), as previously described (Gurovich et al., 2019). Frontal facial 2D images were collected for three cohorts: individuals with *ZNF462* loss of function variants, Noonan syndrome, and healthy controls. Noonan syndrome was used as a second control group due the overlapping facial features of ptosis, downslanting palpebral fissures, hypertelorism, and low set ears in a subset of individuals. All facial images were fully de-identified through the use of the DeepGestalt facial analysis (Gurovich et al., 2019). Controls were matched for age, gender, and ethnicity.

RESULTS

Clinical

Figure 1 shows the single nucleotide variant and small insertion/deletion (indel) locations on *ZNF462* for both the 14 newly reported cases and previously reported cases. All variants are predicted to result in loss of function, including a canonical splice variant in patient 6 that is predicted to result in abnormal splicing (Table 1; Figure 1). Most of these variants are in exon 3, which makes up 54% of the coding region of *ZNF42*. .

Table 1 summarizes the clinical features of all 24 affected individuals with 96% of individuals being Caucasian. Seventeen of 21 families (86%) have *de novo* variants, the other four families include unknown, mosaic, and autosomal dominant inheritance (Table 1). The two families with autosomal dominant inheritance demonstrated that the *ZNF462* variant segregated with the phenotype characterized in this study: patient 5's father had

ptosis surgery and patients 15–17 are from the same family, and previously described (Weiss et al., 2017). The one case of mosaicism was in the mother of patient 1 who had 175 reference reads and 35 alternate reads on WES from a peripheral blood sample [alternate allele frequency = 35/(35+175) = 17%], compared to 120 reference reads and 79 alternate reads in the proband [alternate allele frequency = 79/(79+120) = 40%]. The majority of individuals had developmental delay (79%) and 33% reported autism spectrum disorders (Table 1). The most common facial features were ptosis (83%), down slanting palpebral fissures (58%), exaggerated Cupid's bow/wide philtrum (54%), arched eyebrows (50%), and short upturned nose with bulbous tip (46%). Feeding issues (50%) and hypotonia (50%) were common. Less than half of affected individuals reported metopic ridging or craniosynostosis (38%) or dysgenesis of the corpus callosum (25%). Less common characteristics included structural heart defects (21%) and minor limb anomalies (25%). The clinical analysis of the individuals in this study was heterogenous and not all individuals received brain and heart imaging (Supplementary Clinical Information), thus the above fractions may be an underestimation of brain and heart malformations. Figure 2 shows facial images of individuals with loss of function variants in *ZNF462*.

Facial analysis technology

Binary comparison between individuals with loss of function variants in *ZNF462* and controls was resulted in two statistics: the mean results involved the computation of the average of the AUC of each of the 10 results, and secondly, the aggregated results consist of a score distribution curve and a receiver-operating-characteristic (ROC) curve for the aggregated results for each photo used in the validation set. The binary comparison between *ZNF462* (n=21) and healthy controls (n=21) yielded an AUC of 0.96 (STD 0.03), demonstrating good separation between these two cohorts (Supplementary Table 1). Similarly, the comparison between the *ZNF462* cohort (n=21) and the Noonan syndrome cohort (n=16) yielded an AUC of 0.97 (STD 0.02) which is also good separation (Supplementary Table 1). The aggregated binary comparison for the *ZNF462* group versus health controls yielded an AUC of 0.955 (P=0.006) and for the *ZNF462* group versus health Noonan syndrome yielded an AUC of 0.972 (P=0.001) (Supplementary Figure 1).

Applying DeepGestalt, the confusion matrix/multi-class comparison of the 58 frontal images of the *ZNF462* group and both control groups yielded a mean accuracy of 82.88% (STD 11.79%) which is significantly better than randomly expected (36.21%).

DISCUSSION

We report 24 individuals with loss of function variants in *ZNF462* which includes 14 previously unpublished individuals and 10 individuals reported in the medical literature. Based on this larger assembled cohort of individuals, the phenotype of loss of function in *ZNF462* is now a distinct multiple congenital anomaly syndrome. We show that ptosis (83%), developmental delay (79%), and down slanting palpebral fissures (58%) are three most reported phenotypic features (Table 1). In the previous case series of 6 families and 8 individuals, metopic ridging/craniosynostosis (63%) was a major phenotypic feature. In this report, we show that metopic ridging/craniosynostosis is still important, but less prevalent

(25%) in this syndrome. Consistent with the previous report by Weiss et al. 2017 (Table 1: patients 15–17), loss of function in *ZNF462* appears to have variable expressivity and complete penetrance as demonstrated by patient 5 in the present study with a paternally inherited variant and a father requiring surgery for his ptosis (Table 1; Supplementary Clinical Information). Facial analysis technology was able to accurately differentiate individuals with loss of function in *ZNF462* from Noonan syndrome and healthy controls. We predict that the widespread use of facial analysis technology will result in an increase in the number of cases diagnosed this syndrome.

Based on the prevalence of developmental delay, corpus callosum anomalies, congenital heart defects, and hearing loss, we recommend a comprehensive multidisciplinary evaluation of individuals with loss of function variants in *ZNF462*. This evaluation includes at a minimum: a developmental evaluation, a cardiac exam with echocardiography, brain imaging, hearing evaluation, and consultation with a clinical geneticist and genetic counselor. Other evaluations specific to an individual's presentation such as neurosurgery consultation for craniosynostosis may be appropriate. At this time, treatment of complications associated with *ZNF462* related syndrome are not different from the general population. As more individuals are studied, future specific management recommendations for *ZNF462* related syndrome may be needed.

Pathogenicity of variants in *ZNF462* is presumed to be haploinsufficiency based on individuals having loss of function variants only, and this is reinforced by theGenome Aggregation Database (gnomAD) constraint metric of observed/expected loss of function (o/e) value (Karczewski et al., 2019). Values less than 0.35 (o/e) are considered under selection against LOF (https://gnomad.broadinstitute.org) and *ZNF462* is well below this threshold with an o/e value of 0.03 (90%CI, 0.01–0.09). As noted in the introduction, *ZNF462* is important to embryonic development in multiple species. *ZNF462* contains 23 C2H2-type zinc finger domains, making DNA binding a likely function (Chang, Stoykova, Chowdhury, & Gruss, 2007). We now know that ZNF462 is involved in chromatin remodeling. Using histone peptide pull down assays in mouse brain and kidney, Eberl et al. showed that ZNF462 binds H3K9me3, identifying Znf462 as a chromatin reader involved in heterochromatin modification (Eberl, Spruijt, Kelstrup, Vermeulen, & Mann, 2013). Additionally, Eberl et al. report an interaction with Heterochromatin Protein 1α (HP1α) (Eberl et al., 2013). As hallmarks of heterochromatin, HP1α and H3K9me3 are critical for transcriptional silencing of gene and repetitive DNA and for the maintenance of genome integrity (Almouzni & Probst, 2011; Beisel & Paro, 2011; Ren & Martienssen, 2012), further supporting ZNF462's role in chromatin remodeling. Masse et al. used short hairpin RNA knockdown of pluripotent mouse cells, demonstrating a disruption of pericentromeric domains and redistribution of HP1α proteins, giving evidence that Znf462 is instrumental in maintaining heterochromatin in pluripotent cells (Masse et al., 2010).

In summary we present 24 individuals with loss of function variants in *ZNF462*, and we define a multiple congenital anomaly syndrome that is recognizable from phenotype elements and by using facial analysis technology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

We thank the affected individuals and their families for participating in this study. This project was supported by the National Human Genome Research Institute's Intramural Research Program (P.K. and M.M.); NEI grant R01EY027421 and NHLBI grant X01HL132377 (E.C.E), and a CIHR Foundation Grant (FDN-154279) (K.M.B.). Sequencing and analysis for the Broad Institute of MIT and Harvard Center for Mendelian Genomics (Broad CMG) and was funded by the National Human Genome Research Institute, the National Eye Institute, and the National Heart, Lung and Blood Institute grant UM1 HG008900 to Daniel MacArthur and Heidi Rehm.

REFERENCES

Almouzni G, & Probst AV (2011). Heterochromatin maintenance and establishment: lessons from the mouse pericentromere. Nucleus, 2(5), 332–338. doi:10.4161/nucl.2.5.17707 [PubMed: 21941119]

Beisel C, & Paro R (2011). Silencing chromatin: comparing modes and mechanisms. Nat Rev Genet, 12(2), 123–135. doi:10.1038/nrg2932 [PubMed: 21221116]

Chang YS, Stoykova A, Chowdhury K, & Gruss P (2007). Graded expression of Zfp462 in the embryonic mouse cerebral cortex. Gene Expr Patterns, 7(4), 405–412. doi:10.1016/j.modgep.2006.11.009 [PubMed: 17207666]

Cosemans N, Vandenhove L, Maljaars J, Van Esch H, Devriendt K, Baldwin A, … Peeters (2018). ZNF462 and KLF12 are disrupted by a de novo translocation in a patient with syndromic intellectual disability and autism spectrum disorder. Eur J Med Genet. doi:10.1016/j.ejmg.2018.02.002

Eberl HC, Spruijt CG, Kelstrup CD, Vermeulen M, & Mann M (2013). A map of general and specialized chromatin readers in mouse tissues generated by label-free interaction proteomics. Mol Cell, 49(2), 368–378. doi:10.1016/j.molcel.2012.10.026 [PubMed: 23201125]

Gurovich Y, Hanani Y, Bar O, Nadav G, Fleischer N, Gelbman D, … Gripp KW (2019). Identifying facial phenotypes of genetic disorders using deep learning. Nat Med, 25(1), 60–64. doi:10.1038/s41591-018-0279-0 [PubMed: 30617323]

Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q, … Gauthier (2019). Variation across 141,456 human exomes and genomes reveals the spectrum of loss-of-function intolerance across human protein-coding genes. BioRxiv, 531210.

Laurent A, Masse J, Omilli F, Deschamps S, Richard-Parpaillon L, Chartrain I, & Pellerin I (2009). ZFPIP/Zfp462 is maternally required for proper early Xenopus laevis development. Dev Biol, 327(1), 169–176. doi:10.1016/j.ydbio.2008.12.005 [PubMed: 19111535]

Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, … Exome Aggregation C (2016). Analysis of protein-coding genetic variation in 60,706 humans. Nature, 536(7616), 285–291. doi:10.1038/nature19057 [PubMed: 27535533]

Masse J, Laurent A, Nicol B, Guerrier D, Pellerin I, & Deschamps S (2010). Involvement of ZFPIP/Zfp462 in chromatin integrity and survival of P19 pluripotent cells. Exp Cell Res, 316(7), 1190–1201. doi:10.1016/j.yexcr.2010.02.024 [PubMed: 20219459]

Nagase T, Nakayama M, Nakajima D, Kikuno R, & Ohara O (2001). Prediction of the coding sequences of unidentified human genes. XX. The complete sequences of 100 new cDNA clones from brain which code for large proteins in vitro. DNA Res, 8(2), 85–95. doi:10.1093/dnares/8.2.85 [PubMed: 11347906]

Ramocki MB, Dowling J, Grinberg I, Kimonis VE, Cardoso C, Gross A, … Millen KJ (2003). Reciprocal fusion transcripts of two novel Zn-finger genes in a female with absence of the corpus callosum, ocular colobomas and a balanced translocation between chromosomes 2p24 and 9q32. Eur J Hum Genet, 11(7), 527–534. doi:10.1038/sj.ejhg.5200995 [PubMed: 12825074]

Ren J, & Martienssen RA (2012). Silent decision: HP1 protein escorts heterochromatic RNAs to their destiny. EMBO J, 31(15), 3237–3238. doi:10.1038/emboj.2012.172 [PubMed: 22705945]

Shashi V, Pena LD, Kim K, Burton B, Hempel M, Schoch K, … Kortum F (2016). De Novo Truncating Variants in ASXL2 Are Associated with a Unique and Recognizable Clinical Phenotype. Am J Hum Genet, 99(4), 991–999. doi:10.1016/j.ajhg.2016.08.017 [PubMed: 27693232]

Sobreira N, Schiettecatte F, Valle D, & Hamosh A (2015). GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. Hum Mutat, 36(10), 928–930. doi: 10.1002/humu.22844 [PubMed: 26220891]

Talisetti A, Forrester SR, Gregory D, Johnson L, Schneider MC, & Kimonis VE (2003). Temtamy-like syndrome associated with translocation of 2p24 and 9q32. Clin Dysmorphol, 12(3), 175–177. doi: 10.1097/01.mcd.0000072161.33788.56 [PubMed: 14564155]

Wang B, Zheng Y, Shi H, Du X, Zhang Y, Wei B, … Xu X (2017). Zfp462 deficiency causes anxiety-like behaviors with excessive self-grooming in mice. Genes Brain Behav, 16(2), 296–307. doi: 10.1111/gbb.12339 [PubMed: 27621227]

Weiss K, Wigby K, Fannemel M, Henderson LB, Beck N, Ghali N, … Kruszka P (2017). Haploinsufficiency of ZNF462 is associated with craniofacial anomalies, corpus callosum dysgenesis, ptosis, and developmental delay. Eur J Hum Genet, 25(8), 946–951. doi:10.1038/ejhg.2017.86 [PubMed: 28513610]

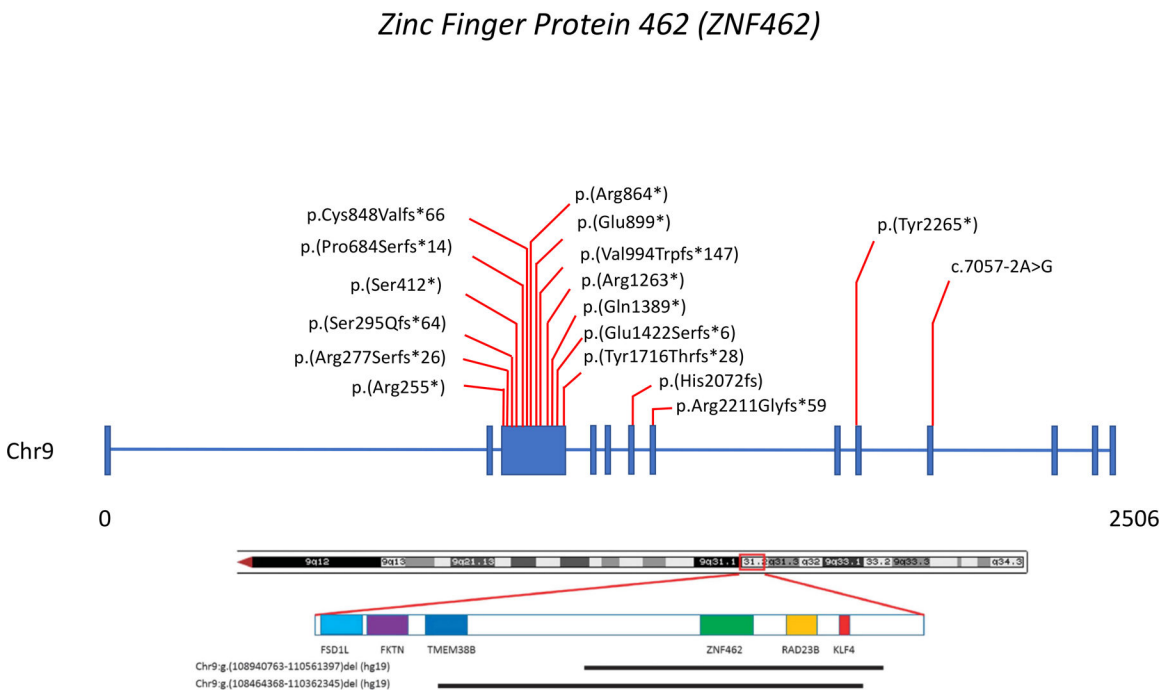

Figure 1.

ZNF462 variant locations. Variants from the present study are in blue and variants from previous publications are shown in black. Thirteen of the seventeen variants are on exon 3 which makes up 54% of *ZNF462*. Note that there are two unrelated individuals with the p.(His2072Tyrfs*8) variant (patients 4 and 11 in Table 1).

Figure 2.

A. Patient 1; B. Patient 2; C. Patient 3; D. Patient 4; E. Patient 5 at 8 years; F. Patient 6 at two and 7 months; G. Patient 7; H. Patient 8 at 3 months and 2.5 years; I. Patient 9 at ages 8 and 15 years; J. Patient 12; K. Patient 13; L. Patient 14; M. Patient 15; N. Patient 16; O. Patient 17; P. Patient 18; Q. Patient 19; R. Patient 20; S. Patient 21; T. Patient 22; U. Patient 24; (Figures M-T are from Weiss et al., 2017 and Figure U is from Cosemans et al., 2018)

Table 1.

Phenotype characteristics of individuals with loss of function variants in *ZNF462*

Patient	Age	Sex	*ZNF462* variant (NM_021224.5)	Inheritance	DD	ASD	Ptosis	Down slanting palpebral fissures	Arched eyebrows	Short upturned nose with bulbous tip	Exaggerated cupid bow/ wide philtrum	Feeding issues	Epicanthal folds	Ears	Craniosynostosis / metopic ridging	Hypotonia	Hypertelorism	Corpus callosum dysgenesis	CHD	Limb anomalies (minor)
1	16m	M	c.2590C>T p.(Arg864*)	maternal (mosaic)	motor/ speech	–	+	–	+	–	+	+	+	low set	–	+	+	normal MRI	not reported	fifth finger clinodactyly
2	10y	M	c.2542del p.(Cys848Valfs*66)	*de novo*	motor/ speech	+	+	+	+	+	+	+	+	–	–	–	+	normal MRI	not reported	not reported
3	6y	M	c.831_834del p.(Arg277Serfs*26)	*de novo*	motor/ speech	–	–	–	–	+	–	+	–	inner ear malformation	–	+	+	normal MRI	bicuspid aoritc valve; VSD	not reported
4	2y 7m	M	c.6214_6215del p.(His2072Tyrfs*8)	*de novo*	speech	–	+	–	–	–	+	+	+	small, lowset	+	–	–	not tested	not reported	not reported
5	14y	F	c.763C>T p.(Arg255*)	paternal	IEP/special education	–	+	–	+	–	–	–	+	hearing loss	+	–	–	not tested	not reported	not reported
6	7m	F	c.7057–2A>G	*de novo*	early intervention for DD	–	+	+	+	+	+	+	+	horizontal crus helix	–	+	+	normal MRI	VSD	prominent creases on hands and feet
7	13y	M	c.6794dup p.(Tyr2265*)	*de novo*	cognitive impairment	+	–	–	+	–	+	–	–	prominent ears/ear pits/ hearing loss	+	–	–	not tested	not reported	not reported
8	2y	M	c.882dup p.(Ser295Glnfs*64)	*de novo*	speech delay	–	+	+	–	–	–	–	–	–	–	–	–	ACC	not reported	not reported
9	15y	M	c.4165C>T p.(Gln1389*)	*de novo*	global	–	+	+	+	–	+	+	–	lowset	–	+	–	not tested	not reported	not reported
10	8y	M	c.1234_1235insAA; p.(Ser412*)	unknown	speech delay; motor apraxia; IEP	–	+	–	–	–	–	+		mildly cupped ears	–	–	–	normal MRI	not reported	not reported
11	2 y 5 m	F	c.6214_6215del p.(His2072Tyrfs*8)	*de novo*	–	–	+	–	–	–	–	+			–	+	–	not tested	not reported	not reported
12	9m	M	c.2049dup p.(Pro684Serfs*14)	*de novo*	motor	–	+	+	+	+	+	–	+	–	–	+	+	normal MRI	not reported	not reported
13	8y 7m	M	c.6631del p.(Arg2211Glyfs*59)	*de novo*	–	–	+	+	–	+	+	–	–	–	–	–	+	not tested	not reported	5th finger clinodactyly
14	8y	F	c.2695G>T; p.(Glu899*)	mother negative/ father unknown	cognitive impairment	–	–	–	+	–	–	+	–	–	–	+	–	normal MRI	not reported	not reported
15[a]	2y	F	c.3787C>T p.(Arg1263*)	paternal	–	–	+	+	+	+	+	not reported	–	–	+	–	+	ACC; colpocephaly	not reported	not reported
16[a]	4y	F	c.3787C>T p.(Arg1263*)	paternal	–	–	+	+	–	+	+	not reported	+	–	+	–	–	normal prenatal ultrasound	not reported	not reported
17[a]	34y	M	c.3787C>T p.(Arg1263*)	maternal	–	–	+	–	–	–	–	not reported	–	–	+	–	–	not tested	not reported	not reported

Patient	Age	Sex	*ZNF462* variant (NM_021224.5)	Inheritance	DD	ASD	Ptosis	Down slanting palpebral fissures	Arched eyebrows	Short upturned nose with bulbous tip	Exaggerated cupid bow/ wide philtrum	Feeding issues	Epicanthal folds	Ears	Craniosynostosis / metopic ridging	Hypotonia	Hypertelorism	Corpus callosum dysgenesis	CHD	Limb anomalies (minor)
18[a]	2y	M	c.2979_2980delinsA p.(Val994Trpfs*147)	*de novo*	speech	+	+	+	+	+	−	+	+	left overfolded ear	+	−	−	− normal MRI	not reported	single palmar crease ;5th finger clinodactyly
19[a]	32m	M	c.4263del p. (Glu1422Serfs*6)	*de novo*	motor/ speech	+	+	+	−	+	−	−	+	lowset	+	+	+	hypoplastic corpus callosum and ventriculomegaly	D-TGA	not reported
20[a]	5y	F	Chr9:g.(108940763–110561397)del (hg19)	*de novo*	−	−	+	+	+	+	+	not reported	+		−	+	−	hypoplastic corpus callosum	not tested	not reported
21[a]	15y	F	Chr9:g(108464368–110362345)del (hg19)	*de novo*	motor/ intellectual	+	−	−	−	−	+	not reported	−		−	−	+	not tested	VSD	not reported
22[a]	9y	M	c.5145delC p. (Tyr1716Thrfs*28)	*de novo*	motor/ speech delay	+	+	−	−	−	−	not reported	−	−	−	+	−	normal MRI	not reported	not reported
23[b]	5y	F	t(2;9)(p24;q32); disrupting *ZNF462* and *ASXL2*	*de novo*	intellectual disability	+	+	+	+	+	+	+	−	lowset; hearing loss	−	+	−	ACC; dilated venricles	VSD; left ventricular hypertrophy	single palmar crease; hypoplastic finger nails
24[c]	24y	M	t(9; 13)(q31.2; q22.1) disrupting *ZNF462* and *KLF12*	*de novo*	intellectual disability	+	+	+	−	−	−	+	+	lowset	+	+	−	hypoplastic corpus callosum	none reported	small hands and feet; proximally placed thumbs
Cohort prevalence[d]					79%	33%	83%	58%	50%	46%	54%	50%	46%	50%	38%	50%	25%	25%	21%	25%

[a]Weiss et al., 2017;

[b]Talisetti et al., 2003;

[c]Cosemans et al., 2018

[d]In order to avoid overestimating phenotype prevalence, we divided each positive phenotype report by the entire cohort (n=24).

Abbreviations: ACC (agenesis of the corpus callosum); ASD (autism spectrum disorder); CHD (congenital heart disease); DD (developmental delay); D-TGA (D-transposition of the great arteries); F (female); IEP (individualized education program); M (male); MRI (magnetic resonance imaging); ventricular septal defect (VSD);

Am J Med Genet A. Author manuscript; available in PMC 2019 December 28.