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Chromosomal abnormalities related to fever of unknown origin in a Chinese pediatric cohort and literature review

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Abstract

Background: Fever of unknown origin (FUO) has been difficult to diagnose in pediatric clinical practice. With the gradual change in the disease spectrum, genetic factors have received increasing attention. Limited studies have shown an association between FUO and chromosomal abnormalities. In this study, we investigated the clinical and genetic characteristics of patients with FUO presenting with chromosomal abnormalities in a Chinese pediatric cohort.

Results: Chromosomal abnormalities were detected in 5.5% (8/145) of the patients with FUO. Six patients with inflammatory fever presented with pharyngitis/amygdalitis (4/6), oral aphthous ulcer (2/6), digestive symptoms (3/6), developmental delay (4/6) and elevated C-reactive protein levels (6/6) during fever. These patients were often considered to have systemic inflammatory diseases, such as Behcet's disease or systemic juvenile idiopathic arthritis. Trisomy 8, 7q11.23 dup, 3p26.3-p26.1 del/17q12 dup, 22q11.21 del, and 6q23.3-q24.1 del were identified in patients with inflammatory fever. The *TNFAIP3* gene was included in the 6q23.3-q24.1 deletion fragment. Two patients with central fever were characterized by facial anomalies, developmental delay, seizures and no response to antipyretic drugs and were identified as carrying the de novo 18q22.3-q23 del. By performing a literature review, an additional 19 patients who had FUO and chromosomal abnormalities were identified. Trisomy 8, 6q23.2-q24.3 del and 18q22.3-q23 del were reported to present as fever, similar to the findings of our study.

Conclusions: We emphasized the important role of detecting chromosomal abnormalities in patients with FUO, especially in patients with systemic inflammatory manifestations or developmental delay. Identifying chromosomal abnormalities may change the diagnosis and management of patients with FUO.

Keywords: Fever of unknown origin, Chromosomal abnormalities, Copy number variations, Autoinflammatory, Central fever

Background

Fever is a common manifestation of various diseases in children. Fever of unknown origin (FUO) has also been particularly difficult to specifically diagnose in pediatric clinical practice since Petersdorf and Beeson first defined it in 1961 [1]. Previous reports suggested that 40%-50% of pediatric patients with FUO were identified as having an infection. Noninfectious etiologies, including autoimmune disease, malignancy, environmental stressors or

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drugs, also play important roles in FUO diagnosis [2]. However, the causes of fever in some children are still unexplained.

Autoinflammatory and thermic dysregulation may be potential causes of FUO. Autoinflammatory diseases mainly present as early-onset recurrent fever and systemic inflammation and involve various organs and tissues, such as the skin, mucosa, joints, gastrointestinal tract, and central nervous system. This type of disease is characterized by dysregulation of the innate immune system with antigen-independent immune pathway hyperactivation and the development of sterile inflammation [3, 4]. After the identification of the first gene, *MEFV*, which results in familial Mediterranean fever [5], more than 50 new monogenic autoinflammatory diseases have been discovered due to advances in genetic sequencing and are known as important causes of unexplained fever in children. Central fever is caused by abnormalities in the thermoregulatory center and therefore an elevated body temperature range. The outstanding characteristics of central fever are a lack of infectious symptoms and lack of response to antipyretic treatment. An abnormal thermoregulatory center may result from craniocerebral diseases, such as cerebral hemorrhage, traumatic brain injury, and brain surgery [6]. In pediatric patients, central fever often occurs with the following diseases: cerebral dysplasia, seizures and developmental delay [7], which often have significant genetic factors.

Despite remarkable progress in understanding the pathogenesis of FUO, its accurate diagnosis and management remain very difficult. Previous studies have mainly focused on the clinical etiology of FUO rather than genetic factors. In addition to single genes, genetic factors include chromosomal abnormalities. Chromosomal abnormalities are further categorized as numerical or structural. Copy number variation (CNV) is a type of chromosomal deletion or duplication variation ranging in size from 1 kb to several Mb. Chromosomal abnormalities, especially CNVs, have been indicated to be linked to a number of diseases, such as neurological disorders and congenital malformations [8]. In a few studies, CNVs have been recognized as an important cause of inflammatory fever [9]. Recent discoveries suggest that pathogenic CNVs using next-generation sequencing (NGS)-based methods have provided genetic characteristics of many previously undiagnosed patients with systemic autoinflammatory diseases, especially if single genes have not been confirmed [10].

However, the specific proportions and characteristics of CNVs in patients with FUO remain unclear and should not be ignored by clinicians. In this study, patients with FUO who were treated at a single center in China were enrolled, their genetic backgrounds were analyzed,

especially for chromosomal abnormalities, and the relationship between chromosomal abnormalities and fever was explored to improve our understanding of the influence of genetics on FUO.

Methods

This study was approved by the Ethics Committee of Human Experimentation in Children's Hospital of Fudan University. Written informed consent was obtained from the parents of all patients.

Patients

Children with fever of unknown origin (FUO) and recurrent fever who were admitted to our hospital between January 2016 and June 2021 were included in this study. We excluded patients who were clinically diagnosed with infections, connective tissue disease and malignant tumors. FUO was defined as an elevated temperature ($>38.3^{\circ}\text{C}$ measured by the rectal route or $>37.3^{\circ}\text{C}$ by the axillary route) for more than 3 weeks or a failure to determine a diagnosis after 1 week of inpatient investigations [11]. Recurrent fever was defined as at least three episodes of unexplained fever in a six-month period, with a minimum interval of seven days between episodes [12, 13]. The relevant data are summarized in detail. Data from patients with FUO who had chromosomal abnormalities and were previously reported in PubMed Medline (<https://www.ncbi.nlm.nih.gov/pubmed/>) were reviewed.

Genomic DNA extraction

Genomic DNA was extracted from peripheral blood samples collected from patients and their parents using a TINGEN genomic DNA purification kit (TINGEN, Tianjin, China) according to the manufacturer's instructions. The DNA concentration and absorbance were determined using a NanoDrop spectrophotometer (NanoDrop Technologies, Berlin, Germany).

Next-generation sequencing

Genomic DNA fragments were enriched using the Agilent SureSelect XT Human All Exon V5 kit. After enrichment, DNA libraries were sequenced with the HiSeq 2000 platform according to the manufacturer's instructions (Illumina, San Diego, CA) with an average on-target sequencing depth of $120\times$. More information about sequencing and data analysis, particularly of single nucleotide variations, can be found in a previous study [14]. The pipeline for clinical NGS-involved CNV detection (PICNIC) was used to detect CNVs from whole-exome sequencing (WES) data. PICNIC filters out high-frequency gene deletions/duplications. The detailed process for CNV analysis was presented in published studies [15,

16]. CNVs were further verified by performing comparative genomic hybridization (CGH) or multiplex ligation-dependent probe amplification (MLPA).

Array CGH

We applied an Agilent custom Human Genome CGH microarray 4 × 180 K (Agilent Technologies, Santa Clara, CA, USA) for CNV analysis. Array CGH experiments were performed using standard protocols provided by the manufacturer. The analysis was performed using Agilent CytoGenomics software (Agilent Technologies, version 2.5.8.11) [15].

MLPA

An MLPA probe set (MRC Holland, P029-C1) was used to detect chromosomal imbalances. This kit contained probes of genes mapped to the 7q11.23 critical region (*CLIP2*, *ELN*, and *LIMK1*). MLPA was carried out according to the standard protocol (MRC Holland, MLPA[®] General Protocol MDP-v007, Amsterdam, Netherlands). The electrophoresis was performed using an ABI 3130 Genetic Analyzer, and data were analyzed by GeneMarker software (SoftGenetics, State College, United States, version 1.95). The results were considered abnormal when the relative peak height ratio was below 0.7 or above 1.30.

Results

Chromosomal abnormalities in FUO

One hundred forty-five patients with FUO or recurrent fever were enrolled in our cohort, and trio-WES was performed. Ultimately, 33.1% (48/145) of patients had single gene mutations of primary immunodeficiency diseases. Notably, in addition to monogenic diseases, 8 patients (P1-P8) had chromosomal abnormalities, which accounted for 5.5% (8/145) of the total patients with FUO in our cohort (Fig. 1). The characteristics of these 8 children (P1-P8) with chromosomal abnormalities are further summarized in Table 1. The patients with chromosomal abnormalities were further divided into 6 patients with inflammatory fever and 2 patients with central fever.

Clinical manifestations of patients with inflammatory fever

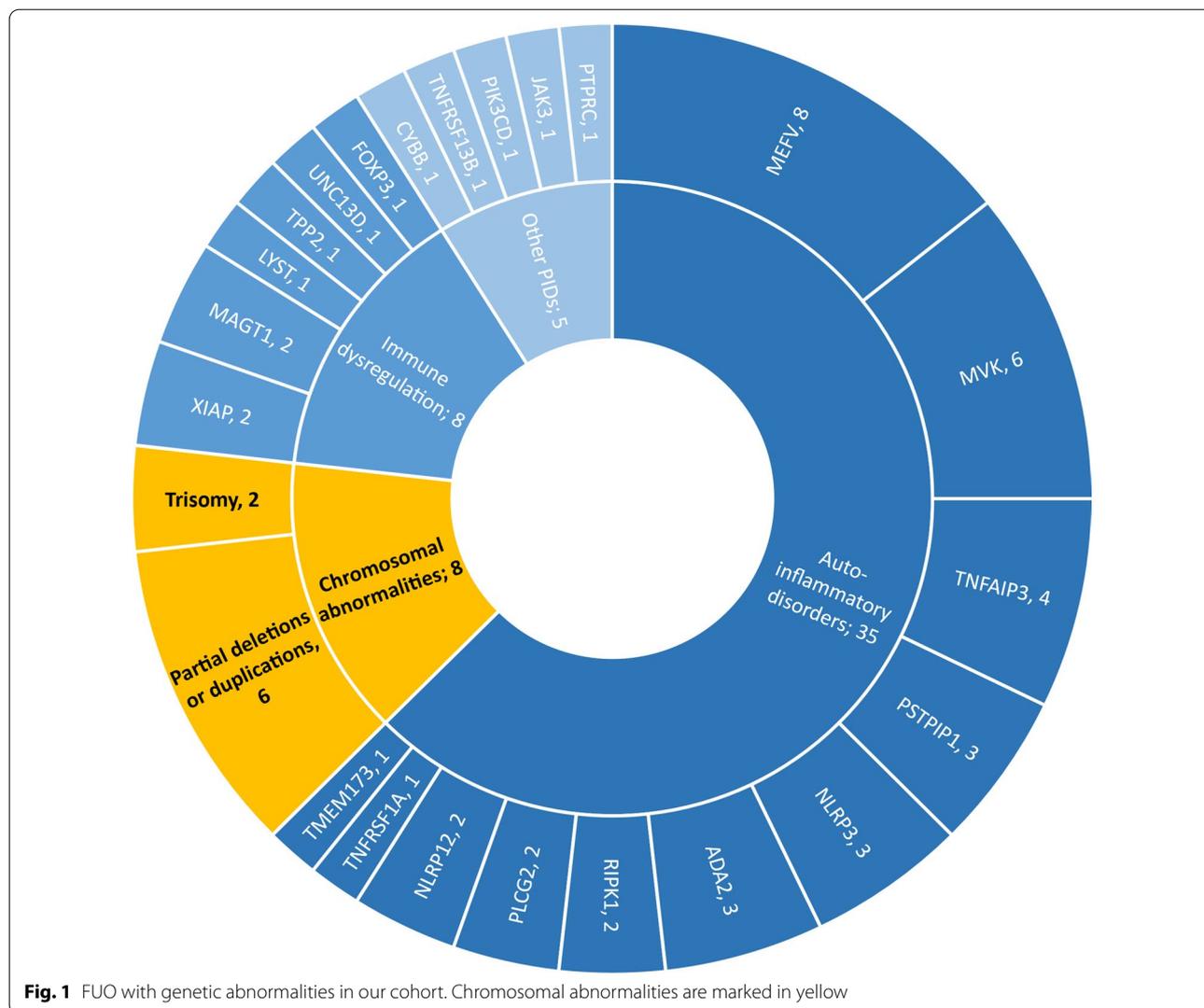
Six patients (P1-P6) presented with inflammatory fever, with a median onset age of 10 months (range, 2 months-36 months). Five (P1-P5) of them had periodic fever, lasting for 3–7 days and usually recurring at intervals of 1–2 months, while one patient (P6) had intermittent fever with a recurrence frequency of 7–8 times per year. These patients suffered from hyperpyrexia with a temperature spike > 38.5 °C (axillary temperature).

Abnormal inflammation in other organs during fever was prominent. Pharyngitis and tonsillitis were the most

frequent concomitant symptoms, which occurred in 4/6 patients (P1-P4). The affected skin and mucosa were characterized by recurrent oral aphthous ulcers (P4 and P5) and red maculopapules during fever (P6). Digestive symptoms included vomiting, chronic diarrhea and abdominal pain. Moreover, a thickened terminal ileum wall was discovered in P4 by abdominal CT, and perianal, colon and intestinal ulcers were detected in P5 using enteroscopy. Four patients had lymphadenectasis (P3-P6), three of whom had hepatosplenomegaly (P4-P6). Two patients had hematological involvement during fever. P5 presented with moderate anemia and thrombocytopenia that was able to rise to normal after temperature control, and the peripheral blood smear was normal at the beginning of the disease. She subsequently had persistent thrombocytopenia and a progressive decline in hemoglobin and was eventually diagnosed with acute myeloid leukemia (AML) at 69 months of age. Moreover, P6 was suspected to have hemophagocytic lymphohistiocytosis (HLH) at the age of 2 years because of pancytopenia, and her blood cell counts increased after corticosteroid therapy. In addition to the inflammatory manifestations, four patients (P1-P3 and P6) had developmental delay in motor or language, and two of them (P3 and P6) also presented growth retardation.

During fever attacks, the levels of inflammatory markers were markedly increased (Table 2). Elevated white blood cell count (WBC) (5/6), C-reactive protein (CRP) (6/6), erythrocyte sedimentation rate (ESR) (6/6) and serum amyloid A (SAA) (2/2) were detected. The acute phase reactants plummeted to normal levels when the body temperature stabilized. Routine immunological function tests, in comparison with the reference values in healthy children in China [17], showed that T lymphocytes decreased and immunoglobulin E (IgE) levels increased significantly in P5, and the CD4/CD8 ratio of P6 was reversed. The etiological examinations, such as blood culture, urine culture, and throat swab culture, were all negative. The possibility of *Mycobacterium tuberculosis*, Epstein–Barr virus, and cytomegalovirus infection was excluded after pathogen antibody and DNA detection.

Therefore, several inflammatory diseases were initially considered, and anti-inflammatory therapy was given to these patients due to their inflammatory manifestations. For example, P3 had periodic fever, tonsil exudation, cervical lymph node enlargement, and increased inflammatory indicators, which were consistent with the main manifestations of periodic fever, aphthous stomatitis, pharyngitis, and adenitis (PFAPA). Single-dose corticosteroids were effective in the abrupt cessation of acute attacks. Systemic juvenile idiopathic arthritis (s-JIA) was suspected in P6 at the age of 3.6 years based on the



presence of fever, rash, lymphadenectasis, hepatosplenomegaly, polyarthritits and a positive (1:100) titer of antinuclear antibody (ANA). Although she received prednisone, diclofenac and naproxen treatment, recurrent fever and joint swelling were still intermittent. Unfortunately, she died of systemic inflammatory response syndrome at the age of 4.5 years.

Clinical manifestations of patients with central fever

Two patients (P7 and P8) presented with persistent fever attacks with a fluctuating body temperature of approximately 1.5 °C (P7 between 37.2–38.6 °C and P8 between 37.2–38.8 °C). Fever developed beginning at the age of 6–9 months and was not apparently associated with any infectious or inflammatory manifestation. Acute phase reactants remained normal. A transient respiratory

syncytial virus, *Haemophilus influenzae* and *Staphylococcus aureus* lung infection occurred in P7, while cytomegalovirus-DNA was detected in peripheral blood and *Clostridium difficile* toxin was positive in stool samples from P8; however, adequate anti-infection therapy was administered without any efficacy against the fever. Both patients had facial and congenital abnormalities, for example, P7 presented with cryptorchidism, macrocephaly and frontal bossing, and P8 presented with congenital aural atresia (CAA), ocular hypertelorism, low-set ears and high arched palate. They also presented with growth retardation and developmental delay. The Ages & Stages Questionnaire, Third Edition (ASQ-3 scale) score of P7 showed a suspected overall developmental delay. Epileptic seizures, myelinated dysplasia and ventriculomegaly were further separately confirmed

Table 1 Baseline characteristics of FUIO patients with chromosomal abnormalities

| Patient | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 |
|--|---|---|--|--------------------------------------|---|---|--|-------------------------------------|
| Fever phenotype | Periodic fever | Periodic fever | Periodic fever | Periodic fever | Periodic fever | Recurrent fever | Central fever | Central fever |
| Age at fever presentation | 10 m | 36 m | 8 m | 10 m | 24 m | 2 m | 9 m | 6 m |
| Age at diagnosis | 56 m | 67 m | 21 m | 44 m | 60 m | 45 m | 22 m | 14 m |
| Gender | F | F | M | F | F | F | M | M |
| Fever interval | 1 m | 1 m | 1 m | 0.5–1 m | 2 m | irregular | persistent | persistent |
| Facial abnormality | - | - | - | - | - | irregular frontal bossing | + | + |
| Congenital anomalies | VSD/ASD | - | - | flexion deformity of finger | - | PDA | + | congenital aural atresia |
| Growth retardation | - | - | + | - | - | + | + | + |
| Developmental delay | + | + | + | - | - | + | + | + |
| Pharyngitis/amygdalitis | + | + | + | + | - | - | - | - |
| Lymphadenectasis/hepatosplenomegaly | - | - | + | + | + | + | - | - |
| Skin/mucous | - | - | - | Oral aphthous perianal abscess | Oral aphthous | rash | - | - |
| Gastrointestinal | Vomiting | - | - | Diarrhea | Abdominal pain | - | - | - |
| Arthritis | - | - | - | - | - | + | - | - |
| Blood | - | - | - | - | anemia, thrombocytopenia | HLH | - | - |
| Central nervous system | Tourette syndrome | - | - | Seizure | - | Seizures | Seizures | Hypotonia; abnormal EEG |
| Abnormal chromosome and gene | 7q11.23 dup | 3p26.3–p26.1 del;17q12 dup | 22q11.21 del | Trisomy 8 | Trisomy 8; PTPN11: c.1508G>C | 6q23.3–q24.1 del | 13q32.3–q34 dup;18q22.3–q23 del | 18q22.3–q23 del |
| RefSeq genes included in abnormal chromosome | NCF1, LAT2, PTPN12, MERM1 | TRNT1, CCL5, CCL2, CCL16, etc | SCARF2, etc | IKBK8, TNFRSF11B, etc | IKBK8, TNFRSF11B, etc | TNFAIP3, IFNGR1, etc | TNFSF13B, GALR1, TSHZ1, etc | GALR1, TSHZ1, etc |
| Treatment | Antibiotic, colchicine, bacterial lysates | Antibiotic, temporary corticosteroid, bacterial lysates | Antibiotic, single-dose corticosteroids during attacks | Antibiotic, temporary corticosteroid | Antibiotic, mesalazine, bacterial lysates | Antibiotic, long-term corticosteroids, NSAIDs | Antibiotic, thymopolyptides, antiepileptic | Antibiotic, rehabilitation training |

m months; F female; M male; VSD ventricular septal defect; ASD atrial septal defect; PDA patent ductus arteriosus; HLH hemophagocytic lymphohistiocytosis; EEG electroencephalogram; NSAIDs nonsteroidal anti-inflammatory drugs

Table 2 Immunophenotypes and inflammatory markers of FUO patients with chromosomal abnormalities in our cohort

| Patient | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | Reference range |
|---------------------|---------|---------|----------|----------|-----------|-----------|---------|---------|--|
| Age | 52 m | 67 m | 21 m | 48 m | 60 m | 51 m | 22 m | 12 m | |
| Gender | F | F | M | F | F | F | M | M | |
| WBC (cells/ul) | 23,300↑ | 5700 | 23,210↑ | 23,400 ↑ | 18,000↑ | 15,700↑ | 8300 | 10,700 | 4000–12,000 |
| NEUT (%) | 71.4↑ | 55 | 74.4↑ | 55 | 85.4↑ | 57 | 59.2 | 25.8 | 30–70 |
| CD19 (%) | 25.35↑ | 15.38 | 24.38 | 18.2 | 9.31↓ | 19.05 | 35.7↑ | 18.82 | 12-24 m: 13.23–26.39; 48-72 m: 10.46–21.77 |
| CD19 (cells/ul) | 788.55↑ | 483.06 | 1475.59↑ | 495.95 | 142.81↓ | 678.93 | 1006.77 | 917.87 | 12-24 m: 461–1456; 48-72 m: 303.52–777.25 |
| CD3 (%) | 65.62 | 74.35 | 63.69 | 66.63 | 69.94 | 67.36 | 45.38↓ | 61.32 | 12-24 m: 53.88–72.87; 48-72 m: 59.50–75.56 |
| CD3 (cells/ul) | 2041.2 | 2335 | 3854.4 | 1815.9 | 1072.6↓ | 2400.8 | 1279.8↓ | 2990 | 12-24 m: 1794–4247; 48-72 m: 1480–2847 |
| CD4 (%) | 38.47 | 42.00↑ | 38.26 | 38.69 | 53.14↑ | 17.62↓ | 31.03 | 36.82 | 12-24 m: 24.08–42.52; 48-72 m: 28.49–41.07 |
| CD4 (cells/ul) | 1196.79 | 1319.18 | 2315.53↑ | 1054.46 | 814.94 | 628.14↓ | 875.25↓ | 1795.57 | 12-24 m: 902–2253; 48-72 m: 767–1592 |
| CD8 (%) | 21.96 | 25.5 | 21.74 | 21.95 | 16.3↓ | 46.56↑ | 10.08↓ | 22.17 | 12-24 m: 19.00–32.51; 48-72 m: 19.70–32.04 |
| CD8 (cells/ul) | 683.00 | 800.81 | 1315.9 | 598.19 | 249.91↓ | 1659.24↑ | 284.28↓ | 1080.9 | 12-24 m: 580–1735; 48-72 m: 553–1127 |
| CD16CD56 (%) | 6.74↓ | 8.84 | 11.24 | 13.61 | 18.72 | 12.83 | 16.54 | 18.65 | 12-24 m: 7.21–20.9; 48-72 m: 7.83–20.99 |
| CD16CD56 (cells/ul) | 209.56↓ | 277.64 | 680.25 | 370.2 | 287.17 | 457.14 | 466.52 | 909.37 | 12-24 m: 270–1053; 48-72 m: 227–668 |
| IgG (g/L) | 7.60 | 13.2 ↑ | 7 | 10.5 | 19.8 ↑ | 6.5 | 7.1 | 10.2↑ | 12-24 m: 5.52–11.46; 48-72 m: 4.95–12.74 |
| IgM (g/L) | 1.55 | 2.27 ↑ | 1.01 | 1.81 | 1.96 | 1.59 | 0.7 | 0.37 | 12-24 m: 0.6–2.12; 48-72 m: 0.65–2.01 |
| IgA (g/L) | 0.52 | 1.19 | 0.48 | 0.67 | 2.8 ↑ | 0.85 | 0.3 | 0.21 | 12-24 m: 0.06–0.74; 48-72 m: 0.33–1.89 |
| IgE (KU/L) | 171.6 ↑ | 32.55 | 34.18 | 31.13 | 3006.67 ↑ | 37.42 | 7.1 | 29.72 | < 100 |
| CRP (mg/L) | 87 ↑ | 16 ↑ | 27.18 ↑ | 70 ↑ | 40 ↑ | 132 ↑ | < 8 | < 0.5 | < 8 |
| ESR (mm/h) | 48 ↑ | 32 ↑ | 64 ↑ | 44 ↑ | 54 ↑ | 107 ↑ | 35↑ | 2 | 0–26 |
| SAA (mg/L) | NA | 259.6 ↑ | 256.4 ↑ | NA | NA | NA | < 2.5 | < 2.5 | < 10 |
| IL-2 (pg/ml) | NA | 1.3* | 0* | NA | 1.71 | NA | 0 | 2.3 | 0–11.4 |
| IL-4 (pg/ml) | NA | 1.35* | 0* | NA | 2.32 | NA | 0 | 0.8 | 0–12.9 |
| IL-6 (pg/ml) | 109.6 ↑ | 2.86* | 0.8* | 7.6* | 44.79 ↑ | 667.7 ↑ | 0 | 5.9 | 0–16.6 |
| IL-8 (pg/ml) | NA | NA | NA | NA | NA | 4956.86 ↑ | NA | NA | 0–21.4 |
| IL-10 (pg/ml) | NA | 3.56* | 2.1* | NA | 10.64 ↑ | 12.95 ↑ | 1.07 | 6 | 0–5.9 |
| TNF-α (pg/ml) | NA | 2.27* | 0* | NA | 90.96 ↑ | 11.03↑ | 0 | 3.6 | 0–5.5 |
| IFN-γ (pg/ml) | NA | 2.64* | 0* | NA | NA | NA | 0 | 1.5 | 0–17.3 |

m months; F female; M male; WBC white blood cells; NEUT Neutrophil count; CRP C-reactive protein; ESR erythrocyte sedimentation rate; SAA Serum amyloid A; IL interleukin; TNF tumor necrosis factor; IFN interferon; NA not available

The percentage and numbers of lymphocyte subsets in the peripheral blood reference to the literature [17]

*Without fever

by electroencephalogram examination and head magnetic resonance imaging (MRI). The ASQ-3 score of P8 showed abnormalities in the gross motor and fine motor domains. His sleep electroencephalogram revealed sharp waves, and MRI revealed small softening foci in the left centrum semiovale, although no visible convulsions occurred. Nonsteroidal anti-inflammatory drug (NSAID) treatments, such as ibuprofen and acetaminophen, did not exert any effect on fever in these patients. They were eventually diagnosed with central fever. The temperature changes in both patients are shown in Fig. 2.

Abnormal chromosome structure and number

CNVs were detected using trio-WES in these eight patients and verified with CGH (Fig. 3) or MLPA (Fig. 4c). Their parents had no FUO, and no chromosomal abnormalities were detected. In patients with inflammatory

fever, we identified de novo CNVs: a 7q11.23 duplication in P1; a deletion at 3p26.3-p26.1 and a duplication at 17q12 in P2; a 0.7 Mb deletion at 22q11.21 (20,733,478–21,461,788) without involvement of the *TBX1* gene in P3; and a 3.576 Mb deletion at 6q23.3-q24.1 in P6 (Fig. 4a). The *TNFAIP3* gene was involved in the deleted fragment in P6 (Fig. 4b). Two patients (P4 and P5) were identified to have trisomy 8. In addition, P5 had another mutation, c.1508G > C, in *PTPN11*.

De novo 18q22.3-q23 del was identified in patients (P7 and P8) with central fever. Meanwhile, P7 also carried another CNV (13q32.3-q34 dup).

Literature review

We searched PubMed using the search criteria "Chromosome Disorders" [MeSH Terms] OR "Copy number variation" OR "chromosome abnormality" OR

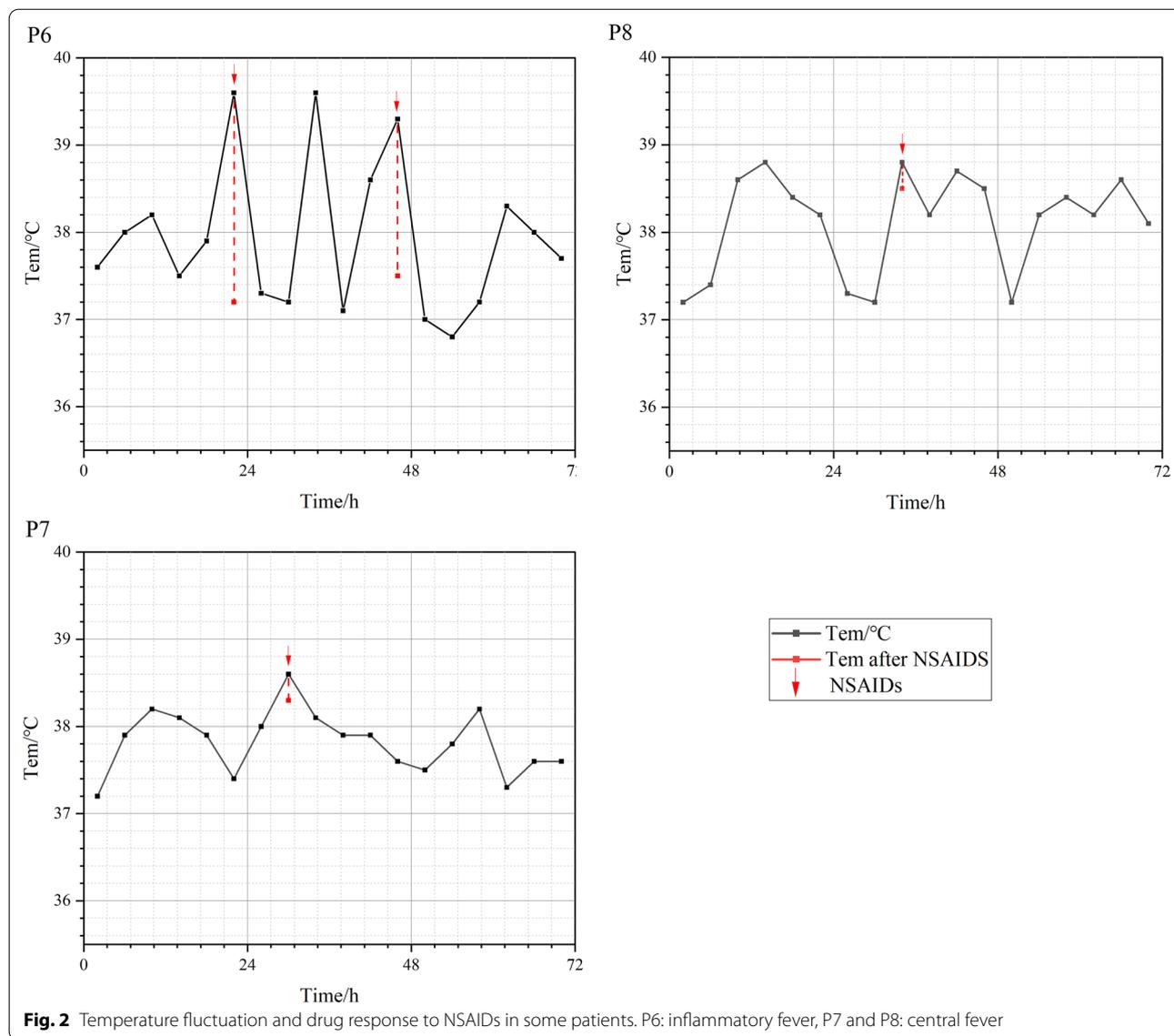


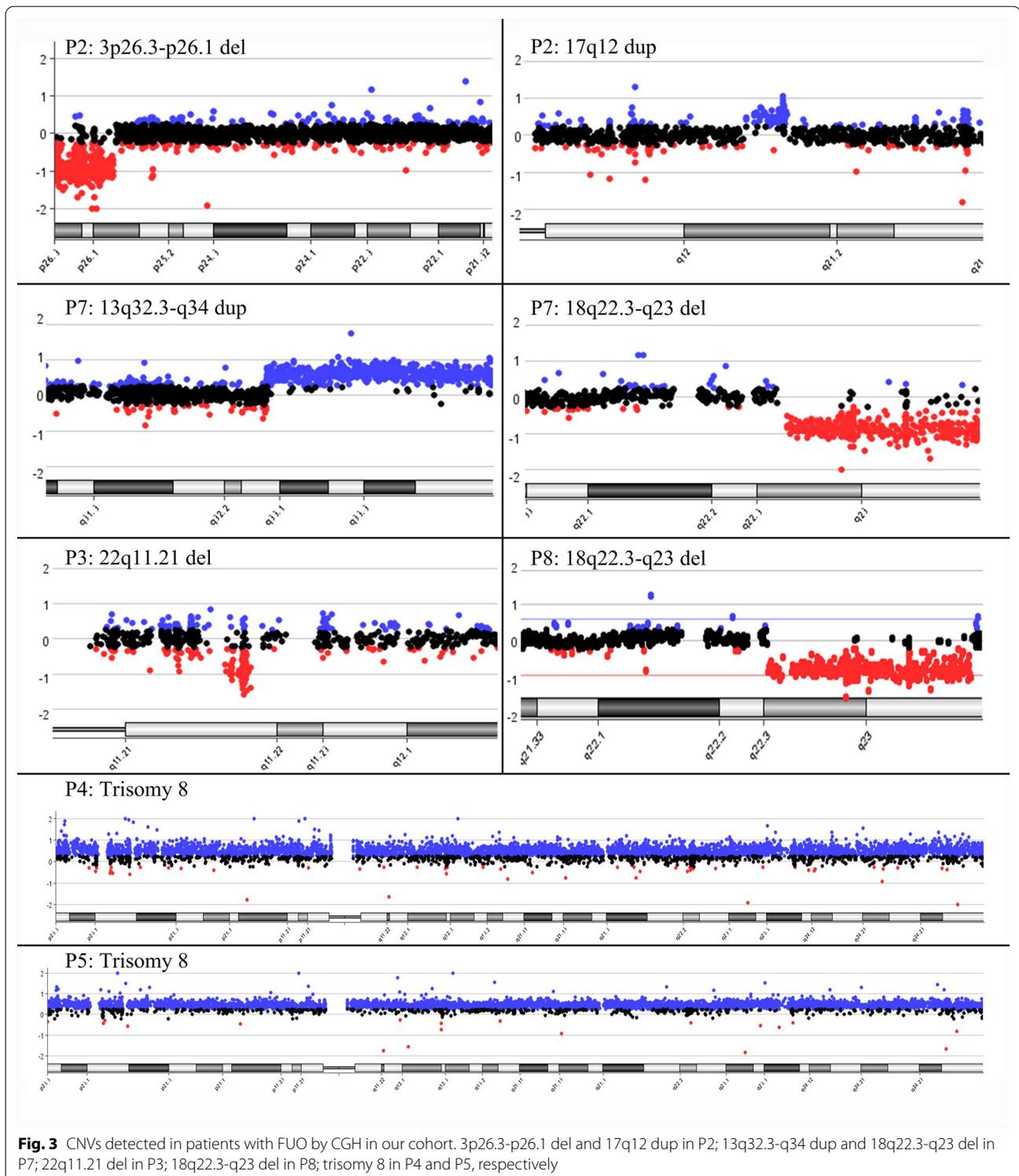
Fig. 2 Temperature fluctuation and drug response to NSAIDs in some patients. P6: inflammatory fever, P7 and P8: central fever

"chromosome deletion" OR "chromosome duplication" OR "trisomy" AND "fever"[All Fields] AND "(humans)". Ultimately, 19 patients with FOU were found to have chromosomal abnormalities or CNVs, which might explain or partially explain the cause of fever [9, 18–31]. The data are summarized in Table 3.

In previous reports, trisomy 8 was detected most frequently in inflammatory fever, often leading to periodic fever and Behcet's-like disease similar to P4 and P5 in our study. Patients with 6q23.2-q24.3 del, 16p13.3 del, chromosome 18 rearrangement (18q21.3-q22.2 dup/18q22.2-qter del) and 19q13.42 dup were described as having recurrent fever, which was considered systemic inflammatory diseases, for example, s-JIA. Seven patients with 15q del (2 maternal

and 5 paternal) and one patient with 18q22.3-q23 del were reported to have central fever. Central fever was usually accompanied by facial anomalies and neurological involvement, including developmental delay, microcephaly, hypotonia, and epileptiform discharges. Additionally, one patient reported to have 22q13.33 del suffered from an acute and transient psychotic disorder accompanied by irregular fever, which was not classified as inflammatory fever or central fever.

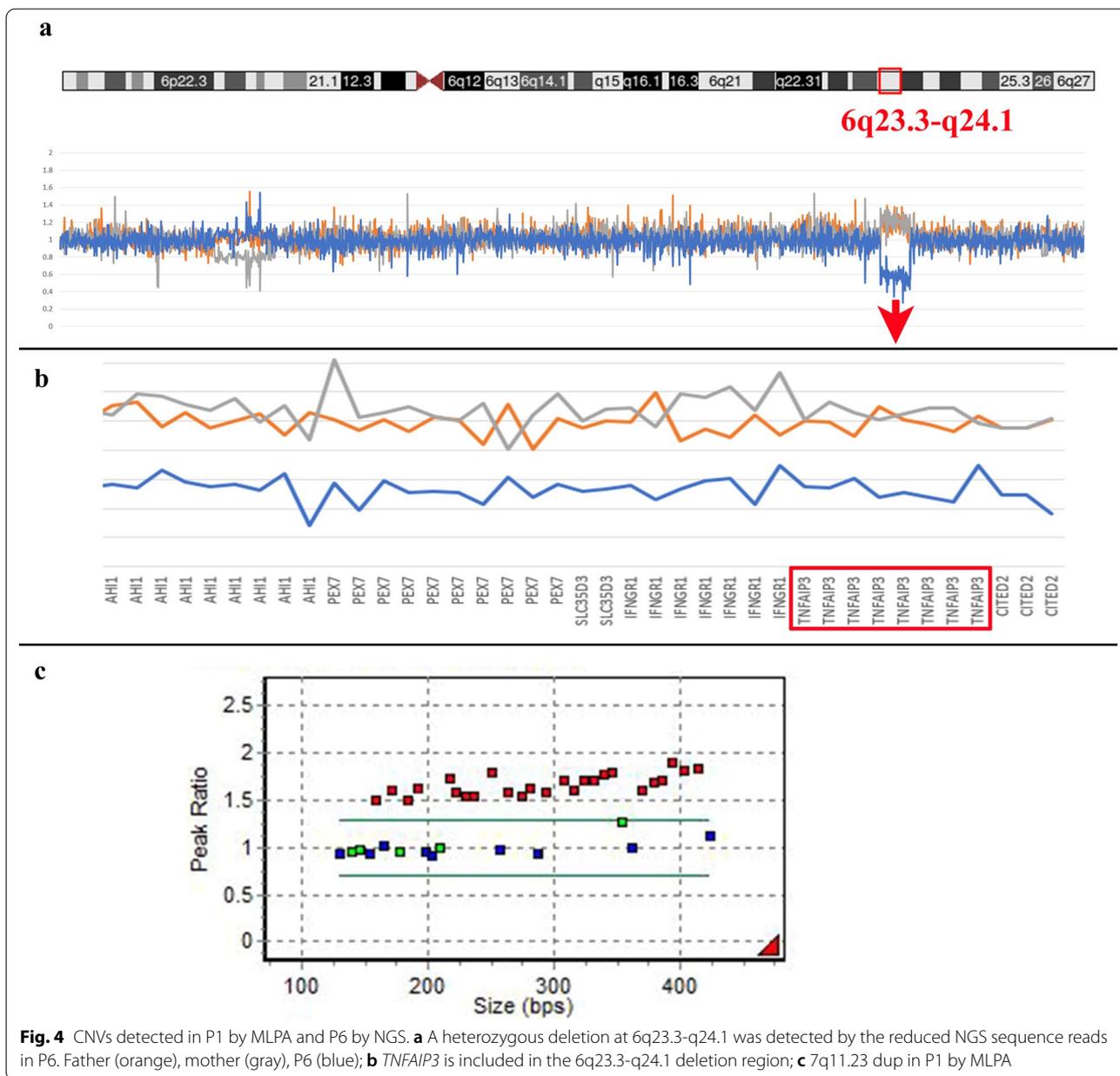
In addition to trisomy 8, 6q23.3-q24.1 del and 18q22.3-18q23 del, our study also revealed additional CNVs that may be related to FOU, such as 7q11.23 dup, 3p26.3–26.1 del/17q12 dup, and 22q11.21 del. A chromosome map related to FOU was drawn (Fig. 5).



Discussion

FUO is a challenging disease. Recent studies have shown the importance of performing NGS in adult patients with FUO [32]. However, limited genetic data are available in

pediatric patients [33]. In our study, 145 patients presenting with FUO during the study period were enrolled, and 24.1% (35/145) of these patients were diagnosed with monogenic autoinflammatory diseases. However,



some cryopyrin-associated periodic syndromes caused by low-frequency somatic *NLRP3* variants, especially the p. E567K variant, may have been missed by WES due to the depth of coverage [34]. Moreover, the detection of chromosomal abnormalities in patients with FUO was 5.5%, which should not be ignored. No previous reports have focused on the overall proportion of chromosomal abnormalities in patients with FUO. The proportion of chromosomal abnormalities in our FUO cohort was significantly higher than that in the general pediatric population, ranging from 0.43% to 0.83% in live births [35–37], and there were also chromosomal spectrum differences,

suggesting the significance of chromosomal-related tests in FUO.

Recently, several studies have begun to focus on chromosomal abnormalities leading to inflammatory fever. Hiromi Tadaki et al. reported recurrent fever in a patient with s-JIA who carried 19q13.42 dup, which contains the *NLRP* family associated with the inflammatory pathway [30]. Chromosomal rearrangement (18q21.3-q22.2 dup/18q22.2-qter del) encompassing a duplication of *TNFRSF11A* has been reported to be associated with recurrent fever, rash and lymphadenopathy [9]. CNV detection approaches are recommended as routine

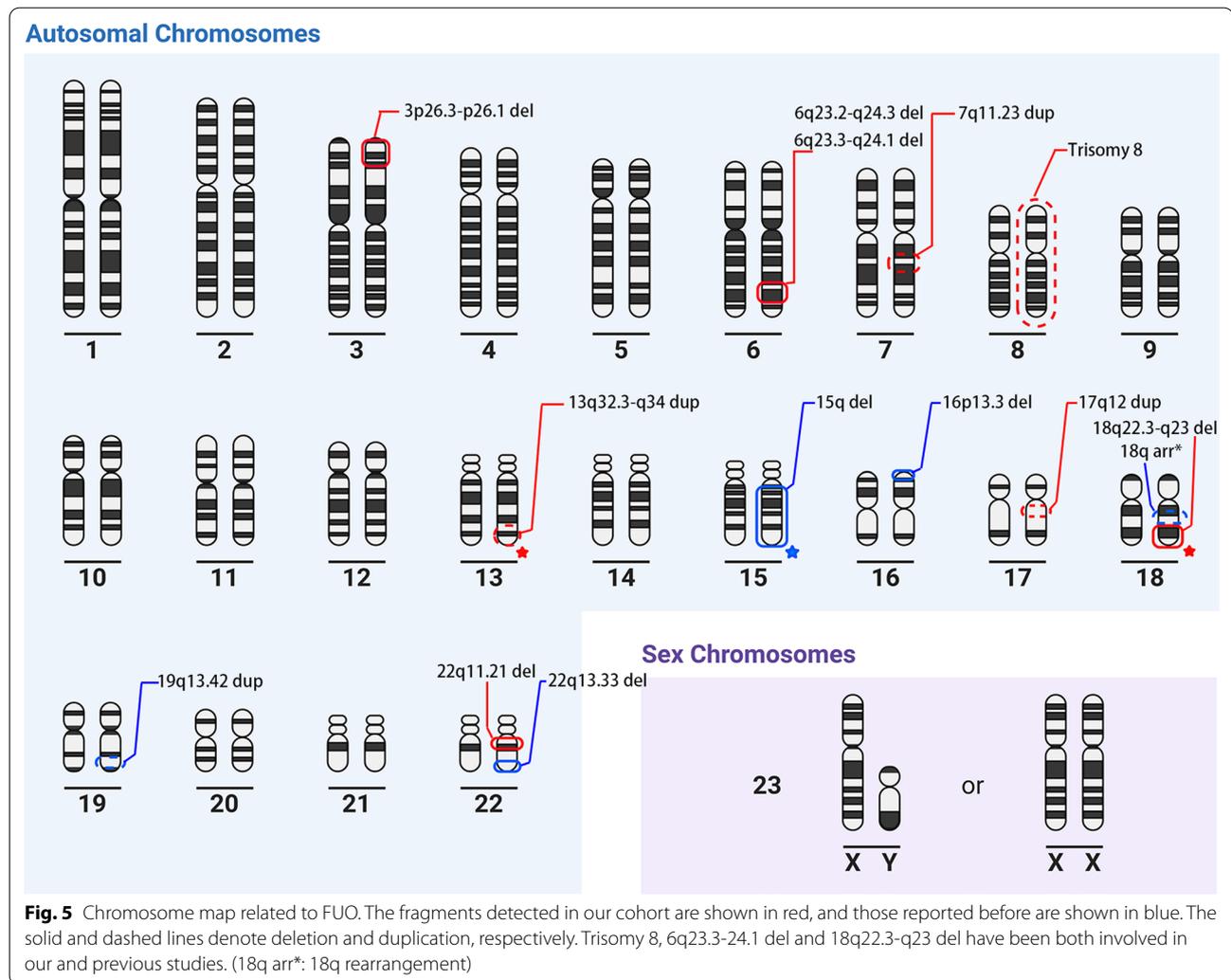
Table 3 FUO related chromosomal abnormalities reported in the literature

| Patients with chromosomal abnormalities | Classification of fever | Fever features | Age at fever | Sex | Concomitant symptoms | Inflammatory biomarkers (during fever) | Treatment | Congenital anomalies | Growth retardation | Developmental delays | Others | Refs. |
|---|-------------------------|--|--------------|-----|---|---|--|----------------------|--------------------|----------------------|--|-------|
| 6q23.2-q24.3 del | Inflammatory fever | recurrent fever, several FUO during first year | < 1y | M | Neutrophilic dermatosis, oral aphthae, diarrhoea and perianal ulcers | Increased CRP, ESR | Methylprednisolone, etanercept | - | + | + | inverted CD4/CD8 ratio, ANA: 1:160 + | [18] |
| Trisomy 8 | Inflammatory fever | recurrent fever, high fever | 69y | M | Sweet's syndrome, MDS(RA), convulsion | Increased neutrophils, CRP, IL-6, G-CSF | NSAIDS, prednisone | - | NA | NA | - | [19] |
| | Inflammatory fever | periodic fever, for 2 years | 1.5y | F | Chronic diarrhea, skin lesion, hydropericardium, MDS | Increased SolIL2R | Dexamethasone, colchicine | - | NA | NA | PTPN11 mutation | [20] |
| | Inflammatory fever | periodic fever, high fever | 53y | F | Intestinal Behcet's-like disease, MDS | - | Colchicine, Azacitidine, prednisolone | - | NA | NA | MEFV E148Q mutation | [21] |
| | Inflammatory fever | periodic fever, for 1 year | 66y | M | GIBD, macrocytic anemia | Increased WBC | Colchicine, mesalamine | - | NA | NA | - | [22] |
| | Inflammatory fever | periodic fever, high fever | 72y | M | Erythema nodosum, MDS(RA) | Increased WBC, CRP | Colchicine, prednisolone | - | NA | NA | - | [23] |
| | Inflammatory fever | periodic fever, high fever | 68y | M | Erythema nodosum, rhabdomyolysis and acute renal failure, diarrhea, MDS(RA), oral ulcer | Increased WBC, CRP | prednisolone | - | NA | NA | - | |
| 15q del (maternal) | Central fever | persistent fever, 37.8–39.0 °C | 1 m | F | - | - | No response to antibiotics and antipyretic medications | Facial anomalies | - | + | EEG: epileptiform discharges, seizures, microcephaly, hypotonia | [24] |
| | Central fever | persistent fever, 37.5–39.0 °C | 2 m | M | - | - | No response to antibiotics and antipyretic medications | Facial anomalies | - | + | EEG: epileptiform discharges, microcephaly, hypotonia, spastic tetraplegia | |

Table 3 (continued)

| Patients with chromosomal abnormalities | Classification of fever | Fever features | Age at fever | Sex | Concomitant symptoms | Inflammatory biomarkers (during fever) | Treatment | Congenital anomalies | Growth retardation | Developmental delays | Others | Refs. |
|---|-------------------------|--|-----------------|-----|--|--|--|---|--------------------|----------------------|--|-------|
| 15q del (paternal) | Central fever | persistent fever, 38–39.0 °C | 13y | M | Hypotension, hyperpyrexia, rhabdomyolysis, acute kidney injury | – | External cooling | Hypospadias | – | NA | obesity, obstructive sleep apnoea, asthma, hypertension, fatty liver disease, diabetes | [25] |
| | Central fever | persistent fever, 37.2–39.6 °C | 15d | M | – | – | No response to antibiotics medications | Facial anomalies | – | NA | hypotonia, feeding difficulty | [26] |
| | Central fever | persistent fever, 37.6–39.6 °C | 6 m | M | – | – | NA | Facial anomalies | – | NA | hypotonia, hypogonadism | |
| | Central fever | persistent fever, 38.5–39.5 °C | 7 m | F | – | – | NA | Facial anomalies | – | + | Microcephaly, hypotonia | |
| | Central fever | persistent fever, high fever | Neonatal period | F | Rhabdomyolysis, MODS | – | Fluid resuscitation | Facial anomalies | NA | NA | deep venous thrombosis, seizure, hypotonia | [27] |
| 16p13.3 del | Inflammatory fever | recurrent fever, 2–5/year | 18y | M | Abdominal pain, arthritis | Increased CRP, ESR | Colchicine | Facial anomalies | + | + | MEFV M694V mutation | [28] |
| 18q22.3-q23 del | Central fever | long-term fever, up to 40 °C | 5 m | M | Hyporexia, poor weight gain | – | No response to antibiotics and antipyretic medications | Facial anomalies, congenital aural atresia, congenital vertical talus | + | + | delayed myelination, hypotonia | [29] |
| 18q21.32-q22.2 dup; 18q22.2-qtter del | Inflammatory fever | recurrent fever, frequency > 1/month | 3w | M | Rash, abdominal pain, lymphadenopathy, constipation | NA | NA | Facial anomalies, cleft palate, TOF | NA | + | – | [9] |
| 19q13.42 dup | Inflammatory fever | recurrent fever associated with s-JIA, spiking fever | 2y | F | s-JIA | NA | Refractory to conventional treatment, Tocilizumab | – | NA | NA | – | [30] |
| 22q13.33 del | Undetermined | irregularly fever, 37–38 °C, approximately 1/month | 23y | F | Acute and transient psychotic disorder | NA | Olanzapine, valproic acid, haloperidol, oxazepam | Facial anomalies | – | + | – | [31] |

m months; y years; d days; w weeks; F female; M male; NA not available; FUO fever of unknown origin; CRP C-reactive protein; ESR erythrocyte sedimentation rate; ANA anti-nuclear antibodies; MDS myelodysplastic syndrome; RA refractory anemia; IL-6 interleukin-6; G-CSF granulocyte colony-stimulating factor; WBC white blood cells; NSAIDs non-steroidal anti-inflammatory drugs; SoliL2R: soluble IL-2 receptor; GIBD gastrointestinal Behcet's disease; EEG electroencephalogram; MODS multiple organ dysfunction syndrome; TOF tetralogy of fallot; s-JIA systemic onset juvenile idiopathic arthritis



diagnostics in patients with systemic autoinflammatory diseases for whom a confirmatory genotype is unavailable [10, 18]. Chromosomal imbalances involving either the loss or gain of large genomic regions may be detectable with molecular cytogenetic techniques such as CGH, and smaller CNVs can also be detected by MLPA [10].

The discovery of chromosomal abnormalities in patients with FUO can guide diagnosis and treatment for better management strategies. In our cohort, six patients with inflammatory fever had chromosomal abnormalities, two of which were trisomy 8. Both patients with constitutional trisomy 8 [22, 38, 39] and patients with trisomy 8 in their bone marrow [19–21, 23, 40, 41] have been reported to exhibit Behcet's disease (BD) or other inflammatory disorders. CRP and interleukin (IL)-6 levels were significantly increased in patients with trisomy 8 during acute inflammation [20, 42]. Inflammatory manifestations related to trisomy 8 in children are rarely reported. Two patients in our study diagnosed

with trisomy 8 had symptoms similar to BD, along with increased CRP/ESR levels and elevated IL-6 levels. Treatment with anti-inflammatory medications, such as systemic steroids, can improve clinical symptoms [20, 39]. Trisomy 8 is also thought to increase the risk of the development of malignancy, especially myelodysplastic syndrome (MDS) symptoms [40, 43]. Associations between intestinal BD, MDS and trisomy 8 have been described. In addition, P5 also carried the gene mutation c.1508G>C in *PTPN11*. *PTPN11* encodes the protein tyrosine phosphatase SHP2, a signal protein involved in the RAS/MAPK pathway [20]. The mutation c.1508G>C, either germline or somatic, has been reported to be associated with juvenile myelomonocytic leukemia [44, 45], which requires hematopoietic stem cell transplantation.

TNFAIP3 is located in the 3.576 Mb deletion region on chromosome 6q23.3-q24.1, which was identified in P6. *TNFAIP3* mutation may cause insufficient production of A20, which finally results in negative feedback

inhibition of the NF- κ B signaling pathway. This mutation has been reported to be the genetic factor underlying many autoinflammatory and autoimmune diseases, such as BD and systemic lupus erythematosus [46, 47]. One patient with 6q23.2-q24.3 del was previously described to have a systemic autoinflammatory disease characterized by recurrent fever, oral aphthae and perianal ulcers [18]. P6 clinically manifested with early-onset s-JIA and showed a poor response to corticosteroids and NSAIDs. Cytokine inhibitors such as tumor necrosis factor (TNF) inhibitors can be further considered to inhibit inflammatory responses [47]. Loss of the tumor suppressor gene *TNFAIP3* may lead to B-cell lymphomas [48, 49]. Deletions in 6q23.3-6q24.1 have recently been identified by aCGH in ~30% of primary mediastinal B-cell lymphomas [48]. Long-term attention should be given to the occurrence and development of tumors in patients with chromosomal abnormalities.

Furthermore, some CNVs in our study have not been previously reported to be related to inflammatory fever, such as a duplication at 7q11.23 in P1, a deletion of 3p26.3-p26.1 and a duplication at 17q12 in P2, and a 0.7 Mb deletion of 22q11.21 in P3. Common CNVs in four chromosomal “hot spots” can result in deletions or duplications in the 22q11.2, 7q11.23, 17p11.2, and 16p11.2 regions [8]. The population frequency of 7q11.23 duplication is relatively high (1:7,500–20,000), and children can mainly present with developmental delays/intellectual disabilities and behavioral problems, which is consistent with P1 [50]. Moreover, P1 was hospitalized repeatedly due to fever and tonsillitis. In addition to autoinflammatory symptoms that may be consistent with PFAPA, P3 experienced growth retardation and developmental delay, which are considered exclusion criteria for PFAPA, according to the modified Marshall's criteria [51]. A central 22q11.2 deletion was detected in P3, which was relatively rare compared to the typical 3 Mb 22q11.2 deleted region. The central 22q11.2 deletion can result in developmental problems [52], such as developmental delay. It is possible that those high-frequency chromosomal abnormalities are found incidentally in FUO. Subsequent case reports are required to clarify the potential association between CNVs detected and fever.

No clear statistics are available on the association between central fever and genetic factors. Deletion of the long arm of chr18 was previously described as a common autosomal syndrome with an incidence of 1:40,000 live births [29]. Patients with this syndrome are characterized by short stature, facial dysmorphism, foot deformities, CAA, variable intellectual disability and neurological abnormalities [53]. Neurological abnormalities may include hypotonia, seizures, nystagmus, poor coordination, tremor and abnormal signals on brain MRI. After

a literature review, we identified one patient who presented with thermic dysregulation with one of the smallest 18q22.3-q23 interstitial deletions described thus far [29]. Two more patients (P7 and P8) with fever and 18q deletion syndrome analyzed in our study indicated that thermal dysregulation may be a symptom related to this disease rather than an occasional finding. Deficiency or dysfunction of the involved gene, *GALRI*, which encodes the galanin receptor GALR1 and mediates the hyperpolarization of warm sensitive neurons in the preoptic area of the hypothalamus, was speculated to be the cause of fever. In addition, P7 carried a duplication at 13q32.3-q34, which was associated with congenital malformation of the brain, kidney, limb and lung in previous reports [54]. Overall, most patients (6/8) in our cohort had developmental delay, highlighting the need for chromosomal testing in children with FUO and developmental delay. Long-term rehabilitation training is necessary for these patients.

Conclusions

In conclusion, we summarized the genetic characteristics of children with FUO at a single Chinese center and emphasized the role of chromosomal abnormalities in FUO. The characteristics of chromosomal abnormalities leading to fever were systematically summarized. Our study revealed several chromosomal abnormalities known or potentially related to central and inflammatory fever. We suggest that CNV detection approaches should be routinely performed in patients with FUO, especially in patients with obvious signs of systemic inflammatory manifestations or developmental delay, which may reveal the genetic basis of FUO in these patients. Identifying chromosomal abnormalities may change the diagnosis and care of these patients.

Abbreviations

AML: Acute myeloid leukemia; a-CGH: Array comparative genomic hybridization; ANA: Antinuclear antibody; CAA: Congenital aural atresia; CNV: Copy number variation; CRP: C-reactive protein; BD: Behcet's disease; ESR: Erythrocyte sedimentation rate; FUO: Fever of unknown origin; HLH: Hemophagocytic lymphohistiocytosis; IL: Interleukin; MDS: Myelodysplastic syndrome; MLPA: Multiplex ligation-dependent probe amplification; MRI: Magnetic resonance imaging; NGS: Next-generation sequencing; NSAIDs: Nonsteroidal anti-inflammatory drugs; PFAPA: Periodic fever, aphthous stomatitis, pharyngitis, and adenitis; SAA: Serum amyloid A; s-JIA: Systemic juvenile idiopathic arthritis; TNF: Tumor necrosis factor; WBC: White blood cell count; WES: Whole exome sequencing.

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Author contributions

BS and MY contributed to the study design and drafted the manuscript. JH, WW, WY, XH, QZ, and HY contributed to the study design and data collection. JS and XW provided academic feedback and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used or analyzed during the current study are all included within the article and are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the ethics committee of Children's Hospital of Fudan University (No. 2019 017). The patients and their parents provided written informed consent for enrollment in this study.

Consent for publication

Not applicable.

Competing interests

All the authors declare that they have no conflicts of interest.

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