

Salbutamol benefits children with congenital myasthenic syndrome due to *DOK7* mutations

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Abstract

Congenital myasthenic syndromes due to *DOK7* mutations cause fatigable limb girdle weakness. Treatment with ephedrine improves muscle strength. Salbutamol, a β_2 -adrenergic receptor agonist with fewer side effects and more readily available, has been effective in adult and anecdotal childhood cases. This study reports the effects of salbutamol on motor function in childhood *DOK7* congenital myasthenic syndrome. Nine children (age range 5.9–15.1 years) were treated with oral salbutamol, 2–4 mg TDS. The effect on timed tests of motor function, pre- and up to 28 months post-treatment, was audited retrospectively. All 9 reported functional benefit within 1 month, with progressive improvement to a plateau at 12–18 months. Within the first month, all 3 non-ambulant children resumed walking with assistance. Although improvements were seen in some timed tests (timed 10 m, arm raise time, 6 min walk time) this did not fully reflect the observed functional benefits in daily living activities. No major side effects were reported. We conclude that oral salbutamol treatment significantly improves strength in children with *DOK7* congenital myasthenic syndrome and is well tolerated. Outcome measures need to be refined further, both to accurately reflect functional abilities in children and to document progress and treatment response.

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1. Introduction

Mutations in *DOK7*, encoding the skeletal muscle adaptor protein Dok-7, result in congenital myasthenic syndrome (CMS) associated with small, simplified neuromuscular junctions [1]. The resulting phenotype is variable,

but typically patients present in childhood with a limb-girdle pattern of weakness and ptosis. Ophthalmoplegia is rare but progressive respiratory impairment frequently occurs. Treatment with cholinesterase inhibitors is ineffective and response to 3,4-diaminopyridine variable [2–4]. Ephedrine has been shown to be helpful, with patients reporting an improvement over a 1–24 month period [5,6]. However, ephedrine has both α and β -adrenergic effects and concern remains regarding central and cardiac adverse effects, particularly with long term use in children. Salbutamol, a selective β_2 agonist, has been used

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successfully for many years in paediatrics as a bronchodilator to treat asthma and, more recently, to improve muscle strength in SMA [7] and some congenital myopathies [8,9], with a good safety profile. In the USA, where ephedrine is no longer available, it was postulated that salbutamol may have similar benefit to ephedrine in CMS and this has recently been confirmed in an open label study of predominantly adults with *DOK7* synaptopathy, with patient reported functional improvement on albuterol, the USA equivalent of salbutamol [10]. Although ephedrine is still available in the UK, we offer salbutamol as first line treatment to children (including infants) with *DOK7* CMS, to reduce the potential for α -adrenergic side effects. In our multidisciplinary paediatric myasthenia clinics, children are assessed regularly with objective timed measures of fatigue and functional motor ability, in addition to detailed clinical review. The timed tests used are targeted to be brief, to maintain interest and motivation in younger children and are easily administered in the clinic setting. Here we present a retrospective review of the effect of salbutamol on functional ability and timed tests in nine children with *DOK7* mutations followed in our clinics for up to 28 months.

2. Patients and methods

2.1. Patients

This is a retrospective case review of clinical data and timed tests of muscle fatigability recorded at routine clinic visits. The study was approved by the Clinical Audit Team at Great Ormond Street Hospital. Nine children from eight unrelated families with genetically confirmed *DOK7* mutations were included. Molecular analysis of the *DOK7* gene was performed by the Oxford NCG service as previously described [1]. All mutations have been previously reported.

Patient 8 had received pyridostigmine from the age of 17 months with some improvement initially but she remained non-ambulant. The pyridostigmine was stopped 3 months after salbutamol was started, with no decline in functional ability. Patient 2 had received ephedrine from the age of 8.3 years but due to parental choice it was changed to salbutamol after 7 months. None of the other children had received pharmaceutical treatment for their congenital myasthenia before commencing salbutamol.

Prior to starting oral salbutamol, each child had detailed clinical review, cardiac examination, baseline BP and 12 lead ECG. Three had transthoracic echocardiograms. Children aged 5–8 years were prescribed salbutamol liquid, 1 mg TDS for 1 week, increasing to 2 mg TDS thereafter. Older children were offered slow release salbutamol tablets, 4 mg once daily, increasing after 1 week to 4 mg twice daily. The 15 year old boy, unable to swallow tablets, took salbutamol liquid 4 mg TDS. Each child was longitudinally assessed clinically, using the medical research council (MRC) scale of muscle strength, BP, heart rate and spirometry to assess vital capacity.

2.2. Timed assessments

The following timed tests of motor function and muscle fatigability were performed where possible: timed rise from sitting on the floor, timed 10 m walk, maximum time maintaining 90° forward flexion of the right arm at the shoulder (up to 120 s), number of repeated sit to stand from a chair (in 1 min) and grip myometry (Newtons) using a Citec hand held dynamometer (C.I.T. Technics, Netherlands). During the study period we also introduced a 6 min walk time assessment, performed according to standard protocols, in those clinics where the facility was available and the children were able to walk that distance. Accordingly, full results including baseline pre-treatment were only achieved for patient 4.

Children were evaluated at baseline pre-treatment, at 1–3 months, 6–8 months and approximately 6 months post-treatment thereafter. ECG was repeated at 1–3 months, then yearly.

3. Results

Nine children (6 females and 3 males) from eight unrelated families were included in the study. Patients 4 and 5 are sisters. Clinical demographics are shown in Table 1. Their age at starting salbutamol ranged from 5.9 years to 15.1 years. Six had symptoms from birth but genetically confirmed diagnoses were delayed in all. All had limb girdle weakness and three were non-ambulant at baseline (patients 6, 7 and 8). The non-ambulant children had knee and hip flexion contractures and patient 7 had undergone spinal surgery for scoliosis. In addition to limb girdle weakness, facial weakness (8/9) and ptosis (6/9) were common, but ophthalmoplegia (2/9) was rare and consisted of limited upgaze only. This is consistent with the phenotype described by others [2,3].

3.1. Effect of salbutamol on functional ability

All families reported functional improvement within one month of starting salbutamol. Onset of improvement was noted as early as 2 weeks, with a gradual increase to a plateau at 6–18 months. Ambulant children reported improved endurance for distances, ability to run and climb stairs (previously not possible in some) and fewer falls. One no longer had fluctuation in motor abilities. The three non-ambulant children resumed walking: within 2 weeks of starting salbutamol patient 5 could rise from the floor, walk with a crutch and go upstairs – he now walks 30 m unaided. Patient 6 was able to stand and walk assisted after 1 month of treatment, took 30 steps unaided at 7 months and now can walk at least 10 m unaided. Patient 8 at 1 month of treatment was able to take 23 steps with hands held and use the bathroom unaided. At 5 months she climbed stairs with a rail and continues to improve. Walking was achieved in all 3 despite ongoing knee flexion contractures. Improvement in functional ability was often

Table 1
Clinical demographics of *DOK7* cohort.

Patient	Age at onset; age salbutamol started	Sex	Parental consanguinity	<i>DOK7</i> mutation analysis
1	Birth; 5.9 year	f	No	c.325G>T c.596delT
2	18 m; 8.9 year	f	No	c.101–24_141del: 176_206delinsAG c.1124_1127dupTGCC
3	Birth; 5.8 year	f	No	c.101–24_141del: 176_206delinsAG c.1124_1127dupTGCC
4	Birth; 10.9 year	m	Yes	c.415G>C c.415G>C
5	Birth; 13.8 year	m	No	c.1124_1127dupTGCC c.513C>T
6	Birth 15.1 year	m	No	c.1124_1127dupTGCC c.533–37_c.533–11del27
7	3 m; 10.5 year	f	No	c.1124_1127dupTGCC c.512G>A
8	Birth; 9.9 year	f	No	c.54+25_39del15intron1 c.1263delC
9	18 m; 8 year	f	No	c.601.C>T c.1142_1127dupTGCC

remarkable, described by one parent as ‘amazing like a different child’. Ptosis, when present, appeared to respond less well to treatment with only 2 reporting an improvement.

3.2. Effect of salbutamol on timed tests

Changes in functional measures (timed arm raise, timed rise from sitting or lying and timed 10 m) for each individual are illustrated in Fig. 1(a–c). Sustained arm raise improved over the first year in all children (Fig. 1a). The

later decline in some did not correlate with parental reports of stable functional abilities. Other tests varied in their usefulness according to pre-existing muscle strength. Children who were most weak or non-ambulant before treatment showed improvement in their timed rise (Fig. 1b) and timed 10 m (Fig. 1c). However, in the more able children, values for timed rise and timed 10 m were normal at baseline and throughout the study period. The reported improvement in endurance in these children (e.g. ‘he can keep going for longer’) with reduced falls, was not reflected in the 10 m

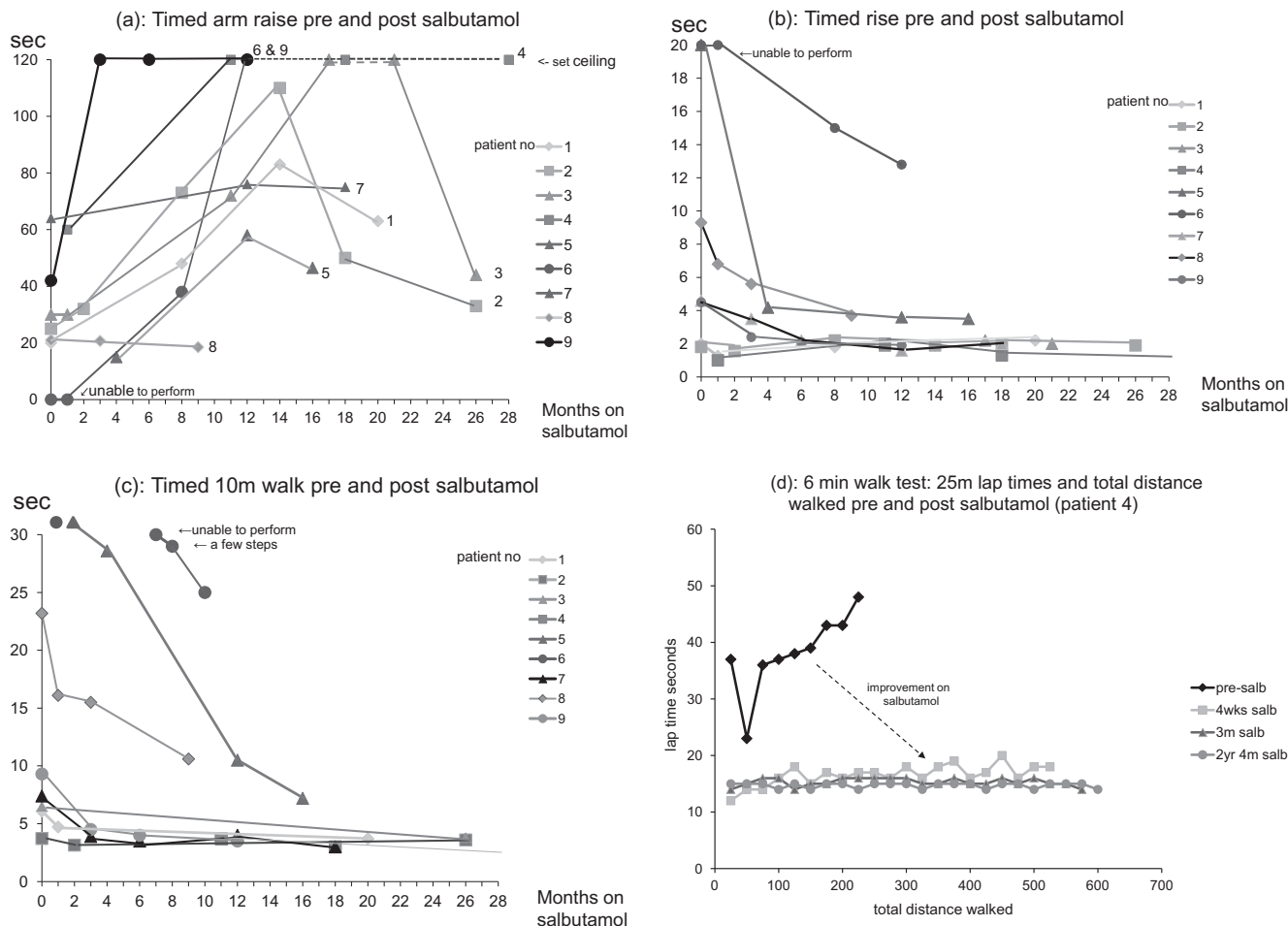


Fig. 1. Effect of salbutamol on timed tests in *DOK7* CMS: (a) timed arm raise in forward flexion to 90 degrees at the shoulder, (b) timed rise to stand from sitting on the floor, (c) timed 10 m walk, and (d) 6 min walk test (patient 4): total walking distance and time for each 25 m lap.

walk or timed rise. In later assessments we introduced the 6 min walk test (6MWT). Results from patient 4 at baseline and after 1, 3 and 28 months on salbutamol are shown in Fig 1(d). There was improved walking distance and reduced fatigue for individual laps following 1 and 3 months of salbutamol which was still sustained at follow up after 28 months on treatment. Unfortunately, baseline values were not available pre-salbutamol for 3 other children in whom 6MWT was subsequently performed. Results of the number of repeated 'sit to stand' in 1 min showed a dramatic improvement in the 3 non-ambulant children, who were unable to perform this test pre-salbutamol, but completed 19, 15 and 32 'sit to stands', respectively at their last assessment. For 4 ambulant children in whom serial data were available, the number of 'sit to stand' achieved in 1 min showed a range of improvement over treatment, with the percentage increment from the initial to the latest number achieved ranging from 16% to 65% as follows: patient 1, 25–34 (36%); patient 2, 18–21 (16%); patient 3, 20–33 (65%); patient 8, 15–18 (20%). Grip myometry was measured in 7/9 children, with serial values in 5. Results were variable, with some younger children having difficulty using the myometer. Repeating the grip myometry test 8 times in succession at each assessment demonstrated a decrease between the first and eighth values (repeated as a possible indication of fatigue) in only 4 of 18 measurements: patient 5: a decrease of 15% at 16 m; patient 6, a decrease of 33% at 8 months; patient 7, a decrease of 15% and 33%, at 5 and 12 months post salbutamol, respectively. Maximum myometry strength (the highest of 8 scores at each assessment) improved marginally with salbutamol treatment in four children who had serial measures, the percentage increment from the initial to last assessment ranging from 4% to 7% for patients 1,2,3 and 7. Maximum grip myometry force in patient 5 increased by 95% (from 65 to 127 Newtons) between 12 and 16 months on salbutamol (aged 13 years 9 months and 14 years 1 month, respectively). Mean myometry scores (an average of the 8 scores at each assessment) increased by a percentage of 14% in patient 1, 18% in patient 2, unchanged in patient 3, 91% in patient 5 and 2% in patient 7. Likewise, timed upgaze gave variable results, ranging from not possible at all due to ptosis (patient 3) to sustained for a full minute (patients 2,6), with other children showing fluctuation in values over treatment with no consistent pattern.

3.3. Adverse reactions during therapy

All children remained on treatment without noteworthy adverse effects. One child reported occasional palpitations after 18 months of treatment, but cardiovascular examination and ECG monitoring were normal. The two sisters had muscle cramps on exercise and at night prior to salbutamol treatment. This became more marked on salbutamol. Reduction of the salbutamol dose was considered, but rejected by the family as the benefit in functional improve-

ment was felt to outweigh the disadvantage of increased cramps.

4. Discussion

Oral salbutamol administered for at least one month was found to improve muscle function in nine children with *DOK7* congenital myasthenic syndrome (CMS), without notable adverse effects. All reported an increase in stamina and function, and this was confirmed on the timed functional assessments. Improvement was reported at 1 month but continued for a further 5–17 months. Remarkably, children who had been non-ambulant for many years acquired the ability to walk independently after salbutamol was administered. The functional improvement observed is not typical of the natural history of the disease. Mutations in *DOK7* result in an endplate synaptopathy often associated with a slow, progressive decline in walking and running ability during childhood [2], possible loss of ambulation in childhood, adolescence or adulthood [2–4] and characterised by small, simplified and unstable neuromuscular junctions [1,4]. It is difficult to deduce from this small series and the short time-frame of study whether introduction of salbutamol at a younger age will improve mobility over the long term. However it is noteworthy that two of our non-ambulant patients (5 and 6) became wheelchair dependent between the ages of 10 and 12 years. By contrast, all of the ambulant children in this series on salbutamol are now over 9 years of age (three are aged 12, 13 and 14 years) and none of them require wheelchair assistance even for distances. The development of contractures in our weaker, non-ambulant children has also limited resumption of mobility, so that introduction of salbutamol at an early age, before decline in mobility and especially before the pubertal growth spurt, would be a pragmatic recommendation. Future studies with muscle MRI may help determine whether fixed myopathy develops over time in *DOK7* CMS and whether this can be influenced by early salbutamol treatment.

The improvement in muscle function that we observed with salbutamol is similar to that reported with ephedrine [4,5] and is likely to be due to β_2 adrenergic effects. When β_2 agonists are used experimentally in a variety of inherited neuromuscular disorders muscle function is improved [5–10]. β_2 receptors act through the G-protein second messenger pathway to induce an increase in intracellular cyclic AMP, and subsequently activate protein kinase A and potentially inhibit proteolytic pathways. A second mechanism of action is through the phosphatidylinositol 3-kinase pathway leading to the activation of the serine-threonine kinase Akt that phosphorylates numerous intracellular targets [11,15].

Dok-7 is a key component of the muscle-specific tyrosine kinase (MuSK) signalling pathway and is essential for neuromuscular synaptogenesis [12] and for maintaining synaptic structure. Activation of MuSK depends on autophosphorylation initiated by neural derived Agrin but is

largely facilitated by the binding of a Dok7 dimer to two MuSK monomers [16]. One of the downstream effects of MuSK itself is the phosphorylation of the acetylcholine receptor β subunit, which facilitates binding of the Rapsyn anchor protein. β 2 agonists activate cAMP-protein kinase A that is thought to feed into the MuSK signalling pathway at the neuromuscular junction [13,14]. The salbutamol-induced increase in kinase activity may partially compensate for the reduced MuSK signalling resulting from impaired Dok-7 function and thus provide a compensatory mechanism to stabilise the endplate. Indeed, β 2 agonists appear to work best in the congenital myasthenic syndromes associated with disrupted endplate structure such as in the *DOK7* synaptopathy or endplate acetylcholinesterase deficiency [10].

CMS are rare disorders and therefore small patient numbers available for randomised controlled trials will always limit studies of treatment efficacy. As reported in a previous study of ephedrine in *DOK7* CMS, outcome measures may not fully reflect functional benefit [5]. Outcomes in children also need to take account of muscle function changes with age due to natural motor development and more variable motivation than in adults. In autoimmune myasthenia there are well-established outcome measures from questionnaires (e.g. MG-ADL) and timed tests of muscle function (e.g. QMG score) but none have been validated in children or in CMS. We explored the use of several timed tests, which are used commonly in paediatric neuromuscular assessments to reflect functional motor ability (timed rise from sitting or lying, timed 10 m run) and the compounding effect of muscle fatigue (repeated sit to stand in 1 min, timed arm raise in forward flexion at the shoulder, timed upgaze and repeated grip myometry). We selected these tests as a minimum screen to use in the outpatient setting where time and motivation of younger children may be limited.

Timed arm raise was helpful in most, but the timed rise from floor and 10 m timed walk were only useful in those who were weak at baseline. We found repeated grip myometry to be of limited value in young children, who found it difficult to grip the myometer adequately. Although values increased during the treatment period in others, this may reflect age-related maturation. We were unable to consistently demonstrate fatigue of grip strength on repeated myometry, but we have done so in children with other CMS or autoimmune myasthenia, so that this may reflect the predilection for weakness and fatigue to affect proximal limb girdle muscles in *DOK7* CMS. Timed upgaze was likewise of limited value in assessing the effect of treatment in *DOK7* children – some had no ptosis or upgaze fatigue at baseline and, when ptosis was present, it proved less responsive to treatment than limb girdle weakness and fatigue. All families reported improved endurance and fewer falls with salbutamol treatment, this was reflected in a substantial improvement in the 6MWT in the one child tested longitudinally. The 6MWT may therefore prove to be a

better objective marker for future CMS treatment studies and we have now introduced this as part of our clinic assessment. We noted fluctuations in timed tests due to difficulties with motivation, mild intercurrent illness and fatigue induced by travel on the clinic day. The development of validated timed tests for parents of CMS children to use at home to monitor progress should provide a useful adjunct to clinic testing.

This study found that oral salbutamol appears to be well tolerated and improves muscle function in children with *DOK7* CMS. Although results should be interpreted with caution because of the relatively small sample size and lack of placebo control, our findings reinforce the report of similar beneficial effects of albuterol in adults with *DOK7* CMS [10].

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