Author's response to reviews

Title: Whole-exome sequencing of a rare case of familial childhood acute lymphoblastic leukemia reveals putative predisposing mutations in Fanconi anemia genes.

Authors:

Jean-François Spinella (jfspinella@gmail.com)
Jasmine Healy (jasmine.healy@umontreal.ca)
Virginie Saillour (vr.saillour@gmail.com)
Chantal Richer (cricher.hsj@gmail.com)
Pauline Cassart (cassart.pauline@gmail.com)
Manon Ouimet (Manon.Ouimet@recherche-ste-justine.qc.ca)
Daniel Sinnett (daniel.sinnett@umontreal.ca)

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Author's response to reviews: see over
Re.: MS: 1526296555152165 – Revised Manuscript

Dear Editor,

We are resubmitting this revised version of the manuscript entitled "Whole-exome sequencing of a rare case of familial childhood acute lymphoblastic leukemia reveals putative predisposing mutations in Fanconi anemia genes." by Spinella and colleagues for publication in BMC Cancer as a Research article.

In the current study, we performed whole-exome sequencing of a childhood ALL family consisting of mother, father and two affected non-twinned siblings diagnosed with pre-B hyperdiploid ALL and carrying a maternally inherited rare allelic form of PRDM9, a meiosis-specific histone H3 methyltransferase that is suggested to be involved in the definition of Holliday junction branch migration boundaries. Based on the observed concordant hyperdiploid phenotype of both siblings, we postulated that inherited rare disadvantaging DNA variants in predisposing cancer genes from the Fanconi anemia (FA) pathway, leading to defects in Holliday junction resolution and aneuploidy when altered, could affect overall genomic instability in combination with the rare form of PRDM9. Though the family was apparently nonsyndromic, we identified rare mutations in Fanconi anemia genes FANCA, FANCP/SLX4 and in GEN1, coding for an endonuclease member of the FANCP/SLX4 complex, that were co-inherited by the siblings and that could account for their increased predisposition to hyperdiploid ALL. Interestingly, the FA gene variants identified here have previously been identified in familial breast cancer cases but their pathological effects in cancer predisposition remain unknown. We propose that rare disadvantaging DNA variants in predisposing cancer genes such as FANCA, FANCP/SLX4 and GEN1 could affect overall genomic instability and favour nondisjunction of chromosomes leading to increased risk of hyperdiploid pre-B ALL within this family.

We thank the reviewers for their comments, which were very helpful for the improvement of our manuscript. We were pleased with the positive feedback and the interest in our work. Here is a point-by-point response to the reviewers':

Reviewer 1

Revisions

1. The manuscript by Spinella et al., is clearly structured, well focused and gives a comprehensive overview over the topic. However, since they reported the case of a single nonsyndromic pre-B childhood ALL family with two non-twinned siblings diagnosed with ALL, I suggest to publish this article as a case report rather than a research article.

We thank Reviewer 1 for the positive feedback and will follow the editor’s recommendation regarding the paper’s format.
Reviewer 2

**Major compulsory revisions**

1. several genes are shown to carry potentially deleterious variants that segregate with the disease, only FANCA and FANCP/SLX4, together with PRDM9, are considered to have the potential to drive ALL in this family. It is unclear why the authors choose to focus only in the Fanconi anemia proteins and disregard the others. For example, variants found in GEN1 (a Holliday junction resolvase) or CEP55 and PDE4DIP (centrosomal proteins) could also contribute to this process and should be discussed in the paper.

We agree with Reviewer 2 that our rational behind the Fanconi anemia (FA) gene selection was not clear enough. The sibs were diagnosed with nonsyndromic childhood ALL three years apart. We previously identified a rare \textit{PRDM9} allele segregating within the family [11]. \textit{PRDM9} is a histone H3 methyltransferase involved in crossing-over at recombination hotspots and Holliday junctions. To further characterize the underlying inherited genetic contribution to this familial case of ALL, we focused on genetic variants in leukemia predisposing pathways that were co-inherited by both brothers. Though the sibs were not diagnosed with FA, this recessive disorder is linked to hematopoietic dysfunction, chromosomal instability and increased susceptibility to childhood ALL. Based on the observed concordant hyperdiploid phenotype, we hypothesized that co-inheritance of disadvantaging variants in FA genes, along with the rare \textit{PRDM9} allele, could affect genomic stability and lead to increased risk of hyperdiploid ALL within this family. Under a recessive disease model, we screened our exome data, in an unbiased manner, for non-synonymous compound heterozygous variants and homozygous variants shared by both brothers (Table 1, below). Based on our hypothesis, we focused on rare variants in FA pathway genes, to identify functionally relevant candidates (see Figure 2). Among the identified combinations was a rare compound heterozygous variant in the FA gene \textit{FANCP/SLX4}, corroborating the assumption of FA pathway destabilization. A more thorough investigation of the other FA pathway genes led then to the identification of a rare heterozygous variant in \textit{FANCA} (rs61753269) that was also shared by the sibs.

However, as the Reviewer pointed out, we were too quick to exclude additional underlying genetic contributions, and as mentioned in the original manuscript (line 223), we cannot exclude the possibility that functional variants outside of the FA pathway could contribute to ALL onset within the family. Of particular interest is the rare homozygous variant in \textit{GEN1} (rs16981869). \textit{GEN1}, although not a FA gene per se, is a member of the \textit{FANCP/SLX4} complex and is involved in Holliday junction resolution, and could indeed participate in the observed phenotype. We appreciate the opportunity to improve our manuscript and have discussed additional genes such as \textit{GEN1} in the revised version of the manuscript (see below). \textit{PDE4DIP}, \textit{CEP55} and other genes presented in Table 1 were not retained as candidates due to their high MAF or because they are not expressed in hematopoietic cells or their variants are predicted to have benign effects on protein function. This was more clearly stated in the manuscript however we did not discuss these genes further.

- Starting line 39: "Though the family was nonsyndromic, we identified a combination of rare variants in Fanconi anemia (FA) genes \textit{FANCP/SLX4} (compound heterozygote – rs137976282, rs79842542) and \textit{FANCA} (rs61753269) and a rare homozygous variant in the
- Starting line 47: "FANCP/SLX4 and GEN1 are involved in the cleavage of Holliday junctions and their mutated forms, in combination with the rare allele of PRDM9, could alter Holliday junction resolution leading to nondisjunction of chromosomes and segregation defects."

- Starting line 52: "Taken together, these results suggest that concomitant inheritance of rare variants in FANCA, FANCP/SLX4 and GEN1 under the specific genetic background of this particular case, could lead to increased genomic instability, hematopoietic dysfunction, and higher risk of childhood leukemia."

- Starting line 155: "The sibs were diagnosed with nonsyndromic childhood ALL three years apart. We previously identified a rare PRDM9 allele segregating within the family [11]. PRDM9 is a histone H3 methyltransferase involved in crossing-over at recombination hotspots and Holliday junctions. To further characterize the underlying inherited genetic contribution to this childhood ALL family in an unbiased manner, we performed whole exome sequencing of the siblings and both parents. Though the family was nonsyndromic and asymptomatic for FA, this recessive disorder is linked to hematopoietic dysfunction, chromosomal instability and increased susceptibility to childhood ALL. Based on the observed concordant hyperdiploid phenotype of both siblings, we postulated that inherited rare disadvantaging DNA variants in leukemia predisposing pathways like the FA pathway could affect overall genomic instability and, in combination with the rare allelic form of PRDM9, favour nondisjunction of chromosomes leading to increased risk of hyperdiploid pre-B ALL within this family. Under a recessive disease model, we interrogated our exome data and identified shared non-synonymous mutations that were either compound heterozygous or homozygous variant (Table 1) and specifically screened genes associated with the leukemia predisposing syndrome FA (FANCA, FANCB, FANCC, FANCD1/BRCA2, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ, FANCL, FANCM, FANCN/PALB2, FANCO/RAD51C, FANCP/SLX4, FANCO/XPF and FANCS/BRCA1). Among the identified variants, we identified a combination of nonsense variants in the FA gene FANCP/SLX4 (compound heterozygous at rs137976282 and rs79842542), corroborating the assumption of FA pathway destabilization (Figure 2). A more thorough investigation of the other FA pathway genes led then to the identification of a rare heterozygous variant in FANCA (rs61753269) that was also shared by the sibs. Although this variant was heterozygous, restricting the analysis to extremely rare variants allowed us to identify potentially deleterious non-synonymous variations in FA genes that could be contributing to inherited susceptibility to ALL in the sibs. For FANCP/SLX4, both parents transmitted a putatively damaging allele to their affected offspring who were therefore compound heterozygous at rs137976282 (ESP and 1000 Genomes general population MAF <0.001) and rs79842542 (MAF =0.059 and 0.071 in 1000 Genomes and ESP general populations respectively). While two of the three in silico algorithms predicted that the compound heterozygous variants in FANCP/SLX4 were likely deleterious (Sift score =0 for both alleles and Polyphen2 score =1 and 0.964 for rs79842542 and rs137976282 respectively), only Fathmm predicted rs61753269 in FANCA to be damaging (Fathmm score =-1.78) (Table 1). Nevertheless, the high conservation score at FANCA rs61753269 (SiPhy =12.742), combined with its extreme rarity in the population (MAF <0.001 in 1000 Genomes and ESP), suggest that this variant is under strong functional constraint and therefore could have a specific role on protein conformation. Although not a Fanconi anemia gene per se, our exome data also revealed a rare non-synonymous homozygous variant in GEN1 (rs16981869, MAF =0.145394, ESP general population homozygous frequency q^2 =0.025),
that was predicted to be deleterious by all three algorithms. GEN1 is a member of the FANCP/SLX4 complex involved in Holliday junction resolution [27], and in conjunction with PRDM9 and the FA genes identified here, could be contributing to genomic instability in the sibs."

- Starting line 224: "GEN1 codes for an endonuclease, and is a member of the FANCP/SLX4 complex [27] shown to play a role in the maintenance of centrosome integrity [39]. Along with PRDM9, GEN1 and the FANCP/SLX4 complex are involved in the definition of Holliday junction branch migration boundaries and the cleavage of static and migrating Holliday junctions [12,27,37]. Efficient DNA damage repair and simultaneous regulation of cell cycle progression is critical for genomic stability. Interestingly, a rare recessive homozygous variant in GEN1 has been associated with bilateral breast cancer [40] and the depletion of GEN1 or FANCP/SLX4 in Bloom’s syndrome cells results in defects in chromosome condensation and severe chromosome abnormalities, such as nondisjunction of sister chromatids and abnormal mitosis leading to aneuploidy [41,42], highlighting their important role in maintaining genome stability. Thus, mutated FANCP/SLX4 and GEN1, in combination with the rare allele of PRDM9 also segregating within this family, could alter Holliday junction resolution leading to nondisjunction of chromosomes and segregation defects."

- Starting line 263: "Finally, though our rare variant analysis strongly suggests FANCP/SLX4 and FANCA as the most likely candidates, we cannot exclude the possibility that additional inherited genetic variants, rare or common, outside of the FA pathway could contribute to ALL onset within the family. For example, we identified common non-synonymous variants in PDE4DIP and CEP55 (Table 1). Though these centrosomal proteins have been involved in myeloproliferative disorder [45] and carcinogenesis [46] and could promote abnormal cell division and hyperdiploidy, as evidenced recently by Paulsson et al. [47], the identified variants had high MAFs and were predicted to have benign effects on protein function, making them unlikely candidates here. Furthermore, the sibs carry common ALL susceptibility alleles at known GWAS loci [3-6,28] (Table 2), that under an additive effects model could lead up to a 2- to 10-fold increase in risk [9]. Given the male-specific inheritance, we also looked for shared deleterious variants on the X chromosome but found no evidence of X-linked genes contributing to ALL in this family. The exomes of the siblings were also screened for shared de novo mutations that could result from gonadal mosaicism. Putative de novo events were defined as private mutations shared by both siblings, and therefore unknown in public databases, and showing no evidence of heritability from either parent, i.e. no reads supporting the variation in the parental exomes considering a minimum coverage of 8X at the given position in the exome sequencing data. Although no candidate de novo mutation fitting our criteria was identified, the limited coverage of parental exomes may have hindered this analysis. The investigation of more complex genetic models including gene-gene and eventually gene-environment interactions could also reveal additional ALL risk factors."
Supplementary Table S1 has been modified to include additional information regarding homozygosity and the predicted impact of the identified variants (see response #3 below) and is now included in the main manuscript as Table 1 as suggested:

Table 1. Non-synonymous homozygous variants and compound heterozygous shared by both childhood pre-B ALL siblings.

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP ID</th>
<th>Chr</th>
<th>Position</th>
<th>Ref</th>
<th>Sibs</th>
<th>Father</th>
<th>Mother</th>
<th>AA change</th>
<th>1000G MAF</th>
<th>ESP MAF / $q^3$</th>
<th>Sift</th>
<th>Polyphen2</th>
<th>Fathmm</th>
<th>SiPhy</th>
</tr>
</thead>
<tbody>
<tr>
<td>FANCA</td>
<td>rs117465420</td>
<td>16</td>
<td>59514868</td>
<td>AA</td>
<td>GA</td>
<td>TG</td>
<td>TG</td>
<td>A</td>
<td>0.035041</td>
<td>0.031594</td>
<td>0.013075</td>
<td>0.127716</td>
<td>0.999</td>
<td>4.64</td>
</tr>
<tr>
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<td>16</td>
<td>59514868</td>
<td>AA</td>
<td>GA</td>
<td>TG</td>
<td>TG</td>
<td>A</td>
<td>0.035041</td>
<td>0.031594</td>
<td>0.013075</td>
<td>0.127716</td>
<td>0.999</td>
<td>4.64</td>
</tr>
<tr>
<td>FANCP</td>
<td>rs117465420</td>
<td>16</td>
<td>59514868</td>
<td>AA</td>
<td>GA</td>
<td>TG</td>
<td>TG</td>
<td>A</td>
<td>0.035041</td>
<td>0.031594</td>
<td>0.013075</td>
<td>0.127716</td>
<td>0.999</td>
<td>4.64</td>
</tr>
<tr>
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<td>AA</td>
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<td>0.035041</td>
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<tr>
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<td>AA</td>
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<td>TG</td>
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<td>0.035041</td>
<td>0.031594</td>
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<td>0.127716</td>
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<td>0.127716</td>
<td>0.999</td>
<td>4.64</td>
</tr>
</tbody>
</table>

(-) represents missing or not relevant information. For these genes, either or both parents transmitted a putatively damaging allele to their affected offspring, who were therefore compound heterozygous or homozygous, respectively. Genotype calls are provided for each sample (Sibs, Father and Mother) along with corresponding amino acid (AA) changes. Minor allele frequencies (MAF) were derived from the 1000 Genomes (general population, updated in October 2014) and the NHLBI GO Exome Sequencing Project (general population, ESP6500). The frequencies of homozygous variants ($q^2$) were obtained from ESP6500 and were presented when relevant. The putative effect of these substitutions on the protein function was assessed in silico using Sift ($\leq0.05$) [23], Polyphen2 ($\geq0.957$) [24] and Fathmm (<1.5) [25]. SiPhy was used to identify bases under selection (larger is the score, more conserved is the site) [26].

3. The paper revolves around the variants found in FANCA and FANCP/SLX4. However, whether these variants have a deleterious effect is unknown. Although the authors claim that the variants are predicted to have a deleterious effect by in silico analyses, other reports have stated the opposite (Bakker et al. 2012 and Litim et al. 2012). Several algorithms or a more conclusive assessment of variant pathogenicity (such as functional assays) should be used.

We appreciate the Reviewer’s concern regarding the pathogenicity of the identified variants. As mentioned in the original manuscript (line 196) the pathological effects of the identified variants in cancer predisposition remain unknown and further functional investigation is required to substantiate the role of these variants in hyperdiploid pre-B childhood ALL (line 249).

We suspect that the potential effects on FA pathway destabilization that could lead to genomic instabilities and thus to an increased risk of leukemia within this family, rely on the combination of several inherited variants with limited individual penetrance. Functional assays mimicking these combinatorial effects are beyond the scope of this paper. However, as
requested by the Reviewer, we assessed the putative deleterious effect of the variants using additional in silico algorithms. We used Polyphen2 and Sift that predict the possible functional impact of a substitution based on the degree of conservation of the mutated position and physical structure modification of the protein [23,24]. We also used Fathmm that combines sequence conservation within hidden Markov models (HMMs), representing the alignment of homologous sequences and conserved protein domains, with "pathogenicity weights", representing the overall tolerance of the protein/domain to mutations [25]. Finally, we used SiPhy to detect bases under selection and identify elements that are potentially important for protein conformation and function [26].

The variant in FANCA rs61753269 was predicted to have damaging effects by Fathmm only however its high conservation score (SiPhy =12.742) and rare MAF <0.001 suggest that this variant is under strong functional constraint and therefore could have a specific role on protein conformation. Similarly, Litim et al. identified tolerated or benign effects of rs61753269 on FANCA protein function according to in silico analysis and showed no further impact of other missense variations in FANCA on protein function in vitro. We agree with the Reviewer that the functional role of FANCA rs61753269 in cALL warrants further investigation and this has been clearly stated in the paper (see below). FANCP/SLX4 variants were predicted to be deleterious by two of the three algorithms used here (Sift and Polyphen2) and rs79842542 also had a high conservation score (SiPhy =12.895). While Bakker et al. tested 4 SLX4/FANCP variants (p.P378T, p.E787K, p.R1550W and p.R1814C) in a Mitomycin C-induced growth inhibition assay and found neither to be distinguishable from wildtype, the variants reported in our study (p.G141W and p.R204C) have not, to the best of our knowledge, been tested in vitro. The rare GEN1 rs16981869 was predicted to be damaging by all three algorithms used here. Overall, these data support a functional role for these variants in disrupting the FA pathway and Holliday junction and as a result, they could lead to genomic instability and increased risk of ALL within this family, however functional assays are indeed required to confirm these observations.

The results of these analyses have been included in Table 1 (see above) and discussed in the revised manuscript:

- Starting line 145: "Variant frequencies were assessed using 1000 Genomes [20] and NHLBI GO Exome Sequencing Project (ESP) [21] databases. ANNOVAR [22] was used for non-synonymous SNV annotation. The effect of non-synonymous variants on protein conformation and function was assessed using Sift [23], Polyphen2 [24] and functional analysis through hidden markov models (Fathmm, version 2.3) [25]. Sift, Polyphen2 and Fathmm consider a variant as putatively damaging when it presents a score ≤0.05, ≥0.957 and <-1.5, respectively. SiPhy [26] was used to detect bases under selection using multiple alignment data from 29 mammal genomes; larger is the score, more conserved is the site."

- Starting line 172: "Among the identified variants, we identified a combination of missense variants in the FA gene FANCP/SLX4 (compound heterozygous at rs137976282 and rs79842542), corroborating the assumption of FA pathway destabilization (Figure 2). A more thorough investigation of the other FA pathway genes led then to the identification of a rare heterozygous variant in FANCA (rs61753269) that was also shared by the sibs. Although this variant was heterozygous, restricting the analysis to extremely rare variants allowed us to identify potentially deleterious non-synonymous variations in FA genes that could be contributing to inherited susceptibility to ALL in the sibs. For FANCP/SLX4, both parents
transmitted a putatively damaging allele to their affected offspring who were therefore compound heterozygous at rs137976282 (ESP and 1000 Genomes general population MAF <0.001) and rs79842542 (MAF =0.059 and 0.071 in 1000 Genomes and ESP general populations respectively). While two of the three in silico algorithms predicted that the compound heterozygous variants in FANCP/SLX4 were likely deleterious (Sift score =0 for both alleles and Polyphen2 score =1 and 0.964 for rs79842542 and rs137976282 respectively), only Fathmm predicted rs61753269 in FANCA to be damaging (Fathmm score =-1.78) (Table 1). Nevertheless, the high conservation score at FANCA rs61753269 (SiPhy =12.742), combined with its extreme rarity in the population (MAF <0.001 in 1000 Genomes and ESP), suggest that this variant is under strong functional constraint and therefore could have a specific role on protein conformation. Although not a Fanconi anemia gene per se, our exome data also revealed a rare non-synonymous homozygous variant in GEN1 (rs16981869, MAF =0.145394, ESP general population homozygous frequency q^2 =0.025), that was predicted to be deleterious by all three algorithms.

- Starting line 213: "Interestingly, the rare variants FANCP/SLX4 rs137976282 and FANCA rs61753269 have previously been identified in familial breast cancer cases [31-34], however their pathological effects in cancer predisposition remain unknown."

4. The siblings are reported to have a non-syndromic form of ALL. However, they are carriers of potentially deleterious variants in both alleles of FANCP/SLX4 which is consistent with Fanconi anemia diagnostic. ALL is rare in Fanconi anemia patients, but not unheard of and the lack of other symptomatology does not rule out the diagnostic. Therefore, the possibility that the siblings are Fanconi anemia patients exits and should be addressed.

We agree with the Reviewer that the possibility of an underlying FA condition exists and that we cannot fully exclude an undiagnosed disorder, however, according to medical investigation, both siblings were asymptomatic and have not been diagnosed with any ALL-linked genetic disorder. Therefore, this ALL family was considered as nonsyndromic. Following thorough genetic investigation, we identified potentially functional variants in FA genes segregating within the family and shared by the sibs. One may argue that pure, nonsyndromic ALL families are unlikely and that genetic interrogation of such families will ultimately reveal underlying inherited disorders associated with increased risk of ALL. Our results show that the study of familial (non-twinned siblings affected by ALL) or inherited forms of ALL can further our understanding of the genetic causes underlying more common, sporadic forms and shed light on otherwise asymptomatic genetic syndromes.

We have clarified this in the current version of the manuscript (starting line 254):

"Despite the fact that both siblings were asymptomatic and were not diagnosed with an ALL-linked genetic disorder, the possibility of an underlying FA condition exists and an undiagnosed disorder, although rare, cannot be excluded. One may argue that pure, nonsyndromic ALL families are unlikely and that genetic interrogation of such families will ultimately reveal underlying inherited disorders associated with increased risk of ALL. Indeed, our results show that the study of familial or inherited forms of ALL can further our understanding of the genetic causes underlying more common, sporadic forms and shed light on otherwise asymptomatic genetic syndromes."
The authors state that heterozygous carriers of mutations in FA genes can present malformations (line 193), although no bibliography is cited. This sentence should be revised, FA mutation carriers are not known to have any symptomatology.

While autosomal recessive FA patients are known to present with malformations [43,44], Welshimer and Swift reported that heterozygous carriers of a FA gene may be predisposed to some of the same congenital malformations or developmental abnormalities that are common among homozygotes. Given the consanguinity on the paternal side, resulting in multiple miscarriages and polymalformation syndrome in surviving offspring, we may speculate that homozygous or perhaps even segregating heterozygous FA mutations are contributing to the observed phenotypes; however this remains highly speculative without further genotype information on the extended family.

reference is now included in the revised manuscript (starting line 239):

"While autosomal recessive FA patients are known to present with malformations [43], it has been reported that heterozygous carriers of a FA gene may be predisposed to some of the same congenital malformations or developmental abnormalities that are common among homozygotes [44]. Although the sibs had no apparent physical abnormalities, family history revealed a consanguineous marriage on the paternal side (Figure 1) resulting in multiple miscarriages and polymalformation syndrome in surviving offspring. Given that both rare FANCP/SLX4 rs137976282 and FANCA rs61753269 variants were paternally inherited we could hypothesize an underlying recessive disorder affecting the FA pathway; however this remains highly speculative without further genotype information on the extended family."

Minor essential revisions


The modification has been made accordingly (starting line 166):

"Under a recessive disease model, we interrogated our exome data and identified shared non-synonymous mutations that were either compound heterozygous or homozygous variant (Table 1) and specifically screened genes associated with the leukemia predisposing syndrome FA (FANCA, FANCB, FANCC, FANCD1/BRCA2, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ, FANCL, FANCM, FANCN/PALB2, FANCO/RAD51C, FANCP/SLX4, FANCQ/XPF and FANCS/BRCA1)."

7. 180 and 181: Letter “w” is used in the equation and “q” in the explanation.

In the revised version of the manuscript we have chosen to remove this equation, as we do not feel that it adds to the paper. The siblings remain the only 2 individuals among the extended QcALL cohort to carry this particularly rare combination of FANCA rs61753269, FANCP/SLX4 rs137976282, rs79842542 and GEN1 rs16981869 variants. The manuscript has been modified as follows (starting line 196):

"Our in-house exome database of 369 individuals from our childhood ALL cohort (103 patient-
mother-father trios and 60 patients) from the QcALL cohort [27/28] (whole exome sequencing performed on Life Technologies SOLiD System or Illumina HiSeq 2500; data available upon request), revealed a single heterozygote patient at both FANCP/SLX4 positions, 0/369 variant allele carriers at FANCA rs61753269 and 3/369 carriers of the homozygous allele at GEN1 rs16981869 (1 patient and 2 parents). Interestingly, the only 2 other cases harbouring either both variants in FANCP/SLX4 or the homozygous variant in GEN1 were also diagnosed with hyperdiploid pre-B ALL, concordant with the sibship. Overall, the sibs were the only two individuals who carried this particularly rare combination of damaging alleles at FANCA rs61753269, FANCP/SLX4 rs137976282, rs79842542 and GEN1 rs16981869."

8. Line 187: "To date, 15 FA genes...". There are 17 FA genes described.

We thank Reviewer 2 for helping us to improve the accuracy of our manuscript and we updated the text accordingly:

- Starting line 166: "Under a recessive disease model, we interrogated our exome data and identified shared non-synonymous mutations that were either compound heterozygous or homozygous variant (Table 1) and specifically screened genes associated with the leukemia predisposing syndrome FA (FANCA, FANCB, FANCC, FANCD1/BRCA2, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ, FANCL, FANCM, FANCN/PALB2, FANCO/RAD51C, FANCP/SLX4, FANQC/XPF and FANCS/BRCA1)."

- Starting line 209: "To date, 17 FA genes have been identified and mutations within these genes have been shown to cause DNA repair defects leading to genomic instability and aneuploidy, characteristic of FA [29]."


As stated above, the reference has been included in the revised manuscript (starting line 239):

"While autosomal recessive FA patients are known to present with malformations [43], it has been reported that heterozygous carriers of a FA gene may be predisposed to some of the same congenital malformations or developmental abnormalities that are common among homozygotes [44]. Although the sibs had no apparent physical abnormalities, family history revealed a consanguineous marriage on the paternal side (Figure 1) resulting in multiple miscarriages and polyomalformation syndrome in surviving offspring. Given that both rare FANCP/SLX4 rs137976282 and FANCA rs61753269 variants were paternally inherited we could hypothesize an underlying recessive disorder affecting the FA pathway; however this remains highly speculative without further genotype information on the extended family."

10. Supplementary table S1: It is unclear why there are 4 different entries for the gene DNAH2 with different combinations of 2 SNPs.

We agree with Reviewer 2 that this was confusing. There are 4 SNPs in DNAH2 that give rise to 4 different compound heterozygous variants where one parent is heterozygous Aa at position x and homozygous reference BB at position y and the other parent is homozygous reference AA at position x and heterozygous Bb at position y with both sibs inheriting the variant alleles at positions x and y (Aa/Bb). Therefore, the sibs are compound heterozygous
at the following positions in DNAH2:

rs140035306/rs79350244 (GA/CA)
rs140035306/rs117465420 (GA/TA)
rs78354379/rs79350244 (AT/CA)
rs78354379/rs117465420 (AT/TA)

This has been clarified in the revised version and only one entry per variant is presented (see Table 1 above).

We have made extensive efforts to answer all questions and address the specific concerns that were raised. We believe that this manuscript will prove of high interest to the readership of BMC Cancer. We appreciate the opportunity to resubmit our manuscript to your journal and hope that you will find our contribution suitable for publication.

Sincerely,

Daniel Sinnett, Ph.D.
Professor
Department of Pediatrics
University of Montreal
Division of Hematology-Oncology
Sainte-Justine UHC Research Center
3175 Cote-Ste-Catherine
Montreal (Québec) H3T 1C5
Tel: (514) 345-4931 ext. 2990
daniel.sinnett@umontreal.ca