Editorial Comment:

Manuscript ID jmedgenet-2013-102149 entitled "A familial disorder of altered DNA-methylation" which you submitted to Journal of Medical Genetics, has been reviewed.

As you will see, all three external reviewers have raised major points of validity, in view of which the manuscript is not acceptable for publication in JMG in its current form. However, if you would like to submit a revision that satisfactorily addresses these points, we would be willing to re-evaluate your manuscript for publication in JMG. In addition, the editorial committee felt that the paper must be re-written in a more organised fashion, to make it clearer how the findings justify the conclusions. Please note that the revised submission may be sent for further review and there is no commitment to publication at this stage.

We hope that you will be able to resubmit in a timely manner. If we receive your revision after a period longer than three months, the editorial team may decide to treat it as a new submission and send it to new reviewers. In such a case, claims to originality will have to be evaluated in light of literature that has appeared in the interim.

**Answer: We thank the Editor for giving us the opportunity to submit a revised version of our manuscript. We have carefully addressed all comments of the reviewers as outlined in the point-by-point response below. Moreover, we have performed further exome sequencing in one additional affected individual as well as in the father of the affected children as requested. These data are now incorporated into the manuscript. Finally, taking into account the editors request and the reviewers’ comments we have re-written the manuscript in a more organized fashion. We hope that the included modifications lead to a clearer presentation and explain how the findings justify the conclusions.**

Reviewers' Comments to Author:

**Reviewer: 1**

Comments to the Author

A familial disorder of altered DNA-methylation

(Caliebe 2013: Journal of Medical Genetics)

This study investigates genetic and DNA methylation causes for unknown disorder causing complications in pregnancy between apparently healthy Turkish couple with no family history. No obvious chromosomal abnormalities were identified. Direct screening of candidate genes previously associated with imprinting defects found no mutations, apart from a NLRP7 variant. This variant has previously been documented in both heterozygous and homozygous forms in pregnancy complications. Although the variant exists in the dbSNP and 1000 genome. The mother, maternal grandmother, and two of the affected offspring are heterozygous for this variant. The authors suggested that this variant and polymorphic repeat on the other allele may have compound heterozygous effect. Both locus-specific and array-based DNA methylation analyses on the offspring showed defective methylation pattern at both maternally and paternally methylated regions. Although this is an interesting and important study, there are some questionable points that need to be clarified.

**Answer: We thank the reviewer for his/her overall positive evaluation and for considering our work as interesting and important study.**

1. The authors state that "Since the inheritance pattern could be most likely explained by a mutation in a maternal effect gene (p5, line 56)". However, the paternal contribution should not be ignored, especially because the hypomethylation at the paternally methylated regions (ICR1 and IG-DMR) is indicative of paternal effects. Exome sequencing was only carried out on the one affected child, mother and maternal parents, not the father or paternal grandparents. As for maternal side, possibility of the father carrying i) de novo mutation, ii) compound het, and iii) grandmaternally inherited variants not present in the grandfather, should be investigated. In addition, the father is from a consanguineous couple. Can you be sure that the maternal and paternal side of the families are completely unrelated?

**Answer: We agree that the paternal contribution has to be taken into account as well. Therefore, exome sequencing of the father and a second affected child (III-2) has been performed. These novel results are presented now in Suppl Table 9 and the findings are being discussed in a novel chapter introduced into the Results and Discussion section. Nevertheless, these novel data on the father and a second affected child also failed to conclusively unravel the underlying cause of the phenotype in the family. Despite several attempts we were unable to obtain DNA of the paternal grandparents.**

**With regard to a potential relation of the parents the couple was asked several times by different persons whether they are related and it was always denied. They also denied distant relationship of their families. Obviously, this does not ultimately rule out a (distant) relation. In order to unravel such a distant relation not known to the couple we used the data obtained by exome sequencing and calculated the overlap at variant calls between both parents. This was slightly higher (61.73%) as compared to unrelated pairs (range 54.43%-54.90%). Though this might suggest some inbred (or population) background in the couple we do not have control data of known consanguineous couples to further draw conclusions as to an inbred coefficient.**

2. What are the parental ages?

**Answer: In the first pregnancy the mother´s age was 30 years and the father´s age 29 years. In the last pregnancy their ages were 34 years resp. 33 years. This information has been added on the Supplementary Table S1 of the revised manuscript.**

1. P4, line 4: “Such familial occurrence of a multilocus imprinting disorder affecting both paternal and maternal imprints has yet been rarely described[13].”

-In addition to the ref13, Azzi et al 2009 (HMG) also found both maternal and paternal methylation defects and perhaps should be included.

**Answer: We thank the reviewer for this comment and have included the named paper as novel reference, which is now reference 13.**

2. Locus-specific DNA methylation analysis:

- Table 1, p10: Do the numbers in bracket represent the total methylation percentage? and why do some MLPA results not have these numbers?

**Answer: Indeed, the parenthesis gives the methylation values per locus. We have added this information to the legend of Table 1 and also included the missing values.**

-The Table 1 description says "21 imprinted loci" but on the p26 it says "17 known imprinted regions". This should be consistent.

**Answer: In the table the H19 and MEG3 are each shown with 3 loci, but they are each counted as one region. Thus, the 21 loci refer to 17 regions as outlined in the text. We have clarified this in the legend to table 1.**

3. LUMA:

- Supplementary Figure S1, P34: III-2 (pb) is listed twice in the graph. Are they representing technical replicates? or supposed to be a different tissue type?

**Answer: From patient III-2 two independent samples from peripheral blood were available. These have been analyzed separately. This is indicated now in the legend to Supplementary Figure S2.**

- P4, line 10: "performed LUMA in the three affected offspring and the parents". Why does the Fig. S1 show results for only two offspring?

**Answer: We thank the reviewer for pointing us to this inconsistency. As shown in the figure, LUMA has only been performed for patient III-1 and III-2 (and the phenotypically not affected parents). As LUMA is requiring considerable amounts of DNA, we have not performed the analysis in the third offspring due to material limitations. We have changed the text accordingly by stating: “we performed LUMA in two of the three affected offspring and the parents”.**

4. 27K array:

-p4, line23-26: Were your array results consistent with all the results from locus specific methylation analysis at 21 imprinted loci? (genes and direction of methylation)

**Answer: We and others have previously shown that there is a good concordance between the CpG levels determined by the 27K array and by other techniques. The same holds true for the present study. Nevertheless, consistency between the locus specific and array-based results is hard to prove as different CpGs are targeted. Indeed, only a very limited number of CpGs on the 27K array is located in differentially methylated regions of imprinted genes. These in turn do not necessarily overlap with the CpGs targeted by the locus-specific approaches like MS-MLPA or BS-pyrosequencing.**

**To answer the reviewers question we have mapped the CpGs on the 27K array to the CpGs recently described by Court et al. (Genome Research, 2014) as imprinted. A total of 132 of those CpGs were represented on the 27K array. T-test analysis was then performed to identify CpG loci differentially methylated between all patient samples and controls (FDR<0.05). The results are displayed in the following heatmap obtained by use of the Qlucore OMICS explorer (normalized to mean=0). Differential methylation for various imprinted genes can be seen to different extents in the various patient tissues. This is in agreement with the locus specific analyses.**



These results are displayed for the reviewer only as various studies already dealt with concordance of various techniques to determine methylation.

- p4, line 33. DNA methylation can also be age specific. Have your analysis also adjusted for subjects' ages? Monocyte number changes with age and as blood cells have different DNA methylation profiles (Reinius et al 2012) this would be problematic when using peripheral blood.

**Answer: We agree that DNA methylation of some loci is age related. Luckily, for most of our controls, in particular the controls for the embryonic tissues (controls for III-1 and III-3) we were able to analyze age and organ matched samples which were (in part) commercially available. However, getting a blood sample from a healthy (!) fetus or newborn is obviously difficult due to ethical considerations. Consequently, the peripheral blood samples were not age matched. Nevertheless, our approach of analysis across tissues corrects for this to a certain extent. We have included the information, that the peripheral blood controls were not age matched, in Supplementary Methods, chapter A Sample preparation.**

-p5, line15: Where are the Supplementary tables 4-6? Do they correspond to the tables of data starting the page p41 onwards? If so, they should be titled.

**Answer: The requested supplementary tables containing huge amount of data had indeed been included in the original submission. We initially tried to upload them as searchable xls-file for the reviewers’ and readers’ convenience. Nevertheless, this had not been possible. Thus, after some discussions with the editorial office and following the journal’s policies we finally submitted them as pdf. This unfortunately not only destroyed the structure but also resulted in a total of 2000 pages. Therefore you might not have received them. We have contacted the editorial office to find a way to provide the xls-files in addition to / instead of the PDFs.**

-only 18 genes overlapped with combined and tissue-specific analysis. This may just indicate the observed methylation differences in the combined analysis are largely due to tissue specific methylation profiles.

**Answer: We disagree here, as the initial comparison was controlled for the respective tissue. Nevertheless, the lack of overlap might be due to tissue-specific differences in differential methylation which would be well in line with the kind of mosaic methylation defect we describe.**

5. Are there any genotype-epigenotype correlations?

**Answer: We do not really understand which kind of analysis is intended here by the reviewer. We did not look into allele-specific methylation here.**

6. p2, line47: “that time only UPD7 was” to "maternal UPD7".

**Answer: The term “maternal” has been added accordingly.**

7. p25, line 35: “he learned to walked.” To "walk"

**Answer: We excuse for the mistake which has been corrected.**

8. The array table in supplementary contains "10-Sep" and "2-Mar" on the gene name column which looks like a excel typo.

**Answer: Thank you for pointing us to this issue. We have corrected these typos.**

**Reviewer: 2**

Comments to the Author

In this manuscript Almuth et al., describe a family with multiple-imprinting defects. This family has been used in previous studies describing the identification of RB1 imprinting and non-imprinted methylation anomalies. Therefore the work presented here is the third in a series utilizing this interesting family, describing the phenotype and molecular characterization of the parents, two fetuses and a child with suspected SRS/BWS.

 The manuscript contains an impressive amount of clinical data revealing that the two fetuses and child have features consistent with different imprinted syndromes and have high B-HCG characteristic of a molar pregnancy. In addition there is an impressive description of the DNA methylation profile of both imprinted and non-imprinted genes using numerous techniques. However, I feel the paper is let down slightly in the description of the NLRP7 aberrations in the mother (compound heterozygous for p.A719V and an unconfirmed promoter micro-deletion) which authors put much emphasis on.

 I think the manuscript is highly appropriate for the reads of JMG, and will be of interest to the imprinting community and researchers focusing on early miscarriage/molar pregnancies, but I have some queries before the manuscript is suitable for publication.

**Answer: We thank the reviewer for his/her overall positive evaluation and for considering our work as interesting and important study.**

Major comments:

- The p.A719V is listed in the 1000 genome project as a variant (it is rare and we do not know the phenotype of the individuals with the variant in this project). The evidence that it might be pathological is very weak as both IFT and PolyPhen scores are not high enough to be classes as deleterious. However Rima Slim’s group have described cases of women with poor reproductive history with this mutation. Based on these observations the authors should add a sentence arguing that the extent of defective imprinting in various tissues could account for the phenotype variability allowing them to speculate why the three pregnancies had different outcomes, specifically why child 111-2 phenotype seems milder and compactable with a live birth.

**Answer: We thank the reviewer for his/her proposal. Indeed, over the whole manuscript we have now down-tuned the role of the p.A719V. Moreover, in accordance with the reviewer’s proposal we have now included a sentence into the manuscript stating:** **Moreover, the extent and variation of DNA-methylation levels at imprinted loci suggested mosaicism for the DNA-methylation changes which could explain the phenotypic variability of the three offspring.**

- Without molecular clarification of the NLRP7 promoter micro-deletion there is no evidence for recessive NLRP7 mutations in the mother that manifests as maternal effect phenotype in her subsequent pregnancies. I do not understand how a hypomethylated probe (cg16106497) in the mother is indicative of a deletion? The authors maybe able to use the Infinium HumanMethylation27 array data to look at signal intensities (rather than methylation) to suggest a heterozygous deletion as similar approaches are now possible with the Infinium HumanMethylation450 array? Is it possible to use the R Script CHAMP designed specifically for copy-number analysis on their dataset?

**Answer: We thank the reviewer for his/her proposal. Nevertheless, not the hypomethylation detected by the HumanMethylation27 array was indicative for a deletion but the arrayCGH subsequently performed to investigate the reason for the hypometylation. Nevertheless, these analyses were complicated by the highly repetitive nature of the region with almost complete identity of the repeats we failed to obtain unambiguous results. As this fact also hampers the copy number analysis using the HumanMethylation27 array and as the resolution of the latter array would be much lower than that of the tilling array applied we refrained from adapting CHAMP to HumanMethylation27 arrays. To address the criticism we have moved the results and discussion on the potential deletion from the main manuscript into the supplementary files. We modified the main manuscript therefore into: “In this context it is remarkable, that in the array-based methylation analysis we noticed the mother (II-4) of the affected offspring to show a strong hypomethylation (normalised methylation value: -1.2) at one CpG (cg16106497) in the region containing the 5’ ends of both genes *NLRP2* and *NLRP7* as compared to controls (mean: 2.0, range: -0.2 to 3.9, see online supplementary figure S6). Though it is tempting to speculate that this variant reduces *NLRP7* expression so that the mother is functionally compound heterozygous at this locus a maternal dysfunction of *NLRP7* could not ultimately be proven (see online materials).”**

- As the authors mention, the NLRP2/NLRP7 is promoter interval is made of numerous duplication events making primer design/probe specific challenging. For example the cg16106497 probe hybridizes to two locations within the 19q13 interval. The authors state, “despite several experimental attempts we could not unambiguously prove the deletion”, but do not mention what techniques were utilized? Have they tried long-range PCR across the interval with primers located within single copy sequences? Southern blot?

**Answer: Indeed, we spent a huge amount of time and efforts to resolve this. Besides tiling arrayCGH we tried long-range PCR as well as RainDance based re-sequencing of the region. All these attempts failed to unambiguously decipher the underlying change. We refrained from describing all these experiments as essentially they were inconclusive and did not change the message of the manuscript. In accordance with the criticism of reviewer 3 we have now moved the discussion of the putative deletion from the main manuscript into the supplement as at the end it remains inconclusive at this stage (see answer to previous comment).**

- Was the CRCT2 mutation found by exome sequencing confirmed in the family?

**Answer: Indeed, we verified this mutation which appeared heterozygous in the mother. Re-analysis of the exome data including the novel exome data also did not call this mutation anymore homozygous. Thus, the information on this gene was omitted from the manuscript.**

Minor comments:

- The only hypermethylation observed in Table 1 is at the NESP promoter. The methylation status of this interval is dependent upon the primary GNAS imprint, with hypomethylation of the GNAS-DMRs causing the concomitant hypermethylation at the NESP somatic DMR. Therefore the statement in the first and last paragraphs on page 3 should be corrected.

**Answer: We agree with the reviewer and have changed this accordingly. We have included a sentence that the *NESP* hypermethylation is caused by *GNAS* hypomethylation and have substituted the previous statement by: “The only hypermethylation was observed for the *NESP* somatic DMR but this is most likely caused by hypomethylation of the *GNAS* DMR.”**

- The authors should discuss the role of NLRP7 in the establishment vs. maintenance of imprints in light of their data. Since both maternal and paternal imprints are partially affected then it is likely to be a maintenance issue, which is surprising since lack of maternal imprints in NLRP7 FBHM cases were originally hypothesized to be an establishment defect.

**Answer: In line with the comments of the reviewers we have down-tuned the importance of NLRP7. In the light of this, we have not further elaborated the discussion on NLRP7.**

**Reviewer: 3**

Comments to the Author

Comments to the authors:

General Comments:

The authors present an interesting patient with poor reproductive outcome and evidence of methylation abnormalities in products of conception.

While this is an interesting case, some of the research data are of suboptimal quality. Controls for methylation studies are not comprehensive enough (for example matched controls for the tissues of miscarried pregnancies are not presented). The rationale for analyzing NLRP7 and related genes is not clear considering the type of methylation abnormalities found (increased and decreased methylation on maternal and paternal imprints and on low CG content regions). The variant data related to NLRP7 are over-interpreted.

**Answer: While we thank the reviewer for sharing our view that this is an interesting family we strongly disagree on some of the other statements. Indeed, we took much care to use matched controls for the methylation studies regarding age and tissue. It needs to be considered, that some of the investigated fetal tissues are indeed hard to obtain. Despite this fact, we performed a matched analysis of the methylation whenever possible as we fully agree that this is mandatory. Nevertheless, some control tissues were not available in a matched way simply due to ethical issues. The rationale for analyzing NLRP7 is obvious from the respective literature. To the best of our knowledge, the genome-wide extent of methylation changes in developed fetuses / children with NLRP7 defect is completely unknown. Nevertheless, despite this disagreement with the reviewers’ view we have seriously taken the criticism and strongly down-tuned the discussion of the NLRP7 variant throughout the manuscript to avoid any over-interpretation.**

Specific Comments

P 1, line 24: “Targets” should be reworded, not a correct term in this context.

**Answer: We altered the sentence thus omitting “targets”. The sentence reads now: “Indeed, two evolutionary closely related genes located head-to-head in chromosomal region 19q13, *NLRP2* and *NLRP7*, have been associated with such “maternal effect mutations”.”**

 Line 26: Authors should note that in the referenced paper imprinting defects were not limited to 11p15, but also found at some other loci.

**Answer: The fact that one child described by Meyer and co-workers had also partial loss of methylation in PEG1 has been added. The sentence now reads: “A homozygous mutation in *NLRP2* has been described in a woman giving birth to siblings with BWS due to an imprinting defect in 11p15 and partial loss of methylation in *PEG1* in one child.”**

 line 29: Authors should add other references besides the paper by Murdoch et al [7] which is not the only one describing imprinting abnormalities in these pregnancies.

**Answer: We agree that the single reference of Murdoch does not reflect the full spectrum of research in this field. However, inclusion of all relevant references will not be compatible with the journal’s restriction to 20 references. Therefore we included a recent publication (Mahadevan et al., doi 10.1093) which gives a concise overview on the relevant work in this field. This reference has now been included as reference 7 together with the following statement: “for an overview see [7]”**

P 1 lines 41 etc. in the case report description: Please refer to the symbols in the pedigree associated with the described pregnancies for clarity.

**Answer: This has been carried out in the main text und supplement.**

 Line 51: bHCG should be spelled out (first description)

**Answer: Beta-human chorionic gonadotropin has been spelled out in the main text and in the supplementary information.**

 Lines 57 etc. and Figure 1A etc.: the paper would be enhanced by adding images of the histology, considering the described overlap in this family of cases with placental mesenchymal dysplasia and molar pregnancy. This can be as online supplemental data.

**Answer: An additional supplementary figure (now supplementary figure 1) with images of the histology of the pregnancies III-2 and III-3 has been added. We refer to the images both in the main text and the supplement.**

P2 Line 35: Replace the term “achieved” with “interpreted” or another suitable word. The signals are obtained by the hybridization itself, the software is only a tool to visualize and interpret them.

**Answer: Thank you for drawing our attention to this inaccuracy. We use “interpret” instead of “achieve”.**

P2 Line 44: I do not understand why authors refer to a phenotype in “either one of the parent”. This should be rephrased.

**Answer: We used “phenotype in either one of the parent” to underline that such a disorder could be caused by a chromosomal aberration in one of the parents. We rephrased the sentence to: “Conventional chromosome analyses in both parents and all three offspring, molecular karyotyping on both parents as well as individuals III-1 and III-2, and FISH analyses of chromosomes X, Y, 13, 18, and 21 performed on amniotic fluid samples of III-1 and III-2 lacked evidence for a chromosomal aberration as cause of the phenotype in the offspring III-1, III-2, and III-3.”**

P2 Lines 51 etc.: The authors see DNA methylation changes at both maternal and paternal imprints. It is therefore unclear why they considered NLRP7, NLRP2, and KHDC3L as candidate genes. These genes have been associated with DNA methylation changes at maternal imprints only in the vast majority of described cases and families.

**Answer: We agree with the reviewer that mutations in these genes have yet been only linked to changes in maternal imprints. Nevertheless, this does not preclude that this might be different in other families. As there have not yet been described any genes associated with disturbance of methylation at maternally and paternally imprinted or even other genes we still consider the named genes the obvious candidates. To clarify this, we have rephrased into: “Besides *NLRP2, NLRP7, ZFP57* and *KHDC3L* which have yet been predominately linked to changes of maternal marks at imprinted DMRs with high CpG content we considered DNMT1o as potential candidates.”**

P3, Line 19: Clarify to which “above shown molecular studies” you refer. This sentence is ambiguous.

**Answer: The sentence was changed to “In line with previous studies demonstrating the validity of the array(14), there was good agreement of the array-based results and the above shown results of locus specific analyses of imprinted loci.”**

 Line 29: Authors should clarify what GATHER maploc analysis is or add a reference for this.

**Answer: We regret that this was not obvious from the reference [15] which we already had included at the end of the sentence which is: JT Chang and JR Nevins. "GATHER: A Systems Approach to Interpreting Genomic Signatures." Bioinformatics 22(23), 2006. This references refers to GATHER maploc analysis. We have now put the citation immediately after “GATHER maploc”.**

 Lines 38 etc.: “Affected recurrently in more than one comparison” needs to be clarified in the text: is it more tissues in a particular individual, or more individuals, but the same tissue?

**Answer: Both interpretations are correct. Since not from all individuals sample tissue of identical origin was available, the comparisons were performed both, intra- as well as inter-individually.**

 Lines 57 etc.: The observation of both increased and decreased methylation is surprising. If a role for NLRP7, NLRP2 and KHDC3L is considered. As in my related comment above, the rationale for considering these genes needs to be better clarified.

**Answer: With regard to the candidate genes please see our comment above. Moreover, as outlined in our comment to reviewer 2 the *NESP* hypermethylation is caused by *GNAS* hypomethylation**

P4, Lines 7 etc.: same applies to the finding of enrichment for “low CpG content” sites. This would argue against a role for NLRP7/2 and KHDC3L. The regions with altered methylation described in families with identified mutations in these genes are imprinted DMRs with high CpG content.

**Answer: There are only limited (if at all existing) data on the pattern of DNA methylation changes outside well known imprinted loci in late gestational fetuses and born children associated to mutations in NLRP7/NLRP2 or KIHDC3L. We are not aware of any evidence for a global methylation pattern associated with these mutations outside the framework for moles. So we do not see why our findings argue against these genes.**

P4 Lines 19-20: refer to comments above regarding rationale for studying these genes.

**Answer: To refer to the reviewer’s criticism we modified the text to: “Besides NLRP2, NLRP7, ZFP57 and KHDC3L which have yet been predominately linked to changes of maternal marks at imprinted DMRs with high CpG content we considered DNMT1o as potential candidates.”**

 Lines 23 etc.: Although described by Deveault as pathogenic, the p.A719V is most likely a common, non-pathogenic variant. The comments regarding a stochastic effect are not very relevant considering that this is likely a benign polymorphism. Please refer to the following recent reference Prenatal Diagnosis 2013: Sep 17:6. PMID 24105752 for additional evidence that this is not a pathogenic mutation.

**Answer: As outlined above we have down-tuned the discussion of the NLRP7 variant. Moreover, we have included the named reference.**

 Lines 40 etc.: The authors need to better clarify the deletion and how they concluded that it is not on the maternal allele. Considering that it does not affect expression of NLRP2 or NLRP7, it is likely not relevant to the phenotype, and the data are over-interpreted. The interpretation that expression elsewhere may be altered is too much speculation for which there are no data.

**Answer: As outlined above we have down-tuned the discussion of the NLRP7 variant. Moreover, to address the comment of the reviewer and to avoid over-interpretation we moved the part on the possible deletion from the main text into the supplement.**

P5 Lines 10 etc.: The data should be compared to ethnicity matched data and evidence should be provided that variants were actually confirmed by Sanger sequencing (or at least a subset of them). Otherwise this could also be explained by suboptimal quality of the sequencing data.

**Answer: We have validated XXX of the mutations by Sanger Sequencing. Validation rate was …. With regard to the ethnicity we agree that it would be good to have ethnicity matched control data. Nevertheless, the couple originates from the Kurdish part of Turkey and neither population control data are to the best of our knowledge available nor do we have access to a control population.**

Figure 1A: The filled pedigree symbol should be on II, 4, as she is the proband carrying the disorder that causes her abnormal pregnancies or at a minimum use two types of shading.

**Answer: We fully agree with the reviewer that likely a defect in the mother causes the phenotype in the children. Nevertheless, phenotypically aberrant are yet only the offspring. Moreover, one of the other reviewers prompted us to also analyse the exome of the father inplying the hypothesis of a paternal cause of the phenotype. Thus, we have refrained from shading the mother’s symbol.**

Suppl. Methods: The controls in the table are inadequate. Comparing (in some tissues) an “n” of 1 is always going to give differences in methylation. It is also unclear if this was the sample size for all methylation assays or only the array. In the methods on the methylation profiling, it is not described how many replicates were done. This needs to be corrected.

**Answer: We have included as many age- and tissue-matched controls into the analyses as available. In this context it needs to be considered that it is close to impossible to get e.g. a considerable number of (healthy) fetal muscle tissues at a certain gestational stage, as not only tissue but also age needs to be corrected for. In order to correct for the – for some tissues – rare number of controls we have overlapped the independent results from various tissues. This minimizes the number of false positives. Moreover, we would like to point out that some of the results have meanwhile been confirmed by independent technology (Beygo et al., 2013). Thus, in our view the methylation analyses were most carefully conducted and the points raised by the reviewer have a priori been taken into account but were in part limited to achieve.**

In the Supplementary Results, please include information on the histology of the placenta that support the diagnosis of placental mesenchymal dysplasia and comment on this diagnosis in view of molar changes seen by ultrasound.

**Answer: We have added information on the histology including respective figures into the Supplementary Results (see Supplementary Figure 1 in the revised manuscript).**

Ref 5 (Kou et al) is not the reference that describes postulated imprinting of NLRP2. This should be [6] Bjornsson et al, 2008 Genome Res. PMID: 18369178. Authors should recheck accuracy of all references and numbering of references.

**Answer: We have changed the reference and checked the accuracy.**

A schematic figure that shows the relative size and location of the small deletion would enhance the paper (scan be either in main manuscript, which is preferred, or in supplemental data).

**Answer: As we have down-tuned this part and omitted the deletion from the main manuscript we have not included this additional figure.**

Supplementary Fig. 5: It is unclear what the significance of the CG hypomethylation is in view of the deletion, as it also does not seem to affect expression of NLRP7 and NLRP2. Please clarify better and also include this on the suggested schematic figure.

**Answer: Please see our comments to the other reviewers. As we have omitted all data on the putative deletion from the main manuscript and as to space constraints we have not further elaborated the discussion on this point.**