Article

GNB5 Mutations Cause an Autosomal-Recessive Multisystem Syndrome with Sinus Bradycardia and Cognitive Disability

LODDER, Elisabeth M, DE NITTIS, Pasquelena, KOOPMAN, Charlotte D & Collaboration

FISH, Richard (Collab.), *et al*.

Abstract

GNB5 encodes the G protein β subunit 5 and is involved in inhibitory G protein signaling. Here, we report mutations in GNB5 that are associated with heart-rate disturbance, eye disease, intellectual disability, gastric problems, hypotonia, and seizures in nine individuals from six families. We observed an association between the nature of the variants and clinical severity; individuals with loss-of-function alleles had more severe symptoms, including substantial developmental delay, speech defects, severe hypotonia, pathological gastro-esophageal reflux, retinal disease, and sinus-node dysfunction, whereas related heterozygotes harboring missense variants presented with a clinically milder phenotype. Zebrafish gnb5 knockouts recapitulated the phenotypic spectrum of affected individuals, including cardiac, neurological, and ophthalmological abnormalities, supporting a direct role of GNB5 in the control of heart rate, hypotonia, and vision.

Reference

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GNB5 variants cause a novel multisystem syndrome associated with sinus bradycardia and intellectual disability

Elisabeth M Lodder[1]*, Pasquelena De Nittis[2,3]*, Charlotte D. Koopman[4,5]*, Wojciech Wiszniewski[6], Carolina Fishinger Moura de Souza[7], Najim Lahrouchi[1], Nicolas Guex[2,8], Valerio Napolioni[9], Federico Tessadori[5], Teun de Boer[4], Leander Beekman[1], Eline A. Nannenberg[10], Lamiae Boualla[11], Nico A. Blom[12], Wim de Graaff[13], Maarten Kamermans[13,14], Dario Cocciadiferro[3,15], Natascia Malerba[3,15], Barbara Mandriani[3,16], Zeynep Hande Coban Akdemir[6], Richard J. Fish[17], Mohammad K. Eldomery[6], Ilham Ratbi[11], Arthur A. M. Wilde[1], Teun P. de Boer[4], William F. Simonds[18], Marguerite Neerman-Arbez[17], V. Reid Sutton[6,19], Fernando Kok[20], James R. Lupski[6,19,21], Alexandre Reymond[2#], Connie R. Bezzina[1#], Jeroen Bakkers [4,5#], Giuseppe Merla[3#]

[1]Department of Clinical and Experimental Cardiology, Heart Center, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

[2]Center for Integrative Genomics, University of Lausanne, CH-1015 Lausanne, Switzerland

[3]Medical Genetics Unit, IRCCS Casa Sollievo della Sofferenza, viale Cappuccini, 71013, San Giovanni Rotondo, Foggia, Italy

[4]Department of Medical Physiology, Division of Heart and Lungs, University Medical Center Utrecht, Utrecht, The Netherlands

[5]Hubrecht Institute-KNAW, University Medical Centre Utrecht, Utrecht, The Netherlands

[6]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, USA

[7]Medical Genetics Service, Hospital de Clinicas de Porto Alegre, Porto Alegre, Brazil.

[8] SIB-Swiss Institute of Bioinformatics, CH-1015 Lausanne, Switzerland

[9]Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Palo Alto, CA, USA

[10]Department of Clinical Genetics, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands

[11]Département de Génétique Médicale, Mohammed V University of Rabat, Morocco
[12]Department of Pediatric Cardiology, Emma Children's Hospital, Academic Medical Centre, Amsterdam, the Netherlands
[13]Retinal Signal Processing, Netherlands Institute for Neuroscience, Amsterdam, The Netherlands
[14]Department of Genome Analysis, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands
[15]PhD program in Experimental and Regenerative Medicine, University of Foggia, Foggia, Italy
[16]PhD Program, Molecular Genetics applied to Medical Sciences, University of Brescia, Brescia, Italy
[17]Department of Genetic Medicine and Development, University Medical Centre (CMU), 1211 Geneva, Switzerland
[18]Metabolic Diseases Branch/NIDDK, National Institutes of Health, Bethesda, MD, USA
[19]Texas Childrens' Hospital, Houston, TX, USA
[20]Child Neurology Division, Department of Neurology of University of Sao Paulo School of Medicine, Sao Paulo, Brazil
[21]Department of Pediatrics, Baylor College of Medicine, Houston, Texas, USA

*Co-first authors
[#]Co-senior authors

Correspondence should be addressed to:
Connie R. Bezzina, c.r.bezzina@amc.uva.nl
Jeroen Bakkers, j.bakkers@hubrecht.eu
Giuseppe Merla, g.merla@operapadrepio.it

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Abstract

We report a new genetic multi-system disorder caused by mutations affecting inhibitory G-protein signaling. Whole exome sequencing of 9 individuals from 6 unrelated families, from 4 different continents, with overlapping clinical manifestations identified bi-allelic loss-of-function and missense variants in *GNB5* that encodes the G-protein β subunit 5. This autosomal recessive disease combines the very unique finding of early-onset Heart rate disturbance, as well as Eye disease, Intellectual disability, Gastric problems, HypoTonia and Seizures; thus we propose the acronym HEIGHTS. A striking association between the nature of the variants and clinical severity of the syndrome was observed: individuals with loss-of-function alleles had more severe symptoms, including significant developmental delay, speech defects, severe hypotonia, pathological gastro-esophageal reflux, retinal disease and sinus node dysfunction, whereas carriers of a homozygous missense variant presented with a clinically milder phenotype. Zebrafish knocked out for *gnb5* faithfully recapitulated the phenotypic spectrum of affected individuals, including cardiac, neurological and ophthalmological abnormalities, thus providing further experimental evidence for a direct role of *GNB5* in the control of heart rate, hypotonia, and vision.

Main text

Heterotrimeric G proteins trigger a signal transduction cascade composed of α, β, and γ subunits. They are associated with G protein-coupled receptors (GPCRs) in modulating an array of cellular functions, including release of a multitude of hormones and growth factors, regulation of cell contraction and migration, as well as cell growth and differentiation during development [1-3] [4]. G-protein coupled signaling plays a crucial role in neuronal communication, including regulation of the antagonistic effects of the parasympathetic and sympathetic branches of the autonomic nervous system throughout the body. We report a new genetic disorder caused by mutations affecting *GNB5* (MIM: 604447), encoding guanine nucleotide binding protein, subunit beta 5, with disease manifestation in multiple systems.

We identified nine affected individuals (six females and three males) from six unrelated families presenting with a clinical overlap of neurological and cardiac conduction defects. Shared phenotypic features representing the cardinal characteristics of this syndrome include global developmental delay, seizures, generalized hypotonia, retinal disease and the uncommon feature of early-onset **sinus** node dysfunction **(Table 1)**. Additional clinical investigations and diagnostic studies did not show any evidence of structural central nervous system, ocular and cardiac anomalies. Affected individuals from four of the six families (families A-D) demonstrated the severe end of the disease spectrum with significant cognitive deficits, delayed motor development, severe hypotonia, retinal disease, pathological gastro-esophageal reflux, and sinus node dysfunction. Families E and F presented with a milder phenotype including mild intellectual impairment, language delay and bradycardia (**Figure 1, Table 1, Supplemental text**). We suggest describing this syndrome, and aggregate of rare endophenotypes, associated with pathogenic variation in the same gene, with the acronym “HEIGHTS” (Heart and Eye disease, Intellectual disability, Gastric problems, HypoTonia and Seizures).

As no potentially pathogenic genomic structural abnormalities were identified by array-CGH and karyotyping of the affected subjects, we applied whole

exome sequencing (WES) to all the affected individuals and their healthy parents. Families were recruited in Italy (family A), Brazil (B and F), the United States of America (C and D) and the Netherlands (E). The institutional review boards of the IRCCS Casa Sollievo Della Sofferenza Hospital, the "Hospital das Clínicas da Universidade de São Paulo", the Baylor College of Medicine, the Amsterdam Academic Medical Center and the University of Lausanne approved this study. Participants were enrolled after written informed consent was obtained from parents or legal guardians. The clinical evaluation included medical history interviews, a physical examination and review of medical records. To uncover genetic variants associated with the complex phenotype shown by the nine affected subjects we sequenced their exomes and that of their parents. DNA libraries were prepared from blood-derived genomic DNAs by standard procedures. Exomes were captured and sequenced using different platforms to reach 50-120-fold coverage on average (**Supplemental text**). Variants were called as previously described[5-7]. Variants were filtered based on inheritance patterns including autosomal recessive, X-linked and *de novo*/autosomal dominant. Variants with MAF<0.05% in control cohorts (dbSNP, the 1000 genome project, NHLBI GO Exome Sequencing Project, the Exome Aggregation Consortium database and our in-house databases) and predicted to be deleterious by SIFT[8], PolyPhen-2[9] and/or UMD predictor[10] were prioritized.

As some families reported a potential history of consanguinity (family B, C and F, **Figure 1; Table 1**) we filtered variants using Mendelian expectations for the assumption of a rare autosomal recessive trait. We found only the *GNB5* gene compliant with Mendelian expectations and bearing bi-allelic putative deleterious variants in all affected individuals (**Figure 1, Table S1**). Sanger sequencing in each family confirmed the anticipated segregation of the *GNB5* variants. Strikingly, the variants found in the severely affected individuals (families A-D) were predicted to be loss-of-function (LoF) alleles, whereas the more mildly affected individuals from families E and F were homozygous for the same missense variant, c.242C>T (NM_006578.3); p.(S81L) **(Figure 1, S1a)**. In families B, C and D the affected individuals were homozygous for splice variants (c.249+1G>T; p.(D84Lfs31X)) and (c.249G+3A>G;

p.(D84Vfs31X)) and a nonsense variant (c.906C>G; p.(Y302X)), respectively (**Figure 1, S1a; Table S1**). In family A, the affected siblings were compound heterozygous for a maternally inherited nonsense variant (c.994C>T; p.(R332X)) and a paternally inherited splice-site change (c.249G>A; (p.Q83Q)), that are predicted by conceptual translation to likely trigger nonsense-mediated decay of the corresponding transcripts; a hypothesis that was confirmed experimentally (**Figure 1, S1a, S2; Supplemental text**).

The five *GNB5* LoF variants identified in families A-D are either not present or present with AF≤8.25 x 10^{-6} (allele frequency) in ExAC (Exome Aggregation Consortium, Cambridge, MA; Version 0.3.1) (**Table S1**). Correspondingly, LoF variants in *GNB5* are underrepresented compared to expectation (8/19.1 respectively) in ExAC, suggesting selective pressure against such variants. The p.(S81L) missense variant identified in family E of Moroccan ancestry and family F of Brazilian ancestry has an AF<5 x 10^{-5} (6/121,000) in the human population and 4.3 x 10^{-4} in Latinos (5/11,574). A sample of individuals from Morocco identified a prevalence of 1 out of 1260 (7.94 x 10^{-4}) for this allele. Pathogenicity of this variant is further supported by three-dimensional representation of the encoded protein complexed with RGS9, a member of the R7-subfamily of Regulators of G-protein Signaling (RGS) proteins and common binding partner of GNB5. GNB5 is folded into essentially identical seven-bladed β-propellers (WD40 repeated domains) with equivalent N-terminal helical extensions[11]. Replacement of the evolutionary conserved S81 (**Figure S1b**) by Leucine will induce localized structural changes in the immediate vicinity of this residue, which could impair both the central pore of the β-propeller and the binding kinetics of RGS proteins (**Figure 2, S3, S4; Supplemental text**).

In line with the clinical presentation of HEIGHTS syndrome, *Gnb5* ablation in mice resulted in marked neurobehavioral abnormalities, including learning deficiencies, hyperactivity, impaired gross motor coordination, abnormal gait[12], defective visual adaptation[13] and perturbed development and functioning of retinal bipolar cells[14]. Correspondingly, mice lacking *Rgs6*, the *GNB5*-dependent RGS protein enriched in heart tissue, exhibit bradycardia

and hypersensitivity to parasympathomimetics[15; 16]. To independently investigate the functional effects of HEIGHTS-associated variation of *GNB5* in the full phenotypic spectrum of subjects reported herein, we engineered a zebrafish model knocked out for *gnb5*.

CRISPR/Cas9 genome editing was used to generate zebrafish with LoF mutations in *gnb5a* and *gnb5b,* the two *GNB5* paralogs present in the genome of this teleost (**Figure S5**). We identified stable lines with a +7bp insertion in *gnb5a* and a -8bp +15bp deletion/insertion in *gnb5b* causing a frameshift and premature truncation of the encoded proteins, respectively (**Figure S6**). It was anticipated that *gnb5a* and *gnb5b* might have redundant functions, which was confirmed by the absence of overt phenotypes in embryos homozygous for either LoF mutations. In-crosses of *gnb5a/gnb5b* double heterozygous carriers resulted in clutches of embryos containing the expected 6.25% of *gnb5a*$^{-/-}$*/gnb5b*$^{-/-}$ double mutants (henceforth referred to as *gnb5* mutants). Consistent with HEIGHTS syndrome manifestations, zebrafish mutants have no striking dysmorphologic features (**Figure S6d**), but the larvae generally die 7-14 days post fertilization (dpf).

To assess the putative involvement of *GNB5* in autonomic nervous system functions, we investigated the GNB5/RGS/GIRK channel pathway. As GNB5 recruits RGS proteins to G-protein-coupled inward rectifier potassium (GIRK) channels involved in the hyperpolarization of cell membranes[16; 17], we first investigated if LoF of *GNB5* could delay GIRK channel deactivation kinetics, increase hyperpolarization time of cell membranes, and impair cell responsiveness to new stimuli. Carbachol is a parasympathomimetic compound that activates acetylcholine receptors of the heart (NCBI, PubChem) and the GNB5/RGS/GIRK channel pathway. Treatment of *gnb5* mutant larvae with carbachol resulted in a strong decrease of the heart rate, whereas it had little effect on wild-type and sibling larvae (**Figure 3**), consistent with loss of negative regulation of the cardiac GIRK channel by GNB5/RGS. In contrast, treatment with the sympathetic agonist isoproterenol resulted in an increased heart rate that was similar in wild-type, sibling and *gnb5* mutant larvae (**Figure 3**). These results indicate that *GNB5* is crucial for

the parasympathetic control of heart rate, but not for sympathetic control suggesting that lack of *GNB5* is associated with extreme bradycardia at rest. Correspondingly, HEIGHTS individuals present severe bradycardia at rest (minimal observed heart rates of <25bpm (beats per minute)) combined with a normal chronotropic response (max heart rates >150 bpm).

The severe muscle hypotonia reported in HEIGHTS individuals could result from GIRK-mediated hyperpolarization of neurons controlling skeletal muscle tone. *gnb5* mutant embryos hatched normally from their chorion, a process that requires muscle contraction, but their swimming behavior appeared abnormal at 3 dpf. To investigate whether this abnormal behavior was linked to neurologic dysfunction and hypotonia, we examined the touch-evoked escape response. We anticipated that neurons would only become fully hyperpolarized after an initial stimulus and thus presented the embryos with three consecutive tactile stimuli. Whereas wild-type larvae rapidly swam away in response to repeated tactile stimuli, *gnb5* mutants showed a significant decrease in swimming distance and swimming speed at stimulus two ($P \leq 0.0001$) and three ($P \leq 0.01$), but not after the first stimulus (**Figure 4a-c**). Accordingly, *gnb5* mutant larvae were predominantly unresponsive to repeated tactile stimuli (**Supplemental movies**). To test whether this abnormal escape response is the consequence of neurologic dysfunction rather than reduced muscle function, we performed a tail movement assay. 5 dpf larvae were given a strong tactile stimulus while recording the movement of the tail (**Figure 4d-e**). No significant difference was detected in the maximum tail angle between wild-type and *gnb5* mutant larvae (**Figure 4e**). These results indicate that the tail muscles of *gnb5* mutants are fully functional and that the abnormal escape response is associated with neurological dysfunction and possibly muscle hypotonia.

Since HEIGHTS individuals have visual problems, including nystagmus, we investigated the visual system by measuring the optokinetic response (OKR) of *gnb5* mutant larvae. When wild-type larvae were placed in a drum with rotating light stimulus (**Figure S7a**), the OKR consists of smooth pursuit eye movements followed by rapid rest saccades in the opposite direction (**Figure**

S7b, Supplemental movies). In contrast, OKR was completely absent in *gnb5* mutant larvae although their eyes showed no morphological abnormalities and could make eye movements (**Figure S7c, Supplemental movies**). This indicates that the eye muscles are functional in *gnb5* mutants but that proper eye-movement control depends on *GNB5*. Overall these data showed that *gnb5* mutants faithfully recapitulate the phenotypic spectrum of HEIGHTS patients, including cardiac, neurologic and ophthalmologic abnormalities.

These results for the first time provide evidence for a direct role of *GNB5* in the control of heart rate, motor capacity, and vision. Whereas the $G\beta_{1-4}$ are highly homologous and widely expressed[18], $G\beta_5$ exhibits much less homology with the other isoforms and is preferentially expressed in the brain and nervous system[19; 20].

Germline *de novo GNB1* variants cause severe neurodevelopmental disability[21], hypotonia and seizures. *GNB3* bi-allelic LoF has been linked to stationary night blindness in human[22], retinal degeneration in chicken[23] and reduced cone sensitivity and mild bradycardia in mice[24; 25]. A single nucleotide polymorphism (SNP) in *GNB3* was associated with postural tachycardia syndrome[26], incidence of cardiovascular disease and stroke[27]. Similarly, *GNB2* and *GNB4* map to loci governing heart rate on chromosome 7 and 3, respectively[28; 29]. We hereby demonstrate that bi-allelic LoF and missense variants in *GNB5* cause the multisystem HEIGHTS syndrome with features that include global developmental delay, sinus node dysfunction, seizures, eye abnormalities, gastric problems and generalized hypotonia. We highlight the importance of *GNB5* for neuronal signaling, including the regulation of the antagonistic effects of the parasympathetic and sympathetic nervous system.

Supplemental Data

Supplemental Data include Supplemental Material and Methods, Supplemental Text, seven figures and one table.

Competing interest

JRL has stock ownership in 23andMe, is a paid consultant for Regeneron Pharmaceuticals, has stock options in Lasergen, Inc., is a member of the Scientific Advisory Board of Baylor Miraca Genetics Laboratories, and is a co-inventor on multiple United States and European patents related to molecular diagnostics for inherited neuropathies, eye diseases and bacterial genomic fingerprinting. Baylor College of Medicine (BCM) and Miraca Holdings Inc. have formed a joint venture with shared ownership and governance of the Baylor Miraca Genetics Laboratories (BMGL), which performs clinical exome sequencing. The Department of Molecular and Human Genetics at Baylor College of Medicine derives revenue from the chromosomal microarray analysis (CMA) and clinical exome sequencing offered in the Baylor Miraca Genetics Laboratory (BMGL; http://www.bmgl.com/BMGL/Default.aspx website). The remaining authors declare that they have no competing interests.

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Web Resources

1000 Genomes Project Browser, http://browser.1000genomes.org/index.html

Burrows-Wheeler Aligner, http://bio-bwa.sourceforge.net/

dbSNP, http://www.ncbi.nlm.nih.gov/SNP/

Ensembl genome assembly GRCh37, http://grch37.ensembl.org/Homo_sapiens/Info/Index

Exome Aggregation Consortium (ExAC) Browser, http://exac.broadinstitute.org/

ExomeDepth, https://cran.r-project.org/web/packages/ExomeDepth/index.html

GATK, https://www.broadinstitute.org/gatk/

GraphPad Prism, www.graphpad.com

NetGene2, http://www.cbs.dtu.dk/services/NetGene2

NHLBI GO Exome Sequencing Project (ESP) Exome Variant Server, http://evs.gs.washington.edu/EVS/

NNSPLICE, http://www.fruitfly.org/seq_tools/splice.html

OMIM, http://www.omim.org/

PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/

SIFT, http://sift.jcvi.org/

SnpEff, http://snpeff.sourceforge.net/

SOAPsnp, http://soap.genomics.org.cn/soapsnp.html

Swiss-PdbViewer, http://spdbv.vital-it.ch/

UMD-Predictor, http://umd-predictor.eu/

Authors' contribution

GM, AR, CRB, JB conceived and directed the study. D.C, P.D.N., N.M., BM, C.F.M.d.S., FK, W.K.W., V.R.S., Z.H.C.A., J.R.L., M.K.E., V.N., R.J.F., M.N.A., recruited patients, gathered clinical information, prepared samples, performed whole-exome and mutational analysis. D.C, P.D.N., N.M., B.M., E.M.L., La.B., N.L, L.B performed the model organism experiments. NG

performed structural modeling of GNB5 variant. E.M.L. performed statistical analysis, E.M.L., E.A.N., A.A.M.W., and L.B. analyzed the data. C.D.K., F.T., TdB, W.d.G., M.K., J.B. designed and conducted the zebrafish experiments and analyzed the data. N.A.B. and I.R. contributed reagents/materials/analysis tools. W.F.S. provided unpublished *gnb5* KO mouse model data. P.D.N., A.R., G.M., C.R.B., J.B. wrote the manuscript. All authors reviewed and approved the manuscript.

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Figure Legends

Figure 1. Pedigrees from the six families investigated in this study

Affected members of families A to D (upper red-lined panel) and E to F (lower blue-lined panel) show severe and mild manifestation of the core symptoms of the novel HEIGHTS syndrome defined in this study. Filled symbols represent individuals with severe Sinus Sick Syndrome (SSS; top left quarter), intellectual disability (ID; top right quarter), hypotonia (bottom left quarter) and seizures (bottom right quarter), whereas light grey top left quarter indicate the presence of mild ID. Genotypes are specified according to NM_006578.3.

Figure 2. 3D model of the GNB5-RGS complex

(**a**) Three-dimensional representation of the human GNB5 (orange) complexed with RGS9 (red). A loop of RGS9 (blue) blocks the access to the

central pore of GNB5, in which a glycerol molecule (green) can be found in the x-ray structure [11]. The S81 residue is highlighted in cyan. (**b**) Top view of the GNB5 molecular surface. The S81 position (cyan) is emphasized with respect to the central pore. The glycerol molecule is shown in green (RGS9 and C111 have been removed for clarity). (**c, d**) Detailed views of the S81 aminoacidic context **(c)** and the energy minimized S81L variant **(d)**.

Figure 3. Cardiac function in *gnb5* mutant zebrafish

(**a, d**) Box-whisker plots demonstrate the heart rate response and the relative heart rate change of 5 dpf wild-type, sibling and *gnb5* mutant larvae to (**a,c**) 400µM of the parasympathetic agonist Carbachol (CCh) (wild-type N=10, sibling N=39, *gnb5* mutant N=14) and (**b, d**) 100 µM of the sympathetic agonist isoproterenol (ISO) (wild-type N=12, sibling N=22, mutant N=9). The relative heart rate change is the % change between the basal heart rate measured and the heart rate after addition of CCh or ISO. WT, wild-type; SIB, sibling; MT, *gnb5* mutant; bpm, beats per minute.

Figure 4. Neurologic function in *gnb5* mutant zebrafish

(**a, c**) Touch-evoked escape response assay in which three consecutive tactile stimuli were applied. (**a**) Representative responses of 3 dpf wild-type and *gnb5* mutant embryos to a touch stimulus. Scalebar = 0.5cm. Box-whisker plots show quantification of the (**b**) swimming distance and (**c**) swimming speed in TL wild-types (N=19), siblings (N=46) and *gnb5* mutants (N=27). (**d, e**) Analysis of maximum tail movement at 5dpf. (**d**) Representative minimum projection images of tail movement in wild-type and *gnb5* mutant embryos, including tail angle analysis. The tail angle represents the angle between the head-tail midline axis in resting state and a line that was drawn from just caudal of the swimbladder to the tip of the tail at maximal tail movement (**e**) Tail angle quantification is displayed in box-whisker plots (wild-type N=10, *gnb5* mutants N=10).

Figure 1

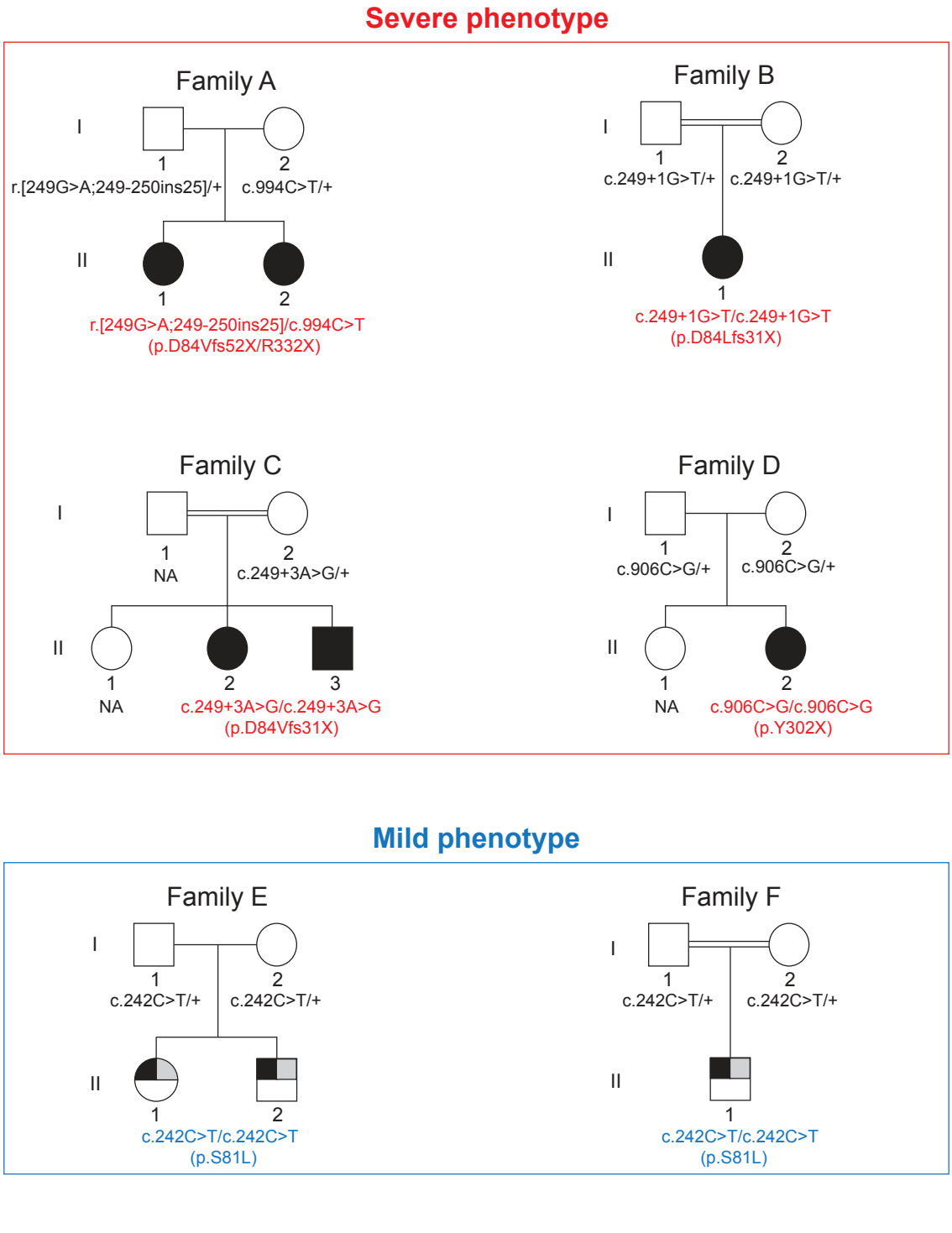

Severe phenotype
Family A
I
1
r.[249G>A;249-250ins25]/+
2
c.994C>T/+
II
1
2
r.[249G>A;249-250ins25]/c.994C>T
(p.D84Vfs52X/R332X)
Family B
I
1
c.249+1G>T/+
2
c.249+1G>T/+
II
1
c.249+1G>T/c.249+1G>T
(p.D84Lfs31X)
Family C
I
1
NA
2
c.249+3A>G/+
II
1
NA
2
3
c.249+3A>G/c.249+3A>G
(p.D84Vfs31X)
Family D
I
1
c.906C>G/+
2
c.906C>G/+
II
1
NA
2
c.906C>G/c.906C>G
(p.Y302X)
Mild phenotype
Family E
I
1
c.242C>T/+
2
c.242C>T/+
II
1
2
c.242C>T/c.242C>T
(p.S81L)
Family F
I
1
c.242C>T/+
2
c.242C>T/+
II
1
c.242C>T/c.242C>T
(p.S81L)
SSS
Severe ID
Mild ID
Hypotonia
Seizures

Figure 2

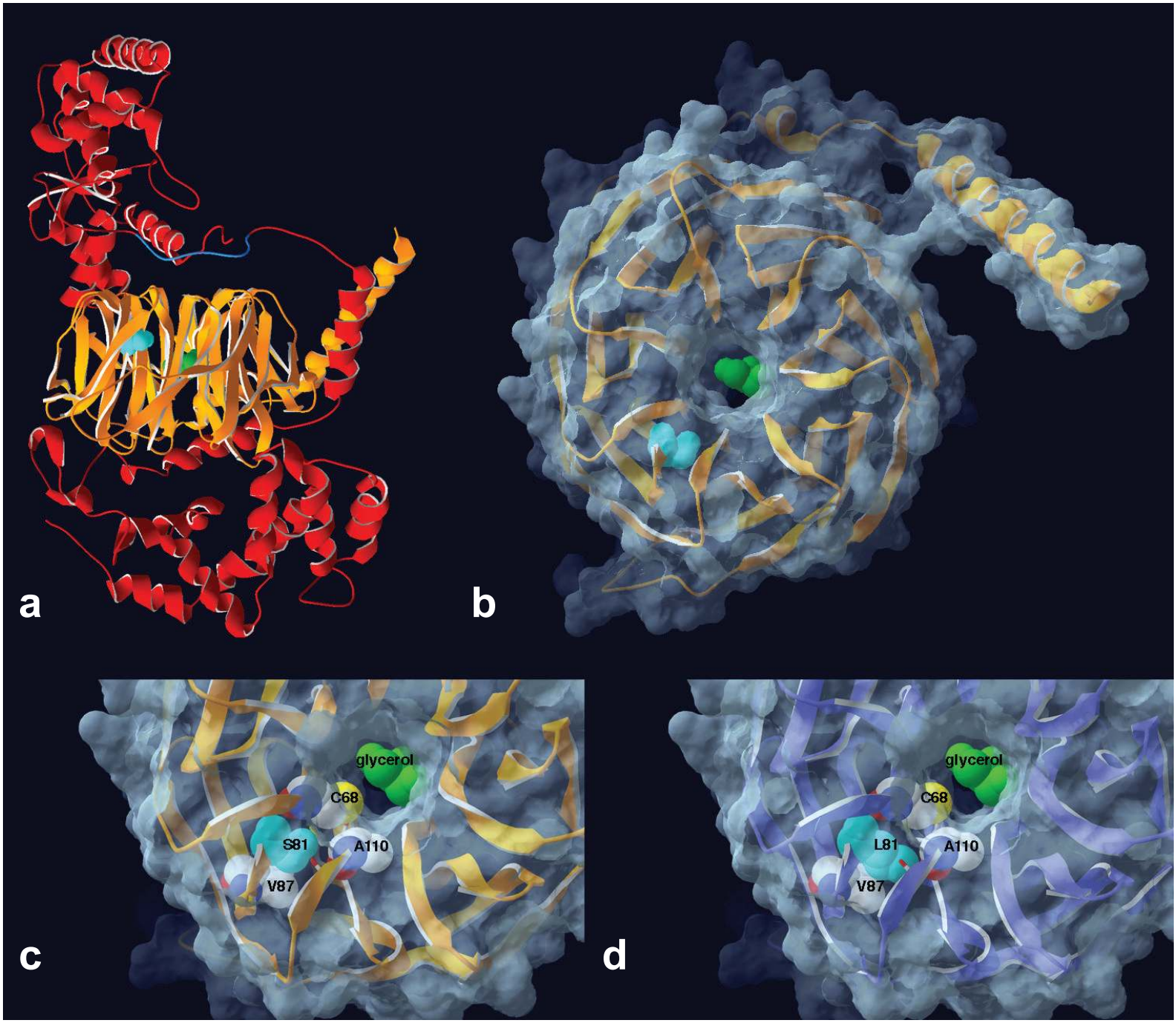


Figure 3

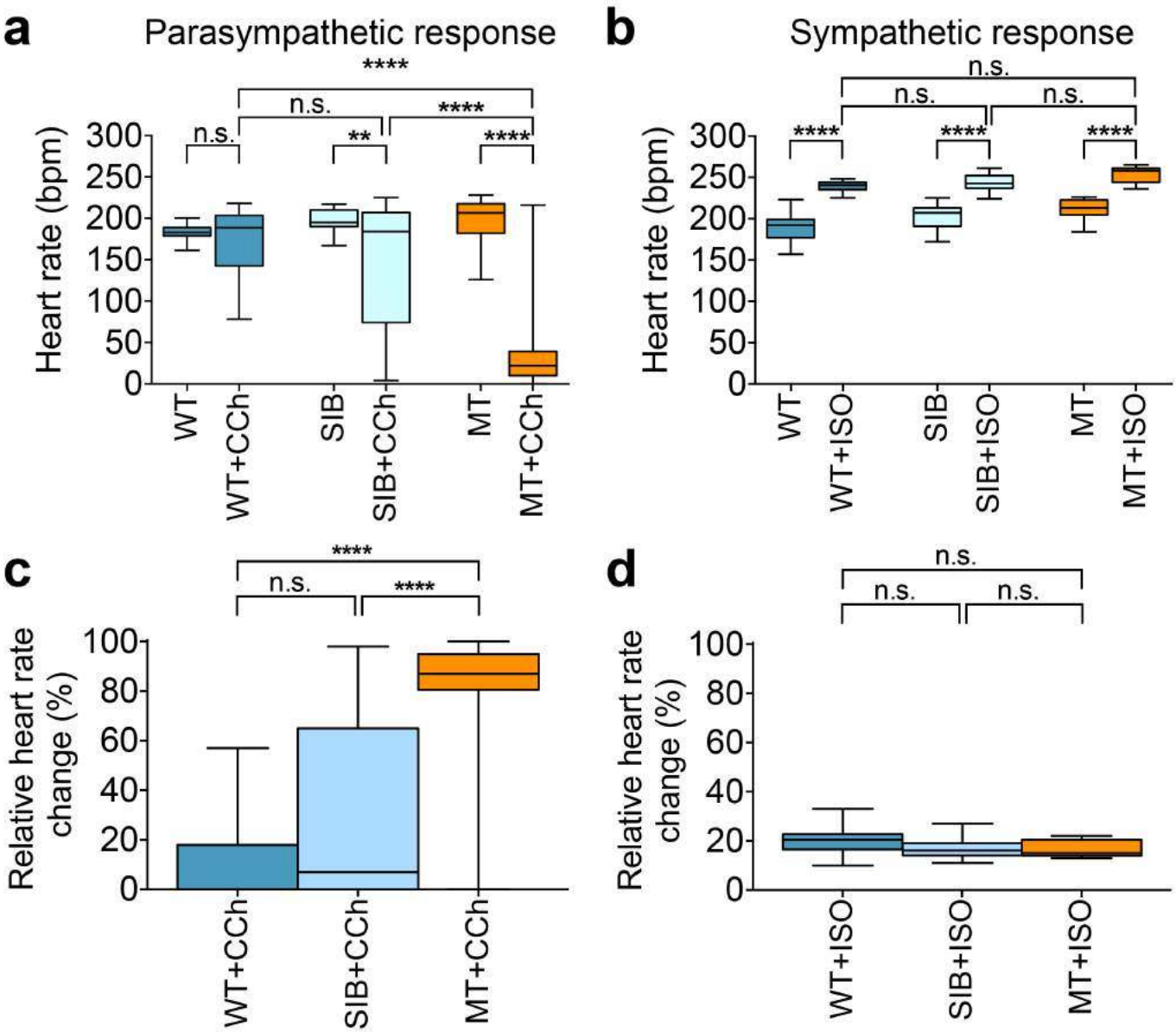
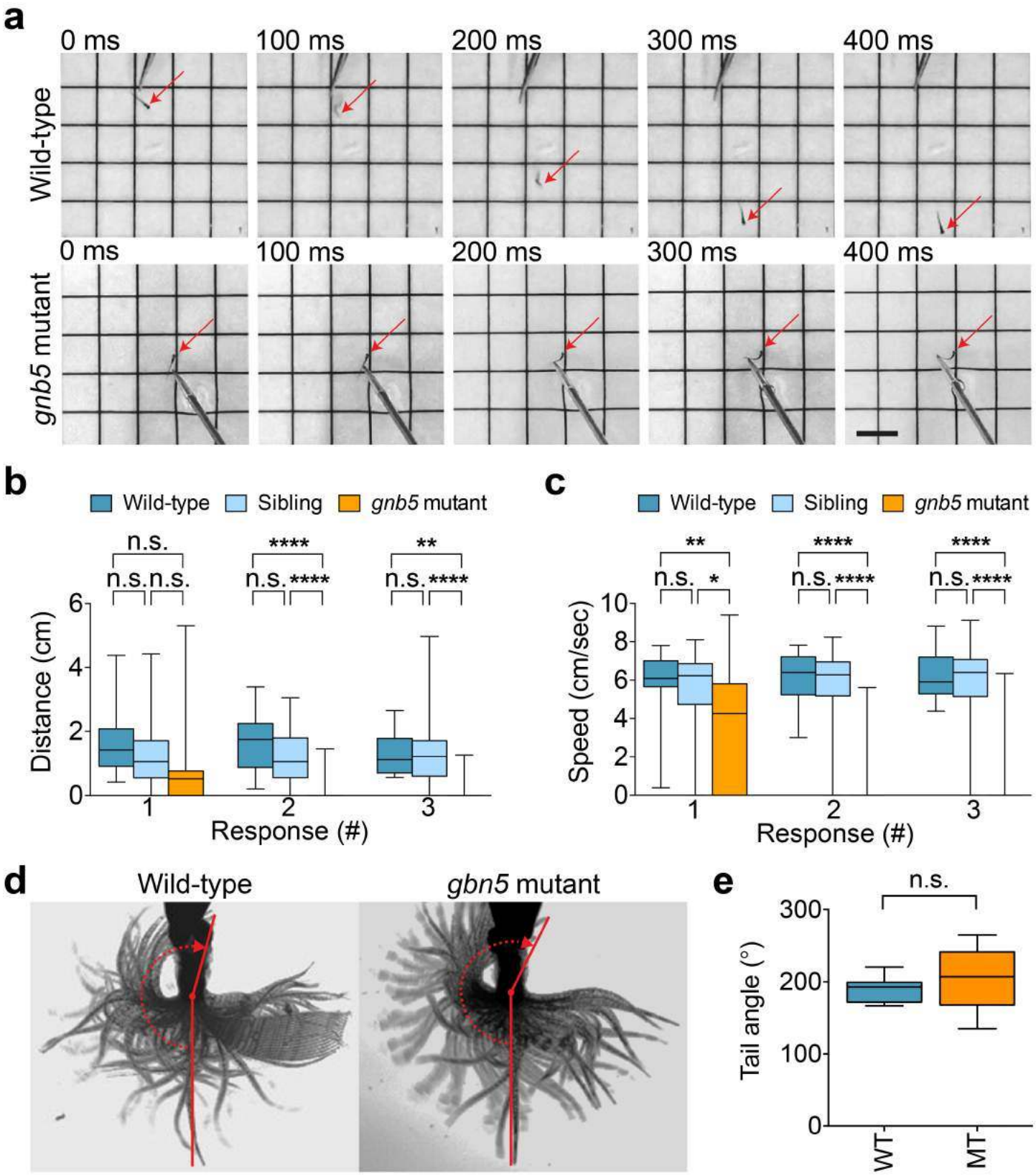

a
Parasympathetic response
Heart rate (bpm)
300
250
200
150
100
50
0
****
n.s.
n.s.
****
**
****
WT
WT+CCh
SIB
SIB+CCh
MT
MT+CCh
b
Sympathetic response
Heart rate (bpm)
n.s.
n.s.
n.s.
****
****
****
WT
WT+ISO
SIB
SIB+ISO
MT
MT+ISO
c
Relative heart rate change (%)
100
80
60
40
20
0
****
n.s.
****
WT+CCh
SIB+CCh
MT+CCh
d
Relative heart rate change (%)
n.s.
n.s.
n.s.
WT+ISO
SIB+ISO
MT+ISO

Figure 4


a
0 ms
100 ms
200 ms
300 ms
400 ms
Wild-type
gnb5 mutant
b
Wild-type
Sibling
gnb5 mutant
Distance (cm)
Response (#)
n.s.
****
**
c
Speed (cm/sec)
Response (#)
*
d
Wild-type
gbn5 mutant
e
Tail angle (°)
WT
MT

Table

Table 1: Overlapping clinical features of individuals with GNB5-related syndrome

	Family A		Family B	Family C		Family D	Family E		Family F
	II.1	**II.2**	**II.1**	**II.2**	**II.3**	**II.2**	**II.1**	**II.2**	**II.1**
Gender, Age	F, 22	F, 20	F, 6	F, 11	M, 9	F, 12	F, 13	M, 8	M, 23
Birth weight	3580 g (50th percentile)	NA	2980 g (15th percentile)	2751 g (15th percentile)	NA	2845 g (15th percentile)	NA	NA	NA
Ethnicity	Italy	Italy	Jordan	Puerto Rico	Puerto Rico	India	Morocco	Morocco	Brazil
Consanguinity	-	-	+	+	+	-	-	-	+
Altered speech development	+	+	+	+	+	+	+	+	NA
- Verbal understanding			nonverbal	unremarkable	unremarkable				
- Lexical production			nonverbal	delayed	delayed	Nonverbal	delayed	delayed	
Intellectual disability (ID)	+	+	+	+	+	+	mild	mild	mild
Epilepsy	+	+	+	-	-	+	-	-	-
Sinus Sick Syndrome (SSS)	+	+	+	+	+	increased PR interval (intermittent Weckenbach)	+	+	+
- Minimum heart rate	24	39	NA	paced	paced	NA	20	16	
- Maximum heart rate	163	192	NA	paced (27% heartbeats on Holter)	paced (20% heartbeats on Holter)	NA	176	180	NA
- Chronotropic response	NA	NA	NA	+	+	NA	unremarkable	unremarkable	NA
- Escape beats	+	+	NA	paced	paced	NA	+	+	NA
- Pacemaker implantation	-	-	-	+	+	-	-	+	NA
- Heart structural abnormalities	-	PFO	NA	-	-	-	-	-	NA
Hypotonia	+	+	+	+	+	+	-	impaired fine motor skills	-
Pathological gastric reflux	+	+	-	+	+	+	-	-	NA
Nystagmus	+	+	+	+	+	+	NA	-	NA
Metabolic work-up									
- Plasma amino acids chromatography	938 µm/l (restored)	+ (restored)	unremarkable	unremarkable	unremarkable	unremarkable	444 µm/l	unremarkable	NA
- Urine organic acids	unremarkable	unremarkable	increased excretion of 3-methyl-glutaconic acid	unremarkable	unremarkable	unremarkable	NA	NA	NA

Patient numbers refer to those of the pedigree in **Figure 1**.

Abbreviations are as follows: M, male; F, female; NA, Not Available; +, present clinical trait; -, not present clinical trait; PFO, Patent Foramen Ovale; RV dilatation, Right Ventricular dilatation.
Complete pedigree charts, consanguinity status, variants, and related homozygous and/or compound heterozygous alleles are reported in **Figure 1** and **Table S2**.

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TouchEvokedResponse_Movie1_wild-type_AJHG.mov

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TouchEvokedResponse_Movie2_gnb5mutant_AJHG.mov