Targeted interventions for patellofemoral pain syndrome (TIPPS): classification of clinical subgroups

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ABSTRACT

Introduction: Patellofemoral pain (PFP) can cause significant pain leading to limitations in societal participation and physical activity. An international expert group has highlighted the need for a classification system to allow targeted intervention for patients with PFP; we have developed a work programme systematically investigating this. We have proposed six potential subgroups: hip abductor weakness, quadriceps weakness, patellar hypermobility, patellar hyponymobility, pronated foot posture and lower limb biarticular muscle tightness. We could not uncover any evidence of the relative frequency with which patients with PFP fell into these subgroups or whether these subgroups were mutually exclusive. The aim of this study is to provide information on the clinical utility of our classification system.

Methods and analysis: 150 participants will be recruited over 18 months in four National Health Services (NHS) physiotherapy departments in England. Inclusion criteria: adults 18–40 years with PFP for longer than 3 months, PFP in at least two predesignated functional activities and PFP elicited by clinical examination. Exclusion criteria: prior or forthcoming lower limb surgery; comorbid illness or health condition; and lower limb training or pregnancy. We will record medical history, demographic details, pain, quality of life, psychomotor movement awareness and knee temperature. We will assess hip abductor and quadriceps weakness, patellar hypermobility and hyponymobility, foot posture and lower limb biarticular muscle tightness.

The primary analytic approach will be descriptive. We shall present numbers and percentages of participants who meet the criteria for membership of (1) each of the subgroups, (2) none of the subgroups and (3) multiple subgroups. Exact (binomial) 95% CIs for these percentages will also be presented.

Ethics and dissemination: This study has been approved by National Research Ethics Service (NRES) Committee North West—Greater Manchester North (11/NW/0814) and University of Central Lancashire (UCLan) Built, Sport, Health (BuSH) Ethics Committee (BuSH 025). An abstract has been accepted for the third International Patellofemoral Pain Research Retreat, Vancouver, September 2013.

ARTICLE SUMMARY

Strengths and limitations of this study

- Currently the largest randomised controlled trial sample size is 176 patients. Our target sample size of 150 patients for this feasibility study indicates the scale and ambition of our programme of work.
- We have also included a comprehensive set of psychosocial and physiological measures as these may also help us to understand differences between potential subgroups. Traditionally patellofemoral research has focused on biomechanical and to some extent pain measures and has paid little attention to the wider holistic picture of a patient’s discomfort.
- We have not included an assessment of cost or resource use in this study. Therefore, any difference in the resource use of different subgroups will remain unknown.
- Qualitative methods would enhance the investigation of psychosocial aspects.
- The study is not longitudinal, but importantly will provide key data to inform such studies.

BACKGROUND

Patellofemoral pain (PFP) can cause significant pain and dysfunction leading to limitations in societal participation and physical activity. Higher body mass indices and higher than expected levels of disability and psychological morbidity have been observed in patients with PFP.1 2 A number of studies provide evidence which challenges the common view that PFP is a relatively trivial and self-limiting condition: 91% of patients had pain and dysfunction at a follow-up of a minimum of 4 years following diagnosis;3 96% reported having problems, a mean of 4 years following diagnosis;4 73% still had pain at an average of 5.7-year follow-up;5 and 94% had ongoing problems for on average 16 years following diagnosis.6

It has also been reported that there is a possibility that PFP predisposes people to
osteoarthritis in later life; Stathopulu and Baildam found that 45% of their patients with PFP, for whom PFP was the first recorded musculoskeletal problem, were later diagnosed with other arthritic conditions. In a study of people with knee pain aged over 50 years, it was found that 507 (64%) had definite radiographic evidence of patellofemoral osteoarthritis, which suggests that there are specific degenerative processes occurring within the patellofemoral joint, which may not be related to the other articular components of the knee. It is unknown how many of these patients had patellofemoral problems when they were younger. Utting et al, reported that 22% of patients (mean age 67) undergoing unicompartimental patellofemoral arthroplasty recalled having PFP as an adolescent. However, a recent systematic review reported that the link between PFP and patellofemoral osteoarthritis should be interpreted with caution due to the paucity of high-quality evidence.

PFP is a condition commonly referred for physiotherapy and PFP recently emerged as the third highest ranked topic out of 185 in the Chartered Society of Physiotherapy Musculoskeletal Research Priority Project. In this national survey, there was 94.9% agreement on the importance of PFP with respect to physiotherapy practice, quality of care, cost-effectiveness and public health. The mean number of National Health Services (NHS) physiotherapy treatment sessions for patients referred with PFP is reported as 8 with the maximum number of sessions reported as 17. The Cochrane Library lists four current reviews and one protocol which are specific to the conservative management of PFP. Collectively these reviews suggest that there is a weak evidence base for conservative management of PFP, including physiotherapy, mainly due to the poor methodological quality of existing studies. In 2012 we found 52 randomised controlled trials (RCTs) recruiting 2667 participants that investigated interventions for PFP. 60% reported results that were not statistically significant. The average sample size was 51 participants and the largest study included 176 participants. As reflected in the number of Cochrane reviews conservative care for PFP is diverse and usually consists of a multimodal package of interventions. The current multimodal approach can include a variety of muscle strengthening and stretching techniques, patellar taping or bracing and foot orthotics. More recent high-quality studies have proposed that a targeted intervention approach for specific subgroups of patients with PFP may produce improved patient outcomes. Crossey et al also discuss the need for developing classification systems of patients with PFP so that “more specific treatment can be designed and evaluated.” The idea of clinically subgrouping patients with PFP and then delivering targeted treatment emerged from the First International PFP Research Retreat. One of the questions posed, as part of the consensus-building process, was “Where do we need to go?” The response was “Identification of sub-groups of people with PFP is needed. This may be achieved through the use of high quality Randomised Controlled Trials (RCTs), which should only investigate a few potential subgroups for which there is a credible rationale...High quality RCTs for different conservative treatments (eg, hip muscle retraining, gait re-education) are strongly encouraged. However, these treatments should be targeted to those sub-groups of people who are most likely to benefit.” The conference proposed three anatomic-based subgroups, proximal (hip and pelvis), local (patella and knee factors) and distal (foot and ankle). The same proximal, local and distal subgrouping approach was adopted by the Second International PFP Research Retreat. This subgrouping provides a rationale for researchers to develop targeted treatment interventions. However, until today there have been no studies which have further investigated this premise. Interestingly a separate process of international consensus building about the future direction of research in the field of primary care musculoskeletal studies has been conducted. This group has also highlighted the need for future studies to adopt a subgrouping targeted approach in order to improve our understanding of the mechanisms underlying musculoskeletal problems to optimise patient management. They highlight that in previous studies the heterogeneity of patient samples produces a small treatment effect, which masks a wide range of individual responses leading to the conclusion that non-pharmacological interventions in musculoskeletal conditions lead to little patient benefit.

WORK PLAN
We have developed a work programme consisting of a number of phases to investigate subgrouping and the targeted intervention approach in PFP. Phase 1: was the theoretical classification of patients with PFP into distinct clinical groups based on clinical assessment tests, which could be used to target intervention in clinical practice, through the development of a clinical practice framework. Phase 2 (the current phase): is developing and testing the feasibility of using a clinical practice framework to assign patients with PFP into subgroups. Phase 3: RCT evaluating the cost-effectiveness of using the clinical practice framework compared to usual care to improve quality of life of patients with PFP. This will incorporate an internal pilot study, to check assumptions about outcome variability which will inform sample size estimates.

Results of phase 1: identification and development of clinical assessment tests
We have completed phase 1 of this work. This was a literature review, evidence synthesis and clinical mapping undertaken by the targeted interventions for PFP research team to establish supporting evidence for the existence of subgroups. Subgroups were derived from the literature which conformed to the following criteria: (1) they could
potentially be identified by simple evidence-based clinical assessment tests; (2) the tests could be used routinely by physiotherapists in a variety of clinical practice settings ranging from primary care facilities to tertiary teaching hospitals; (3) minimal expertise and training was required for competent performance of the tests; (4) any equipment required for the tests needed to be low cost; (5) published thresholds for potentially assigning patients to subgroups had to be available; and (6) any potential subgroup then had to be matched to a specific and credible treatment intervention. Using the First International PFP Research Retreat subgroups as a starting point, we found that there was often more than one clinical problem at the proximal, local and distal sites, and that there were also multiple and sometimes complex clinical assessment tests and multiple interventions. For example, a number of different strength factors have been proposed proximally at the hip.26 27 Patients with proprioceptive deficits have been identified locally at the patella and knee.28 29 However, the problem with this subgroup is that as yet there are no simple and cheap methods to accurately identify proprioceptive deficit in clinical centres that do not have access to dynamometry. Two clinical prediction rules for the likely success of orthotic intervention have been proposed distally at the foot and ankle; however, there is no agreement between the two studies as to the individual clinical items. The first30 lists three items: forefoot valgus alignment; great toe extension; navicular drop test and the second31 lists four different items: age, height, worst pain measured using a visual analogue scale; midfoot width difference from weight bearing to non-weight bearing. Therefore, at the end of this work we have proposed six rather than three subgroups each of which has a specific clinical test which yields a score from which a threshold has previously been published. The threshold scores will be used to assign patients to subgroup membership (figure 1). However, the literature was unable to provide any evidence of the relative frequency with which patients with PFP fell into each of these subgroups and whether these subgroups were mutually exclusive. Therefore, the next stage of our developmental work is an investigation of the distribution of patients into the subgroups when the clinical assessment tests and subsequent threshold scores are applied in routine physiotherapy practice (phase 2). This is the feasibility study which forms the basis of this paper. The main aims of this feasibility study are to assess the relative frequency with which patients fall into each of the subgroups and whether or not the subgroups are mutually exclusive. By the end of this study we would expect to have greater clarity as to whether all, some or none of our proposed subgroups could potentially be useful in clinical practice to form the basis of targeted treatment.

Phase 2: methods

Research question

Do clinically important subgroups of patients with PFP exist?

Aim of study

To provide information on the clinical utility of subgrouping patients with PFP.

Study objectives

This study is designed to provide clinical evidence for theoretically derived subgroups of patients with PFP, which may be appropriate for targeted treatment. In this study we will apply evidence-based routine clinical assessment tests to a representative sample of patients with PFP referred for physiotherapy in order to examine: (1) the relative frequency with which they fall into each of the subgroups; (2) whether the subgroups are mutually exclusive or whether, and how frequently, patients fall into two or more subgroups; (3) whether there are any subgroups which may not be clinically important in the context of targeted treatment because insufficient patients fall into these subgroups; (4) whether patient and clinical characteristics vary between the subgroups. The collection of study data will also allow us to explore the potential for better methods of classifying subgroups by including patient and/or clinical characteristics or by the use of different test thresholds.

Study design

Observational study, at one time point (start of physiotherapy), of adults age 18–40 years with a clinical diagnosis of unilateral or bilateral PFP present for longer than 3 months (for full eligibility criteria see box 1).

Setting

Four NHS physiotherapy departments in England: Central Manchester University Hospitals NHS Foundation Trust; Harrogate and District NHS Foundation Trust; Lancashire Care NHS Foundation Trust; NHS Solent.

Patient recruitment

One hundred and fifty potential participants in total will be recruited over an 18-month period across the four collaborating centres. A research physiotherapist based within each of the four physiotherapy departments will check eligibility and obtain informed consent. Each patient that agrees to take part will be assessed once only by a research physiotherapist (one at each participating centre). The research assessment for this feasibility study will consist of two parts. Part 1: assessment of demographic, clinical and psychosocial patient characteristics which will take approximately 20 min to complete (table 1). Data will be collected on characteristics known to have an impact on outcome. These data may help us to further understand differences between potential subgroups or suggest new subgroups. Previous studies have used some of these tools, however no other study has attempted to systematically investigate psychosocial issues in patients with PFP in the comprehensive manner proposed here. Part 2: clinical assessment tests which take approximately 25 min to complete (table 2).
Thresholds, for assigning participants to subgroups, for each test are based on normative data from healthy populations ±1 SD (figure 1).

**Clinical assessment tests**

*Dynamometer measurement of quadriceps muscle strength* using a Lafayette Manual Muscle Test System (range 0–136 kg): The participant will be in a seated position and the hips and knees flexed to 90°. Muscle strength of the knee extensors will be assessed with a portable dynamometer mounted against a stabilisation strap positioned perpendicular to the tibia just above the malleoli. The force exerted against the dynamometer in this position will be recorded and the moment arm of this force around the extension/flexion axis of the knee joint will be measured using a tape measure as the distance from the level of the dynamometer on the tibia to the centre of the knee joint (assumed to coincide with the most prominent point on the femoral epicondyle identified through palpation). These two measurements will be used to calculate the maximum knee extensor moment (Nm) during an isometric maximal voluntary contraction (MVC) test as the product of the force in Newtons (N) and moment arm in metres (m).³²

*Dynamometer measurement of hip abductors muscle strength:* The participant will be in side lying with the tested leg uppermost in the neutral anatomical position. The participant will be asked to abduct their leg sideways (ie, towards the ceiling) from this position; the portable dynamometer mounted against a stabilisation strap will be held perpendicular to the side of the leg at a level just above the knee joint. To ensure that abductor muscle strength is tested and that the lower limb does not rotate externally, the participants will be instructed to ensure their toes are pointed horizontally during the contraction. The force exerted against the dynamometer in this position will be recorded and the moment arm of this force around the adduction/abduction axis of the hip joint will be measured using a tape measure as the distance from the level of the dynamometer on the thigh to the centre of the hip joint. These two measurements will be used to calculate the maximum hip abductor moment (Nm) during an MVC test as the product of the force (N) and moment arm (m).³² Strength testing will be performed to assess functional capacity, in particular we are interested in the relative balance between the quadriceps and hip abductors. This simple clinical test procedure will not enable the peripheral and central components of muscle dysfunction to be identified but will provide a useful indication of contractile performance.

*Patellar glide:* With the participants in supine, the quadriceps muscles relaxed and the knees in extension, the clinician will apply a medially and then a laterally directed force to the patella. The total displacement of the...
Inclusion criteria

- Men and women aged 18–40 years able to give informed written consent
- Clinical diagnosis of unilateral or bilateral patellofemoral pain longer than 3 months
- Anterior or retropatellar pain reported on at least two of the following activities: prolonged sitting, ascending or descending stairs, squatting, running, kneeling and hopping/jumping
- In addition to the above, at least two of the following clinical examination findings:
  - pain during resisted isometric quadriceps contraction
  - pain with palpation of the posterior borders of the patella
  - pain during squatting

Exclusion criteria

- Previous knee surgery and participants awaiting surgery for another lower limb joint problem(s)
- Ligamentous instability and/or internal derangement
- History of patella subluxation or dislocation
- Joint effusion when the midpatellar girth is 5% or more than the non-involved knee
- True knee joint locking and/or giving way
- Coexistent acute illness or chronic disease
- Bursitis, patella or iliobial tract tendinopathy, Osgood-Schlatter’s disease, Sinding-Larsen-Johansson Syndrome, muscle tears or symptomatic knee plicae
- Participants already involved in active lower limb training programmes
- Pregnancy or breast feeding
- Men and women aged 40 years able to give informed consent

Foot posture index: There are six component assessments (1) talar head position, (2) supralateral and infralateral malleolar curvature, (3) calcaneal frontal plane position, (4) medial malleolar curvature, (5) talar sagittal plane position, (6) calcaneal sagittal plane position.

## Table 2 Clinical assessment tests

<table>
<thead>
<tr>
<th>Proposed clinical groups</th>
<th>Tests</th>
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<tbody>
<tr>
<td>Hip abductor weakness</td>
<td>Hand-Held Dynamometry&lt;sup&gt;32&lt;/sup&gt;</td>
</tr>
<tr>
<td>Quadriceps weakness</td>
<td>Hand-Held Dynamometry&lt;sup&gt;32&lt;/sup&gt;</td>
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<tr>
<td>Patellar hypomobility</td>
<td>Patellar Glide Test&lt;sup&gt;33&lt;/sup&gt;</td>
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<tr>
<td>Patellar hypermobility</td>
<td>Patellar Glide Test&lt;sup&gt;33&lt;/sup&gt;</td>
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<tr>
<td>Pronated foot posture</td>
<td>Foot Posture Index&lt;sup&gt;35&lt;/sup&gt;</td>
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<tr>
<td>Lower limb biarticular muscle tightness</td>
<td>Hamstrings length test&lt;sup&gt;34&lt;/sup&gt;</td>
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<td></td>
<td>Gastrocnemius length test&lt;sup&gt;33&lt;/sup&gt;</td>
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## Table 1 Patient characteristics assessment

<table>
<thead>
<tr>
<th>Domain</th>
<th>Questionnaire/items</th>
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<tbody>
<tr>
<td>Clinical characteristics</td>
<td>Time since onset</td>
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<tr>
<td></td>
<td>Previous treatment</td>
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<tr>
<td></td>
<td>History of trauma</td>
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<tr>
<td></td>
<td>Physical activity (International Physical Activity Questionnaire IPAQ)&lt;sup&gt;38&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Physical functioning (Modified Functional Index Questionnaire (MFQ))&lt;sup&gt;39&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Socioeconomic characteristics</td>
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<td>Gender</td>
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<td>Anthropometry</td>
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<td>Height</td>
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<td></td>
<td>Weight</td>
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<td></td>
<td>Body mass</td>
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<td></td>
<td>Leg length</td>
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<td></td>
<td>Skin fold over the patellae</td>
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<tr>
<td>Psychosocial pain measures</td>
<td>Nociceptive pain</td>
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<td></td>
<td>Short Form McGill Pain Questionnaire (SF-MPQ-2)&lt;sup&gt;40&lt;/sup&gt;</td>
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<td></td>
<td>Numeric Pain Rating Scale (NPRS) for average pain during the past week</td>
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<td></td>
<td>Neuropathic pain</td>
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<td></td>
<td>Leeds Assessment of Neuropathic Symptoms and Signs (S-LANSS) Questionnaire&lt;sup&gt;41&lt;/sup&gt;</td>
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<tr>
<td>Quality of life</td>
<td>WHO Disability Assessment Scale 2 (WHODAS 2.0)&lt;sup&gt;42&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>EQ-5D-5 L&lt;sup&gt;43&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Hopkins Symptom Checklist 25 (HSCL-25)&lt;sup&gt;44&lt;/sup&gt;</td>
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<tr>
<td>Psychomotor movement awareness</td>
<td>Movement Specific Reinvestment Questionnaire&lt;sup&gt;45&lt;/sup&gt;</td>
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<tr>
<td>Physiological parameters</td>
<td>Self-reported indicators of cold knee&lt;sup&gt;46&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Skin temperature measurement (over centre of patella and muscle belly of tibialis anterior)</td>
</tr>
</tbody>
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Foot posture index: There are six component assessments (1) talar head position, (2) supralateral and infralateral malleolar curvature, (3) calcaneal frontal plane position, (4) medial malleolar curvature, (5) talar sagittal plane position, (6) calcaneal sagittal plane position.
(4) prominence in the region of the talonavicular joint,
(5) congruence of the medial longitudinal arch,
(6) abduction/adduction of the forefoot on the rear-foot. Each of the component assessments or observations are graded 0 for neutral, with scores of −2 for clear signs of supination and +2 for clear signs of pronation. Unless the criteria outlined for each of the features are clearly met then the more conservative score will be awarded. When the scores are combined, the aggregate value gives an estimate of the overall foot posture. Large positive aggregate values indicate a pronated posture.35

Training of research therapists
All research physiotherapists will undertake a full day training session during the first month of the study, when they will be provided with training on the research processes and on how to undertake the standardised clinical assessment tests. During the training sessions, all the therapists will be observed by the principal investigator and MC performing each of the clinical test procedures and provided with peer feedback. Intertherapist variability will be examined during these sessions and although it will not be possible to conduct a formal inter-rater reliability assessment during the training it will provide an opportunity to observe any variability in performance and address it. All the physiotherapists will be provided with a comprehensive manual including the standard operating procedures, along with a data recording proforma.

Sample size
Given the nature of the study, power calculations are not applicable. One hundred and fifty participants will enable us to estimate, with 95% CI, the numbers and percentages of participants who meet the criteria for membership of (1) each of the six subgroups individually; (2) none of the subgroups; (3) multiple subgroups (for each represented subgroup combination) to within ±7.5% for well-represented (30% prevalence) subgroups and to within ±3.5% for sparse (5% prevalence) subgroups (or multiple subgroups).

Analysis plan
As the main purpose of the study is to describe the distribution of patients with PFP into the different subgroups following application of the clinical assessment test criteria, including whether patients meet the criteria for multiple subgroups or fail to meet the criteria for any of the subgroups, the primary analytical approach will be descriptive.

We shall present numbers and percentages of participants who meet the criteria for membership of
1. Each of the six subgroups individually;
2. None of the subgroups;
3. Multiple subgroups (for each represented subgroup combination).

Exact (binomial) 95% CIs for these percentages will also be presented. We shall also present descriptive statistics (mean (SD), median (IQR), count (%), as appropriate) of the patient characteristics for each subgroup (including the no clinical subgroup) to indicate how these characteristics vary across subgroups. The data will also enable some further exploratory analyses to be performed. The nature of these analyses will depend on the patterns of the distribution of participants into subgroups. However, we expect that they will include an exploration of the sensitivity of the distribution of subgroup membership to the choices of thresholds, particularly if substantial numbers of patients fall into either multiple subgroups or no subgroup; they are also likely to include explorations of the joint effects of patient characteristics on the distribution of patients into subgroups, using techniques including multiple logistic regression. Demographic, clinical and psychosocial characteristics as previously described may also be included as covariates in later exploratory model-based analyses.

DISCUSSION
Recent literature has strongly promoted the idea of subgrouping patients with PFP and delivering targeted treatment, as it is believed that this may be more beneficial than the current multimodal therapeutic approaches.20–22 Despite these recommendations, this premise has not yet been investigated. The main aims of this feasibility study are therefore to assess the relative frequency with which patients fall into each of the subgroup and whether or not the subgroups are mutually exclusive.

As outlined above, the study has a number of strengths and addresses key gaps in current knowledge. It is ambitious in terms of scale and scope. There is often controversy and lack of consensus within the field of patellofemoral research, due to two related factors associated with the nature of the current evidence base. First, there are a relatively large number of normative data; studies conducted on very small samples of healthy participants that do little to enhance our understanding of this complex chronic condition.36 Second, as already discussed, there is a limited number of high-quality, large scale clinical trials. Set against this context, where currently the largest RCT sample size is 176 patients,20 our target sample size of 150 patients for this feasibility study indicates the scale and ambition of our programme of work. In terms of the scope of this study, we have also included a comprehensive set of psychosocial and physiological measures as these may also help us to understand differences between potential subgroups. Traditionally, patellofemoral research has focused on biomechanical and to some extent pain measures and has paid little attention to the wider holistic picture of a patient’s discomfort. One study has indicated that psychological morbidity may be important in PFP.2 The more comprehensive data being obtained in the present study will provide us with a unique insight into the patient’s experience of the condition which may also
help further our understanding of future treatment options. There are also potential limitations in the scope of this study. We have not included an assessment of cost or resource use in this study. Therefore, any differential in the resource use of different subgroups will remain unknown; we plan to address this in phase 3 of our programme of work. In the current feasibility study, there is however, a health economics component as we are collecting EQ-5D-5L data so we will gain some insight into the potential health consequences of different subgroups. Qualitative methods would enhance the investigation of psychosocial aspects. We envisage using mixed methods, as well as more patient and public involvement in subsequent studies. The study is not longitudinal, but importantly will provide key data to inform such studies. The results of the present study are expected in 2014. They will provide answers to a number of questions about the validity and relevance of subgrouping in PFP in clinical practice and will inform future trials.

Status
The study has currently recruited 101/150 patients and is scheduled to be completed by 29 November 2013.

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Contributors
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Competing interests
None.

Ethics approval
This study has been approved by National Research Ethics Service (NRES) Committee North West—Greater Manchester North, REC reference: 11/NW/0814 and University of Central Lancashire (UCLan) Built, Sport, Health (BuSH) Ethics Committee Reference Number: BuSH 025. All relevant Research and Governance approvals have been secured at the 4 NHS Trusts where data collection is taking place and all licenses obtained for the questionnaire instruments were required.

Provenance and peer review
Not commissioned; internally peer reviewed.

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Correction

Selfe J, Callaghan M, Witvrouw E, et al. Targeted interventions for patellofemoral pain syndrome (TIPPS): classification of clinical subgroups. BMJ Open 2013;3:e003795. Figure 1 of this article was published incorrectly. The correct figure 1 is below.

**Figure 1** Proposed clinical sub-groups and clinical test thresholds for sub-group assignment.