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ORIGINAL ARTICLE

# EPHA7 haploinsufficiency is associated with a neurodevelopmental disorder

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## Abstract

Ephrin receptor and their ligands, the ephrins, are widely expressed in the developing brain. They are implicated in several developmental processes that are crucial for brain development. Deletions in genes encoding for members of the Eph/ephrin receptor family were reported in several neurodevelopmental disorders. The ephrin receptor A7 gene (*EPHA7*) encodes a member of ephrin receptor subfamily of the protein-tyrosine kinase family. EPHA7 plays a role in corticogenesis processes, determines brain size and shape, and is involved in development of the central nervous system. One patient only was reported so far with a de novo deletion encompassing *EPHA7* in 6q16.1. We report 12 additional patients from nine unrelated pedigrees

Bérénice Schell, Hala Nasser, and Myriam Rachid or Alain Verloes and Anne-Claude Tabet contributed equally to this study.

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with similar deletions. The deletions were inherited in nine out of 12 patients, suggesting variable expressivity and incomplete penetrance. Four patients had tiny deletions involving only *EPHA7*, suggesting a critical role of *EPHA7* in a neurodevelopmental disability phenotype. We provide further evidence for *EPHA7* deletion as a risk factor for neurodevelopmental disorder and delineate its clinical phenotype.

**KEYWORDS**
6q16.1 microdeletion, EPHA7, intellectual disability, microcephaly, neurodevelopmental disorder, speech and language development

## 1 | INTRODUCTION

Ephrin receptors (Eph) are the largest family of transmembrane receptor tyrosine kinases. They have a single kinase domain and an extracellular region containing a Cys-rich domain and two fibronectin type III repeats.[1] Eph interact with membrane bound ephrins (Efn) ligands: binding Efn activates the tyrosine kinase activity of the Eph through receptor clustering. Ephrins can be attached by a glycosylphosphatidylinositol linkage (Efn-A) or a transmembrane domain (Efn-B). Based on the similarity of their extracellular domain sequences and affinities, the EPH receptors are sorted in two subclasses: the EphA preferentially bind Efn-A, EphB preferentially bind the Efn-B. The Eph/Efn functions as a contact-mediated bidirectional signaling system between neighboring cells: forward signaling is propagated into the Eph-expressing cell, and reverse signals are propagated into the Efn-expressing cell. The system has been implicated in mediating developmental events, noteworthy body segmentation, central nervous system patterning (axon guidance), neural crest migration and angiogenesis.[2,3]

In human pathology, deletions of EPH/EFN genes have been implicated in several form of syndromic neurodevelopmental disability (NDD). A 1.4 Mb de novo 1q21.3 deletion encompassing *EFNA1*, *EFNA3* and *EFNA4* was reported in a 2.5 years old girl with microcephaly, dysmorphic features and intellectual disability (ID).[4] Recently, we delineated the NDD phenotype associated with a 610 kb 13q33.3 deletion encompassing *EFNB2* in a family with developmental delay (DD), ID, seizures, hearing impairment, and congenital heart defect.[5]

A de novo 6q16.1 deletion encompassing *EPHA7* was also described in a 16-month-old patient presenting mild DD, microcephaly and dysmorphic features.[6]

The ephrin receptor A7 (*EPHA7*, mapped in 6q16.1) encodes a member of ephrin receptor subfamily of the receptor tyrosine kinase family. Among glycosylphosphatidylinositol-anchored ephrin-A ligands, EphA7 binds ephrin-A5 with high affinity and their interaction regulates brain development modulating cell–cell adhesion and repulsion.[7,8] EphA7 regulates dendrite morphogenesis restricting dendritic elaboration in early corticogenesis and promotes dendritic spine formation later in neuronal development.[8,9] EphA7 signaling also drives neuronal maturation and synaptic function suggesting the involvement of *EPHA7* in NDD.[6,9]

In the present study, we report 12 patients from nine unrelated families with 6q16.1 deletions encompassing *EPHA7*, including four patients with a deletion involving only *EPHA7*, in order to further support the role of *EPHA7* haploinsufficiency as a risk factor for NDD.

## 2 | MATERIAL AND METHODS

An informed consent for genetic testing was obtained from all patients and their parents.

Data from Patients 1 to 9 and 11 were collected through the French network AChro-Puce https://acpa-achropuce.com/.

Patients 10 and 12 were enrolled through the Chromosome 6 Project,[10] Patient 12 was published before[10] and parents of both patients gave consent during the enrolment procedure.

ID panel of 450 genes (Data S1) was performed in Patients 1–4 and 9 and identified no pathogenic variant.

No additional pathogenic variant has been identified by trio exome sequencing in Patient 10.

### 2.1 | Molecular cytogenetic analysis

#### 2.1.1 | Chromosomal microarray analysis

Microarray studies were done in all 12 patients. DNA was extracted using standard procedures from peripheral blood lymphocytes. Patients 1–5 (P1–P5) were investigated using Illumina OmniExpress (Illumina Inc., San Diego, CA) SNP microarray, which contains over 700 000 markers (mean resolution of 30 kb). Data were processed with the Infinium assay. The results were analyzed with Illumina GenomeStudio software, facilitated by the CNV partition algorithm. SNP profiles were analyzed by comparing the Log R ratio, ln (sample copy number/reference copy number), and the B allele frequency.

Other patients were analyzed with the following oligonucleotide arrays: Agilent 180 K (Agilent Technologies, Santa Clara, CA) in Patients 6–9, and 12, Agilent 60 K in Patient 11 and Affymetrix CytoScan 750 k (ThermoFisher, MA) in patient 10. Results were analyzed according to the Human Feb. 2009 (GRCh37/hg19) assembly (UCSC Genome Browser, http://genome.ucsc.edu).

**TABLE 1** Clinical features of patients with 6q16.1 deletions

| General caracteristics | Traylor et al. (2009) | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | P9 | P10 | P11 | P12 | Summary |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sex | M | M | F | M | M | M | F | M | M | F | F | F | F | 7 M / 6F |
| Array results | del 6q16.1 | del 6q16.1 | del 6q16.1 | del 6q16.1 | del 6q16.1 (+ *del 7q11.23)* | del 6q15q 16.1 | del 6q16.1 | del 6q16.1 | del 6q16.1 | del 6q15q16.1 | del 6q15 q16.1 | del 6q15q 16.1 | del 6q14.3q 16.1 | 6q14.3–6q16.1 |
| Breakpoints | 92 780 274–94 849 199 | 93 973 569–98 435 125 | 93 973 569–98 435 125 | 93 973 569–98 435 125 | 93 973 569–98 435 125 | 91 385 628–94 529 099 | 94 028 579–94 181 308 | 94 024 705–94 483 810 | 93 101 362–94 038 302 | 89 949 668–94 292 552 | 89 431 855–95 879 201 | 88 569 372–94 783 161 | 87 927 105–94322474 | 87 927 105–98 435 125 |
| Deletion size | 2.1 Mb | 4.5 Mb | 4.5 Mb | 4.5 Mb | 4.5 Mb | 3.1 Mb | 152 kb | 486 kb | 936 kb | 4.3 Mb | 6.4 Mb | 6.2 Mb | 6.4 Mb | 152 kb–6.4 Mb |
| Inheritance | de novo | pat | pat | pat | pat (+ de novo) | ND | ND | mat | pat | mat | mat | mat | de novo | 9 inherited / 2 de novo |
| Confirmation | FISH (RP11-270O11) | FISH (RP11-47D17) | FISH (RP11-47D17) | FISH (RP11-47D17) | FISH (RP11-47D17) | FISH (RP11-47D17) | FISH (CTD-2248A96) | qPCR | qPCR | qPCR | qPCR | FISH (RP11-608F5) | ND | 8 FISH / 4 qPCR |
| Birth Measurements | | | | | | | | | | | | | | |
| Gestational age in WG | 38 | 39 | 39 | ND | 39 | ND | 37 + 5 | 37 | ND | 40 | 39 | 39 | 39 + 2 | 39 WG (38–40) |
| Weight in g (percentile) | ND (3 pc) | 3080 (27 pc) | 2900 (23 pc) | ND | 3120 (30 pc) | ND | 2975 (49 pc) | 3440 (86 pc) | 3730 (ND) | 3435 (52 pc) | 2850 (20 pc) | 3725 (83 pc) | 2500 (4 pc) | 38 pc (3–100) |
| Length in cm (percentile) | ND (50 pc) | 50 (46 pc) | 48 (26 pc) | ND | 48 (16 pc) | ND | 47 (30 pc) | 51 (87 pc) | 49.5 (ND) | 51 (60 pc) | 47 (14 pc) | 51 (73 pc) | 47 (11 pc) | 41 pc (11–87) |
| Head Circumference in cm (percentile) | ND | 33 (15 pc) | 34 (45 pc) | ND | 36 (85 pc) | ND | 35.5 (93 pc) | 37.2 (100 pc) | 36.5 (ND) | 34 (31 pc) | 33 (20 pc) | 34 (45 pc) | ND | 54 pc (15–100) |
| Latest growth parameters | | | | | | | | | | | | | | |
| Age | 1 year 3 months | 8 years | 7 years | 6 years | 3 years | 2 years | 7 years | 10 years | 4 years | 1 year 5 months | 22 months | 5 years | 7 years | 5 years (15 monts–11 years) |
| Weight in Kg (SD) | 8.1 (−3.1 SD) | 26.7 (+ 1 SD) | 23 (+0 SD) | 30.8 (+3 SD) | 10.8 (−2 SD) | 9.75 (−2 SD) | 38.1 (+3 SD) | ND (+0 SD) | 16.5 (+0 SD) | 5.4 (−4.2 SD) | 11 (−0.9 SD) | 16 (−1 SD) | 31 (−1.7 SD) | −0,6 SD (−4.2 to +3) |
| Height in cm (SD) | 74.1 (−1 SD) | 128 (+0 SD) | 119 (−0.5 SD) | 125 (+2 SD) | 89 (−1.5 SD) | 81 (−1.5 SD) | 126.5 (+1 SD) | ND (+0 SD) | 103 (+0.5 SD) | ND | 87 (0 SD) | 103 (−1 SD) | 147 (+0.3 SD) | −0.39 SD (−1.5 to +2) |
| Head Circumference in cm (SD) | 43.3 (−2 SD) | 51.5 (+0 SD) | 49 (−2.3 SD) | 53.5 (+2 SD) | 50.5 (+1 SD) | 44.5 (−3 SD) | 56 (+3 SD) | 57.3 (+2 SD) | 49.5 (+0 SD) | 43.2 (−2 SD) | 46 cm (−0.6 SD) | 46 (− 3 SD) | 53 cm (0 SD) | −0.37 SD (−3 to +3) |
| Dysmorphic facial features | Yes | No | No | Yes | Yes (Williams Beuren Syndrome) | No | Yes, mild | Yes | ND | Yes, mild | No | No | Yes | 7/13 (54%) |

(Continues)

**TABLE 1** (Continued)

| General caracteristics | Traylor et al. (2009) | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | P9 | P10 | P11 | P12 | Summary |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Description | Triangular-shaped face, cupped and posteriorly rotated ears and periauricular tags | No | No | Cupped ears, right preauricular pit | Bilateral bifid tragus, short helix | No | Hypertelorism | Low set ears, hypertelo-rism, bulbous nose | ND | Round face, long philtrum, thin upper lip, low-set ears, large ear lobes | No | No | Plagiocephaly | |
| Neurological features | | | | | | | | | | | | | | |
| Hypotonia | ND | ND | ND | ND | Yes | ND | No | Yes | ND | Yes | Yes | Yes | No | 5/7 (71%) |
| Developmental delay | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | 13/13 (100%) |
| Age at walking | ND | 15 months | 13 months | 20 months | Not walking at 36 months | ND | 24 months | 14 months | ND | 30 months | 36 months | 17 months | 18 months | 13–>36 months |
| Intellectual disability | ND | Mild | Mild | Mild | Severe | Yes | Mild | Mild | Yes | Mild | Yes | Mild | Moderate | 10/10 (100%) |
| Speech delay | ND | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | 12/12 (100%) |
| Age at talking | ND | 24 months | 30 months | 30 months | Not talking at 3 years | Not talking at 2 years | First sentences at 36 months | ND | ND | Five words at 30 months | Not talking at 3 years 6 months | ND | 28 months, stopped talking at 4 years, now absent speech | 24–>42 months |
| Behavior | ND | Lack of inhibition | ND | Hyperkinesia | ND | Very calm, shows no fear | Impulsivity, attention-deficit | Attention-deficit | Aggressive behavior, communi-cation difficulties | Attention-deficit | ND | Hyperactivity | Easily upset, hyperac-tive, autism | Abnormal in 9/9 (100%) |
| Sleep disturbance | ND | No | No | Yes | No | No | Yes | Yes | Yes | No | Yes | Yes | No | 6/12 (50%) |
| Microcephaly | Yes | No | Yes | No | No | Yes | No | No | No | Yes | No | Yes | No | 5/13 (38%) |
| Brain MRI | Questionable brain atrophy/ hemorrhage | Normal | Normal | ND | Normal | ND | Normal | ND | ND | Olfactory bulb agenesis | ND | Normal | Normal | Abnormal in 2/8 (25%) |
| Others features | | | | | | | | | | | | | | |
| Eye anomalies | mild ptosis on the left side | No | No | No | No | No | Ptosis | Strabismus | ND | No | No | Astigmatism | CVI, iris coloboma | 5/12 (42%) |
| Cardiac anomalies | ND | No | No | BAP without stenosis or regurgita-tion | Supravalvular aortic stenosis | No | No | No | ASD | VSD | No | No | No | 4/12 (25%) |

 

**TABLE 1** (Continued)

| General caracteristics | Traylor et al. (2009) | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | P9 | P10 | P11 | P12 | Summary |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Other | | | Precocious puberty | 2 café-au-lait spots | Right renal cyst and bilateral ureteral dilatation | | | Hypomineralization of tooth enamel | | Clinodactyly 5th finger, joint hypermobility | Feeding difficulties, chewing difficulties, excessive drooling, constipation, recurrent infections, immune system abnormality | | Feeding difficulties, epilepsy | |

*Note*: Clinical information is given for individuals 1–12 with 6q16.1 deletions plus one case from Traylor et al.[6] Patient 12 was also published before.[10]

Abbreviations: ASD, atrial septal defect; BAV, bicuspid aortic valve; CIV, cortical visual impairment; del, deletion; F, Female; kb, kilobases; M, Male; Mb, megabases; mat, maternal; ND, not described; pc, percentile; pat, paternal; VSD, ventricular septal defect; WG, weeks of gestation.

### 2.1.2 | Fluorescence in situ hybridization analysis

Fluorescence in situ hybridization on metaphase spreads using a specific probe located at 6q16.1: RP11-47D17 was used to confirm deletions for Patients 1–5, CTD-2248A96 for P6 and RP11-608F5 for P9 (RainbowFish, Amplitech).

### 2.1.3 | qPCR

qPCR was used to confirm the 6q16.1 deletion for Patients 7–10 and their parents.

We used the Universal Probe Library system (Roche, Indianapolis, IN). Probes and primers were chosen using Probe Finder v2.45 software (Roche, http://www.universalprobelibrary.com) for P7 and P9.

Qiagen Multiplex PCR kit (Qiagen, Courtaboeuf, France), ABI Prism 3130 XL sequencer (Applied Biosystems, Foster City, CA) and GeneMarker V2.7.0 (Soft Genetics) software were used for P8.

## 2.2 | Exome sequencing

Genomic DNA was extracted from peripheral blood lymphocytes of the Patients P1 and P2 (Family A), P10 (Family H), and P11 (Family G). IntegraGen SA (Evry, France) carried out the library preparation, exome capture, sequencing, and data analysis. Genomic DNA was captured using a Twist Human Core Exome Enrichment System (Twist Bioscience, San Francisco, CA) and IntegraGen Custom. Sequence capture, enrichment, and elution were performed in accordance with the manufacturer's instruction and protocols (Twist Bioscience) without modification, except for library preparation performed with NEBNext® Ultra II kit (New England Biolabs, Beverly, MA). For library preparation, 150 ng of each genomic DNA was fragmented by sonication and purified to yield fragments of 150–200 bp. Paired-end adaptor oligonucleotides from the NEB kit were ligated on repaired, a-tailed fragments, and then purified and enriched by seven polymerase chain reaction (PCR) cycles. Next, 500 ng of these purified Libraries was hybridized to the Twist oligo probe capture library for 16 h in a single plex reaction. After hybridization, washing and elution, the eluted fraction was PCR-amplified for eight cycles, and then purified and quantified by a quantitative PCR to obtain sufficient DNA template for downstream applications. Each eluted-enriched DNA sample was then sequenced on an Illumina HiSeq4000 (Illumina, San Diego, CA) as single-end 75-bp reads. Image analysis and base calling were performed using Real-Time Analysis, version 3.4.4 (Illumina) with default parameters.

Base-calling was performed using the Real-Time Analysis software sequence pipeline (version 2.7.7) with default parameters. Sequence reads were mapped to the human genome build (hg38) using the Burrows-Wheeler Aligner tool (http://bio-bwa.sourceforge.net). The duplicated reads were removed (sambamba tools; https://lomereiter.github.io/sambamba). Variant calling was performed via the Broad Institute's GATK Haplotype Caller GVCF tool (3.7) (https://

gatk.broadinstitute.org). Annotation and variants filtering was performed using Sirius platform (https://sirius.integragen.com/). Only rare variants (allele frequency <1% and an absence of homozygotes in the GnomAD database), with a potential effect on proteins (non-synonym, and synonym or intronic variants within 5 bp of a splice site), or already described in the HGMD database (Qiagen, Valancia, CA), were kept for analysis.

# 3 | RESULTS

## 3.1 | Clinical features of patients with 6q deletions

Detailed clinical descriptions are available in Data S1 and are summarized in Table 1.

We describe seven male and five female patients from nine unrelated families with 6q16.1 deletions sharing a partial or full deletion of the *EPHA7* gene. Current ages ranges from 15 months to 11 years.

All patients were born at term with normal birth parameters and normal postnatal growth. Postnatal microcephaly (−2 to −3 SD) was reported in 38% (5/13). All patients presented with a NDD associating DD/ID, various behavioral disorders and speech delay.

Other features included hypotonia (71%), non-specific facial dysmorphism (54%) sleep disturbance (50%), eye abnormalities (40%) such as ptosis, strabismus, astigmatism or iris coloboma, and cardiac defects (25%) including bicuspid aortic valve, atrial or ventricular septal defects.

## 3.2 | Chromosomal microarray analysis results

Microdeletions of our patients are compared to previous reports in Figure 1.

Deleted genes are listed in Table 2. Deletion sizes ranged from 152 to 6.4 Mb.

Four patients had tiny deletions involving only *EPHA7* (P5–P8).

The deletions were inherited in nine out of 12 patients: paternally inherited for P1–P4 (Family A, Figure 2) and P8 and maternally inherited for P7 and P9–P11. Inheritance was not specified in two out of 12 patients (P5 and P6). P12 carried the largest 6q14.3q16.1 deletion, the only deletion that occurred de novo in our patients.

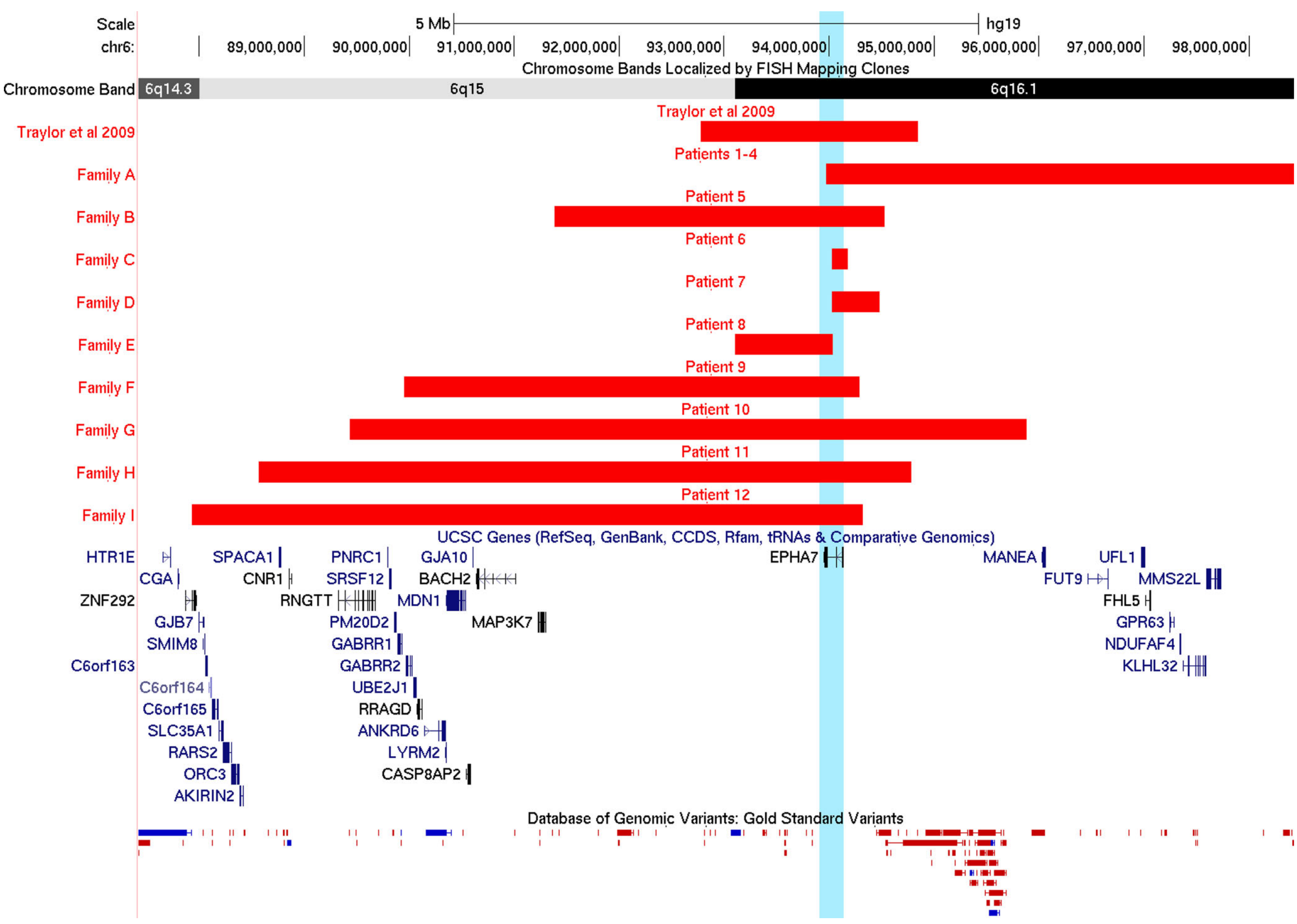


**FIGURE 1** Mapping of the *EPHA7* deletion (highlighted in blue) in our patients and in the patient previously reported (red bar). This figure was generated from data in the UCSC database (UCSC Genome Bioinformatics, genome.ucsc.edu/), Genomic coordinates are based on GRCh37/hg19 [Colour figure can be viewed at wileyonlinelibrary.com]

**TABLE 2** Lists of genes included in the 6q15q16.1 deletions

| Chr. band | Deleted genes | Patient | Description | MIM number | HI score | pLI | Phenotype | Inheritance |
|---|---|---|---|---|---|---|---|---|
| **6q14.3** | *ZNF292* | **P12** | Zinc finger protein 292 | 616 213 | 26.55 | **1** | ZNF92-Related Developmental disorder | AD |
| | *GJB7* | | Gap junction protein, beta 7, 25 kDa | 611 921 | 62.1 | 0 | | |
| **6q15** | *SLC35A1* | | Solute carrier family 35 | 605 634 | 23.12 | 0.68 | Congenital disorder of glycosylation, type lif | AR |
| | *RARS2* | | Arginyl-tRNA synthetase 2, mitochondrial | 611 524 | 21.71 | 0 | Pontocerebellar hypoplasia type 6 | AR |
| | *ORC3* | | Origin recognition complex, subunit 3 | 604 972 | 16.76 | 0.92 | | |
| | *ANKIRIN2* | | Akirin 2 | 615 165 | 17.80 | 0.92 | | |
| | *SPACA1* | **P11, P12** | Sperm acrosome associated 1 | 612 739 | 66.54 | 0.02 | | |
| | *CNR1* | | Cannabinoid receptor 1 (brain) | 114 610 | **1.58** | 0.51 | | |
| | *RNGTT* | **P10–P12** | RNA guanylyltransferase and 5′-phosphatase | 603 512 | **2.89** | 0.37 | | |
| | *PNRC1* | | Proline-rich nuclear receptor coactivator 1 | 606 714 | 23.15 | 0.91 | | |
| | *PM20D2* | | Peptidase M20 domain containing 2 | 615 913 | 45.56 | 0 | | |
| | *GABRR1* | | Gamma-aminobutyric acid (GABA) A receptor, rho 1 | 137 161 | 31.63 | 0 | | |
| | *GABRR2* | **P9–P12** | Gamma-aminobutyric acid (GABA) A receptor, rho 2 | 137 162 | 29.38 | 0 | | |
| | *UBE2J1* | | Ubiquitin-conjugating enzyme E2, J1 | 616 175 | 29.26 | 0.44 | | |
| | *RRAGD* | | Ras-related GTP binding D | 608 268 | 24.06 | 0.82 | | |
| | *ANKRD6* | | Ankyrin repeat domain 6 | 610 583 | 42.81 | 0 | | |
| | *MDN1* | | MDN1, midasin homolog (yeast) | 618 200 | 38.89 | 0 | | |
| | *CASP8AP2* | | Caspase 8 associated protein 2 | 606 880 | NA | NA | | |
| | *GJA10* | | Gap junction protein, alpha 10, 62 kDa | 611 924 | 64.09 | 0 | | |
| | *BACH2* | | BTB and CNC homology 1, basic leucine zipper transcription factor 2 | 605 394 | **7.83** | **1** | Immunodeficiency 60 | AD |
| | *MAP3K7* | | Mitogen-activated protein kinase kinase kinase 7 | 602 614 | **2.75** | **1** | Cardiospondylocarpofacial syndrome | AD |
| | | | | | | | Frontometaphyseal dysplasia 2 | AD |
| **6q16.1** | **EPHA7** | **All** | **patients** | **EPH** | | | **receptor A7** | **602 190** |
| | **2.76** | **1** | | | | | | |
| | *MANEA* | **P1–P4** | Mannosidase, endo-alpha | 612 327 | 26.82 | 0 | | |
| | *FUT9* | | Fucosyltransferase 9 (alpha (1, 3) fucosyltransferase) | 606 865 | 17.17 | 0.08 | | |
| | *UFL1* | | UFM1-specific ligase 1 | 613 372 | 24.42 | 0 | | |
| | *FHL5* | | Four and a half LIM domains 5 | 605 126 | 40.56 | 0 | | |
| | *GPR63* | | G protein-coupled receptor 63 | 606 915 | 55.03 | 0.2 | | |
| | *NDUFAF4* | | NADH dehydrogenase (ubiquinone) complex I, assembly factor 4 | 611 776 | 68.35 | 0.55 | Mitochondrial complex I deficiency, nuclear type 15 | AR |

Abbreviations: AD, autosomal dominant; AR, autosomal recessive. Bolded terms indicate loss-of-function intolerant genes (pLI) and/or sensitive to haploinsufficiency (HI score).

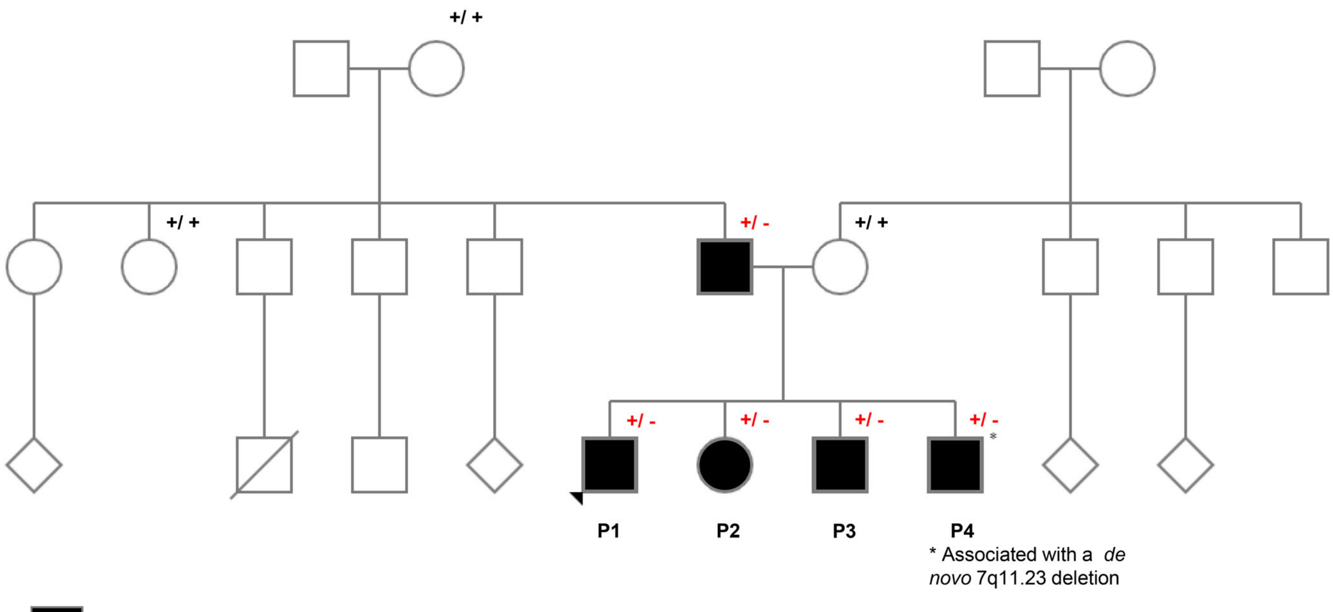


**FIGURE 2** Pedigree of the Family A. A 4.5 Mb-deletion at 6q16.1 in P1–P4 was inherited from the father who presented mild to moderate intellectual deficiency. * P4 also had a de novo 1.4-Mb recurrent 7q11.23 microdeletion of the WBS critical region [Colour figure can be viewed at wileyonlinelibrary.com]

P4 further had Williams–Beuren syndrome (WBS) caused by the recurrent 1.4 Mb de novo heterozygous deletion at 7q11.23.

No additional pathogenic variants have been identified by trio exome sequencing in P1, P2, P10, and P11 (Data S1).

## 4 | DISCUSSION

To date, only one de novo 6q16.1 deletion including *EPHA7* has been reported in a child with mild DD, microcephaly and dysmorphic features. We report 12 new cases of patients with 6q deletion including *EPHA7*, most of them inherited from an unaffected parent (six out seven families). Incomplete penetrance, variable expressivity, and diversity of size and breakpoints makes genotype–phenotype correlation difficult in 6q15q16.1 deletion.[10] In our study, the smallest region of overlap encompasses *EPHA7* only. *EPHA7* is located in 6q16.1, in a gene-poor region, which may explain why patients with larger deletions extending upstream and/or downstream of *EPHA7* show phenotypes similar to patients with narrow deletions involving only *EPHA7* (Figure 1).

The main core phenotype of patients with *EPHA7* includes a NDD with DD/ID, speech delay and behavioral disorders. Most deletions were inherited (9/12) from unaffected parent suggesting that haploinsufficiency of *EPHA7* could act as a risk factor for NDD with variable expressivity and incomplete penetrance. In Family A, the deletion was inherited from the affected father with mild to moderate intellectual deficiency but with a history of cranial concussion (Family A, Figure 2). Two patients (P4, P12) had a more severe phenotype. P4 also had the 1.4 Mb recurrent 7q11.23 microdeletion of the WBS critical region. Most individuals with WBS present mild ID.[11] The additional 6q16.1 deletion could be responsible for his unusually severe NDD phenotype. The 6q16.1 deletion here could act as a second hit that modulates the WBS phenotype. P12 had a larger deletion including exons 4–8 of the *ZNF292* gene that could explain the more severe phenotype with autism, absent speech, and epilepsy. Indeed, de novo and inherited loss-of-function *ZNF292* variants were associated with a neurodevelopmental disorder with ID, speech delay, autism, epilepsy and ocular features. No patients with isolated *ZNF292* deletions have been described thus far.[12]

The haploinsufficiency score of *EPHA7* (HI score: 2.76) indicates that this gene is dosage sensitive and could have a significant effect on the phenotype.[13] According to GnomAD v2.1.1 (https://gnomad.broadinstitute.org), *EPHA7* has a probability of being loss of function (LOF)-intolerant (pLI score: 1). LOF variants in *EPHA7* and deletions

including *EPHA7* are extremely rare in the gnomAD SVs v2.1. No patient with *EPHA7* deletion is reported in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and DGV (http://dgv.tcag.ca/dgv/app/home) (Figure 1).

Eph/Efn signaling is involved in the control of brain size through the regulation of cortical proliferation.[14] EphA7 inhibits dendritic growth early and promotes dendritic spine formation later in development, participating in intercellular signaling during synaptogenesis.[15] Haploinsufficiency of *EPHA7* could affect central neural circuits and explain the NDD of our patients.

Exome sequencing in P1, P2, P10, and P11 showing no other known pathogenic variants also support the causative effect of the *EPHA7* deletion.

In conclusion, our study suggests *EPHA7* deletion should be considered as a risk factor for NDD combining ID, behavioral disorders, speech delay and microcephaly with variable expressivity and incomplete penetrance. Additional patients with *EPHA7* haploinsufficiency and functional studies are still necessary to refine the phenotypic description and precise the genotype–phenotype correlation.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/cge.14017.

## DATA AVAILABILITY STATEMENT

Data supporting the findings from this study are available from the corresponding author on request.

## ETHICS STATEMENT

Written informed consent was received from the patients. The authors adhere to the Declaration of Helsinki Principles.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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