Contiguous mutation syndrome in the era of high-throughput sequencing

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Keywords
AP4 deficiency syndrome, intellectual deficiency, obesity, whole-exome sequencing, zinc-a2-glycoprotein

Abstract
We investigated two siblings, born to consanguineous parents, with neurological features reminiscent of adaptor protein complex 4 (AP4) deficiency, an autosomal recessive neurodevelopmental disorder characterized by neonatal hypotonia that progresses to hypertonia and spasticity, severe intellectual disability speech delay, microcephaly, and growth retardation. Yet, both children also presented with early onset obesity. Whole-exome sequencing identified two homozygous substitutions in two genes 170 kb apart on 7q22.1: a c.1137+1G>T splice mutation in AP4M1 previously described in a familial case of AP4-deficiency syndrome and the AZGP1 c.595A>T missense variant. Haplotyping analysis indicated a founder effect of the AP4M1 mutation, whereas the AZGP1 mutation arose more recently in our family. AZGP1 encodes an adipokine that stimulate lipolysis in adipocytes and regulates body weight in mice. We propose that the siblings’ phenotype results from the combined effects of mutations in both AP4M1 and AZGP1 that account for the neurological signs and the morbid obesity of early onset, respectively. Contiguous gene syndromes are the consequence of loss of two or more adjacent genes sensible to gene dosage and the phenotype reflects a combination of endophenotypes. We propose to broaden this concept to phenotypes resulting from independent mutations in two genetically linked genes causing a contiguous mutation syndrome.

Keywords
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ingly, the power of NGS has also fostered the identification of families with pathogenic mutations in more than one disease gene. Several examples illustrated the role for digenic inheritance in deafness, long QT, or nephrotic syndromes (Schaffer 2013). Similarly, recent publications illustrate how WES can help identifying genetic modifier of a Mendelian trait (Lemmers et al. 2012; Prokudin et al. 2014).

Homozygous or compound heterozygous mutations in any of the four genes encoding the subunits of the adaptor protein complex 4 (AP4) have been found to cause neurological disease underscoring the existence of a recognizable AP4 deficiency syndrome. The phenotype is characterized by microcephaly, severe intellectual disability (ID) with delayed or absent speech, progressive spasticity leading to wheelchair dependence in early adolescence, and growth retardation. We report here on two siblings (one female and one male) presenting the association of typical AP4-deficiency neurological presentation and severe obesity with early onset and provide evidence that this condition is a contiguous mutation syndrome.

Patients II.1 and II.2 (Table 1 and Fig. 1B–C) are brother and sister born to first cousin parents originating from Algeria. They were term-born after an uneventful pregnancy and delivery with normal birth parameters. Neonatal hypotonia was reported for both patients. At their last visit at 4 (II.2) and 7 (II.1) years of age, II.1 had a small head circumference (-1.5 standard deviation [SD]) and II.2 was microcephalic with occipitofrontal circumference on -2 SD (Table 1). They had severe intellectual deficiency with only a few single words. Eye contact was sustained and they both had purposeful hand skills. Patient II.2 presented unmotivated laughs and episodes of heteroaggressivity. Patient II.1 could walk with wall support from 15 months of age and then lost the ability to walk at 4 years of age. Patient II.2 never achieved independent walking. Today, both patients are wheelchair-bound. Patient II.1 experienced a unique episode of prolonged hyperthermic at 10 months of life and received valproate until the age of 3 years without new convulsions. Patient II.2 presented pyramidal syndrome with hyperreflexia, Babinski sign, and spasticity. Excessive weight gain started at 18 months of life for patient II.1 and 6 months of life for patient II.2. At 7 years of age, the weight of patient II.1 is over +4 SD and over +5 SD for patient II.2 at 4 years of age while height remained on the normal range (+1 SD and +2 SD, respectively). Currently, the father has a body mass index (BMI) of 24 and the mother has a BMI of 31.5, with a gain of weight in motherhood.

### Table 1. Clinical findings in patients with mutations in adaptor protein complex 4 (AP4) subunits.

| Sex       | Age at evaluation (years) | ID            | Speech                          | Stereotypes                  | Character      | Neonatal hypotonia | Pyramidal syndrome | Deambulation | Seizures                        | MRI examination                                                                 |
|-----------|--------------------------|---------------|---------------------------------|------------------------------|----------------|-------------------|--------------------|--------------|---------------------------------|--------------------------------------------------------------------------------|
| II.1 M    | 7                        | Severe        | Less than 10 words, echolalia   | Hand flapping                | Calm           | +                 | +                  | Achieved then lost, wheelchair | Cerebral atrophy, incomplete corpus callosum agenesis, and lipoma, delayed myelinisation |
| II.2 F    | 4                        | Severe        | Never acquired                  | Unmotivated laughter, hand flapping | Episodes of heteroaggressivity | +                 | +                  | Never achieved, wheelchair      | 19/20 thin corpus callosum; ventriculomegaly; thinning and abnormal signal of the periventricular white matter; atrophy of the inferior vermis with cortical atrophy, dilated ventricles, prominent cisterna |
| Previously reported AP4-deficiency patients |                        |                |                                 |                              |                |                   |                    |                          |                                                                                  |
| Sex ratio | –1                       |               |                                 |                              |                |                   |                    |                          |                                                                                  |
| From 2 to 24 |                        |               |                                 |                              |                |                   |                    |                          |                                                                                  |
| ID        | Severe                   |               |                                 |                              |                |                   |                    |                          |                                                                                  |
| Speech    | Less than 10 words, echolalia |               |                                 |                              |                |                   |                    |                          |                                                                                  |
| Stereotypes | Hand flapping          |               |                                 |                              |                |                   |                    |                          |                                                                                  |
| Character | Calm                     |               |                                 |                              |                |                   |                    |                          |                                                                                  |
| Neonatal hypotonia | +                  |               |                                 |                              |                |                   |                    |                          |                                                                                  |
| Pyramidal syndrome | +                  |               |                                 |                              |                |                   |                    |                          |                                                                                  |
| Deambulation | Achieved then lost, wheelchair |               |                                 |                              |                |                   |                    |                          |                                                                                  |
| Seizures | A unique episode of prolonged hyperthermic seizure at 10 months of life, treated with valproate until the age of 3 years without new convulsions |               |                                 |                              |                |                   |                    |                          |                                                                                  |
| MRI examination | Cerebral atrophy        |               |                                 |                              |                |                   |                    |                          |                                                                                  |
| Head circumference | –1.5 SD                  | –2 SD         |                                 |                              |                |                   |                    |                          |                                                                                  |
| Stature   | +1 SD                    | +2 SD         |                                 |                              |                |                   |                    |                          |                                                                                  |
| Early onset of severe obesity | Starting at 18 months of life, weight >4 SD at 7 years |               |                                 |                              |                |                   |                    |                          |                                                                                  |
|          |                          |               |                                 |                              |                |                   |                    |                          |                                                                                  |

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No dysmorphic features were observed except a short columella. The neck was short with acanthosis nigricans. Brain magnetic resonance imaging showed cerebral atrophy for patient II.1 (Fig. 1C, left panel), and partial agenesis of the corpus callosum with a lipoma as well as delayed myelinization for patient II.2 (not shown). Multiple ENT infections motivated amygdalectomy for both patients. Endocrine and metabolic screening showed no abnormalities except a raised parathyroid hormone (PTH) (57 ng/L, N = 10–46) with normal calcemia (2.57 mmol/L, N = 2.2–2.7) in II.2 who was supplemented with vitamin D. Array-CGH (SpectralChip CC4-V0.3, Perkin Elmer, Waltham, Massachusetts, USA) showed no pathologic copy number variant (CNV).

To identify the disease-causing mutation of this undiagnosed condition, we performed WES on peripheral blood DNA from all family members. We first focused on novel homozygous variants cosegregating with the disease and corresponding to either nonsynonymous (NS) variants, splice acceptor and donor site mutations (SS), or coding insertions/deletions (indels). We regarded variants as novel if they were absent from all publically available control databases. To identify the disease-causing mutation of this undiagnosed condition, we performed WES on peripheral blood DNA from all family members. We first focused on novel homozygous variants cosegregating with the disease and corresponding to either nonsynonymous (NS) variants, splice acceptor and donor site mutations (SS), or coding insertions/deletions (indels). We regarded variants as novel if they were absent from all publically available control databases.
data sets, including those of dbSNP138 (http://www.ncbi.nlm.nih.gov/SNP), the 1000 Genomes Project (http://browser.1000genomes.org/index.html), the NHLBI ESP Exome Variant Server (http://evs.gs.washington.edu/EVS/), and from in-house exome data containing information for over 600 patient samples. Three candidate variants fulfilled these criteria (Table S1) and were confirmed by Sanger sequencing: a splice variant in the AP4M1 gene (NM_004722.3: c.1137+1G>T), a misense variation in the AZGP1 gene (NM_001185.3: c.595A>T, p.Asn199Tyr), and a misense variation in exon 81 of HERC2 (NM_004667.4: c.12445G>A, p.Ala4149Thr) (Fig. 1D). Filtering WES data for compound heterozygous mutations or heterozygous “de novo” mutations shared by the siblings did not provide any additional candidate variants.

The AP4M1 splice variant affects the donor splice site of intron 14 (Fig. 1D) and was described previously in five affected siblings from a consanguineous Moroccan family with a highly similar neurological presentation but no obesity was reported (Verkerk et al. 2009). As previously demonstrated by Verkerk and colleagues, this variant leads to skipping of exon 14 in the AP4M1 transcript extracted from patients’ cultured skin fibroblast (Fig. 1E). AP4M1 encodes one of the subunit (μ) AP4, an evolutionary conserved heterotetrameric complex consisting of two large (α or β), a medium (μ), and a small (σ) adaptin. Mutations affecting all four subunits of AP4 (AP4M1, AP4E1, AP4S1, and AP4B1) have been found to cause autosomal recessive AP4 deficiency syndrome characterized by severe ID, microcephaly, progressive spastic paraplegia, speech delay, and growth retardation (Table 1 and Table S2) (Verkerk et al. 2009; Abou Jamra et al. 2011; Blumkin et al. 2011; Moreno-De-Luca et al. 2011; Najmabadi et al. 2011; Bauer et al. 2012; Philippe 2012; Kong et al. 2013; Tuyuz et al. 2014; Abdollahpour et al. 2015).

The p.Ala4149Thr substitution in HERC2 affects an absolutely conserved amino acid residue located within the Regulator of Chromosome Condensation (RCC1) protein domain and is predicted to be damaging by various in silico tools (PolyPhen-2 score = 0.968, Sorting Intolerant from Tolerant (SIFT) score = 0, and disease causing according to MutationTaster, Charité University, Germany). The HERC2 gene is located on 15q13.1 and encodes a large ubiquitin ligase protein. A distinct HERC2 founder mutation (c.1781C>T, p.Pro594Leu) has been previously associated in the Amish community with a disorder characterized by mild developmental delay, autism spectrum disorder, and Angelman-like features (MIM:615516) (Puffenberger et al. 2012; Harlalka et al. 2013). Functional studies of the p.Pro594Leu variant demonstrated that it induces protein aggregation and decreased HERC2 abundance.

The third variant (c.595A>T, p.Asn199Tyr) affects the AZGP1 gene, encoding the zinc-α2-glycoprotein (ZAG).

The c.595A>T variant does not affect a highly conserved amino acid residue and its consequences on protein function is unclear (PolyPhen-2 score = 0.946, SIFT score = 0.01, and polymorphism according to MutationTaster). AP4M1 and AZGP1 are located on chromosome 7q22.1, 170 kb aside from each other (Fig. 1F). To discriminate between a hot spot and a founder mutation, we performed genotyping analysis in all family members as well as in two affected siblings from the Moroccan family using microsatellite markers encompassing the AP4M1 locus. As shown in Table S3, both families share a common haplotype supporting the hypothesis of a founder effect of the AP4M1 mutation. The c.595A>T AZGP1 mutation was not present in the previously described Moroccan family (Table S3). These data strongly support the hypothesis that the AZGP1 mutation arose more recently in our family and occurs secondarily to the AP4M1 mutation and on the same haplotype.

Our patients are severely retarded. They have some features of the autistic spectrum but marked and prolonged ocular contacts (Table 1). Although two AP4 patients have been described with a shy and anxious character (Abdollahpour et al. 2015), the majority of patients with AP4 deficiency are described as shy, amicable, and calm (Table 1) (Verkerk et al. 2009; Abou Jamra et al. 2011). Collectively, these data predicted only a minor effect of the p.Ala4149Thr HERC2 variant identified in our family and demonstrated that the c.1137+1G>T AP4M1 variant clearly accounts for the majority of neurological features observed in our patients.

However, none of the 33 previously reported AP4 deficiency cases are reported as obese, while obesity was described as a feature of the two siblings’ condition as it started very early in both (before 1 year of age, table and sup clinical data). Similarly, no obesity has been reported in HERC2-mutated patients. While we cannot exclude that the HERC2 variant may contribute to the neurological presentation of our cases, it is unlikely that it accounts for the obesity. By contrast, ZAG is an adipokine secreted by the adipose tissue and playing an important role in the mobilization and utilization of stored lipids (Balaz et al. 2014). Furthermore, several studies support the role of the ZAG protein in the regulation of body weight in both animal models and humans. Reduced plasmatic ZAG levels have been observed in ob/ob mice and Agrp1 deficient mice showed increased body weight and decreased adipocytic lipolysis (Rolli et al. 2007; Mracek et al. 2010b). Moreover, oral administration of human ZAG to ob/ob mice resulted in progressive loss of body weight (Russell and Tisdale 2012). Linkage analyses showed linkage disequilibrium mapping of genes influencing human obesity, insulin resistance, and type 2 diabetes in the 7q22.1 region and the rs4215 SNP in AZGP1 gene is...
associated with obesity in Chinese population (Sell et al. 1998; Li et al. 2003; Sim et al. 2011; Huang et al. 2012; Sanghera and Blackett 2012; Zhu et al. 2012). Finally, both lower serum concentrations of ZAG and significantly lower ZAG expression in the adipose tissue and liver have been observed in obese subjects (Selva et al. 2009; Mracek et al. 2010a; Garrido-Sanchez et al. 2012; Balaz et al. 2014). Further investigations will be needed to evaluate the ability of the mutant p.Asn199Tyr ZAG protein to induce lipolysis, but these results make AZGP1 an excellent candidate gene for very early onset obesity in humans and may provide interesting clues for novel therapeutic interventions in obese patients.

Altogether, our results suggest that the phenotype observed in our patients results from the additional effects of AP4M1 and AZGP1 mutations accounting for the neurological signs on one hand and the precocious morbid obesity on the other hand. Additional subtle effects of the HERC2 variant on the neurological presentation, particularly on the communicative skills, cannot be excluded. The current report further demonstrates how consanguinity could increase intrafamilial clustering of multiple hereditary diseases and how WES has considerably empowered our ability to detect such complex events.

The term contiguous gene syndrome (CGS) has been proposed in 1986 to explain the association of unrelated clinical features due to the deletion of multiple genes lying in close proximity to one another on a single chromosome (Schnickel 1986). The phenotypes observed arise as a result of the combination of the endophenotypes from each deleted gene sensitive to haploinsufficiency. While CGS is caused by a single mutational event (i.e., a chromosomal deletion), the phenotype we describe is the consequence of two independent mutational hits in two genetically linked genes. The mode of inheritance we describe is also different from a digenic inheritance that refers to the alteration of two interacting genes to cause a phenotype. While the final demonstration of our hypothesis is awaiting the identification of AZGP1 mutations in obese patients with normal neurodevelopment as well as the demonstration of the impaired function of the mutant p.Asn199Tyr ZAG protein, we propose to use the name contiguous mutation syndrome to describe this complex and clinically challenging phenotype caused by independent mutations in genetically linked genes.

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Conflict of Interest

None declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Supplementary Materials and Methods

Table S1. Number of variants identified by whole-exome sequencing.

Table S2. Literature review of patients with mutation in one of the four AP4 genes.

Table S3. Genotypes of the two families carrying the c.1137+1G>T mutation (Verkerk et al. 2009).