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Baraitser-Winter Cerebrofrontofacial Syndrome

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Conflict of interest

The authors declare no competing interests.

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Keywords: *ACTB*, *ACTG1*, Baraitser-Winter Cerebrofrontofacial Syndrome, Baraitser-Winter Syndrome, Coloboma, Pachygyria.

Abstract

Baraitser-Winter Cerebrofrontofacial syndrome (BWCFF) [BRWS; MIM #243310, 614583] is a rare developmental disorder affecting multiple organ systems. It is characterised by intellectual disability (mild to severe) and distinctive facial appearance (metopic ridging/trigonocephaly, bilateral ptosis, hypertelorism). The additional presence of cortical malformations (pachygyria/lissencephaly) and ocular colobomata are also suggestive of this syndrome. Other features include moderate short stature, contractures, congenital cardiac disease and genitourinary malformations. BWCFF is caused by missense mutations in the cytoplasmic beta- and gamma-actin genes *ACTB* and *ACTG1*. We provide an overview of the clinical characteristics (including some novel findings in four recently diagnosed patients), diagnosis, management, mutation spectrum and genetic counselling.

Introduction

The Baraitser-Winter syndrome [BRWS; MIM #243310, 614583] was first described in 1988¹. It is a rare, autosomal dominant, developmental disorder. The main features include a distinctive facial appearance (metopic ridging/trigonocephaly, bilateral ptosis, hypertelorism), intellectual disability and structural brain abnormalities, predominantly pachygyria. Causative missense mutations in the *ACTB* [MIM 102630] and *ACTG1* [MIM 102560] genes were identified in 2012². Two other disorders, Fryns-Aftimos syndrome [MIM #243310]³ and Cerebrofrontofacial syndromes types 1 and 3 [MIM #243310]⁴ were previously thought to be separate clinical entities. It has now been demonstrated that they are also caused by *ACTB* & *ACTG1* mutations, and Baraitser-Winter Cerebrofrontofacial syndrome (BWCF) has been put forward as a unifying name⁵. More recently, fetal cases with microlissencephaly have been described, further extending the phenotypic spectrum⁶. Here we report the first instance of parent-child transmission, review the phenotypic and molecular characteristics of the approximately sixty cases published to date, illustrating key points with two additional patients. We discuss diagnosis, management, mutation spectrum and genetic counselling.

Clinical overview

Craniofacial characteristics

Individuals with BWCFF have a characteristic craniofacial appearance (Table 1), but there is a wide spectrum in severity. Hypertelorism, which may be significant⁷, and congenital non-myopathic ptosis is present in almost all cases. The eyebrows are often arched, which can increase with age. This may be partly due to an effort to raise the eyelids, although it is also seen in the absence of ptosis. The palpebral fissures are usually long and may be downslanting⁷⁻⁸. Epicanthic folds can be present^{1,9}. Partial absence of the upper eyelashes has been reported^{8,10}.

Trigonocephaly or metopic ridging, associated with premature closure of the metopic suture, is common. A recently diagnosed female with a novel mutation had sagittal craniosynostosis, not previously reported, which required surgical correction (Fig. 1a, Patient 2). There is a round, flattened appearance to the face in infants. A progressive coarsening of facial features develops with age. The nose is usually short with a broad nasal bridge, anteverted nares and depressed nasal tip. A long philtrum, thin vermilion border and macrostomia are common⁵. Micro- or retrognathia may be present^{6,10}. The palate is usually high arched. Cleft lip and palate are seen in some patients. The ears are often posteriorly rotated, small and dysplastic⁵.

Ocular features

Uni – or bilateral colobomata are present in about one third of patients, affecting the iris or retina^{2,5-6}. Eyelid colobomata have been reported⁸. Microphthalmia is seen, but is uncommon^{5,9}. There have been some reports of cataracts, myopia and strabismus^{8,11-12}. A recently diagnosed male with a novel mutation presented with a unilateral congenital glaucoma, not previously reported in BWCF (Fig. 1b, Patient 4).

Hearing

Hearing loss is present in some cases, usually sensorineural, and can be progressive^{2,11,13}.

Growth and musculoskeletal manifestations

Mild to moderate short stature in adulthood is common^{2,5}. The neck is often short and may be webbed. Pterygia may affect one or more of the axillae, elbows and popliteal regions⁷⁻⁸. Pectus deformities are common and the nipples are often wide

spaced, hypoplastic and may be inverted. The shoulder muscle bulk may be reduced to a variable extent. Many patients develop an unusual posture with anteverted shoulders, flexed elbows and knees⁵. Camptodactyly and clinodactyly are rarer features^{3,10-11}. The hallux may be broad and, uncommonly, duplex^{5,7}. Talipes equinovarus is seen infrequently⁵.

Neurology

Cortical brain malformations have been reported in 60-70% of patients, and are most consistently seen in those with an *ACTG1* mutation⁵. Pachygyria, frontal or perisylvian, is most common (Fig. 2)⁵⁻⁶ (personal observation). Lissencephaly with diffuse agyria has also been reported^{6,9}. A few have periventricular nodules. The corpus callosum often appears short and thick or may be hypoplastic or absent. Prominent perivascular spaces are common (Fig. 2a & b). Infratentorial structures are usually normal⁵. However, recently a fetus with an *ACTG1* mutation, microlissencephaly and underdeveloped brain stem and cerebellum has been reported⁶.

Microcephaly can be a feature. This is mostly mild with postnatal onset. However, Poirier et al⁶ reported two fetal cases with *ACTG1* mutations and severe microcephaly, associated with lissencephaly/agyria. They described a third patient

with features of BWCF, who presented with microcephaly and perisylvian pachygyria, who also had an *ACTG1* mutation. The case of diffuse agyria previously described by Ramer et al⁹ with an *ACTB* mutation², also had significant microcephaly.

Developmental delay and learning difficulties are present in the vast majority of patients. Mild to moderate developmental delay may be found to a variable extent in those with no structural brain anomalies. Pachygyria and other structural cerebral changes are associated with mild to profound intellectual disability (ID), usually in keeping with the severity of the cortical malformation^{2,5,7}.

Epilepsy has so far only been seen in association with a structural brain anomaly and age of onset is very variable. The seizure disorder may be refractory to treatment.

Other neurological features include generalised hypotonia or spasticity of the lower limbs^{2,5,7}.

Gearing et al.¹⁴ reported monozygotic twins with severe, progressive dystonia from the age of twelve years, with a unique p.Arg183Trp *ACTB* mutation. This has not been found in any other cases, and it remains to be seen whether this is a feature specific to this family.

Cardiovascular

Congenital cardiac disease is a feature in around one third of patients. A patent ductus arteriosus is the most frequent finding. Ventricular or atrial septal defects have been reported^{7,10,13} and rarely bicuspid aortic valve and aortic stenosis⁹, mitral valve regurgitation⁵ and tricuspid regurgitation¹⁰.

Genitourinary

Genitourinary tract abnormalities include hydronephrosis, which is mostly bilateral^{5,10}. Ectopic kidneys may also be a feature⁵. Renal duplication is found more rarely^{10,13}. Undescended testes have been reported^{9,13}.

Gastrointestinal

Umbilical hernias are relatively common, inguinal hernias less so^{8,13}. Achalasia and bulbar palsy requiring gastrostomies were present in the twins reported by Gearing et al¹⁴. However as previously noted, these features may be unique to this family.

Antenatal

Pregnancy is usually uneventful with normal growth. Two cases had hydrops fetalis with marked polyhydramnios⁷. Of note, these patients also had a more severe craniofacial phenotype.

Malignancy

Only two previously reported BWCFF patients have presented with haematological malignancies^{2,5}. One of them, with an *ACTB* mutation, developed a precursor B-cell acute lymphatic leukaemia (ALL) at the age of eight years. The other, with an *ACTG1* mutation, developed a cutaneous lymphoma at the age of nineteen years.

Mutation spectrum & genotype-phenotype correlation

Baraitser-Winter Cerebrofrontofacial syndrome is caused by heterozygous missense mutations in *ACTB* (Chr 7p22.1) and *ACTG1* (Chr 17q25.3), which demonstrate clustering (Fig. 3) suggesting gain-of-function, but a dominant negative effect is also a possible mechanism^{2,15}. This is supported by the observation that no similar phenotype has been found with deletions or duplications of these genes². *ACTB* mutations predominate so far. It is notable that amino acid 196 is most often affected,

with sixteen recurrent cases thus far (p.Arg196His, p.Arg196Cys)⁵, (and unpublished data). There is clinical heterogeneity amongst this group, even in those with the same mutation, suggesting the involvement of modifier genes⁵.

ACTB and *ACTG1* have a highly similar structure¹⁶, and it could therefore be surmised that mutations in either would have similar phenotypic effects. However, a single case with an *ACTG1* mutation (p.Thr120Ile) demonstrated a milder craniofacial phenotype than several with an analogous *ACTB* variant^{2,7}. Therefore, it has been suggested that *ACTB* mutations result in a more severe phenotype^{2,7}. Our observations indicate that a severe craniofacial phenotype has so far been associated with *ACTB* variants, whereas *ACTG1* mutation carriers are more likely, although not consistently¹⁷, to have a cortical brain malformation, hence also more severe intellectual disability and/or epilepsy. Indeed, the two fetuses described by Poirier et al.⁶ with microlissencephaly and facial features of BWCF had novel mutations in *ACTG1* (p.Ile75Leu & p.Pro343Ile). However, it should be noted that parental studies could not be undertaken in these cases, and therefore it could not be proven that the mutations were *de novo*. An analogous *ACTB* p.Ile75Thr mutation has been described⁵. Unfortunately, the patient was lost to follow-up, but was not thought to have comparable severe cerebral anomalies. In the same publication Poirier et al also reported a nine year old male with BWCF and an *ACTG1* mutation (p.Met153Ile), who had pachygyria. It should be noted that a case of agyria has also been documented with an *ACTB* mutation (p.Leu65Val)^{2,9}.

Overall, it appears that *ACTB* mutations can be associated with a more severe craniofacial phenotype, whereas those in *ACTG1* are more likely to result in significant brain structural abnormalities. However, given the small number of cases involved, it is not yet possible to definitively determine the differences in phenotype based on the gene affected. The phenotypic spectrum is likely to broaden with an increasing number of affected individuals being identified.

Genetic and molecular basis

Actin has six different protein isoforms: $\alpha_{\text{skeletal-actin}}$, $\alpha_{\text{cardiac-actin}}$, $\alpha_{\text{smooth-actin}}$, and $\gamma_{\text{smooth-actin}}$ are expressed only in muscle cells. The cytoplasmic actin genes, *ACTB* ($\beta_{\text{cyto-actin}}$) and *ACTG1* ($\gamma_{\text{cyto-actin}}$), however, are ubiquitously expressed in vertebrates. These two isoforms share an almost identical structure, differing by only four amino acids¹⁶. Actins polymerise in vivo (F-actins), and these polymers play a number of crucial roles in the cellular cytoskeleton, for example maintenance of cell shape and mediation of cell-cell signalling. The ability of actin to rapidly polymerise and depolymerise allows for an adaptable and constantly changing cytoskeletal structure to regulate the intracellular environment and interactions with other cells¹⁸. There is some redundancy between cyto-actin isoforms, which may be expected, given the similarity of protein sequence, and they can easily co-polymerise¹⁹. For

example, *Actg1*-null mice fibroblasts show no reduction in overall actin levels due to increased levels of other isoforms, including β_{cyto} -actin²⁰.

However, β_{cyto} -actin and γ_{cyto} -actin have differential intracellular localisation patterns²¹, as well as being highly conserved across mammal and bird species, suggesting non-redundant roles. *Actb*-null mice do not survive to birth²². Loss of *Actb* exclusively in neurons results in abnormal cortical folding in the cerebellum and hippocampus, as well as partial agenesis of the corpus callosum²³. Expression of *Actb* can also vary in a tissue-specific manner, i.e. miRNA-mediated regulation of translation via an alternative transcript affecting the 3' UTR increases expression in neuronal cells²⁴. In addition, post-translational modification by arginylation of β_{cyto} -actin aids cellular movement and increases actin polymerisation²⁶.

Actg1-null mice, in contrast, are viable, albeit with reduced survival in the immediate post-natal period²⁶. Specific knockout of *Actg1* in skeletal muscle results in progressive myopathy and muscle cell death in mice²⁷. *Actg1* regulation also differs: arginylated γ_{cyto} -actin is marked for ubiquitin-mediated degradation²⁸. Adjustment of *Actg1* levels can additionally be achieved by nonsense-mediated decay through production of an alternative transcript including a premature termination codon²⁹.

There is some evidence of tissue-specific cyto-actin functions, with relevance to the pathogenesis of BWCF. Actin polymers play an important role in brain development. The actin cytoskeleton contributes to axonal growth via myosin interactions. It also acts as a guidance mechanism for the developing axon mediated by actin-binding proteins responding to chemical cues in the external environment³⁰. As well as cytoplasmic functions, actin can regulate gene expression in neurons, controlling neurite outgrowth³¹. This appears to be mediated through the serum response factor (SRF) pathway.

In auditory development, cyto-actin is required for stereocilia hair cell development in the ear in mice, but only one isoform needs to be present. However, loss of either *Actb* or *Actg1* results in distinct types of progressive hearing loss over time³². Of note, familial sensorineural hearing loss with no other systemic features has been associated with mutations in *ACTG1* (DFNA 20/26- MIM #604717). These are missense mutations, but are non-overlapping with those found in BWCF³³⁻³⁴.

Recurrent somatic mutations in *ACTB* have been found in large B-cell lymphoma³⁵, and a somatic mutation has been shown in a gastric adenocarcinoma³⁶. γ_{cyto} -actin is upregulated in sporadic lung and colon carcinomas, with an associated increase in invasiveness of the cancer cells³⁷. Actin polymers (F-actin) can increase the transcriptional activity of the transcriptional co-activator β -catenin, known to be dysregulated in many cancers³⁸.

Diagnosis and counselling

A diagnosis of Baraitser-Winter syndrome should be considered in patients presenting with characteristic dysmorphism (metopic ridging/trigonocephaly, arched eyebrows, hypertelorism, congenital ptosis, short nose) together with intellectual disability of varying severity. Other suggestive findings include ocular coloboma and pachygyria. The age of presentation needs to be taken into account, as facial appearance changes significantly over time.

Differential diagnoses may include Noonan syndrome [MIM # 163950], which shares some facial similarities, as well as pectus abnormalities and neck webbing.

Coloboma is very rare in Noonan syndrome and it is not normally associated with cortical brain malformations. The long palpebral fissures may be reminiscent of Kabuki syndrome [MIM #147920; MIM #300867]. Similarities may be found with other syndromes associated with hypertelorism (Teebi hypertelorism syndrome [MIM #145420], Aarskog syndrome [MIM #305400]) and at the extreme end, conditions with frontonasal dysplasia. Ptosis and pterygia overlap with features seen in Escobar syndrome [MIM #265000].

Molecular diagnosis is made by sequencing of the *ACTB* and/or *ACTG1* genes. This may be a single gene approach, or as part of NGS based targeted or whole exome sequencing. Mutations are usually *de novo*, however, in this review we report for the first time a parent with BWCFE and an affected child (Fig 1a, Patient 1, and 2a and 2b; detailed clinical data will be presented elsewhere).

Several patients with a presentation suggestive for BWCFE syndrome with no *ACTB/ACTG1* variants have been identified⁵, therefore there may be further molecular heterogeneity.

As BWCFE is usually associated with *de novo* mutations, the recurrence risk for siblings is low. Prenatal testing can be offered for known variants.

Investigations, management and surveillance

Suggested investigations in a patient with probable or definite BWCFE should include: formal ophthalmological examination, audiometry, echocardiogram, and renal ultrasound scanning. Brain MRI should be considered in the presence of moderate to severe intellectual disability and/or seizure disorder. In patients with mild intellectual disability and without epilepsy, cerebral neuroimaging can be considered on a case by case basis. In cases of significant trigonocephaly,

hypertelorism, or premature fusion of other cranial sutures, referral to a craniofacial surgery department is indicated.

Surveillance of psychomotor development and cognitive function will be required in all patients. The possible occurrence of a hearing impairment and its progression will need to be monitored. Some patients will need surgical correction of their ptosis, and those with coloboma or microphthalmia will need screening for glaucoma as this can be a long term complication³⁹. Treatment may be required for congenital cardiac disease, renal tract malformations and epilepsy. Cleft lip and palate will require surgical repair.

So far, the occurrence of malignancy in BWCFE appears to be rare, and it is yet to be determined whether this condition is associated with a predisposition to cancer.

Therefore, screening is currently not recommended.

Conclusion

BWCFE is becoming a defined clinical entity, however with a wider spectrum of severity than previously thought, with milder and more severe phenotypes having been identified. Typical craniofacial features are crucial in diagnosis, whereas pachygyria/lissencephaly and colobomata are important, but not consistent, clues (Table 1). There are also a number of features unique to individual patients, and it

remains to be determined whether these form part of the syndrome. Future challenges lie in understanding the functional consequences of the underlying actin mutations and the resulting phenotype. Given the ubiquitous expression of cytoplasmic actins, this will be a complex task.

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Figure 1a. BWCF mother (2a&b; ages 3.5y and 31y)-daughter (1; age 6.75y) pair. Features common to both include arched eyebrows, bilateral colobomata, short nose with broad root, long philtrum and thin upper lip. Patient 1 has metopic ridging. Patient 2 had sagittal craniosynostosis, which has not been previously reported. Note coarsening of facial features with age in patient 2. The familial novel *ACTB* mutation is shown.

Figure 1b. Three further BWCF patients. Note typical facial appearance including arched eyebrows, ptosis (patients 3 & 5), hypertelorism, short nose, long philtrum and thin upper lip. Patient 4 (8y) has **left sided** unilateral, congenital glaucoma, not previously reported in BWCF. Each patient's mutation is shown. The **mutation** in patient 3 (8y) has been identified in one other patient⁴⁰. Patient 4 has a novel mutation. The **mutation** in patient 5 (13y) is the most common in BWCF (this patient was previously published⁵).

Figure 2. Neuroimaging features: A & B axial T2 weighted images, C axial and D sagittal T1 weighted images. Frontal pachygyria shown in A & B (black arrows) and more widespread pachygyria (black arrows) in C. Short thick corpus callosum shown in D. **Prominent perivascular spaces are shown in A & B.**

Figure 3. Recurrent mutations in BWCF. *ACTB* (Refseq accession NM_001101.3) and *ACTG1* (Refseq accession NM_001199954.1) genes are combined due to almost identical exon structure. Light grey: coding exons; dark grey: non coding exons. The first amino acid of each exon is shown. Functional domains: Divalent cation (Mg²⁺/Ca²⁺) binding domains (black arrows); Adenosine nucleotide binding domains (white arrows) and DNase binding loop (triangles). Mutations with two or more documented cases are shown.

Figure legends

Table 1. Clinical features of BWCF. Characteristics are divided according to frequency. Cardinal features of BWCF highlighted in bold. Features with less than 10% occurrence in published literature not included.

*Pachygyria/lissencephaly (60-70% of patients) and colobomata (25-30%)



Figure 1 - legend as per manuscript text

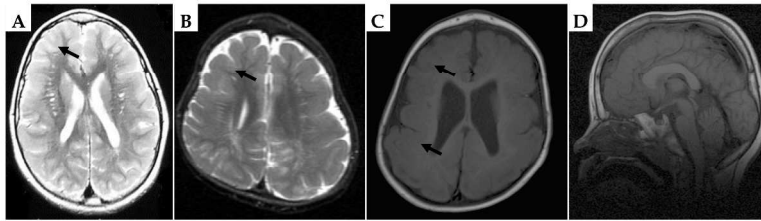


Figure 2 - legend as per manuscript text

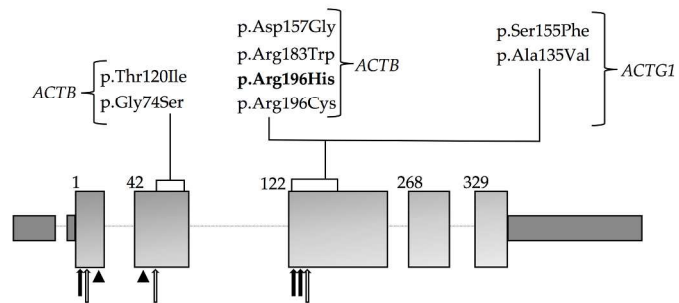


Figure 3 - legend as per manuscript text

Cardinal Features <ul style="list-style-type: none">• Intellectual disability (mild/moderate/severe)• Trigonocephaly/metopic ridging• Hypertelorism• Ptosis• Arched eyebrows
Features present in >50% <ul style="list-style-type: none">• Pachygyria / lissencephaly*• Epilepsy• Long palpebral fissures• Short nose, anteverted nares, wide nasal tip, long philtrum• Thin upper lip, macrostomia, prominent lower lip• Dysplastic ears• Short, webbed neck, pectus
Features present in <50% <ul style="list-style-type: none">• Coloboma*• Microphthalmia• Deafness• Agenesis / thickened corpus callosum• Cleft lip / palate• Kyphosis / scoliosis• Reduced knee / elbow extension

Table 1 - legend as per manuscript text